KOEN BOLHUIS

ON PSYCHOTIC PHENOMENA AND UNRULINESS:

CAPRICHO 26: YA TIENEN ASIENTO
(NOW THEY'RE SITTING PRETTY).

STUDIES ON THE CHILDHOOD RISK FOR SEVERE MENTAL ILLNESS

BUT I DO KNOW A KIND OF MADNESS THAT LIES LOW IN THE MIND, HALF-BURIED IN CONSCIOUSNESS

STELLINGEN HORENDE BIJ HET PROEFSCHRIFT

ON PSYCHOTIC PHENOMENA AND UNRULINESS:
STUDIES ON THE CHILDHOOD RISK FOR SEVERE
MENTAL ILLNESS

1. CHILDHOOD EMOTIONAL AND BEHAVIOURAL PROBLEMS AS EARLY AS AGE 3 YEARS ARE DEVELOPMENTALLY CONTINUOUS WITH SUBSEQUENT PSYCHOTIC EXPERIENCES IN PRE-ADOLESCENT CHILDREN. *(THIS THESIS)*

2. BOTH MATERNAL AND PATERNAL CANNABIS CONSUMPTION ARE ASSOCIATED WITH A HIGHER BURDEN OF OFFSPRING PSYCHOTIC EXPERIENCES AT AGE TEN YEARS, SUGGESTING A COMMON AETIOLOGY FOR CANNABIS USE AND PSYCHOTIC SYMPTOMS. *(THIS THESIS)*

3. ELEVATED GENETIC VULNERABILITY FOR SCHIZOPHRENIA IS ASSOCIATED WITH AN INCREASED RISK OF EXPOSURE TO EARLY-LIFE ADVERSITY. (THIS THESIS)

4. INCORPORATING THEIR MULTI-DIMENSIONALITY IS ESSENTIAL FOR ADVANCING THE SEARCH FOR THE NEUROBIOLOGICAL CORRELATES OF DISRUPTIVE BEHAVIOUR PROBLEMS IN CHILDHOOD. *(THIS THESIS)*

5. CALLOUS TRAITS IN CHILDREN ARE CHARACTERIZED BY WIDESPREAD MACRO- AND MICROSTRUCTURAL DIFFERENCES ACROSS THE BRAIN. (THIS THESIS)

6. ALL PEOPLE ARE NOT CREATED EQUAL. SOME HAVE REAL GIFTS AND TALENTS, AND SOME HAVE REAL PROBLEMS RIGHT OUT OF THE STARTING BLOCK. ONCE WE ACCEPT THAT, WE CAN'T DODGE THE RESPONSIBILITY FOR SOCIAL ACTION (TERRIE MOFFITT, 2018).

7. PROSPECTIVE STUDIES IN GENERAL POPULATION, HIGH-RISK AND CLINICAL SAMPLES CAN COMPLEMENT EACH OTHER IN THE DEVELOPMENT OF CREDIBLE CAUSAL INFERENCE ABOUT DETERMINANTS OF PSYCHOPATHOLOGY, IF FINDINGS ARE TRULY CONSISTENT ACROSS DESIGNS.

8. IT IS SHOCKING THAT SO LITTLE FINANCIAL OR POLITICAL PRIORITY IS GIVEN TO IMPROVING THE MENTAL WELL-BEING OF A GENERATION OF CHILDREN GROWING UP TODAY, A DISADVANTAGED GENERATION SUFFERING FROM DE-MEDICALISATION, BUDGET CUTS, AND CONTINUED SOCIETAL SITGMATISATION.

9. THE DIVISION OF PSYCHIATRY INTO CHILD PSYCHIATRY AND ADULT PSYCHIATRY IS ARBITRARY, AND TRANSITION PSYCHIATRY SHOULD BE A KEY FOCUS FOR BOTH CHILD AND ADULT PSYCHIATRISTS IN ORDER TO ACHIEVE BETTER PATIENT OUTCOMES.

10. IN ORDER TO ADDRESS DISPARATIES IN MENTAL HEALTH OUTCOMES ACROSS DISADVANTAGED MINORITIES, IT IS HIGH TIME FOR PSYCHIATRY TO ACKNOWLEDGE THAT IT IS NOT ONLY A NEUROBIOLOGICAL SCIENCE, BUT ALSO A SOCIAL SCIENCE.

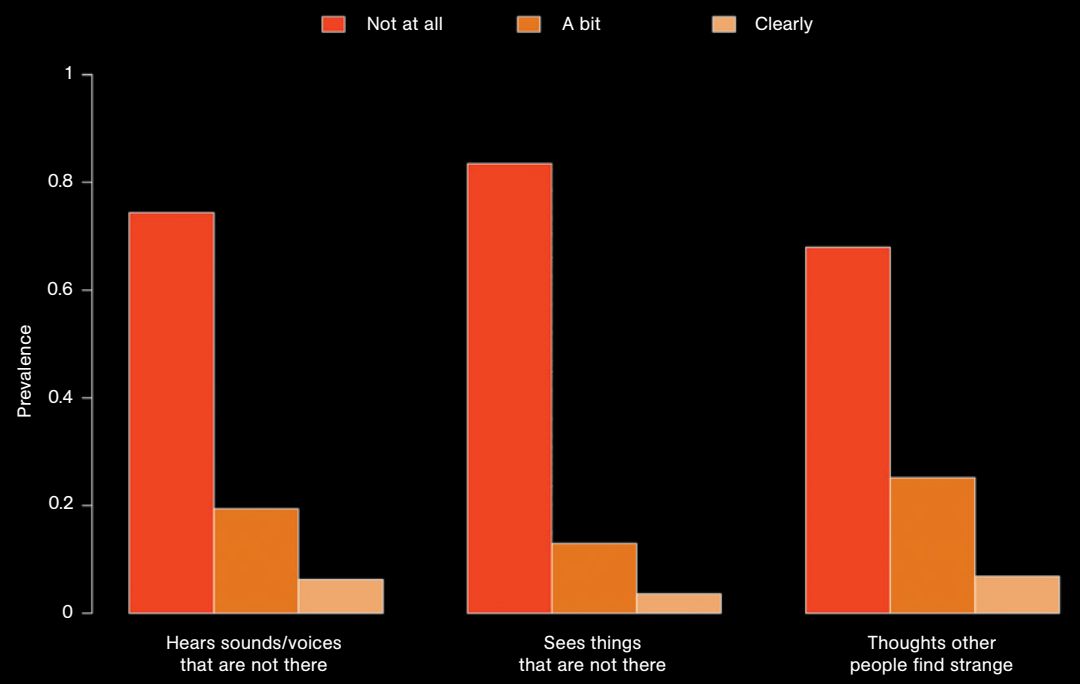
11. LE DÉFI AUQUEL NOUS FAISONS FACE AUJOURD'HUI, C'EST D'IMAGINER DES PERSPECTIVES D'AVENIR CENTRÉES SUR LES ÊTRES HUMAINS, QUI NOUS PARLENT DAVANTAGE ET RÉPONDENT À NOS ATTENTES. (AMINATA TRAORÉ, 2008, *TRANSLATION:* THE CHALLENGE THAT FACES US TODAY IS TO IMAGINE FUTURE PROSPECTS CENTERED ON HUMAN BEINGS, WHICH SPEAK TO US MORE AND WHICH MEET OUR EXPECTATIONS).

De Keisnijding (The Extraction of the Stone of Madness), 1494

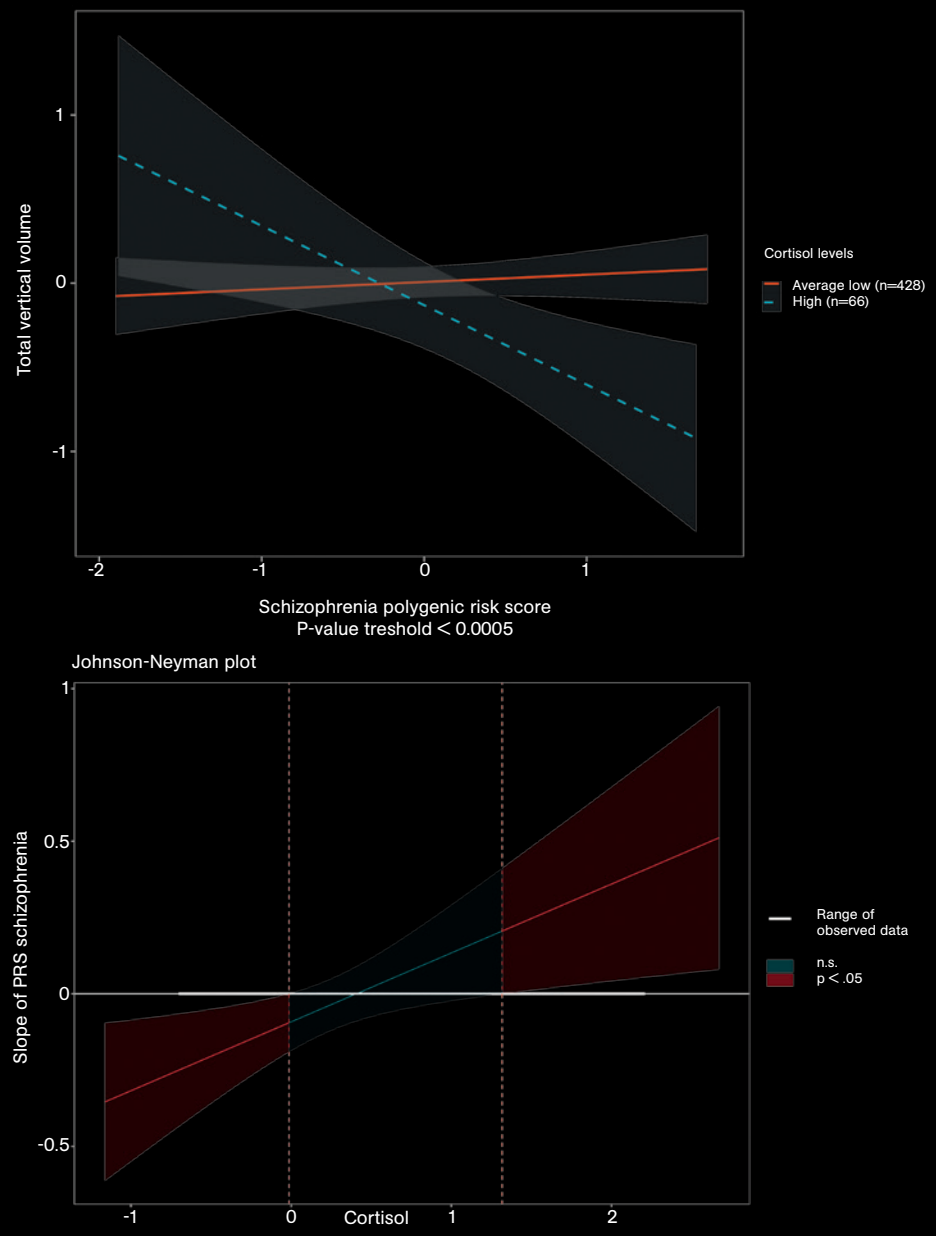
HIERONYMUS BOSCH

Ya Tienen Asiento (Now They're Sitting Pretty), 1799

FRANCISCO DE GOYA



Pertaining to Chapter 2 (page 57); Figure 2: Endorsement rates of the three self-reported psychotic-like experiences (N = 3984).



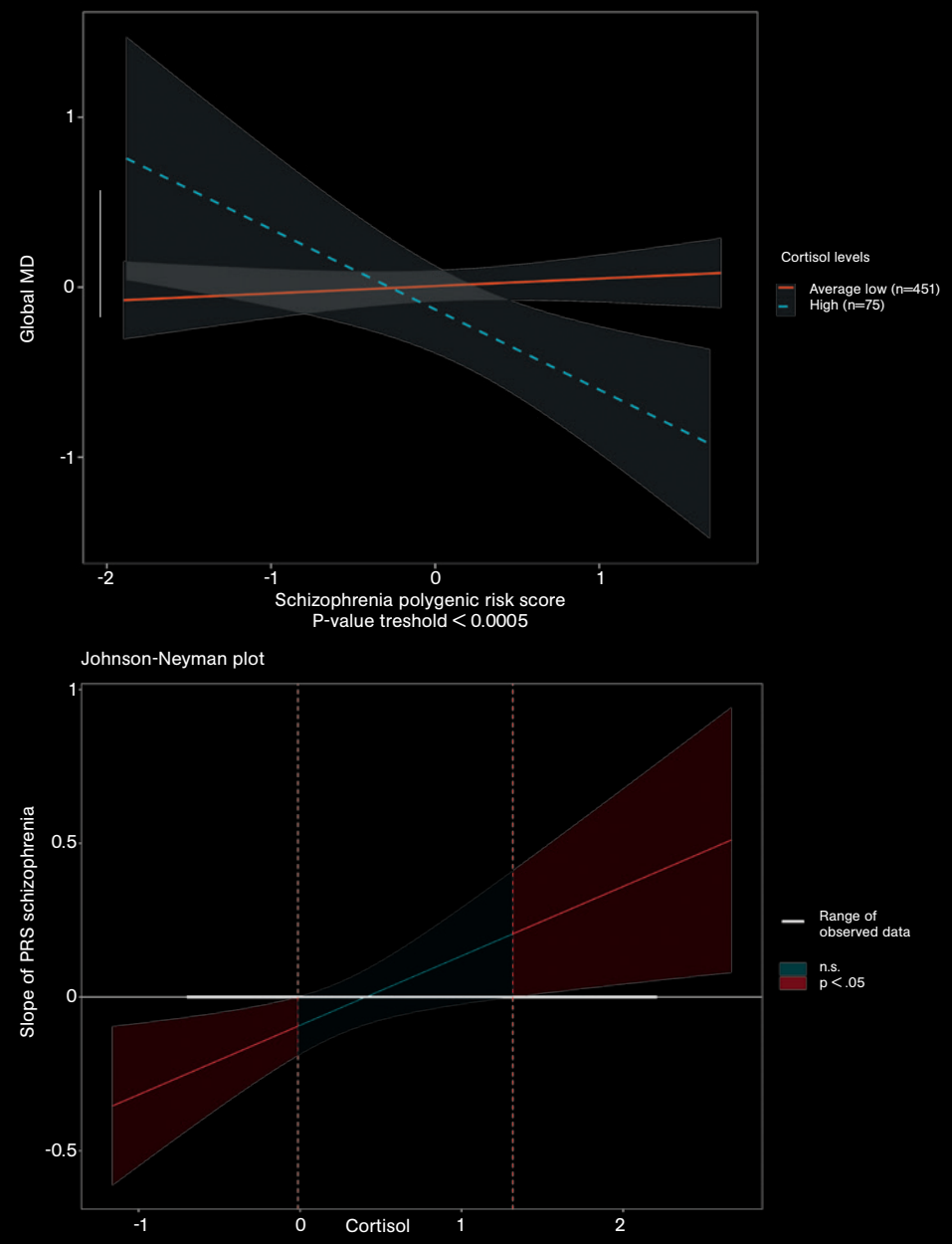
Pertaining to Chapter 5 (page 125); Figure 1: Relationship between schizophrenia polygenic risk score (PRS) and total ventricular volume as a function of hair cortisol level (left) and corresponding Johnson-Neyman plot (right).

Note: For simplicity of visualization, hair cortisol level was categorized into two levels: high (n = 66), one standard deviation above the total sample mean; average-low (n = 428), one standard deviation above mean and lower. The gray-shaded areas denote 95% confidence intervals. P-value threshold for the schizophrenia PRS is shown at Pt < 0.0005. Both schizophrenia PRS (x-axis) and total ventricle volume (y-axis) are standardized. Total ventricle volume was taken as a fraction of total intracranial volume. The right graph shows the Johnson-Neyman plots, which indicates at values of (log-transformed) cortisol below 0.36, the slope of schizophrenia PRS is significantly different from zero, and negative (turquoise shaded area).

Casa de Locos (The Madhouse; detail), 1812–1819.

Portrait d'une Jeunesse (Portrait of Youth), c. 1795.

ANNE-LOUIS GIRODET DE ROUSSY-TRIOSON

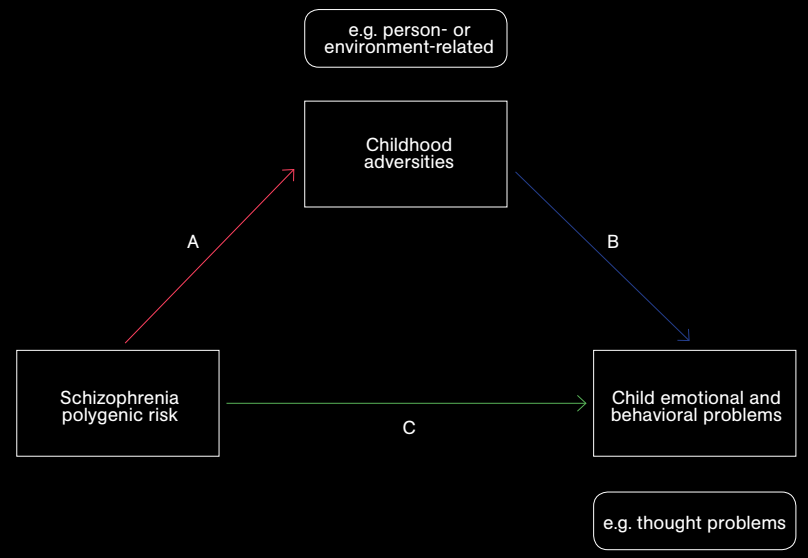


Pertaining to Chapter 5 (page 125); Figure 2: Relationship between schizophrenia polygenic risk score (PRS) and global mean diffusivity as a function of hair cortisol level (left) and corresponding Johnson-Neyman plot (right).

Note: For simplicity of visualization, hair cortisol level was categorized into two levels: low (n = 75), one standard deviation below the total sample mean; average-high (n = 451), one standard deviation below the mean and higher. The gray-shaded areas denote 95% confidence intervals. P-value threshold for the schizophrenia PRS is shown at Pt < 0.0005. Both schizophrenia PRS (x-axis) and global mean diffusivity (y-axis) are standardized. The right graph shows the Johnson-Neyman plots, which indicates at values of (log-transformed) cortisol below -0.01 and above 1.32, the slope of schizophrenia PRS is significantly different from zero, and negative respectively positive (turquoise shaded area).

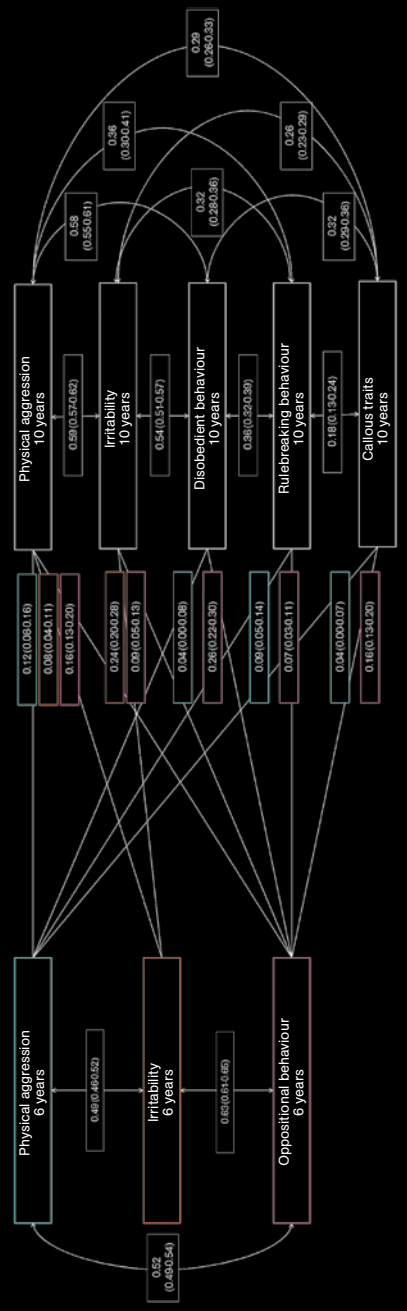
La Monomane de l'Envie (Insane Woman), 1822

THÉODORE GÉRICAULT



Pertaining to Chapter 6 (page 155); Figure 1: Conceptual mediation model

Note: Conceptual model of how childhood adversities might moderate the association of schizophrenia polygenic risk with child emotional and behavioral problems. Path a represent the gene-environment correlation, in which the child's genotype influences their risk for exposure to childhood adversities. Path b is the relationship between environmental exposure (i.e. childhood adversities) and behavior, whereas path c shows the direct effect of genotype on behavior, adjusted for the mediation effect (path a and path b combined).

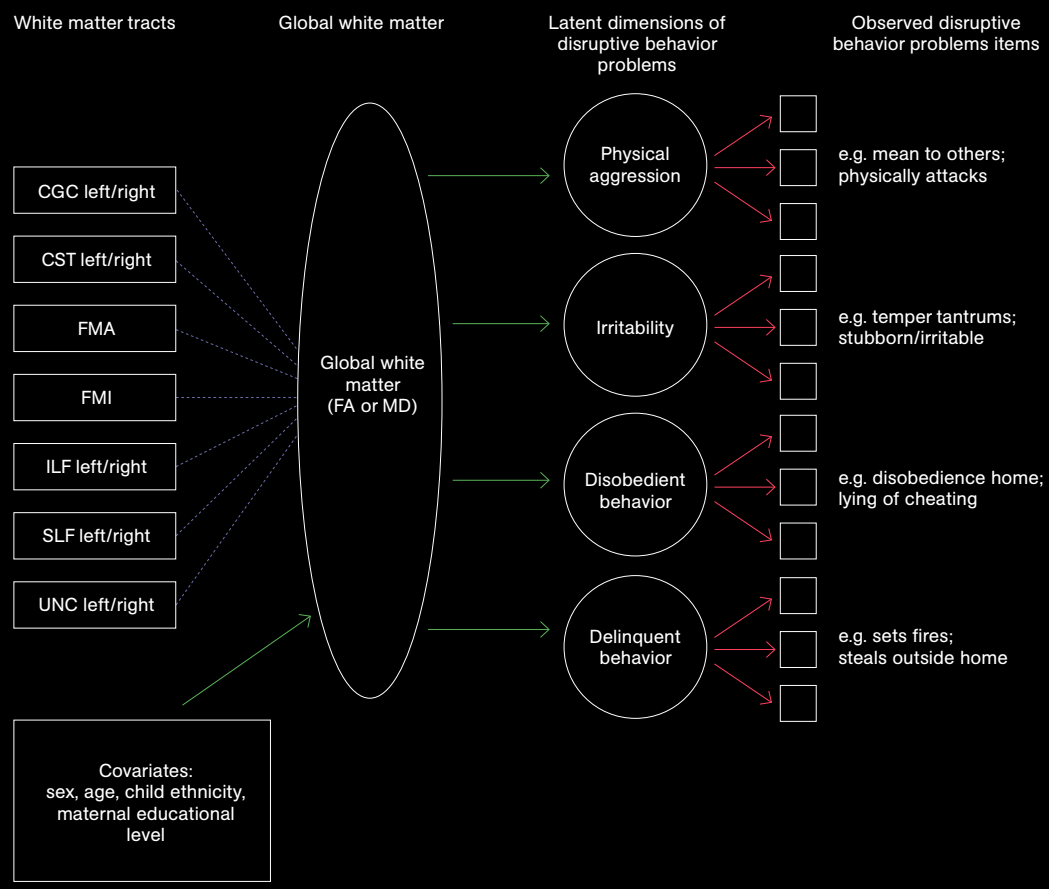


Pertaining to Chapter 7 (page 185); Figure 1: Cross-lagged model of cross-sectional and longitudinal associations between DBPs dimensions in the Generation R sample. Note: significant coefficients with 95% confidence intervals.

No Hubo Remedio (There Was No Help), 1799

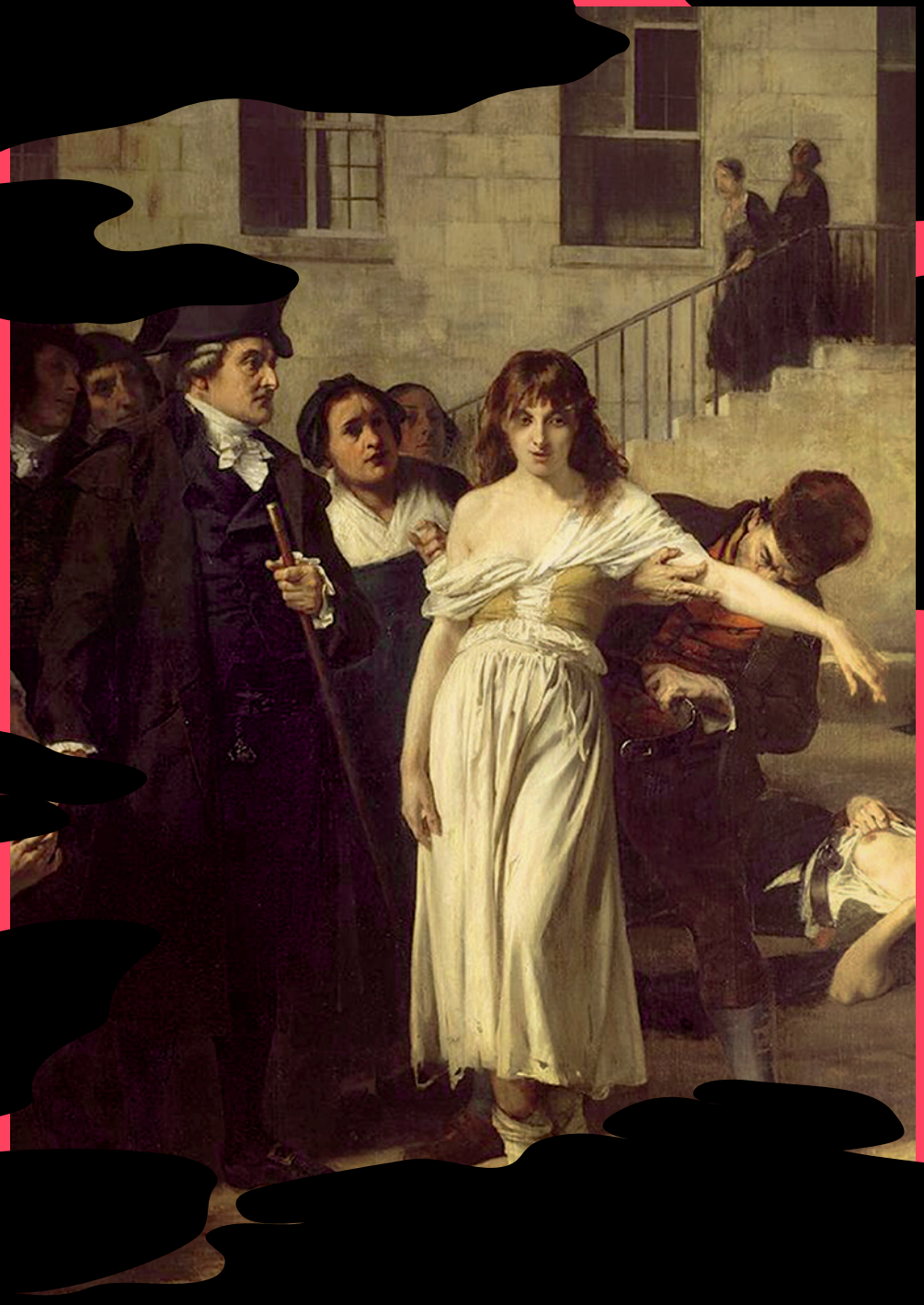
Het Narrenschip (The Ship of Fools), 1490–1500.

HIERONYMUS BOSCH



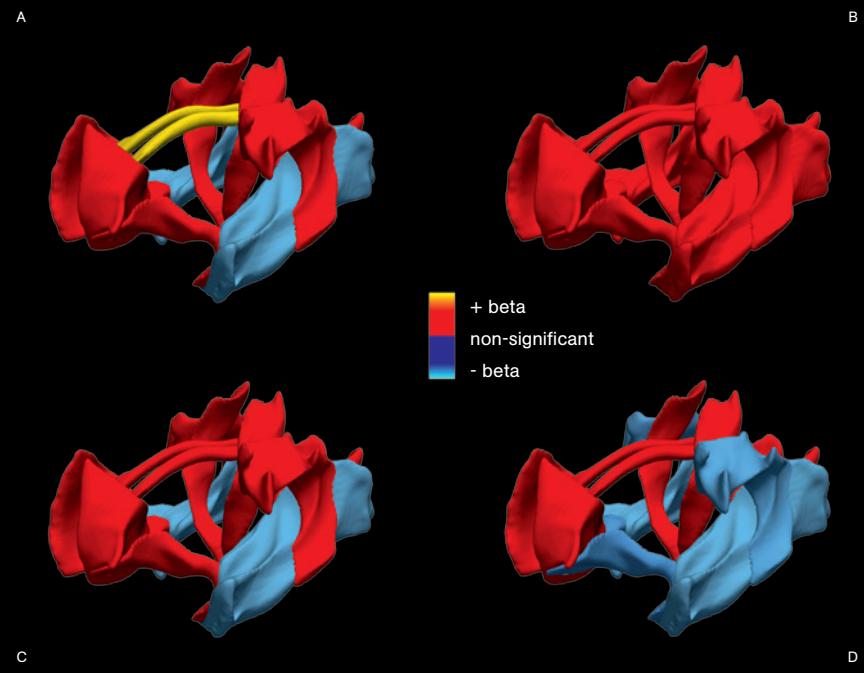
Pertaining to Chapter 8 (page 207); Figure 1: Outline of the structural equation model.

Note: Factor loading paths are depicted with dashed lines and structural equation regression paths are depicted with solid lines. For the sake of simplicity of the figure the interhemispheric correlations between white matter tracts (e.g. left and right uncinate) are not shown. In addition, all four disruptive behavior problems dimensions were allowed to correlate with one another, this is also not shown in the figure. CGC, cingulate gyrus part of cingulum bundle; CST, corticospinal tract; FMA, forceps major; FMI, forceps minor; ILF, inferior longitudinal fasciculus; SLF, superior longitudinal fasciculus; UNC, uncinate fasciculus; FA, fractional anisotropy; MD, mean diffusivity.

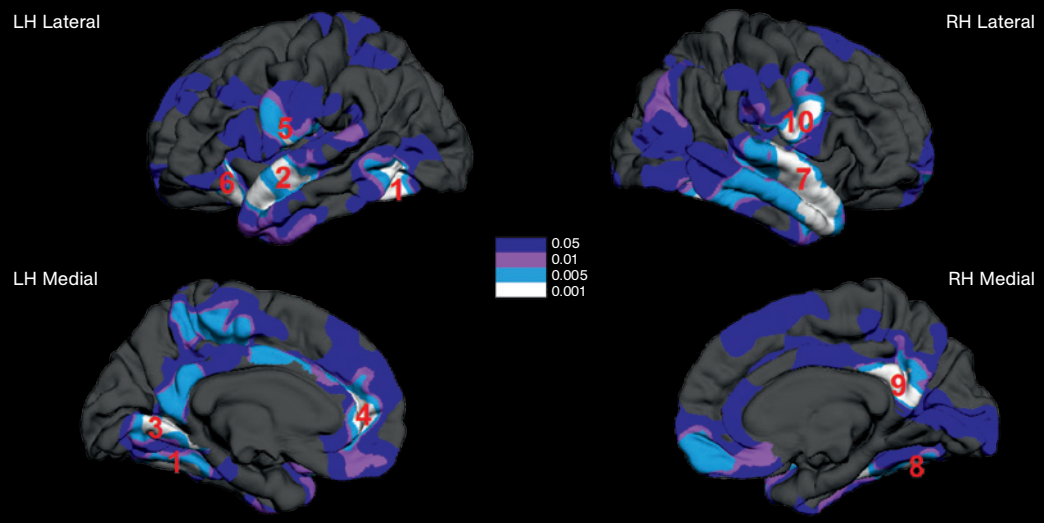


Pinel à la Salpêtrière (Pinel at the Salpêtrière), 1876.

TONY ROBERT-FLEURY

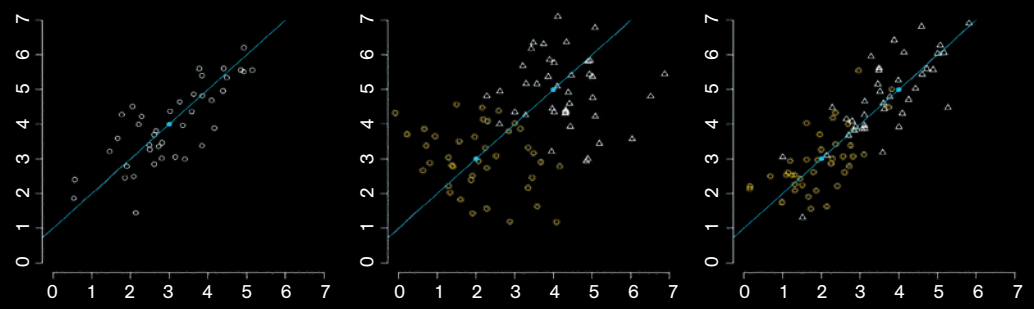


Pertaining to Chapter 8 (page 211); Figure 2: Associations between individual white matter tracts fractional anisotropy and dimensions of disruptive behavior problems: (A) physical aggression, (B) irritability, (C) disobedient behavior, (D) delinquent behavior. Nonsignificant associations are depicted in red, positive associations are depicted in yellow, and negative associations are depicted in blue.



Pertaining to Chapter 10 (page 248); Figure 1: Negative associations between cortical surface area and callous traits (N = 2146)

Note: Analyses are corrected for age, sex, child ethnicity and maternal educational level. Colors represent the cluster forming thresholds. Blue clusters represent a negative correlation between cortical surface area and callous traits at a cluster-wise corrected P-value threshold of <0.05, with transition to light-blue, purple and white for clusters that are negatively correlated with callous traits at more stringent P-value thresholds (i.e. 0.01, 0.005, 0.001, respectively; see legend in Figure). LH: left hemisphere; RH: right hemisphere. Numbers of the clusters correspond to the numbers shown in Table 3.



Pertaining to Chapter 12 (page 305); Figure 1: Covariation due to a continuous latent factor (left panel), due to mean differences between latent classes (middle panel), or due to both continuous factors and latent class differences (right panel). Adapted from Lubke, G. 2012. Mixture Modelling in Mplus.

–Yes, my dear Wilhelm,
nothing on earth is closer to my heart than children. (...)

(...)*When I watch them and see in the smallest of creatures the seeds of all the virtues and strengths* (...)

(...) *they will one day need so badly;* (...)

ON PSYCHOTIC PHENOMENA AND UNRULINESS:
STUDIES ON THE CHILDHOOD RISK FOR SEVERE
MENTAL ILLNESS

OVER PSYCHOTISCHE ERVARINGEN EN WEERBARSTIGHEID:
ONDERZOEKEN NAAR HET KINDERLEEFTIJDSRISICO OP
ERNSTIGE PSYCHIATRISCHE AANDOENINGEN

PROEFSCHRIFT

TER VERKRIJGING VAN DE GRAAD VAN DOCTOR AAN DE
ERASMUS UNIVERSITEIT ROTTERDAM
OP GEZAG VAN DE
RECTOR MAGNIFICUS

PROF.DR. R.C.M.E. ENGELS

EN VOLGENS BESLUIT VAN HET COLLEGE VOOR PROMOTIES
DE OPENBARE VERDEDIGING ZAL PLAATSVINDEN OP

WOENSDAG 20 FEBRUARI 2019 OM 15:30 UUR

DOOR

KOEN BOLHUIS
GEBOREN TE AMERSFOORT, NEDERLAND

ERMASMUS UNIVERSITY ROTTERDAM

PROMOTIECOMMISSIE

PROMOTOREN

PROF. DR. H.W. TIEMEIER
PROF. DR. S.A. KUSHNER

OVERIGE LEDEN

PROF. DR. N.E.M. VAN HAREN
PROF. DR. J. J. VAN OS
DR. I. KELLEHER

PARANIMFEN

ELIZE KOOPMAN-VERHOEFF
JENTIEN VERMEULEN

KOEN BOLHUIS

ON PSYCHOTIC PHENOMENA AND UNRULINESS:

STUDIES ON THE CHILDHOOD RISK FOR SEVERE MENTAL ILLNESS

CAPRICHO 26: YA TIENEN ASIENTO
(NOW THEY'RE SITTING PRETTY)

THE MIND, HALF-BURIED IN CONSCIOUSNESS

BUT I DO KNOW A KIND OF MADNESS THAT LIES LOW IN WHICH LIVES IN PARALLEL TO SANITY, AND GIVEN THE RIGHT CIRCUMSTANCES OR EVEN JUST HALF A CHANCE, CREEPS LIKE A LICK OF FLAME OR A GROWING TUMOUR UP AND AROUND ORDINARY PERCEPTION, CONSUMING IT FOR A WHILE, AND CAUSING ONE, EVEN WHEN NOT AT THE MOVIES, TO QUAKE IN FEAR OF THE WORLD AND PEOPLE AND WHAT THEY – I MEAN, OF, WE – ARE CAPABLE OF.
— JENNY DISKI, 2002, STRANGER ON A TRAIN

(…) *when I see their obstinacy as future resolution and firmness of character,* (…)

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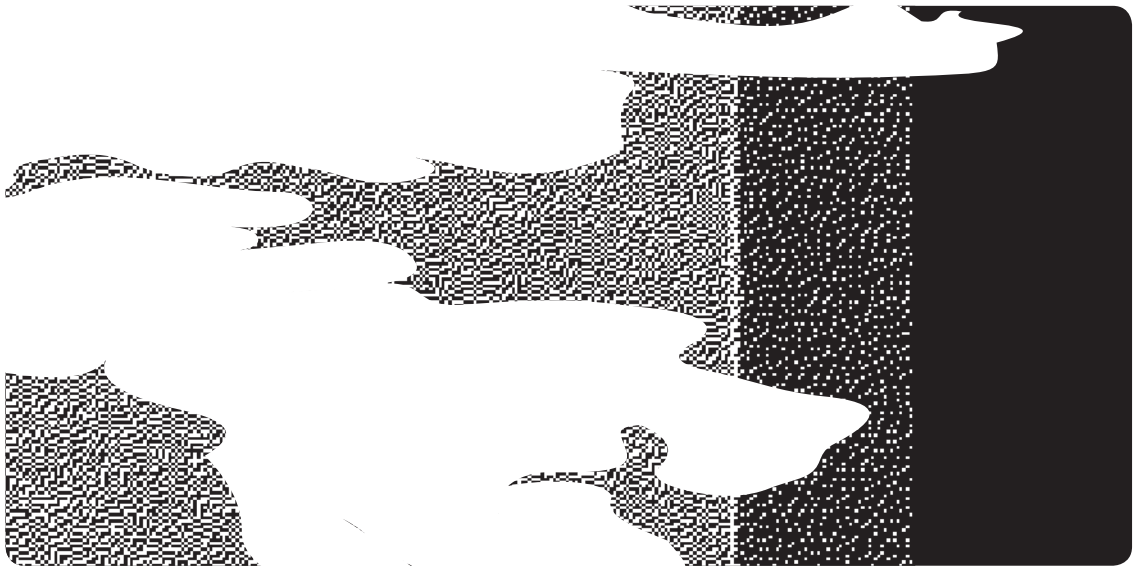


(...) *and their caprice as good humour and that light touch* (...)

MANUSCRIPTS THAT FORM THE BASIS OF THIS THESIS

(...) *which makes it so easy to negotiate the troubles of life, and all of it so unspoilt, so intact!* (...)

CHAPTER 2

Bolhuis K, Koopman-Verhoeff ME, Blanken LME, Cibrev D, Jaddoe VWV, Verhulst FC, Hillegers MHJ, Kushner SA, Tiemeier H. Psychotic-like experiences in pre-adolescence: what precedes the antecedent symptoms of severe mental illness? *Acta Psychiatrica Scandinavica,* 2018, 138 (1): 15–25.

[redacted]

CHAPTER 3

Bolhuis K, Kushner SA, Yalniz S, Hillegers MHJ, Jaddoe VWV, Tiemeier H, El Marroun H. Maternal and paternal cannabis use during pregnancy and the risk of psychotic-like experiences in the offspring. *Schizophrenia Research,* in press.

[redacted]

CHAPTER 4

Koopman-Verhoeff ME, Bolhuis K, Cecil CAM, Kocevska D, Hudziak JJ, Hillegers MHJ, Mileva-Seitz V, Reis IK, Duijts L, Verhulst FC, Luijk MPCM, Tiemeier H. During day and night: childhood psychotic experiences and objective and subjective sleep problems. *Schizophrenia Research,* in press.

[redacted]

CHAPTER 5

Bolhuis K, Tiemeier H, Jansen PR, Muetzel RL, Neumann A, Hillegers MHJ, van den Akker ETL, van Rossum EFC, Jaddoe VWV, Vernooij MW, White T, Kushner SA. Cortisol by schizophrenia polygenic risk moderation and pre-adolescent brain structure. Submitted for publication.

CHAPTER 6

Bolhuis K, Steenkamp LR, Blanken LME, Jansen PR, Hilleger MHJ, Cecil CAM, Tiemeier H, Kushner SA. Schizophrenia polygenic risk scores, childhood adversities and behavior in the general pediatric population: evidence of gene-environment correlation. To be submitted.

[redacted]

CHAPTER 7

Bolhuis K, Lubke GH, van der Ende J, Bartels M, van Beijsterveldt CEM, Lichtenstein P, Larsson H, Jaddoe VWV, Kushner SA, Verhulst FC, Boomsma DI, Tiemeier H. Disentangling heterogeneity of childhood disruptive behavior problems into dimensions and subgroups. *Journal of the American Academy of Child and Adolescent Psychiatry,* 2017, 56(8): 678–686.

[redacted]

CHAPTER 8

Bolhuis K, Muetzel RL, Stringaris A, Hudziak JJ, Jaddoe VWV, Hillegers MHJ, White T, Kushner SA, Tiemeier H. Structural brain connectivity in childhood disruptive behavior problems: a multi-dimensional approach. *Biological Psychiatry,* 2018, in press.

[redacted]

CHAPTER 9

El Marroun H, Bolhuis K, Franken IHA, Jaddoe VWV, Hillegers MHJ, Lahey BB, Tiemeier H. Prenatal cannabis exposure in relation to behavioural and emotional problems in childhood ; a multi-informant and prospective population-based study. *International Journal of Epidemiology,* 2018, in press.

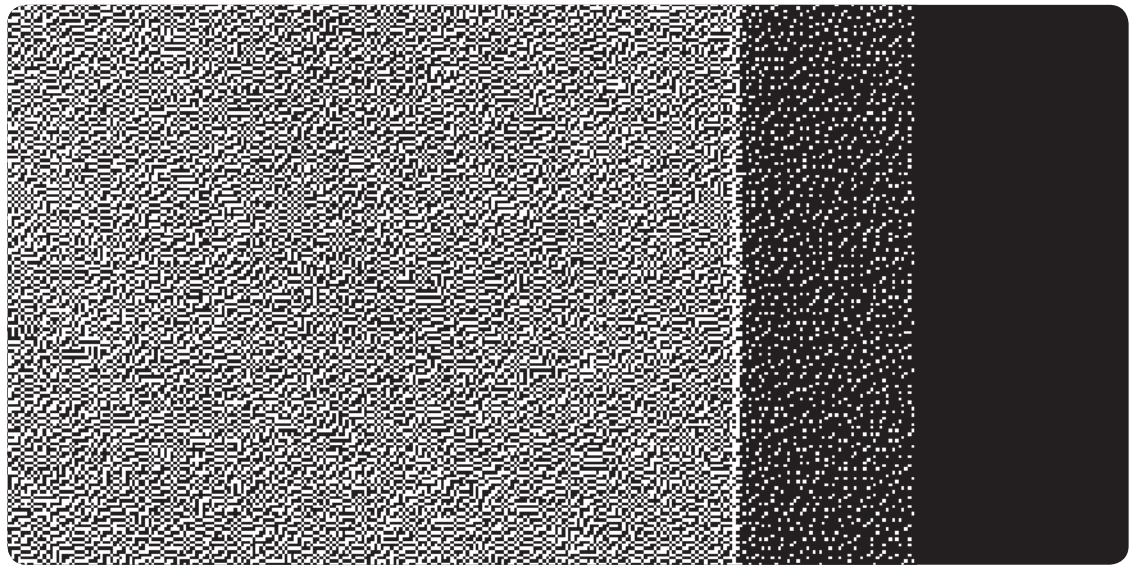
CHAPTER 10

Bolhuis K, Viding E, Muetzel RL, El Marroun H, Kocevska D, White T, Tiemeier H, Cecil CAM. Neural profile of callous traits in children: a population-based neuroimaging study. *Biological Psychiatry,* in press.

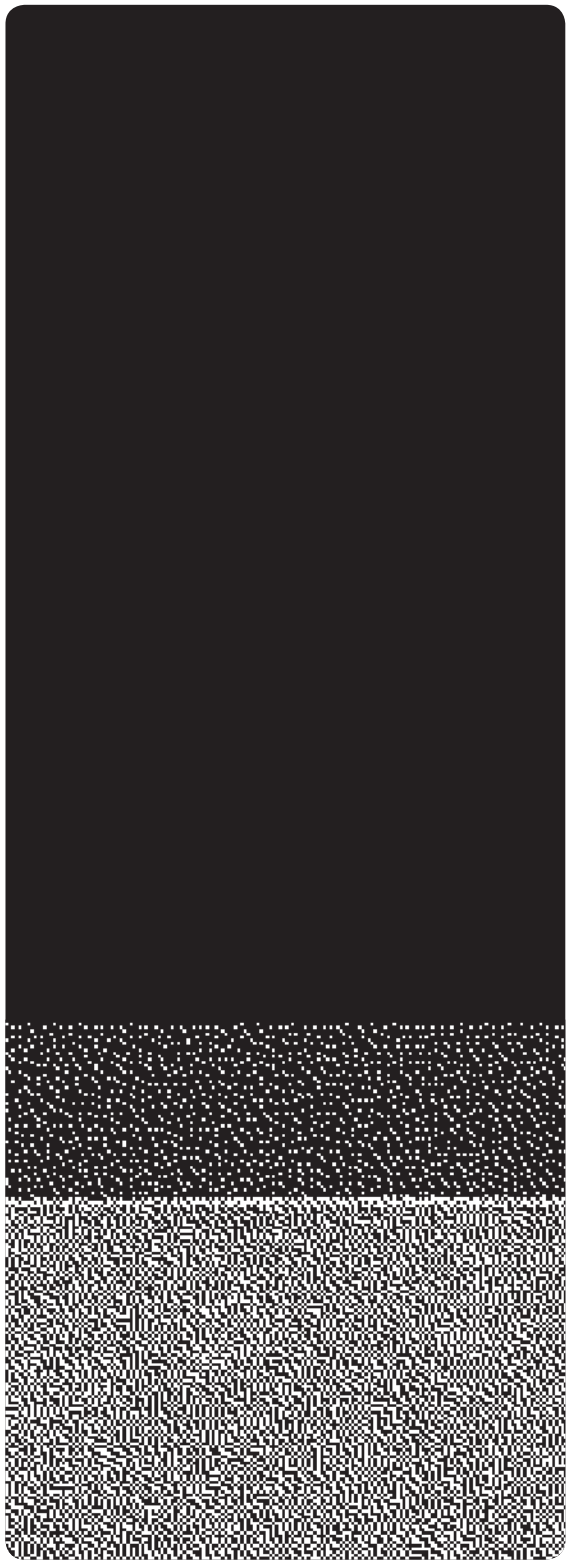
[redacted]

CHAPTER 11

Derks IPM*, Bolhuis K*, Yalcin Z, Gaillard R, Hillegers MHJ, Larsson H, Lundström S, Lichtenstein P, van Beijsterveldt CEM, Bartels M, Boomsma DI, Tiemeier H, Jansen PW. Association between childhood aggression and BMI: results from three population-based cohorts. Conditionally accepted in *Obesity.*
*Contributed equally



(Johann Wolfgang Goethe, 1787, The Sorrows of Young Werther)



CHAPTER 1

GENERAL INTRODUCTION

– Your senses become extraordinarily keen and acute. Your sight is infinite.
Your ear can discern the slightest perceptible sound, even through the shrillest of noises. (...)

RATIONALE

> Yes, my dear Wilhelm, nothing on earth is closer to my heart than children. When I watch them and see in the smallest of creatures the seeds of all the virtues and strengths they will one day need so badly; when I see their obstinacy as future resolution and firmness of character, and their caprice as good humour and that light touch which makes it so easy to negotiate the troubles of life, and all of it so unspoilt, so intact!
>
> (JOHANN WOLFGANG GOETHE, 1787, THE SORROWS OF YOUNG WERTHER)

Many great thinkers have appreciated the significance of childhood development as a stepping stone for how people will be and behave in their adult lives. We know this phenomenon is relevant when development goes well, but this might be even more important when things go wrong. It has been widely acknowledged by many people that those individuals, who suffer from the troubles of the mind, usually had signs of these difficulties very early in life. For example, the same sorrowful young Werther quoted above has been accused by his Lotte, with whom he is madly – and unrequitedly – in love, to have been a difficult man for all of his life: "Oh, why did you have to be *born* [italics added] with this intense spirit, this uncontrollable passion for everything you are close to!". Similarly, Holden Caulfield from J.D. Salinger's *Catcher in the Rye* has been plagued by his emotional difficulties (and has been causing some difficulties…) ever since he was little. What is more daringly, as it is not very self-evident that such topics are discussed among children, is that more examples about the complexities of the mind can also be found in children books, such as in works by Thea Beckman or Astrid Lindgren (who famously said "I want to write for readers who can perform miracles. Only children perform miracles when they read"). Thus, it is safe to say that a large number of writers has stressed the importance of childhood for future well-being and mental health. In parallel, many researchers have also stated the importance of studying mental illness from the perspective of risk in early childhood and adolescence. Their hopes were (and are!) to have a better understanding of human development and to come up with adequate prevention and therapy options for people who need it.

By now it is well-known that a great majority of adults suffering from mental illness has had mental health problems in childhood or adolescence. Studies of clinical adult populations reporting back on their prior mental health (Kessler et al., 2005), as well as prospective studies that have followed children into adulthood demonstrated that approximately 50-70% of adults with a psychiatric disorder had a disorder earlier in life (Copeland, Shanahan, Costello and Angold, 2011; Copeland et al., 2013). Mental disorders such as depression, schizophrenia, and substance use are leading causes of functional disability across the globe (Global Burden of Disease Study, 2015), and a particularly large burden of mental illness is observed in young people (Gore et al., 2011). It is beyond dispute that a more complete comprehension of the developmental pathways of these serious mental health problems is paramount for the improvement of young people's healthy development into adulthood. A better understanding of such serious mental health problems in young people will be beneficial for the implementation of improved service planning, such as early intervention and prevention.

Unsurprisingly, recognition of this burden of impairment has intensified research on potential risk factors and novel treatments for psychiatric disorders in young people. Yet, still relatively little is known about (1) the differences between people who have the same psychiatric diagnosis (i.e. phenotypic heterogeneity), and (2) which genetic and neurobiological factors affect the development of psychiatric problems. Importantly, mental health research still tends to focus on the most severely affected help-seeking individuals with a psychiatric diagnosis. More recent approaches stress the importance of looking beyond (historically defined) clinical categories; studying

mental health across the lifespan; and employing various complementing methodologies in order to more comprehensively study mental health (such as highlighted by the RDoC initiative) (Insel et al., 2010). One way to address these issues would be to study the early symptoms, which not necessarily meet diagnostic thresholds, of severe mental illness at a young age. Such psychiatric symptoms would fall on a continuum over severity, i.e. most children have no or a few symptoms whereas others are more affected. The latter group of children might meet criteria for a clinical psychiatric diagnosis, whereas some children might not meet these criteria even though they exhibit sub-clinical distressing symptoms. Studying these sub-clinical psychiatric symptoms is beneficial as it circumvents selection biases, which are inherent to the examination of clinically recruited help-seeking individuals. This is an important problem that we need to address, as valuable information on the child's full spectrum of behaviours and emotions is lost when research is only focused on children who fulfil criteria for clinical disorders. Two such key phenotypes which cause significant impairment to young people comprise psychotic phenomena and disruptive behaviour problems, and these will constitute the main themes of this thesis.

Psychotic phenomena in children will be discussed in part I of this thesis. Sub-clinical psychotic experiences are very common in childhood, whereas clinical diagnoses of psychosis or schizophrenia are rarely made (Nicolson and Rapoport, 1999). These experiences include hallucinatory phenomena such as hearing or seeing things that are not actually there and delusional thoughts, and they have a general population prevalence of up to 17% in children aged 9-12 years (Kelleher et al., 2012). Psychotic experiences have repeatedly been shown to increase the risk of subsequent psychotic as well as non-psychotic disorders, including severe psychiatric outcomes such as suicidal behaviour (Poulton et al., 2000; Kelleher et al., 2013; McGrath et al., 2016). More than 90% of 11-year-olds who experienced psychotic symptoms were diagnosed with at least one psychiatric disorder by age 38 years (Fisher et al., 2013). And although psychotic experiences generally do not meet criteria for a clinical diagnosis, they signal greater symptom impairment, e.g. higher comorbidity frequency and poorer prognosis. In this thesis we will focus on psychotic experiences in children, with the aim to have a better developmental understanding of which children are at elevated risk for poorer global functioning, beyond what can be explained by a clinical (psychotic) disorder (Healy et al., 2018).

Another illustrative example, which we will address in part II of this thesis, regards disruptive behaviour problems in children. Disruptive behaviour problems include a variety of behavioural symptoms which are directed towards other (i.e. the external environment), such as physical aggression, temper outburst, and rule-breaking. Disruptive behaviours, when they reach the impairment level of clinical diagnoses such as conduct disorder (CD) or oppositional defiant disorder (ODD), are the most prevalent antecedents of psychiatric disorder in adulthood: 25% to 60% of adults with a psychiatric disorder had a disruptive behaviour disorder in childhood (Kim-Cohen et al., 2003). In particular, when sub-clinical symptoms of disruptive behaviours are also considered, the prediction for future impairment is even greater (Tremblay, 2010; Coghill and Sonuga-Barke, 2012). However, most studies on the heterogeneity and aetiology of the broad spectrum of disruptive behaviours problems have to date largely focussed on clinical cases. Here, in this thesis, we will employ a dimensional perspective to investigate the heterogeneity of disruptive behaviour problems and to study their neurobiological correlates.

To summarise, psychotic experiences and disruptive behaviour problems in children thus have high predictive value for future psychiatric impairment. This merits further investigation of their development and aetiology. Hence, in this thesis, we focus on these two manifestations of childhood risk of future severe mental illness, which we will each describe in more detail below.

PART I: ON PSYCHOTIC PHENOMENA

> Hearing voices no one else can hear isn't a good sign, even in the wizarding world.
>
> (J.K. ROWLING, 1998, HARRY POTTER AND THE CHAMBER OF SECRETS)

Psychotic disorders have a typical onset in late adolescence or early adulthood and affect 1-3% of the population. However, hallucinations and delusions, which are classically regarded as symptoms of psychosis, are common continuous occurrences in the general population. Recognition of this phenomenon has followed from large epidemiologic investigations, which have shown that hallucinations and delusions are non-discrete and fall on a continuum varying along dimensions of reality-testing and severity (Strauss, 1969; van Os, Hanssen, Bijl and Ravelli, 2000; Kelleher and Cannon, 2014). Community-based surveys have demonstrated that approximately 5-7% of adults and 8-17% of children & adolescents from the general community report hallucinations and delusions (Linscott and van Os, 2013; Kelleher et al., 2012; van Os and Reininghaus, 2016). In the absence of a psychotic disorder, these hallucinations and delusions are typically referred to as psychotic(-like) experiences or psychotic phenomena. There is evidence that psychotic experiences share overlapping aetiological, genetic as well as environmental risk with florid clinical psychotic disorder (Polanczyk et al., 2010; Zavos et al., 2014; Jeppesen et al., 2015; Pain et al., 2018). This supports the notion of a psychosis continuum both in terms of severity as well as the developmental ontology of psychotic symptoms across the life span. Notably, childhood psychotic experiences are also predictive of various non-psychotic symptoms (e.g. anxiety, depression, mania, suicidality), and increased mental health service use (Kelleher et al., 2013; McGrath et al., 2016; Bhavsar, McGuire, MacCabe, Oliver and Fusar-Poli, 2017), and are characterized by increased functional impairment (Dhossche, Ferdinand, Van der Ende, Hofstra and Verhulst, 2002). Therefore, it is crucial to explore the aetiology of psychotic experiences in children, as a childhood presentation of the extended psychosis phenotype.

GENETIC VULNERABILITY FOR PSYCHOSIS

> Our lives are in truth, owning to heredity, as full of cabalistic ciphers, of horoscopic castings as if sorcerers really existed.
>
> (MARCEL PROUST, 1920, GUERMANTES WAY)

> But I do know a kind of madness that lies low in the mind, half-buried in consciousness, which lives in parallel to sanity, and given the right circumstances or even just half a chance, creeps like a lick of flame or a growing tumour up and around ordinary perception, consuming it for a while, and causing one, even when not at the movies, to quake in fear of the world and people and what they – I mean, of, we – are capable of.
>
> (JENNY DISKI, 2002, STRANGER ON A TRAIN)

Offspring studies have shown that familial risk for severe mental illness increases the risk for psychiatric problems in children (Rasic, Hajek, Alda and Uher, 2014). Recent genetic advances have given us the opportunity to extend the generalisability of high-risk studies to the general population by studying individuals according to their genetic liability for severe mental illness, including schizophrenia. Schizophrenia is a highly heritable psychiatric disorder, mediated through a complex combination of genetic variants. Although not fully without its caveats, polygenic risk scores, composites of genetic risk variants derived from large genome-wide association studies (GWAS), are now considered useful biological indices of genetic vulnerability. The schizophrenia polygenic risk score has been associated with early life emotional and behavioural problems, cognition, social communication difficulties, and brain correlates in the general population (Mistry, Harrison, Smith, Escott-Price and Zammit, 2017; Bogdan et al., 2017). Hence, they provide an avenue for the study of early developmental manifestations of the extended psychosis phenotype and how genetic risk interacts with environmental risks. For example, polygenic risk scores can be used to examine how a genetic liability for schizophrenia interacts with certain environmental stressors (e.g. childhood adversities, stress hormone) in their

collaborative shaping of brain and behaviour. In this thesis, we study various developmental characteristics of the extended psychosis phenotype from the perspective of psychotic phenomena and from the perspective of polygenic risk for schizophrenia.

PART II: ON UNRULINESS

Disruptive behaviour problems in children comprise behaviours such as (physical) aggression, oppositional behaviour, rule-breaking and callousness, and these are among the most common reasons for referral child and adolescent mental health services (Peterson, Zhang, Santa Lucia, King and Lewis, 1996). Children with elevated levels of disruptive behaviour problems greatly impact society in terms of criminal convictions, healthcare and social services costs (Scott, Knapp, Henderson and Maughan, 2001; Rivenbark et al., 2018). Several studies have addressed potential risk factors for childhood disruptive behaviours and important work has been done regarding treatment and preventative measures. However, heterogeneity among disruptive behaviour problems is known to play a crucial role in inconclusive findings regarding their aetiological backgrounds, particularly with respect to neurobiology.

HETEROGENEITY OF UNRULY BEHAVIOUR

> His anger was like a single musical phrase to which in an opera several lines are sung which are entirely different from one another, if one studies the words, in meaning and character, but which the music assimilates by a common sentiment.
>
> (MARCEL PROUST, 1920, THE GUERMANTES WAY)

Many studies using different informants, instruments, and study populations have addressed the heterogeneity and developmental continuities of disruptive behaviour disorders in childhood and

adolescence (Moffitt, 1993; Frick et al., 1993; Moffitt et al., 2008; Lahey and Waldman, 2012). With the operationalisation of new classification schemes such as the DSM-5 and ICD-11, several changes in the criteria for oppositional defiant disorder (ODD) and conduct disorder (CD) were made. For example, it can now be specified whether callous traits are co-occurring with CD, or whether CD had its onset before the age of 10 years, both of which are indicative of a poorer prognosis (Viding, Frick and Plomin, 2007; Moffitt, 1993). Another important change is the possibility to differentiate irritable from oppositional ODD subtypes (Vidal-Ribas, Brotman, Valdivieso, Leibenluft and Stringaris, 2016). However, this heterogeneity of ODD/CD symptoms has not been assessed beyond *a priori* defined diagnostic criteria, which would strengthen our current diagnostic frameworks with an empirical basis. But, more importantly, few studies have disentangled the neurobiological underpinnings of childhood disruptive behaviour problems by taking into account their well-known heterogeneous presentation.

NEUROBIOLOGY OF UNRULY BEHAVIOUR

> Liberate yourself from my vice-like grip!
>
> (J.D. SALINGER, 1951, THE CATCHER IN THE RYE)

Brain grey matter volume reductions have been identified within the insula, amygdala, frontal and temporal regions in young people with disruptive behaviour problems (Rogers and De Brito, 2016). These structures have previously been implicated in reward processing, affect regulation, and behavioural inhibition. Structural white matter networks have rarely been investigated in paediatric neurobiological studies of disruptive behaviour problems. White matter tracts provide high-speed communication of neuronal signals between grey matter regions in the brain, and disruptions in white matter are assumed to affect connectivity between distant brain regions that rely on this communication bridge. It is not well-known how white matter networks are associated with childhood disruptive behaviour, due to inconsistent previous findings (Waller, Dotterer, Murray, Maxwell

and Hyde, 2017). It is believed that the presence of varying levels of distinct disruptive behaviours (e.g. physical aggression, irritability, callous traits) may have contributed to the contradictory results (Blair, White, Meffert and Hwang, 2014). Furthermore, most studies have been primarily based on small, selected samples, so that it remains unclear to what extent brain differences are associated with disruptive behaviours in the general paediatric population. This is a particular gap in the literature on callous traits. In this thesis, we disentangle the heterogeneity of disruptive behaviour problems and callous traits in children, which we further examine in association with structural brain correlates.

THIS THESIS

AIMS

The general aim of this thesis was to gain insights into the neurodevelopmental pathways of children at increased risk for severe mental illness. Here we focussed on two prevalent yet impairing psychiatric phenotypes of childhood: psychotic phenomena and disruptive behaviour problems.

SETTING

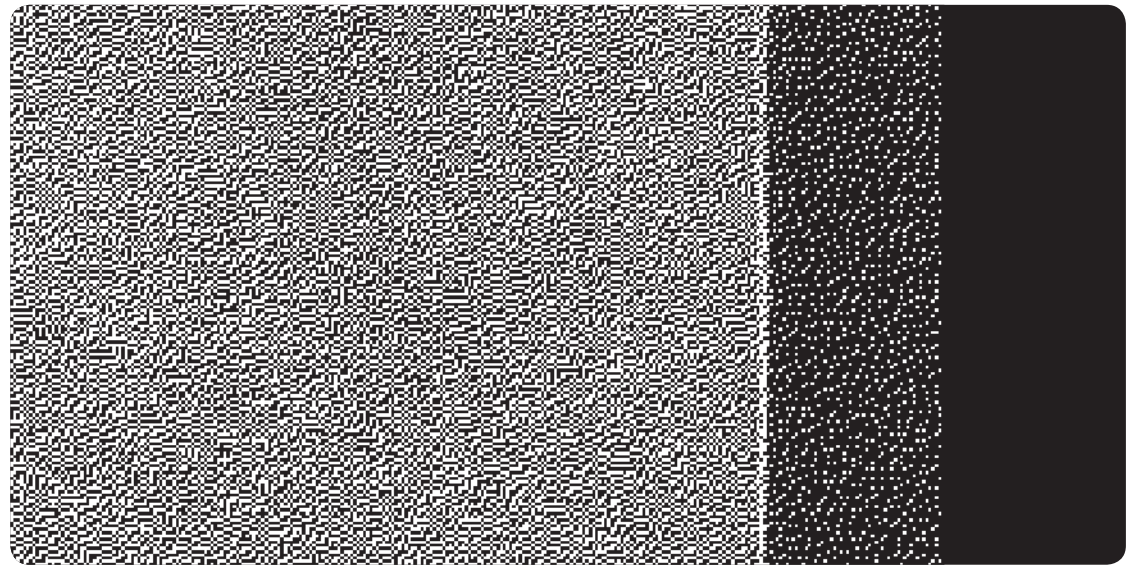
All studies described in this thesis are embedded in the Generation R Study, a prospective population-based cohort from Rotterdam, the Netherlands (Kooijman et al., 2016). The aim of the Generation R Study is to identify early environmental and genetic factors that affect maternal and child health, disease and development. All pregnant women living in the city of Rotterdam with an expected delivery date in the period 2002 to 2006 were eligible for inclusion in the study. At mean age six years and ten years, detailed assessments were conducted, which comprised comprehensive cognitive and behavioural examinations and questionnaires. At the ten-years-of-age data collection wave, 7,393 children and their parents have provided consent for further participation in the study. At age ten years, children were asked for the first time to complete a questionnaire themselves, which included items on psychotic experiences. These children were also invited to participate in the second wave of neuroimaging of the Generation R Study, of whom 4,087 have brain scans available (White et al., 2018). Furthermore, a dedicated home visit data collection project was set up to measure objective sleep parameters through actigraphy assessment in a subgroup of 814 children.

OUTLINE OF THE STUDIES DESCRIBED IN THIS THESIS

This thesis is divided into two parts. The first part has a focus on developmental risk of psychotic phenomena, which are approached from the behavioural and the genetic perspective. In chapter 2, we report a study of the developmental associations from early childhood to subsequent psychotic experiences in pre-adolescence. In chapter 3, we studied the relationship of maternal and paternal cannabis use during pregnancy with psychotic experiences in the offspring. Next, in chapter 4, we examined whether psychotic experiences co-occur with observed and subjective measures of sleep dysfunction. From the genetic perspective, we sought to explore whether stress hormone level moderates the relationship between the genetic liability for schizophrenia and child brain structure in chapter 5. Finally, in chapter 6, we evaluated whether the genetic liability for schizophrenia increases the risk of exposure to childhood adversities, and whether this relationship mediates the increased chance for psychiatric problems in children at elevated genetic risk for schizophrenia.

The second part of this thesis focusses on disruptive behaviour problems in children. In chapter 7, we examined the presence of dimensions and distinct subgroups of childhood disruptive behaviour problems. In chapter 8, we related these dimensions of disruptive behaviour problems to the integrity of brain white matter microstructure. In chapter 9, we addressed the question whether prenatal exposure to maternal and paternal cannabis use increases the risk of externalising behavioural problems in children using a multi-informant approach. In chapter 10, we examined callous traits, which are used to identify a particularly problematic subgroup of children with disruptive behaviour, in relation to grey and white matter brain characteristics. In chapter 11, we explored whether aggressive

behaviour increases the risk for obesity or, conversely, whether higher weight increases the risk for more aggressive behaviour problems. A more general discussion of our findings in the context of the broader literature is provided in chapter 12.



(...) *The slightest ambiguities, the most inexplicable transpositions of ideas take place. In sounds there is colour;* (...)

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(...) *in colours there is a music...*
(Charles Baudelaire, 1860, Les Paradis Artificiels)

PART I:

PSYCHOTIC PHENOMENA

–Hearing voices no one else can hear isn't a good sign, (...)

(ABSTRACT)

OBJECTIVE

Adolescent psychotic-like experiences predict the onset of psychosis, but also predict subsequent non-psychotic disorders. Therefore, it is crucial to better understand the aetiology of psychotic-like experiences. This study examined whether (a) child emotional and behavioural problems at 3 and 6 years, or (b) childhood adversities were associated with psychotic-like experiences at age 10 years.

METHOD

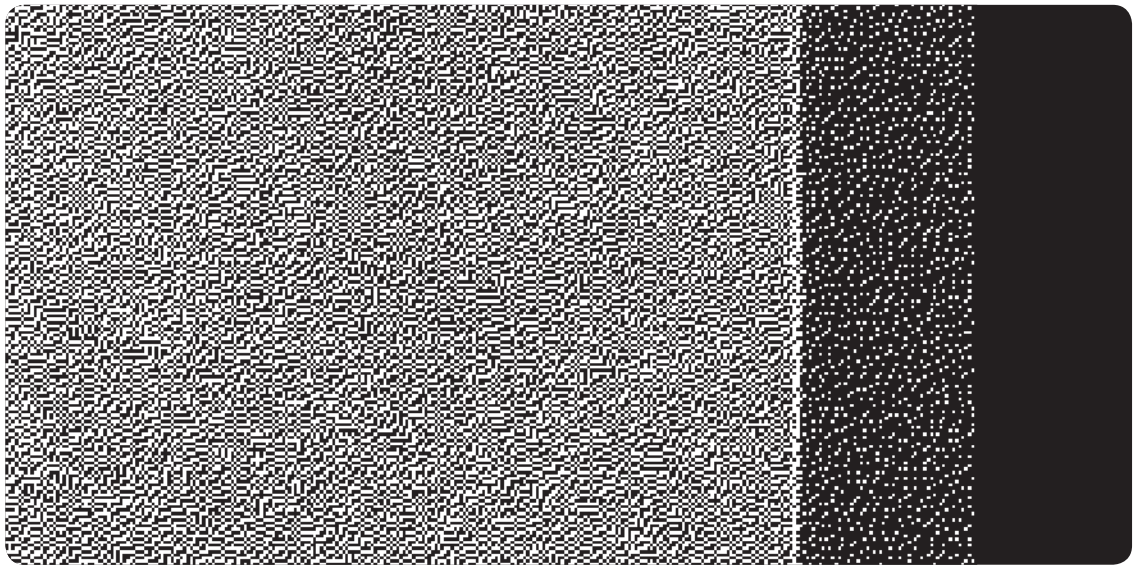
This prospective study was embedded in the Generation R Study; 3984 children (mean age 10 years) completed a psychotic-like experiences questionnaire. Mothers reported problems of their child at ages 3, 6 and 10 years. Additionally, mothers were interviewed about their child's adversities.

RESULTS

Psychotic-like experiences were endorsed by ~20% of children and predicted by both emotional and behavioural problems at three years (e.g. emotional-reactive problems: $OR_{adjusted}$ = 1.10, 95% CI: 1.06-1.15, aggressive behaviour: $OR_{adjusted}$ = 1.03, 95% CI: 1.02-1.05), and six years (e.g. anxious/depressed problems: $OR_{adjusted}$=1.11, 95% CI 1.06-1.15, aggressive behaviour: $OR_{adjusted}$ = 1.04, 95% CI: 1.04-1.05). Childhood adversities were associated with psychotic-like experiences (>2 adversities: $OR_{adjusted}$ = 2.24, 95% CI: 1.72-2.92), which remained significant after adjustment for comorbid psychiatric problems.

CONCLUSION

This study demonstrated associations between early adversities, childhood emotional and behavioural problems and pre-adolescent psychotic-like experiences, which will improve the understanding of children at increased risk for severe mental illness.



(...) *even in the wizarding world.*
(J.K. Rowling, 1998, Harry Potter and the Chamber of Secrets)

CHAPTER 2

PSYCHOTIC-LIKE EXPERIENCES IN PRE-ADOLESCENCE:

WHAT PRECEDES THE ANTECEDENT SYMPTOMS OF SEVERE MENTAL ILLNESS?

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MARIA ELISABETH KOOPMAN-VERHOEFF,
LAURA BLANKEN, DRAGAN CIBREV,
VINCENT JADDOE, FRANK VERHULST,
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HENNING TIEMEIER

Acta Psychiatrica Scandinavica, 2018,

INTRODUCTION

Psychotic disorders occur very rarely in childhood (Nicolson and Rapoport 1999). However, sub-clinical psychotic-like experiences, such as hallucinations and delusional thoughts, are common phenomena in childhood, with a general population prevalence of approximately 17% in children aged 9-12 years (Kelleher, Connor, et al. 2012). Adolescent psychotic-like experiences have repeatedly been shown to predict later psychosis (Zammit et al. 2013; Poulton et al. 2000), although their high prevalence suggests that for many children psychotic-like experiences may constitute normative behaviour (Linscott and van Os 2013). Notably, psychotic-like experiences share overlapping genetic risk with clinical psychotic disorders (Zavos et al. 2014). This supports the notion of a psychosis continuum, both in terms of severity from sub-clinical psychotic-like experiences to clinical psychosis, as well as the developmental ontology of psychotic symptoms across the lifespan (van Os and Reininghaus 2016). Psychotic-like experiences are predictive of various non-psychotic symptoms, including anxiety, depressed mood, and suicidal behaviour (Yung et al. 2007; Wigman et al. 2012; Kelleher, Corcoran, et al. 2013; Dhossche et al. 2002; Kelleher, Keeley, et al. 2012; Fisher et al. 2013; Jeppesen, Clemmensen, et al. 2015). In the presence of non-psychotic disorders, psychotic-like experiences signal greater severity, including higher comorbidity and poorer prognosis (Dhossche et al. 2002; Kelleher, Keeley, et al. 2012). Moreover, more than 90% of 11-year-olds who experienced psychotic symptoms were diagnosed with at least one psychiatric disorder by age 38 years (Fisher et al. 2013). Against the background of the high predictive value of childhood psychotic-like experiences, it is crucial to have a better understanding of their developmental ontology and aetiology (van Os and Reininghaus 2016). However, few studies have been able to integrate early childhood behavioural assessment with exposure to adversities prior to psychotic-like experiences, which would help us to refine the extended psychosis phenotype from a developmental perspective.

Most adult psychiatric disorders, including anxiety, mood, and impulse control disorders, are associated with an elevated risk for subsequent psychotic-like experiences (McGrath et al. 2016).

Previous studies have shown that childhood autistic traits (Sullivan et al. 2013), attention deficit/hyperactivity disorder (Hennig et al. 2016), and other psychiatric disorders (Siebald et al. 2016) are associated with adolescent psychotic-like experiences. However, most studies of the association between childhood psychotic-like experiences and psychiatric comorbidity have been cross-sectional (Jeppesen, Clemmensen, et al. 2015; Kelleher, Keeley, et al. 2012), or investigated which future psychiatric disorders might be predicted by adolescent psychotic-like experiences (Zammit et al. 2013; Fisher et al. 2013; Poulton et al. 2000). These studies cannot inform us about which psychiatric problems were already present in early childhood. Therefore, prospective designs are required to elucidate behavioural and emotional risk indicators preceding the manifestation of psychotic-like experiences. In addition, most studies of the development of psychotic-like experiences do not adjust for the presence of comorbid psychiatric problems to test whether the observed associations are specific for psychotic-like experiences (Achenbach et al. 2016). This is needed as comorbidities are very common in child psychiatry (Rutter and Pickles 2016), and the extended psychosis phenotype is no exception (van Os and Reininghaus 2016).

It has been repeatedly shown that psychotic symptoms and disorders are predicted by childhood adversities (Trotta, Murray, and Fisher 2015; Varese et al. 2012; Read et al. 2005). McGrath et al. hypothesised that childhood adversities increase the risk for adult psychosis through effects on non-psychotic symptoms (McGrath et al. 2017), but whether the association between adversities and psychotic-like experiences can be explained through comorbid psychiatric problems remains unclear. Research on childhood adversity typically relies on recall, even in prospectively designed studies. It has been argued that studies would ideally take into account the degree to which an adversity was subjectively experienced as traumatic (Kelleher, Keeley, et al. 2013; Catone et al. 2015), the age-at-onset and chronicity of the event (McGrath et al. 2017), and the reporting source (Trotta, Murray, and Fisher 2015) to obtain more conservative estimates for the association between childhood adversities and subsequent psychotic-like experiences (Krabbendam 2008).

AIMS OF THE STUDY

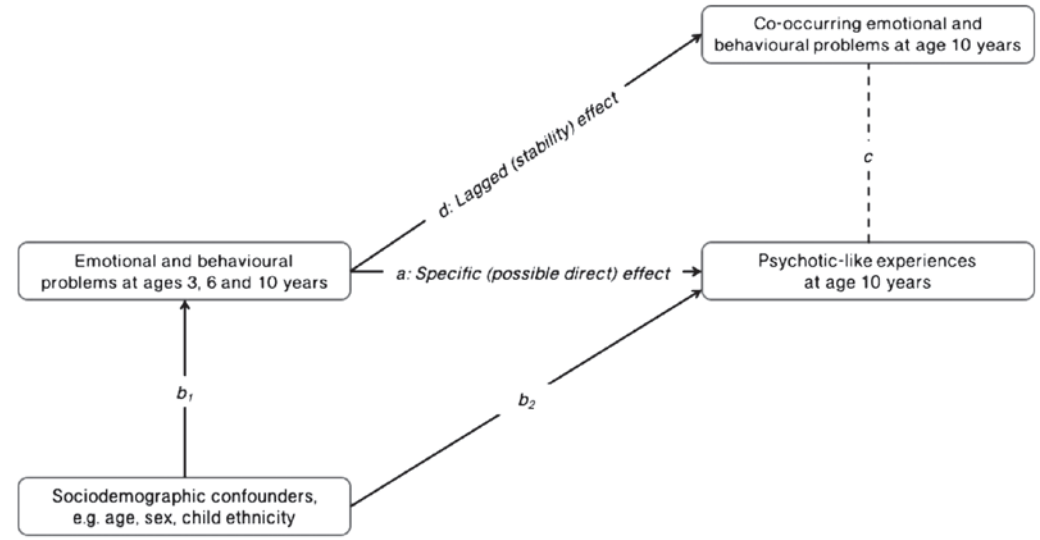
We employed a prospective design to allow for a developmental perspective on the risk indicators for pre-adolescent psychotic-like experiences. First, we aimed to study the emotional and behavioural problems co-occurring with psychotic-like experiences. Second, we examined which problems at ages 3 and 6 years were associated with psychotic-like experiences at age 10 years, while accounting for adversities. To obtain specific estimates, our analyses were adjusted for co-occurring emotional and behavioural problems. See Figure 1 for visualisation of the conceptual model of associations. Third, we examined whether childhood adversities were associated with psychotic-like experiences at age 10 years. Separate analyses were conducted for adversities occurring before vs. after age 5 years to examine vulnerable developmental periods. Subsequently, childhood adversities were partitioned into physical maltreatment, sexual maltreatment, and other adversities. Furthermore, we examined the risk of adversities on psychotic-like experiences independent of other co-occurring emotional and behavioural problems. We expected both emotional problems and behavioural problems at ages 3, 6, and 10 years to be associated with psychotic-like experiences at age 10 years. In addition, we expected childhood adversities to predict pre-adolescent psychotic-like experiences, and this association could be partly explained by co-occurring emotional and behavioural problems.

METHODS

STUDY POPULATION

This study was embedded in the Generation R Study, a prospective cohort from foetal life onwards, which in the period 2002 to 2006 enrolled pregnant women living in Rotterdam, the Netherlands (Kooijman et al. 2016). All women who were pregnant in in that period were eligible for inclusion and approximately 61% of them were included at baseline (N = 9778). The cohort is largely representative of the female population in reproductive age living the in the Rotterdam area. Children who participated at mean age 10 years were more often of Dutch nationality and had older and more

Figure 1: Conceptual association model employed in the current study.



Note: Theoretical model demonstrating the association of early childhood emotional and behavioural problems with pre-adolescent psychotic-like experiences (arrow a: Specific (possible direct) effect). All analyses were adjusted for confounding variables (arrows b1 and b2). Psychotic-like experiences commonly co-occur with comorbid emotional and behavioural problems (dashed line c). This line is not depicted as an arrow as (1) the arrow could be absent (i.e., shared cause); (2) it is possible that the arrow goes from emotional problems at age 10 years to pre-adolescent psychotic-like experiences (i.e., comorbid emotional or behavioural problems at age 10 years are the mediator); (3) theoretically, the arrow could also go from psychotic-like experiences to emotional and behavioural problems, if the latter were secondary to psychotic like experiences (i.e., psychotic-like experiences are the mediator). See Methods and Results for sensitivity analyses with additional adjustment for co-occurring emotional and behavioural problems.

highly-educated mothers (Kooijman et al. 2016). Study protocols were approved by the local ethics committee and written informed assent and consent was obtained from all participants and their parents, respectively.

For the current study, data on self-reported psychotic-like experiences was available in 4342 participants. From this sample, twins and siblings (n = 310), and children without any behavioural assessment at either age three, six or ten years were excluded (n = 48); which left a final sample size of 3984 participants.

MEASURES

MOTHER-REPORTED CHILD EMOTIONAL AND BEHAVIOURAL PROBLEMS

At age 10 years, child emotional and behavioural problems were assessed with the Child Behavior Checklist/6-18 (CBCL), an internationally validated and reliable measure of emotional and behavioural problems (Achenbach and Rescorla 2001). The CBCL/6-18 consists of eight syndrome scales: anxious/depressed, withdrawn/depressed, somatic complaints, social problems, thought problems, attention problems, rule-breaking behaviour, and aggressive behaviour. The first three scales load on internalising (i.e. emotional) problems, and the last two scales load on externalising (i.e. behavioural) problems. The CBCL measures emotional and behavioural problems on a continuous severity scale (Tick, van der Ende, and Verhulst 2007; Basten et al. 2013), and has been shown to predict DSM-based psychiatric disorders in adulthood (Hofstra, van der Ende, and Verhulst 2002; Roza et al. 2003). Items were scored by mothers on a three-point scale (0 = not true; 1 = somewhat or sometimes true; 2 = very or often true), based on behaviour of the past six months.

The preschool version of the CBCL was used to measure child emotional and behavioural problems at ages 3 and 6 years. This version of the CBCL includes seven syndrome scales, which are similar to those from the CBCL/6-18, and items are similarly scored on a three-point scale (0 = not true; 1 = somewhat or sometimes true; 2 = very or often true).

Not all participants were examined at all ages. Of the 3984 participants, mother-reported CBCL data was available in n = 3025 children at age 3 years, in n = 3653 children at age 6 years, and in n = 3846 children at age 10 years.

MOTHER-REPORTED CHILD AUTISTIC TRAITS

At age 6 years, mothers completed the Social Responsiveness Scale (SRS), a valid and reliable measure for capturing clinical and subclinical autistic traits on a quantitative scale (Constantino et al. 2003). We used the 18-item short-form of the scale, which contained the following subscales: social cognition, social communication, and autistic mannerisms. The SRS has been shown to correlate with DSM-based criteria for autism spectrum disorder (Constantino et al. 2003; Charman et al. 2007). For these analyses, 3345 participants were included.

CHILDHOOD ADVERSITIES

When children were on average 10 years, their mothers were interviewed about their offspring's childhood adversities (n = 3822). Mothers were asked about 24 childhood adversities, e.g. parental divorce/separation, transferring schools, and physical or sexual maltreatment (Amone-P'Olak et al. 2009). In case of a positive response, the child's age when the event had happened was registered, and the perceived severity of each event was rated as none, a little, moderate, or a lot. Only events with at least moderate impact were coded as adversities in the present analyses. Lifetime prevalence of the examined adversities are listed in Table S1.

SELF-REPORTED PSYCHOTIC-LIKE EXPERIENCES

At age ten years, psychotic-like experiences were assessed by child self-report questionnaire using three items from the Youth Self-Report (Ivanova et al. 2007): (i) I hear sounds or voices that according to other people are not there; (ii) I see things that other people think are not there; and (iii) I have thoughts that other people would find strange. Children responded to what extent they agreed with the

statement on a three-point scale: not at all (0), a bit (1), or clearly (2), and were summed to create a total score. These questions have previously been shown to adequately capture psychotic symptoms in childhood and adolescence (Kelleher et al. 2011; Kelleher, Keeley, et al. 2013; Linscott and van Os 2013). To facilitate interpretation and reduce positive skewness, the sum score was classified into the following categories: no symptoms (0 points, 54.8% of children), a few symptoms (1-3 points, 39.4% of children), and a lot of symptoms (4-6 points, 5.8% of children). These cut-offs were chosen so that the children in the upper category would have endorsed 'clearly' on at least one of the items. These three categories were treated separately in ordinal logistic regression models, i.e. categories were not collapsed.

The endorsement of child-reported psychotic-like experiences was compared to the endorsement of mother-reported psychotic-like experiences. Mother-reported psychotic-like experiences were derived from the CBCL at age 10 years, which were comparable to the child-reported psychotic-like experience (i.e. (a) "My child hears sounds or voices that aren't there", (b) "My child sees things that aren't there", and (c) "My child has strange ideas").

COVARIATES

The following covariates that have previously been associated with psychotic-like experiences, were taken into consideration in our analyses (Linscott and van Os 2013). Age and sex were obtained from medical records. Child ethnicity was considered as Dutch when both parents were born in the Netherlands, while children were classified as non-Dutch if at least one of the parents was born outside the Netherlands (further specified as 'Other Western' or 'Other Non-Western'). Maternal education was defined by the highest attained educational level and classified into low (primary school or lower), middle (lower and intermediate vocational training) or high (higher vocational education and university). Maternal psychopathology was assessed at 3 months post-natal with the Brief Symptom Inventory (BSI) (Derogatis and Melisaratos 1983), a validated self-reported continuous measure of 53 items encompassing a spectrum of psychiatric symptoms. In addition, children were asked to state whether they had completed the psychotic-like experiences questionnaire alone or with help from others.

Child self-reported problems at age 10 years were assessed with the Brief Problem Monitor (BPM) (Achenbach et al. 2011), which encompasses scales regarding internalising, externalising and attention problems and is a validated abbreviated version of the CBCL.

STATISTICAL ANALYSES

First, the endorsement rates of the individual self-reported psychotic-like experiences were determined; and cross-informant comparisons were made between mother-reported and self-reported psychotic-like experiences of the child. Second, cross-sectional associations between pre-adolescent emotional and behavioural problems and psychotic-like experiences at age ten were examined using ordinal logistic regression. Third, the association between emotional and behavioural problems at ages 3 and 6 years and psychotic-like experiences at age 10 years was studied with ordinal logistic regression. Analyses were adjusted for covariates, as described above, and subsequently also adjusted for the presence of childhood adversities. In a sensitivity step, analyses were additionally adjusted for co-occurring self-reported behavioural and emotional problems to obtain estimates specific for the association with psychotic-like experiences independent of co-occurring child self-reported emotional and behavioural problems. These sensitivity analyses were conducted in order to examine the specific associations with psychotic-like experiences over and above the associations with more global self-reported psychiatric problems.

Fourth, we assessed the association between lifetime childhood adversities and the risk for psychotic-like experiences at age ten with ordinal logistic regression, unadjusted and subsequently adjusted for covariates. Childhood adversities were initially analysed jointly. Next, separate analyses were conducted for adversities occurring before and after age five years. In addition, specific associations were examined between physical maltreatment, sexual maltreatment, and other non-maltreatment adversities with psychotic-like experiences.

Sensitivity analyses were conducted to examine the association between childhood adversities and subsequent psychotic-like experiences, adjusted for the presence of co-occurring emotional and behavioural problems. Given that co-occurring emotional and behavioural problems were measured at the same time point (age ten years) as psychotic-like experiences, emotional and behavioural problems could not be studied as a mediator. Some scholars might consider co-varying for co-occurring emotional and behavioural problems as over-adjustment of the model, but we argue that these sensitivity analyses provide important insight into the specific effects of childhood adversities on psychotic-like experiences independent of emotional and behavioural problems more generally (Achenbach et al. 2016).

Missing data of the covariates were handled by multiple imputations in MICE 2.25 (van Buuren and Groothuis-Oudshoorn 2011; Greenland and Finkle 1995) using 100 imputed datasets, which were pooled for analyses. Analyses were repeated on non-imputed complete-case data and these results were similar to the main findings based on imputed data (results not shown). All analyses were conducted in R statistical software (R Core Team 2015).

RESULTS

PREVALENCE OF PSYCHOTIC-LIKE EXPERIENCES

Sociodemographic characteristics are described in Table 1, with the right column showing the estimates of children with the highest scores of psychotic-like experiences (>4 points, 5.8% of children). Figure 2 shows the endorsement percentages for the three psychotic-like experiences. Auditory and visual perceptive phenomena were reported by 26% and 17% of children, respectively. Strange thoughts were reported by 32% of children (Fig.2).

Co-occurring self-reported internalising, externalising and attention problems were moderately correlated with psychotic-like experiences (range, r=0.25-0.45, Table S2).

Table 1: Child and maternal sociodemographic characteristics.

	N	Total population (N = 3984)	Children with more psychotic-like experiences (n = 232)
Child characteristics			
Age at psychotic-like experiences assessment, mean (SD)	3984 (0.0% missing)	9.82 (0.35)	9.81 (0.35)
Sex, % girls	3984 (0.0% missing)	50.89	50.86
Ethnicity, %	3952 (0.8% missing)		
Dutch		66.04	65.80
Other Western		8.48	9.52
Other Non-Western		25.48	25.68
Total problems score age 3, median (IQR)	3025	17.72 (17.00)	21.00 (16.95)
Total problems score age 6, median (IQR)	3653	15.00 (18.86)	21.00 (18.18)
Total problems score age 10, median (IQR)	3846	13.22 (17.00)	24.00 (21.91)
Autistic traits total score, median (IQR)	3345	0.17 (0.22)	0.22 (0.28)
Psychotic-like experiences sum score, median (IQR)	3984 (0.0% missing)	0.00 (1.00)	5.00 (2.00)
Psychotic-like experiences assessment, % filled out alone	3984 (0.0% missing)	43.80	51.72
Maternal characteristics			
Educational level, %	3666 (8.0% missing)		
High		62.68	63.38
Medium		35.22	34.27
Low		2.10	2.35
Psychiatric problems, median (IQR)	2711 (32.0% missing)	0.12 (0.23)	0.13 (0.29)

Table 2: Cross-sectional associations between mother-reported emotional and behavioural problems and self-reported psychotic-like experiences (n = 3846).

	Self-reported psychotic-like experiences at age 10 years			
	Model 1		Model 2	
Mother-reported problems	OR (95% CI)	P	OR (95% CI)	P
Anxious/Depressed	1.16 (1.13-1.19)	<0.001	1.14 (1.11-1.17)	<0.001
Withdrawn/Depressed	1.17 (1.12-1.21)	<0.001	1.13 (1.09-1.18)	<0.001
Somatic Complaints	1.18 (1.14-1.22)	<0.001	1.16 (1.12-1.20)	<0.001
Social Problems	1.19 (1.15-1.22)	<0.001	1.16 (1.13-1.20)	<0.001
Thought Problems	1.22 (1.18-1.25)	<0.001	1.19 (1.16-1.23)	<0.001
Attention Problems	1.14 (1.12-1.16)	<0.001	1.12 (1.10-1.15)	<0.001
Rule-Breaking Behaviour	1.19 (1.14-1.24)	<0.001	1.16 (1.11-1.21)	<0.001
Aggressive Behaviour	1.09 (1.07-1.11)	<0.001	1.08 (1.06-1.10)	<0.001

Note: Model 1 is adjusted for age at psychotic-like experiences assessment, sex of the child, ethnicity of the child, maternal educational level, maternal psychiatric problems at 3 months post-natal, and whether psychotic-like experiences questionnaire was completed alone or with help from another person. Model 2 is additionally adjusted for childhood adversities up to age 10 years.

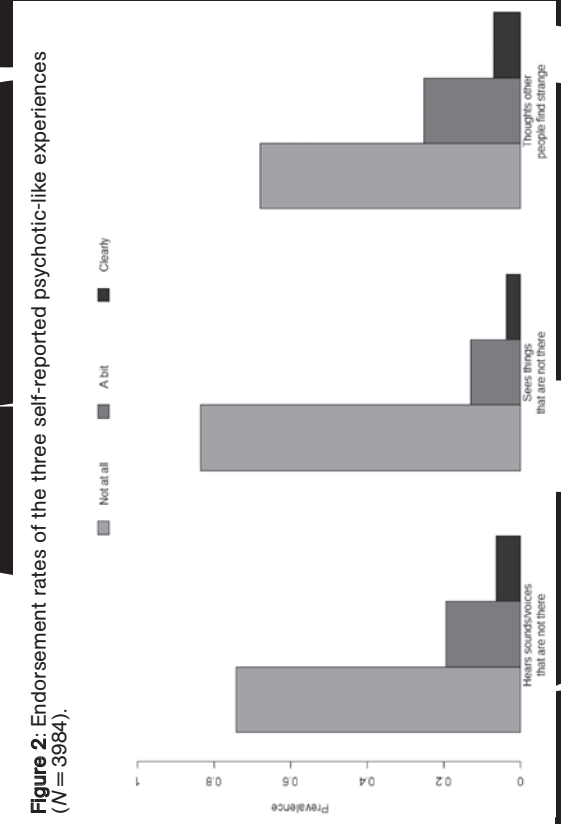


Figure 2: Endorsement rates of the three self-reported psychotic-like experiences (N = 3984).

(Full colour image presented on page 3.)

Psychotic-like experiences endorsement by mother-report and child-report are related, but comparisons indicate that children report a much higher burden of psychotic-like experiences than reported by their mothers (Table S3), as demonstrated by the statistically significant but low kappa values.

CO-OCCURRING EMOTIONAL AND BEHAVIOURAL PROBLEMS

All mother-reported emotional and behavioural problems were associated with psychotic-like experiences at age 10 years in the cross-sectional analyses with adjustment for confounders (Table 2). However, after additional adjustment for co-occurring self-reported problems, only the associations of psychotic-like experiences with anxious/depressed problems, withdrawn/depressed problems, somatic complaints, social problems and thought problems remained (Table S4).

PRECEDING EMOTIONAL AND BEHAVIOURAL PROBLEMS

Almost all age 3 years emotional and behavioural problems were associated with psychotic-like experiences at age 10 years. Similarly, all emotional and behavioural problems at age 6 years, including autistic traits, were associated with increased risk for psychotic-like experiences at age 10.

In sensitivity analyses corrected for co-occurring self-reported emotional and behavioural problems (Table S5), only emotional problems at age 3 years (i.e. emotional reactive problems: $OR_{adjusted}$=1.06, 95% CI 1.01-1.11, somatic complaints: $OR_{adjusted}$=1.05, 95% CI 1.00-1.10, withdrawn problems: $OR_{adjusted}$=1.07, 95% CI 1.00-1.14) and at age 6 years (i.e. anxious/depressed symptoms: $OR_{adjusted}$=1.05, 95% CI 1.00-1.09, somatic complaints: $OR_{adjusted}$=1.05, 95% CI 1.01-1.10, and sleep problems: $OR_{adjusted}$=1.05, 95% CI 1.01-1.09) were associated with psychotic-like experiences at age 10 years. No associations between autistic traits, aggressive behaviour or attention problems and psychotic-like experiences at age 10 years were found.

CHILDHOOD ADVERSITIES

Overall, childhood adversities were associated with psychotic-like experiences (Table 4, one or two adversities: $OR_{adjusted}$=1.77, 95% CI 1.53-2.04; more than two adversities: $OR_{adjusted}$=2.24, 95% CI 1.72-2.92), in analyses adjusted for covariates. Figure S1 demonstrates that children who have experienced childhood adversities reported more psychotic-like experiences.

Childhood adversities occurring before versus after age five years were similarly predictive of psychotic-like experiences (before age 5 years: $OR_{adjusted}$=1.73, 95% CI 1.37-2.19; after age five years: $OR_{adjusted}$=1.80, 95% CI 1.56-2.08). Children who had been physically or sexually maltreated were more likely to have psychotic-like experiences (physical maltreatment: $OR_{adjusted}$=1.62, 95% CI 1.04-2.52; sexual maltreatment: $OR_{adjusted}$=2.65, 95% CI 1.21-5.80). We also found an association between other (non-maltreatment) childhood adversities and psychotic-like experiences ($OR_{adjusted}$=1.28, 95% CI 1.18-1.38).

In sensitivity analyses adjusted for co-occurring child self-reported emotional and behavioural problems (Table S6), the observed associations between lifetime childhood adversities and psychotic-like experiences were attenuated (one or two adversities: $OR_{adjusted}$=1.27, 95% CI 1.08-1.48; more than two adversities: $OR_{adjusted}$=1.21, 95% CI 0.90-1.63), which was mostly explained by additional adjustment for self-reported emotional problems.

DISCUSSION

In this prospective study, we demonstrated that psychotic-like experiences were common in 10-year old children, of whom approximately 20% reported auditory and visual perceptive phenomena. Psychotic-like experiences tended to co-occur with various emotional and behavioural difficulties, such as anxiety, emotional reactivity, and aggressive behaviour. Moreover, we showed that children with psychotic-like experiences were more likely have higher scores of earlier emotional and behavioural problems at ages 3 and 6 years. With additional adjustment for the presence of other co-occurring

Table 3: Longitudinal associations between early childhood emotional & behavioural problems and self-reported psychotic-like experiences at age 10 years.

	Emotional & behavioural problems at age 3 years and self-reported psychotic-like experiences at age 10 years ($n = 3025$)				Emotional & behavioural problems at age 6 years and self-reported psychotic-like experiences at age 10 years ($n = 3653$)			
Mother-reported problems	Model 1 OR (95% CI)	*P*	Model 2 OR (95% CI)	*P*	Model 1 OR (95% CI)	*P*	Model 2 OR (95% CI)	*P*
Emotional Reactive	1.11 (1.06-1.16)	<0.001	1.10 (1.06-1.15)	<0.001	1.12 (1.08-1.15)	<0.001	1.11 (1.07-1.14)	<0.001
Anxious/Depressed	1.05 (0.99-1.10)	0.092	1.04 (0.99-1.10)	0.129	1.12 (1.08-1.17)	<0.001	1.11 (1.07-1.15)	<0.001
Somatic Complaints	1.07 (1.02-1.12)	0.003	1.06 (1.02-1.11)	0.005	1.10 (1.06-1.14)	<0.001	1.09 (1.05-1.13)	<0.001
Withdrawn	1.12 (1.05-1.19)	<0.001	1.12 (1.05-1.18)	<0.001	1.14 (1.09-1.20)	<0.001	1.13 (1.08-1.18)	<0.001
Sleep Problems	1.04 (1.01-1.08)	0.014	1.04 (1.01-1.07)	0.018	1.11 (1.07-1.15)	<0.001	1.10 (1.06-1.14)	0.016
Attention Problems	1.09 (1.04-1.14)	<0.001	1.09 (1.04-1.14)	<0.001	1.13 (1.08-1.17)	<0.001	1.12 (1.07-1.16)	<0.001
Aggressive Behaviour	1.03 (1.02-1.05)	<0.001	1.03 (1.02-1.05)	<0.001	1.04 (1.03-1.06)	<0.001	1.04 (1.03-1.05)	<0.001
Autistic traits					1.92 (1.41-2.63)	<0.001	1.76 (1.28-2.41)	<0.001

Note: For the association between autistic traits and psychotic-like experiences 3345 participants were included in the analyses. Model 1 is adjusted for age at psychotic-like experiences assessment, sex of the child, ethnicity of the child, maternal educational level, maternal psychiatric problems at 3 months post-natal, and whether psychotic-like experiences questionnaire was completed alone or with help from another person. Model 2 is additionally adjusted for childhood adversities up to age of behavioural exposures (i.e. ages 3 and 6 years).

Table 4: Association between childhood adversities and psychotic-like experiences ($n = 3822$).

	Self-reported psychotic-like experiences at age 10 years			
	Unadjusted model ($n = 3822$)		Adjusted model ($n = 3822$)	
Childhood adversities	OR (95% CI)	*P*	OR (95% CI)	*P*
All childhood adversities				
No adversities (ref, n=2652)	-		-	
1 or 2 adversities (n=947)	1.71 (1.47-1.96)	<0.001	1.75 (1.51-2.02)	<0.001
>2 adversities (n=223)	2.11 (1.62-2.76)	<0.001	2.23 (1.70-2.94)	<0.001
Adversities before age 5 years (n=293)	1.69 (1.34-2.12)	<0.001	1.73 (1.37-2.19)	<0.001
Adversities after age 5 years (n=1036)	1.75 (1.52-2.01)	<0.001	1.80 (1.56-2.07)	<0.001
Maltreatment (n=96)	1.78 (1.20-2.64)	0.004	1.84 (1.24-2.74)	0.003
Physical maltreatment (n=79)	1.54 (1.00-2.39)	0.051	1.62 (1.04-2.52)	0.033
Sexual maltreatment (n=23)	2.64 (1.20-5.81)	0.015	2.67 (1.22-5.86)	0.014
All other adversities (n=1030)	1.27 (1.18-1.36)	<0.001	1.28 (1.18-1.38) *	<0.001

Note: The adjusted model is adjusted for age at psychotic-like experiences assessment, sex of the child, ethnicity of the child, maternal educational level, maternal psychiatric problems at 3 months post-natal and whether psychotic-like experiences questionnaire was completed alone or with help from another person. * Additionally adjusted for the presence of physical or sexual maltreatment.

psychiatric problems, we observed that pre-adolescent psychotic-like experiences were specifically predicted by earlier emotional difficulties. Lifetime childhood adversities, in particular physical and sexual maltreatment, were associated with psychotic-like experiences. These findings will help us to better understand the extended psychosis continuum from a developmental perspective, and might aid in the early identification of children with increased risk of psychotic-like experiences and, possibly, subsequent psychiatric illness.

Previous studies have indicated that psychotic-like experiences commonly co-occur with a variety of psychiatric problems in youth aged 11-13 years (Dhossche et al. 2002; Kelleher, Keeley, et al. 2012; Jeppesen, Clemmensen, et al. 2015). Our present cross-sectional findings confirm these studies. Importantly, psychotic-like experiences were also associated with emotional and behavioural difficulties earlier in childhood, including anxiety, depression, aggressive behaviour, and autistic traits, at ages three and six years. This is in line with studies demonstrating that autistic symptoms, attention-deficit/hyperactivity disorder and other DSM-based psychiatric disorders are predictive of adolescent psychotic-like experiences (Siebald et al. 2016; Hennig et al. 2016; Sullivan et al. 2013). Moreover, population-based studies have shown that genetic vulnerability to schizophrenia also confers antecedent risk for emotional and behavioural difficulties in childhood (Riglin et al. 2017; Nivard et al. 2017). Together these findings demonstrate that the vulnerability to psychosis is associated with diverse emotional and behavioural difficulties before and after the onset of psychotic-like experiences, which supports the developmental and trans-diagnostic nature of the extended psychosis phenotype (van Os and Reininghaus 2016; Linscott and van Os 2013).

Our sensitivity analyses with adjustment for co-occurring emotional and behavioural problems revealed developmental associations from early childhood emotional problems to pre-adolescent psychotic-like experiences and indicates a specific role for emotional difficulties, such as anxiety and emotional reactivity. A specific association with psychotic-like experiences can only be determined when adjusting for the presence of co-occurring psychiatric problems (Achenbach et

al. 2016; van Os and Reininghaus 2016). The results also suggest that the longitudinal association of early behavioural problems or autistic traits with later psychotic-like experiences is partly explained by the co-occurrence of emotional problems. Autistic traits and behavioural problems such as aggression did not predict psychotic-like experiences over and above their effects on psychiatric problems more generally. Our findings are in line with adult studies, in which mood instability and anxiety have been particularly associated with psychotic symptoms (McGrath et al. 2016; Yung et al. 2007). Importantly, the interpretation that internalising problems mediated the associations to subsequent psychotic-like experiences is also compatible with our data. In addition, our findings extend earlier studies that suggested a direct association between symptoms attention-deficit/hyperactivity disorder, autism spectrum disorder, aggressive behaviour and subsequent psychotic-like experiences (Sullivan et al. 2013; Hennig et al. 2016; Siebald et al. 2016). The predictive value of emotional problems might reflect a general vulnerability to overlapping networks of affective and emotional psychopathology across development, of which psychotic-like experiences might be a common manifestation in pre-adolescence (Kelleher and Cannon 2014). Alternatively, psychosis risk might present differently across the life span, i.e. as emotional difficulties in childhood, as psychotic-like symptoms in adolescence, and finally as clinical psychiatric illness in early adulthood (McGrath et al. 2017; Riglin et al. 2017). Yet different studies suggest that the same genetic factors (Zavos et al. 2014; Jeppesen, Larsen, et al. 2015) and neurobiological correlates (Bourque et al. 2017; O'Hanlon et al. 2015; Jacobson et al. 2010) underlie both clinical psychotic disorders as well as psychotic-like experiences in the general population. This supports the concept of the trans-diagnostic psychosis continuum and emphasizes the importance of studying psychotic-like experiences across development (van Os and Reininghaus 2016).

Self-reported pre-adolescent psychotic-like experiences could potentially be viewed as a non-specific prognostic markers of psychotic disorders (Fisher et al. 2013). Most childhood psychotic-like experiences remit in adolescence (Linscott and van Os 2013; Bartels-Velthuis et al. 2011), possibly indicating that they might be part of

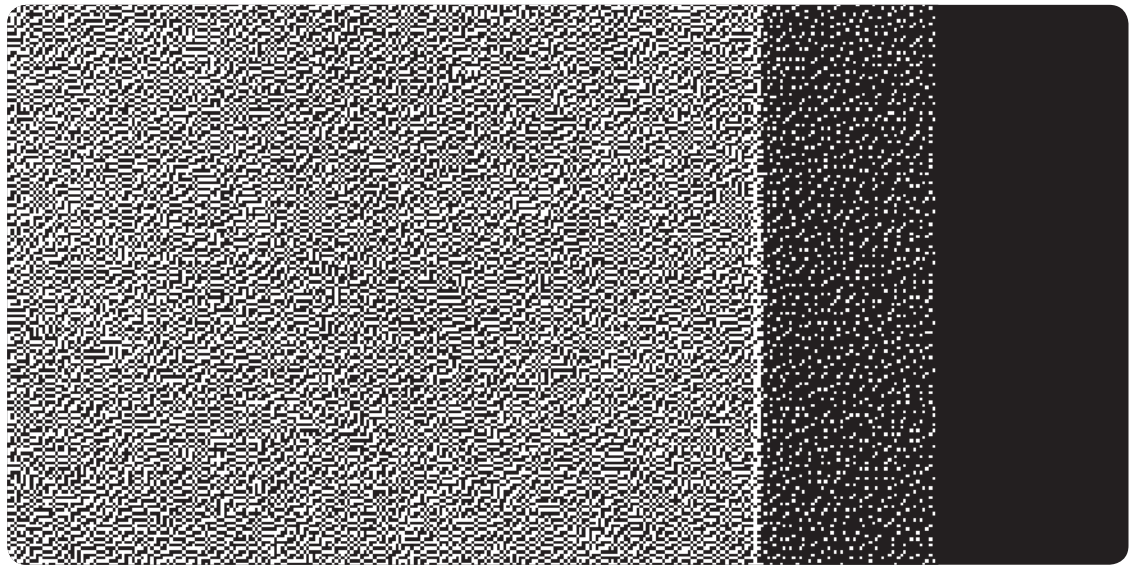
normal childhood development. However, sub-clinical adolescent psychotic symptoms are associated with a poorer prognosis (Wigman et al. 2012; Dhossche et al. 2002), increased comorbidity (Kelleher, Keeley, et al. 2012), and an elevated risk of adult-onset severe mental illness (McGrath et al. 2016; Zammit et al. 2013; Kelleher, Corcoran, et al. 2013). We showed that children reported more psychotic-like experiences than their mothers, which, although over-reporting by the child cannot be excluded, underscores the advantages of integrating different reporting sources. In the present study, psychotic-like experiences were reported by approximately 20% of children between ages nine and twelve years, which is comparable to prevalence rates obtained in studies which used clinical interviews for similarly aged children (Kelleher, Connor, et al. 2012; Kelleher, Keeley, et al. 2012; Polanczyk et al. 2010). The present findings seem to indicate that severe emotional problems might show overlap with psychotic-like experiences, which could be interpreted as children misclassifying emotional difficulties and psychotic-like experiences. However, this might also point towards a phenomenological overlap of different spectrums of childhood emotional and psychotic-like phenomena, which is interesting from a diagnostic classification perspective. Importantly, as psychotic-like symptoms can be very distressing to young people and their families (Maijer, Palmen, and Sommer 2017), evidence-based risk stratification and clinical guidance need to be developed. More specifically, future research should address the developmental characteristics of children with persistent psychotic-like experiences and children with transitory psychotic-like experiences but who might develop other psychiatric symptoms.

Childhood adversities have been robustly associated with the subsequent development of psychotic symptoms and disorders in childhood, adolescence, and adulthood (Trotta, Murray, and Fisher 2015; Kelleher, Keeley, et al. 2013; McGrath et al. 2017; Varese et al. 2012; Read et al. 2005). Using an interview of childhood adversity that ascertained the age and impact of each event, our present results corroborate these previous studies. While our findings are in line with studies demonstrating that children who have been exposed to sexual or physical maltreatment are more likely to develop psychotic symptoms (McGrath et al. 2017), we also observed associations between

other non-maltreatment adversities and psychotic-like experiences. This might signify that the development of psychotic-like experiences is not particular to any specific adversity (Trotta, Murray, and Fisher 2015; Kelleher, Keeley, et al. 2013), although some adversities might associate more strongly with subsequent psychotic-like experiences. Interestingly, similar associations were observed for adversities occurring before and after the age of five years, suggesting that in the general population it is difficult to define a distinct period of sensitivity to adversities regarding the risk of developing psychotic-like experiences. The association between childhood adversities and pre-adolescent psychotic-like experiences attenuated when we also adjusted for co-occurring emotional and behavioural symptoms. Adult studies have also observed the importance of comorbid psychiatric problems in this relationship (McGrath et al. 2017), and future studies need to integrate the mediating or confounding role of co-occurring psychopathology in the relationship between childhood adversities and the psychosis continuum.

This study benefitted from its prospective design with multi-informant measures on childhood emotional and behavioural problems to overcome shared rater bias, and a well-designed interview for childhood adversities. Importantly, it should be noted that the current study was observational in design, restricting any inferences on the purported causality of our findings. A limitation of the present study is that psychotic-like experiences were ascertained through self-report questionnaires with little information on delusional thoughts. This could lead to a suboptimal assessment of psychotic-like experiences as we did not comprehensively measure the full spectrum of psychotic symptoms. Also, child self-reported measures of psychotic-like experiences might result in inflated prevalence estimates compared to clinician-confirmed assessments (Kelleher, Connor, et al. 2012; Linscott and van Os 2013). However, self-reported psychotic-like experiences have been shown to accurately predict clinician-confirmed symptoms of psychotic disorders (Kelleher et al. 2011; van Nierop et al. 2012). In addition, our childhood adversities interview relied on mother-report only. Future research should incorporate multi-informant measures of childhood adversities to address this limitation.

In summary, this prospective study provides evidence for developmental associations from early childhood emotional and behavioural problems to psychotic-like experiences in pre-adolescence. Moreover, children who were exposed to adversity were more likely to later develop psychotic-like experiences, and this was partly independent of co-occurring psychiatric problems. Importantly, this might help us identify children with increased risk for psychotic psychopathology and subsequent psychiatric illness, including psychotic disorders.



—Our lives are in truth, owning to heredity, as full of cabalistic ciphers, of horoscopic castings (...)

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Table S1: Overview of the childhood adversities ascertained through maternal interview and the corresponding lifetime prevalence rates in percentages ($N = 3822$).

Item	Lifetime prevalence (%)
Did your child get seriously sick or did he/she have an accident?	2.9
Did a family member get seriously sick or did someone have a serious accident?	3.3
Did someone else, who is important to the child, get seriously sick or did someone have a serious accident?	4.0
Has the father/mother or other caretaker of your child died?	0.3
Has someone else, who your child cared a lot about, passed away?	4.2
Has a pet, who your child cared a lot about, died?	2.1
Does or did your child have to deal with a high workload at school?	9.5
Has your child ever repeated a grade?	1.0
Are/were there any neighbourhood problems? E.g. vandalism or insecurity.	1.8
Has your family financial difficulties or had your family ever have them?	1.5
Does your child have ongoing conflicts with a family member (or did your child ever have them)?	2.0
Does your child have ongoing conflicts with someone else (or did your child ever have them)?	2.7
Do other family member have ongoing conflicts with each other (or did they ever have them)?	3.2
Are you and your partner divorced or separated?	4.8
Did one of the parents become involuntarily unemployed?	0.9
Did your child lose a good friend due to an argument?	0.8
Did your child ever lose something which was important to him/her? (e.g. through fire, loss, or theft)	1.0
Has someone ever used physical violence against your child? For example, beating him/her up.	0.9
Has someone almost used physical violence against your child? (i.e. this did not actually happen, but your child was frightened of the possibility).	1.4
Has someone made sexual comments or movements towards your child?	0.5
Has your child experienced inappropriate sexual behaviour?	0.2
Has someone spread mean rumours about your child?	1.9
Has your child moved to a different place of residence?	1.2
Has your child changed schools?	1.5

Table S3: Concordance between mother-reported and child-reported psychotic-like experiences, both reported at age ten years. Values given are the numbers of respondents for each category.

			Mother-report		
			Not true	Somewhat true	Very true
			"My child hears sounds or voices that aren't there"		
Child-report	*"I hear sounds or voices that according to other people aren't there"*	Not true	2836	21	2
		Somewhat true	702	37	3
		Very true	204	33	2
		Weighted $\kappa = 0.071$, $P < 0.05$			
			"My child sees things that aren't there"		
Child-report	*"I see things that other people think aren't there"*	Not true	3143	58	1
		Somewhat true	472	24	2
		Very true	116	19	4
		Weighted $\kappa = 0.067$, $P < 0.05$			
			"My child has strange ideas"		
Child-report	*"I have thoughts that other people would find strange"*	Not true	2536	70	1
		Somewhat true	924	39	6
		Very true	236	22	4
		Weighted $\kappa = 0.029$, $P < 0.05$			

Table S2: Correlations between self-reported problems of the child at age ten years ($N = 3984$).

	Mean (*SD*)	Psychotic-like experiences	Internalising problems	Externalising problems
Psychotic-like experiences	0.91 (1.31)			
Internalising problems	2.16 (2.09)	0.45		
Externalising problems	1.93 (1.91)	0.25	0.34	
Attention problems	3.42 (2.49)	0.34	0.38	0.46

Table S4: Cross-sectional associations between mother-reported emotional and behavioural problems and self-reported psychotic-like experiences ($N = 3846$), additionally adjusted for co-occurring self-reported problems.

	Self-reported psychotic-like experiences at age 10 years	
Mother-reported problems	OR (95% CI)	*P*
Anxious/Depressed	1.06 (1.03-1.09)	<0.001
Withdrawn/Depressed	1.05 (1.01-1.10)	0.006
Somatic Complaints	1.11 (1.07-1.15)	<0.001
Social Problems	1.03 (0.99-1.07)	0.095
Thought Problems	1.09 (1.05-1.12)	<0.001
Attention Problems	1.00 (0.98-1.03)	0.907
Rule-Breaking Behaviour	1.02 (0.97-1.07)	0.464
Aggressive Behaviour	0.99 (0.97-1.01)	0.525

Note: Analyses are adjusted for age at psychotic-like experiences assessment, sex of the child, ethnicity of the child, maternal educational level, maternal psychiatric problems at 3 months post-natal, whether psychotic-like experiences questionnaire was completed alone or with help from another person, childhood adversities (similar to main analyses), and additionally adjusted for age ten years Brief Problem Monitor total problems score.

Table S5: Longitudinal associations between early childhood emotional & behavioural problems and self-reported psychotic-like experiences at age ten years, additionally adjusted for co-occurring self-reported problems.

	Emotional & behavioural problems at age 3 years and self-reported psychotic-like experiences at age 10 years ($n = 3025$)		Emotional & behavioural problems at age 6 years and self-reported psychotic-like experiences at age 10 years ($n = 3653$)	
Mother-reported problems	OR (95% CI)	*P*	OR (95% CI)	*P*
Emotional Reactive	1.06 (1.01-1.10)	0.017	1.02 (1.00-1.06)	0.188
Anxious/Depressed	1.03 (0.97-1.09)	0.361	1.04 (1.00-1.09)	0.039
Somatic Complaints	1.05 (1.00-1.10)	0.044	1.05 (1.01-1.09)	0.013
Withdrawn	1.07 (1.00-1.14)	0.037	1.03 (0.98-1.09)	0.202
Sleep Problems	1.01 (0.98-1.05)	0.505	1.05 (1.01-1.09)	0.011
Attention Problems	0.99 (0.95-1.05)	0.829	0.98 (0.94-1.03)	0.410
Aggressive Behaviour	1.00 (0.99-1.02)	0.548	1.00 (0.98-1.01)	0.807
Autistic traits			1.05 (0.75-1.49)	0.769

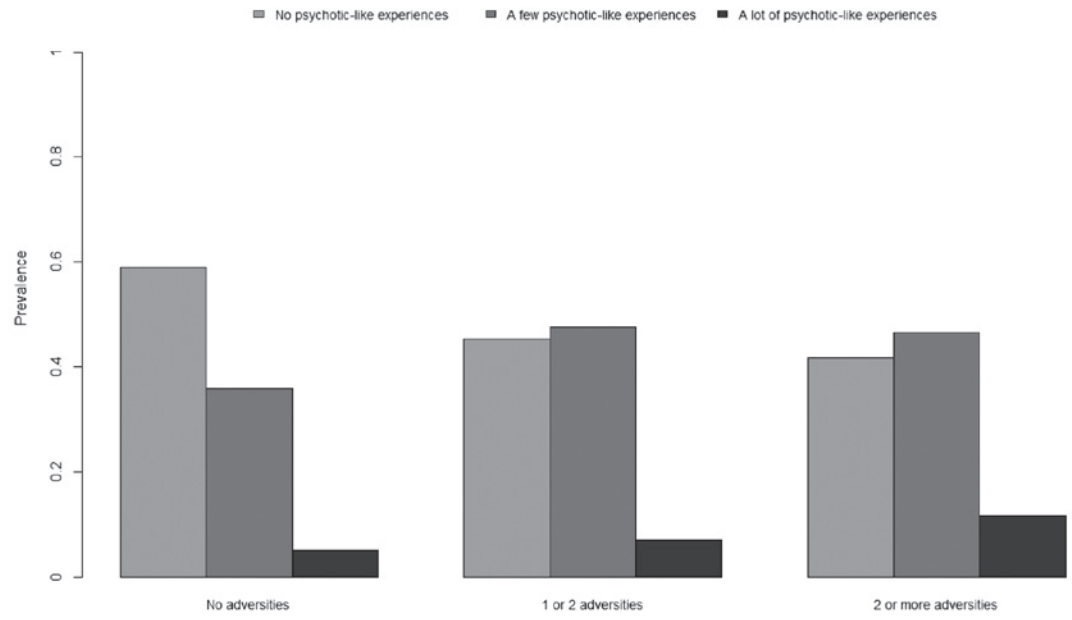
Note: For the association between autistic traits and psychotic-like experiences 3345 participants were included in the analyses. Analyses are adjusted for age at psychotic-like experiences assessment, sex of the child, ethnicity of the child, maternal educational level, maternal psychiatric problems at 3 months post-natal, whether psychotic-like experiences questionnaire was completed alone or with help from another person, childhood adversities (similar to main analyses), and additionally adjusted for age ten Brief Problem Monitor total problems score.

Table S6: Association between childhood adversities and psychotic-like experiences ($N = 3822$), additionally adjusted for co-occurring child self-reported emotional and behavioural problems.

	Full model adjusted for total self-reported problems		Adjusted for self-reported emotional problems only		Adjusted for self-reported behavioural problems only		Adjusted for self-reported attention problems only	
Childhood adversities	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*
All childhood adversities								
No adversities (ref, n=2652)	-	-	-	-	-	-	-	-
1 or 2 adversities (n=947)	1.27 (1.08-1.48)	0.003	1.41 (1.21-1.65)	<0.001	1.57 (1.35-1.83)	<0.001	1.42 (1.22-1.66)	<0.001
>2 adversities (n=223)	1.21 (0.90-1.63)	0.197	1.36 (1.01-1.82)	0.040	1.85 (1.40-2.44)	<0.001	1.56 (1.17-2.07)	0.002
Adversities before age 5 years (n=293)	1.31 (1.02-1.68)	0.035	1.37 (1.07-1.76)	0.012	1.60 (1.26-2.03)	<0.001	1.46 (1.14;1.85)	0.002
Adversities after age 5 years (n=1036)	1.23 (1.06-1.43)	0.008	1.38 (1.18-1.59)	<0.001	1.58 (1.37-1.83)	<0.001	1.43 (1.23-1.66)	<0.001
Maltreatment (n=96)	1.06 (0.70-1.63)	0.776	1.13 (0.74-1.72)	0.565	1.51 (1.00-2.26)	0.048	1.40 (0.93-2.11)	0.104
Physical maltreatment (n=79)	1.05 (0.65-1.68)	0.850	1.08 (0.68-1.73)	0.743	1.42 (0.90-2.23)	0.130	1.26 (0.80-1.99)	0.316
Sexual maltreatment (n=23)	1.07 (0.48-2.42)	0.867	1.37 (0.61-3.10)	0.445	1.84 (0.84-4.04)	0.129	1.64 (0.75-3.59)	0.212
All other adversities (n=1030) *	1.03 (0.95-1.12)	0.484	1.08 (0.99-1.17)	0.090	1.20 (1.11;1.30)	<0.001	1.12 (1.04;1.22)	0.005

Note: All models are adjusted for age at psychotic-like experiences assessment, sex of the child, ethnicity of the child, maternal educational level, maternal psychiatric problems at 3 months post-natal, whether psychotic-like experiences questionnaire was completed alone or with help from another person, and self-reported co-occurring emotional and behavioural problems. * Additionally adjusted for the presence of physical or sexual maltreatment.

Figure S1: Prevalence of psychotic-like experiences stratified by the presence of childhood adversities ($N = 3822$).



(ABSTRACT)

OBJECTIVE

Cannabis use continues to increase among pregnant women. Gestational cannabis exposure has been associated with various adverse outcomes. However, it remains unclear whether cannabis use during pregnancy increases the risk for offspring psychotic-like experiences. In this prospective cohort, we examined the relationship between parental cannabis use during pregnancy and offspring psychotic-like experiences. Comparisons were made between maternal and paternal cannabis use during pregnancy to investigate causal influences of intra-uterine cannabis exposure during foetal neurodevelopmental.

METHOD

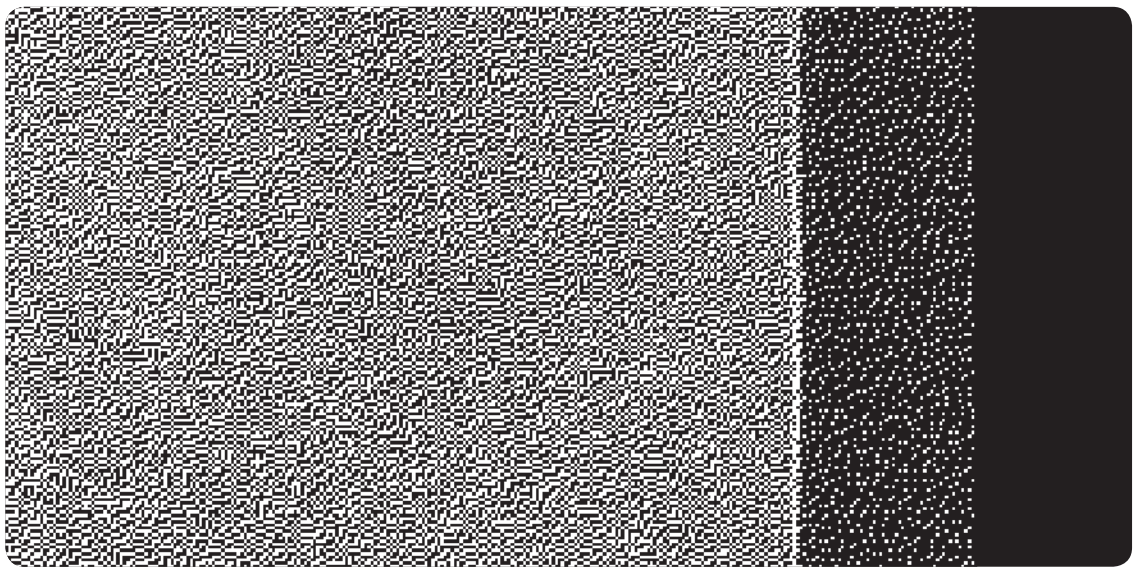
This study was embedded in the Generation R birth cohort and included N = 3692 participants. Maternal cannabis exposure was determined using self-reports and cannabis metabolite levels from urine. Paternal cannabis use during pregnancy was obtained by maternal report.

RESULTS

Maternal cannabis use increased the risk of psychotic-like experiences in the offspring ($OR_{adjusted}$ =1.38, 95% CI 1.03-1.85). Estimates were comparable for maternal cannabis use exclusively before pregnancy versus continued cannabis use during pregnancy. Paternal cannabis use was similarly associated with offspring psychotic-like experiences ($OR_{adjusted}$ =1.44, 95% CI 1.14-1.82).

CONCLUSION

We demonstrated that both maternal and paternal cannabis use were associated with more offspring psychotic-like experiences at age ten years. This may suggest that common aetiologies, rather than solely causal intra-uterine mechanisms, underlie the association between parental cannabis use and offspring psychotic-like experiences. These common backgrounds most likely reflect genetic vulnerabilities and shared familial mechanisms, shedding a potential new light on the debated causal path from cannabis use to psychotic-like phenomena. Our findings indicate that diagnostic screening and preventative measures need to be adapted for young people at risk for severe mental illness.



(...) *as if sorcerers really existed.*
(Marcel Proust, 1920, Guermantes Way)

CHAPTER 3

MATERNAL AND PATERNAL CANNABIS USE DURING PREGNANCY AND THE RISK OF PSYCHOTIC-LIKE EXPERIENCES IN THE OFFSPRING

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Schizophrenia Research, in press.

Childhood psychotic-like experiences are predictive of later psychopathology, including psychotic, affective, and behavioural disorders (Kelleher and Cannon 2011; van Os and Reininghaus 2016; McGrath et al. 2016). Although psychotic disorders are very uncommon in childhood, psychotic-like experiences occur with high prevalence in children from the general population (Kelleher, Connor, et al. 2012; Maijer et al. 2017). Although the majority of psychotic-like experiences remit during development towards adulthood (Thapar et al. 2012; Bartels-Velthuis et al. 2016), children who present with psychotic-like experiences in the context of non-psychotic disorders are characterized by more severe psychopathology, including increased psychiatric comorbid disorders, greater functional and cognitive impairment and poorer prognosis (Dhossche et al. 2002; Kelleher, Keeley, et al. 2012). Importantly, sub-clinical psychotic-like experiences in the general population share genetic risk factors with clinical psychotic disorders (Jeppesen et al. 2015; Zavos et al. 2014). This supports the notion of the psychosis continuum, i.e. a severity continuum with symptoms of psychotic disorder that do not necessarily reach the clinical threshold of psychosis (van Os and Reininghaus 2016). Therefore, it is important to explore the aetiology of psychotic-like experiences, as a childhood presentation of the extended psychosis phenotype (Maijer et al. 2017; van Os and Reininghaus 2016).

Maternal cannabis use during pregnancy has repeatedly been associated with adverse outcomes in the offspring. For example, gestational cannabis use has been associated with fetal growth restriction and lower birth weight (El Marroun et al. 2009), poorer cognitive performance as well as increased behavioral problems in the offspring (El Marroun, Hudziak, et al. 2011; Calvigioni et al. 2014), and differences in offspring cortical thickness of frontal brain areas (El Marroun et al. 2016). Particularly interesting would be to study the effects of cannabis use during pregnancy on offspring psychotic-like experiences, as cannabis use has repeatedly been associated with subsequent psychotic-like symptoms (Arseneault et al. 2004; Moore et al. 2007; Sherif et al. 2016), with a persistent risk of psychopathology beyond the direct effects of exposure to exogenous cannabinoids. Previous studies that investigated whether maternal cannabis smoking during pregnancy is predictive of psychotic-like experiences in offspring (Zammit et al. 2009; Day et al. 2015), were underpowered to observe an association.

Importantly, cannabis use is increasing among pregnant women in Western countries (Hasin et al. 2015; Brown et al. 2017; Volkow, Compton, and Wargo 2017), which warrants further research on the intra-uterine effects of gestational cannabis use on offspring psychiatric problems, including psychotic-like experiences. However, it remains unknown whether parental cannabis use is a risk factor for offspring psychotic-like experiences or whether this relationship is explained by shared etiological factors, such as genetic and environmental vulnerabilities (Gage, Hickman, and Zammit 2016). For example, studies have suggested that similar genetic variants and environmental risks underlie both psychotic symptoms and cannabis use (Power et al. 2014; Verweij et al. 2017; Nesvag et al. 2017). Examining the potential adverse effects of both maternal and paternal cannabis use during pregnancy on psychotic-like experiences in pre-adolescent offspring, which is an age before the risk period of adolescent cannabis use initiation, would help causal inference as comparisons can be made between the observed associations of maternal versus paternal cannabis use during pregnancy and the risk of psychotic-like in the offspring. If the association between cannabis use and psychotic-like experiences is causal, early intra-uterine exposure to cannabis could potentially affect neurodevelopment and contribute to the pathogenesis of psychotic-like phenomena in children who have not yet used cannabis themselves.

Exploring the association between parental cannabis use and offspring psychotic-like experiences might highlight certain parental characteristics that would aid the screening and early detection of childhood psychotic-like experiences, which is predictive of poorer prognosis and greater psychiatric illness severity (Kelleher, Keeley, et al. 2012; Kelleher and Cannon 2011; van Os and Reininghaus 2016). We examined two alternative hypotheses in a large population-based birth cohort from the Netherlands. First, we hypothesized that parental cannabis use increases the risk of offspring psychotic-like experiences through shared genetic or environmental mechanisms. Consequently, we would expect a similar magnitude of association between maternal or paternal cannabis use during pregnancy with offspring psychotic-like experiences.

Alternatively, psychotic-like experiences might arise in children through maternal cannabis use during pregnancy only, suggesting a causal influence of intra-uterine cannabis exposure during foetal neurodevelopmental (El Marroun et al. 2016; Zammit et al. 2009; Calvigioni et al. 2014). In this case, maternal cannabis use during pregnancy would be more strongly associated with offspring psychotic-like experiences than either paternal use or maternal cannabis use that remitted prior to pregnancy.

METHOD

STUDY POPULATION

This study was embedded in the Generation R Study, a population-based birth cohort based in the greater urban area of Rotterdam, the Netherlands (Kooijman et al. 2016). All women living in Rotterdam who were pregnant between April 2002 and January 2006 were eligible for participation, of whom 61% were included. This baseline sample is largely representative of the female population of Rotterdam (Jaddoe et al. 2006). The aim of the Generation R Study is to identify genetic and environmental risk and resilience factors for maternal and child development, including physical and mental health. For the current study, participants were included if data on maternal cannabis use during pregnancy of offspring psychotic symptoms at age ten years were available (N = 3,692). Data on paternal cannabis use and offspring psychotic symptoms was available in 3,371 participants. An overview of the study population flowchart is shown in Figure 1. Compared to the full sample at baseline, mothers who participated at follow-up were more often of Dutch national origin, older and more highly educated (Kooijman et al. 2016). Study protocols were approved by the Medical Ethics Committee of the Erasmus Medical Centre, and informed consent and assent were obtained for all parents and children, respectively.

EXPOSURE TO CANNABIS DURING PREGNANCY

To determine cannabis and tobacco exposure, we used maternal self-reports during pregnancy and measured cannabis metabolite

Figure 1: Inclusion flowchart of the study population

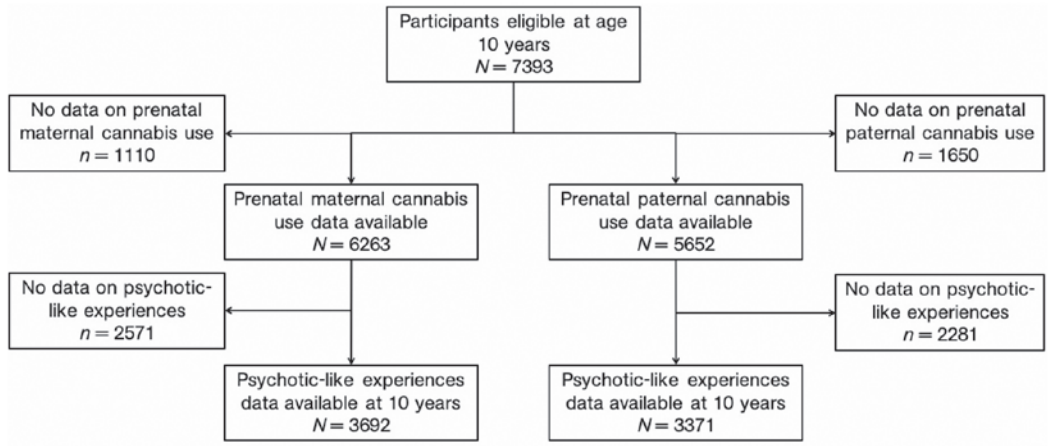


Table 1: Descriptive statistics of the study population

	No exposure group (n = 3123)	Tobacco use throughout pregnancy, (n = 386)		Cannabis use before pregnancy (n = 98)		Cannabis use during pregnancy (n = 85)	
Maternal characteristics		Statistic	P	Statistic	P	Statistic	P
Maternal age at intake in years, mean (SD)	31.45 (4.39)	30.93 (5.44)	0.072	30.28 (5.88|)	0.051	28.53 (6.01)	<0.001
Prenatal psychopathology, median (IQR)	0.22 (0.28)	0.34 (0.39)	<0.001	0.25 (0.28)	0.018	0.46 (0.45)	<0.001
Maternal body mass index, mean (SD)	24.34 (4.00)	25.16 (4.47)	<0.001	23.70 (3.34)	0.067	23.82 (4.56)	0.299
Educational level							
Primary education (%)	4.40	8.99	<0.001	9.28	<0.001	8.54	<0.001
Secondary education (%)	35.80	57.49		48.45		62.20	
Higher education (%)	59.80	33.51		42.27		29.27	
Maternal ethnicity							
Dutch (%)	64.54	60.68	0.310	69.39	0.4697	66.27	0.889
Non-Dutch Western (%)	8.67	9.11		9.18		7.23	
Non-Dutch non-Western (%)	26.79	30.20		21.43		26.51	
Tobacco use							
Never smoked in pregnancy (%)	90.42	0.00	N/A	58.76	<0.001	13.58	<0.001
Smoked in early pregnancy (%)	9.58	0.00		18.56		16.05	
Smoked throughout pregnancy (%)	0.00	100.00		22.68		70.37	
Alcohol use							
Never drank in pregnancy (%)	38.85	30.47	<0.001	29.90	0.025	26.51	0.025
Drinking until pregnancy was known (%)	14.65	13.02		9.28		19.28	
Continued drinking, occasionally (%)	37.28	41.55		45.36		37.35	
Continued drinking, regularly (%)	9.21	14.96		15.46		16.87	
Paternal substance use							
Cannabis use during pregnancy (%)	4.59	11.53	<0.001	64.84	<0.001	84.15	<0.001
Child characteristics							
Gender of the child (% boys)	48.59	51.55	0.296	48.98	1.000	54.12	0.370
Birth weight in grams, mean (SD)	3474 (551)	3322 (542)	<0.001	3426 (487)	0.330	3215 (532)	<0.001
Gestational age at birth in weeks, mean (SD)	39.96 (1.73)	39.81 (1.75)	0.122	40.14 (1.36)	0.198	39.77 (1.51)	0.256
Psychotic-like experiences	45.6	43.8	0.485	55.1	0.179	52.9	0.263
No symptoms (%)	54.37	56.22	0.485	44.90	0.179	47.06	0.263
Mild symptoms (%)	39.51	39.12		47.96		48.24	
Moderate-to-severe symptoms (%)	6.12	4.66		7.14		4.71	

Note: Groups are categorized on maternal cannabis and/or tobacco use during pregnancy. All continues variables are presented as means ± standard deviations; all categorical variables are presented as percentages. There were no missing data on these variables as they were imputed using multiple imputation methods. P-values are derived from ANOVAs for parametric continuous variables, Kruskal-Wallis tests for non-parametric continuous variables and $\chi 2$-tests for categorical variables with reference group as the comparison group. Abbreviations: SD = standard deviation; IQR = interquartile range.

levels from urine (El Marroun et al. 2016; El Marroun et al. 2008). In the first trimester of pregnancy, mothers-to-be were asked whether they had used cannabis (marijuana or hashish) before pregnancy and whether they were still using cannabis. This information was thus not specific for the entire pregnancy. In addition, mothers reported the frequency of cannabis use (i.e. daily, weekly or monthly). Responses were coded as yes or no. Urine samples were also collected in early, mid, and late pregnancy, and the first available sample was used for urinalysis of cannabis metabolites. Urine samples were missing in almost half of the cohort as its collection was performed during a limited period in the prenatal phase of the study. Due to the limited time frame of urine collection, 78.9% (n = 2,375) of the pregnant women filled out the cannabis use questionnaire after urine collection, and the remaining 21.1% filled out the questionnaire before urine sample collection, as described in more detail elsewhere (El Marroun, Tiemeier, et al. 2011). Samples were tested on the presence of 11-nor-Δ9-THC-9-COOH using DRI® Cannabinoid Assay (Microgenics) with a cutoff value of 50 µg/l as recommended by the manufacturer and the Substance Abuse and Mental Health Security Agency. The agreement between self-reported cannabis use and cannabis metabolites from urine using Yule's Y was 0.77, indicating substantial agreement (El Marroun, Tiemeier, et al. 2011). Cannabis use of the biological father during pregnancy (including frequency) was obtained by maternal report during the first trimester of pregnancy, without assessing a specific time frame. We opted for maternal report of paternal cannabis use as few fathers completed questionnaires during pregnancy (data on partner-reported cannabis use was available in n = 2,775 participants in the current sample, as opposed to n = 3,368 participants through mother-report). Maternal report of paternal cannabis use was highly correlated with paternal self-report (r=0.80, P<0.001). Sensitivity analyses pertaining to partner self-reported cannabis use were shown in the Supplement.

OFFSPRING PSYCHOTIC-LIKE EXPERIENCES AT AGE 10 YEARS

At age ten years, children were queried regarding psychotic-like experiences, which were derived from the widely-used and reliable Youth Self Report (Ivanova et al. 2007): (a) "I hear sounds or voices that according to other people are not there", (b) "I see things that other people think are not there", and (c) "I have thoughts that other people would find strange". Previous work has shown that similarly worded questions adequately captured symptoms of the psychosis spectrum, and that they are predictive of clinician-confirmed psychotic symptoms (Kelleher et al. 2011; Kelleher and Cannon 2011). These three items could be scored on a 3-point scale and sum scores of these symptoms were categorized into three groups in order to facilitate interpretation: no symptoms (0 points, n = 1,999, 54.1% of children), mild symptoms (1-3 points, n = 1,473, 39.9%) and moderate-to-severe symptoms (4-6 points, n = 220, 6.0%). These categories were treated separately in our ordinal logistic regression models, i.e. they were not collapsed.

COVARIATES

Several confounders, which have previously been associated with cannabis use or psychotic-like experiences were considered (El Marroun et al. 2008; van Os and Reininghaus 2016). Child age, sex, and maternal age were obtained from medical records. Child ethnicity was considered as Dutch when both parents were born in the Netherlands, while children were classified as non-Dutch if at least one of the parents was born outside the Netherlands (further specified as 'Other Western' or 'Other Non-Western'). Maternal education was defined by the highest educational level attainment and classified into low (primary school or lower), middle (lower and intermediate vocational training) or high (higher vocational education and university). Maternal psychopathology scores were assessed with the Brief Symptom Inventory (BSI) (Derogatis and Melisaratos 1983), a validated self-reported continuous measure of 53-items encompassing a spectrum of psychiatric symptoms. The BSI covers several syndrome scales, including psychoticism, paranoid ideations, depression, anxiety and hostility. Similar to tobacco smoking, maternal alcohol use during pregnancy was assessed through self-reported questionnaires.

STATISTICAL ANALYSES

The study population was categorized into 4 distinctive groups: a) no exposure (*reference*), n = 3,123; b) continued tobacco use throughout

pregnancy, *n* = 386; c) cannabis use exclusively before pregnancy, *n* = 98 and d) continued cannabis use during pregnancy, *n* = 85. First, data were examined and descriptive statistics were presented. Differences between the individuals groups (b, c, and d) and the no exposure group (a) as a reference were tested using ANOVAs for continuous measures and Chi-square tests for categorical variables.

Next, ordinal logistic regression was conducted to investigate whether maternal and paternal cannabis use were associated with offspring psychotic-like experiences. First, maternal cannabis use prior to and continued use during pregnancy are investigated jointly in association with psychotic-like experiences in the offspring. In a secondary analysis, a distinction was made between maternal cannabis use exclusively before versus continued use during pregnancy. All models were adjusted for all relevant covariates, see above. Sensitivity analyses were performed excluding mothers and fathers who were infrequent (i.e. monthly) cannabis users in order to examine dose-response effects. Missing information on the covariates varied between 0 and 12.8%. To avoid bias of complete case analyses, we accounted for missing values on covariates by using multiple imputations in MICE 2.25 (van Buuren and Groothuis-Oudshoorn 2011), and estimates from 100 imputed datasets were pooled and presented throughout the paper. All variables included in the association models were used in the imputation model to avoid artificial attenuation of associations. Data were analysed using R statistical software (R Core Team 2015).

RESULTS

Demographic characteristics of the mother and child are shown in Table 1. Mothers who used cannabis during pregnancy were younger, had higher psychopathology scores and were more likely to have a lower educational level. In addition, mothers who used cannabis were more likely to also use tobacco and alcohol during pregnancy, and more often had partners who also used cannabis (i.e. 84.2%). Children of mothers who used cannabis prior to pregnancy or continuously during pregnancy endorsed psychotic-like experiences slightly more often than non-exposed offspring, although this difference was not statistically significant (*P*=0.18 and *P*=0.26, respectively).

Of the mothers who used cannabis exclusively prior to pregnancy, most were monthly users (*n* = 39, 41.4%), whereas *n* = 25 (26.6%) were weekly users and *n* = 30 (31.9%) were daily users. Mothers who continued using cannabis during pregnancy were most often daily (*n* = 24, 37.5%) or weekly (*n* = 24, 37.5%) users, while *n* = 16 (25.0%) were monthly users. Paternal cannabis use frequency was as follows: daily (*n* = 124, 42.6%), weekly (*n* = 87, 29.9%) or monthly (*n* = 80, 27.5%).

Maternal cannabis use (combined exclusively before and continued during pregnancy) was associated with an increased risk for psychotic-like experiences in their offspring (Table 2, *n* = 183, $OR_{adjusted}$=1.38 [95% CI 1.03-1.85]). Estimates were comparable for cannabis use exclusively before pregnancy versus continued cannabis during pregnancy (cannabis use before pregnancy: *n* = 98, $OR_{adjusted}$=1.39 [95% CI 0.94-2.06]; continued cannabis use during pregnancy: *n* = 85, $OR_{adjusted}$=1.37 [95% CI 0.90-2.08]). Paternal cannabis use was significantly associated with offspring psychotic-like experiences (*n* = 297, $OR_{adjusted}$=1.44 [95% CI 1.14-1.82]). Similar results were observed in the sensitivity analyses using partner self-reported cannabis use (Supplementary Table1), and in sensitivity analyses excluding parents who were infrequent (i.e. monthly) cannabis users (data not shown). On the other hand, maternal gestational tobacco exposure was not associated with offspring psychotic-like experiences (*n* = 386, $OR_{adjusted}$=0.97 [95% CI 0.78-1.20]).

DISCUSSION

Using unique data from a large population-based birth cohort, we demonstrated that maternal and paternal cannabis use were each associated with offspring psychotic-like experiences at age ten years, an age well before the risk period of adolescent cannabis use initiation. Notably, estimates were similar for maternal cannabis use exclusively before pregnancy versus continued cannabis use during pregnancy. Importantly, the association between parental cannabis use and offspring psychotic-like experiences cannot solely be explained through intra-uterine exposure to cannabis. Rather, we find support for the hypothesis that parental cannabis use and offspring psychotic-like experiences share a common aetiology.

Table 2: Association between parental cannabis use and offspring psychotic-like experiences at age 10 years.

	Child psychotic-like experiences at age 10 years				
		Unadjusted model		Adjusted model	
	N	OR (95% CI)	*P*	OR (95% CI)	*P*
Mother					
No exposure	3123	Reference		Reference	
Continued smoking	386	0.91 (0.74;1.12)	0.384	0.97 (0.78;1.20)	0.784
Cannabis use	183	1.34 (1.01;1.79)	0.043	1.38 (1.03;1.85)	0.031
Before pregnancy	98	1.42 (0.97;2.09)	0.075	1.39 (0.94;2.06)	0.097
During pregnancy	85	1.26 (0.83;1.90)	0.273	1.37 (0.90;2.08)	0.145
Father					
No exposure	3074	Reference		Reference	
Cannabis use	297	1.39 (1.10;1.75)	0.005	1.44 (1.14;1.82)	0.002

Note: The adjusted model is corrected for the following covariates: child age, child sex, child ethnicity, maternal age, maternal education level, maternal psychopathology score, maternal drinking during pregnancy. The effect estimate for the association between paternal cannabis use and offspring psychotic symptoms at age 10 years remained unchanged when paternal characteristics (age and educational level) were included as covariates in the adjusted model ($OR_{adjusted} = 1.41$ (95% CI 1.11;1.79), $P = 0.006$).

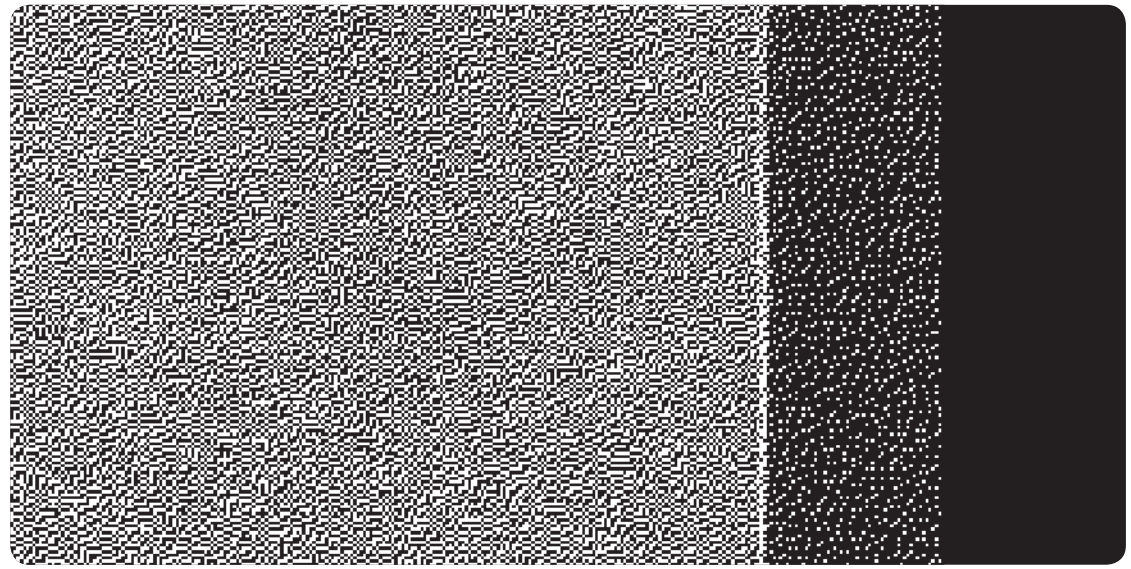
Gestational exposure to cannabis affects fetal growth, offspring brain development, and has been associated with behavioral and cognitive outcomes in the offspring (El Marroun, Hudziak, et al. 2011; El Marroun et al. 2016; El Marroun et al. 2009; Calvigioni et al. 2014). To our knowledge, few studies have examined the effects of prenatal cannabis exposure on psychotic-like experiences in the offspring, for which the few population-based studies to date were underpowered to observe a clear association (Zammit et al. 2009; Day et al. 2015). In the present study, we found that both maternal and paternal cannabis use, as well as maternal cannabis use exclusively before versus continued use during pregnancy increased risk for offspring psychotic-like experiences to a similar extent. Sensitivity analyses with exclusion of parents who infrequently used cannabis further supported our interpretation that the association between parental cannabis use and offspring psychotic-like experiences cannot be fully explained by gestational exposure to cannabis. Rather than solely intra-uterine mechanisms, this relationship can likely be explained through genetic and familial susceptibilities that co-occur in parents as well as their offspring, including epigenetic transgenerational inheritance (such as genomic imprinting) of substance use and psychiatric disorders (Yohn, Bartolomei, and Blendy 2015). In addition, our current findings could possibly be explained by unmeasured confounding factors driving an association between parental cannabis use and offspring psychotic-like experiences. However, our analyses were adjusted for a large number of relevant confounders (Zammit et al. 2009; El Marroun et al. 2008), most notably parental psychiatric problems and socioeconomic factors. Still, the associations observed in this study could have been driven by confounders which were not measured here, most likely overlapping genetic susceptibilities (Nordsletten et al. 2016; Nesvag et al. 2017).

It should be noted that psychotic-like experiences are not equivalent to psychotic disorders, and thus caution is warranted in extrapolating our findings to clinical disorders (Fisher et al. 2013). Yet, even though psychotic-like experiences tend to remit after childhood (Thapar et al. 2012; Bartels-Velthuis et al. 2016), they do appear to show phenomenological and temporal continuity with psychotic disorder (Linscott and van Os 2013), and are reportedly associated with more mental health service use, psychiatric disorder and suicidal behavior (Bhavsar et al.

2017; McGrath et al. 2016; Kelleher et al. 2013). This underscores the importance of studying risk factors for pre-adolescent psychotic-like experiences. There is increasing evidence that cannabis use confers a higher risk for the development of subsequent psychotic symptoms (Belbasis et al. 2018; Gage, Hickman, and Zammit 2016; Arseneault et al. 2004; Vaucher et al. 2017). However, recent Mendelian randomisation studies have observed stronger causal effects of schizophrenia risk on cannabis use initiation than vice versa (Gage et al. 2017; Pasman et al. 2018). Importantly, assortative mating in psychiatric illness and substance use disorders might contribute to the occurrence of more psychotic-like symptomatology in the offspring of cannabis-using parents (Nordsletten et al. 2016). Against this background and by demonstrating the familial co-aggregation of parental cannabis use and offspring psychotic-like experiences, our current findings add novel, albeit preliminary, insights on the discussion of the causal pathway of cannabis use and psychotic-like phenomena.

Strengths of this study included its prospective nature, information on numerous relevant confounders, and the assessment of both maternal and paternal exposure to cannabis. We were limited by our self-report measure of psychotic-like experiences, which might have resulted in elevated prevalence estimates compared to psychotic-like experiences assessed with semi-structured clinical interviews. Nevertheless, psychotic-like experiences assessed through self-reported questionnaires have been demonstrated to have substantial positive predictive value for clinician-confirmed symptoms of psychotic disorder (Kelleher et al. 2011), and our observed prevalence of psychotic-like experiences were similar to those obtained in previous studies using clinical assessments (Polanczyk et al. 2010). In addition, we had limited power to conduct statistical testing on sub-groups of women using cannabis exclusively before versus continued use during pregnancy. However, effect estimates were of the same magnitude as in the analysis on the larger sample of cannabis-using women. Also, we did not have data available of women using cannabis after the first trimester in order to examine trimester-specific associations. Women might have been more likely to discontinue cannabis use after the first trimester. Therefore, we cannot fully rule out the effects of prolonged intra-uterine exposure to cannabis. And although underreporting of cannabis is always possible, neither cannabis possession nor consumption is penalized in the Netherlands (El Marroun et al. 2008). The assessment of maternal as well as paternal cannabis exposure generally relied on maternal report. However, urine metabolites provided independent validation of our cannabis exposure assessment (El Marroun et al. 2016), and the self-reported prevalence of cannabis use in our sample was similar to the national prevalence of cannabis use among pregnant women (Rodenburg et al. 2007). Furthermore, mother-reported and partner-reported cannabis use of the father were highly correlated, and sensitivity analyses with partner-reported cannabis use yielded comparable results. And although age 10 years is early, we cannot fully exclude the possibility of cannabis use by the children. However, data from another Dutch cohort demonstrated that only 0.5% of youth initiated cannabis use before the age of 10 years (van Leeuwen et al. 2011).

In conclusion, the current findings suggest that a common aetiology underlies parental cannabis use and offspring psychotic-like symptomatology, which is most probably a reflection of shared genetic and environmental vulnerabilities present in these families. This has clinically significant implications as diagnostic screening and preventative measures need to be adapted for young people at risk for severe mental illness, and for the families in which these children grow up.



– Hearing voices no one else can hear isn't a good sign, even in the wizarding world.
(J.K. Rowling, 1998, Harry Potter and the Chamber of Secrets)

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(SUPPLEMENT)

Supplementary Table 1: Association between partner self-reported cannabis use and offspring psychotic-like experiences at age 10 years ($N = 2775$).

		Child psychotic symptoms at age 10 years			
		Unadjusted model		Adjusted model	
	N	OR (95% CI)	*P*	OR (95% CI)	*P*
Partner self-report					
No exposure	2546	Reference		Reference	
Cannabis use	229	1.32 (1.02;1.72)	0.035	1.36 (1.05;1.78)	0.022

Note: The adjusted model is corrected for the following covariates: child age, child sex, child ethnicity, maternal age, maternal education level, maternal psychopathology score, maternal drinking during pregnancy. The effect estimate for the association between paternal cannabis use and offspring psychotic symptoms at age 10 years remained unchanged when paternal characteristics (age and educational level) were included as covariates in the adjusted model ($OR_{adjusted} = 1.37$ (95% CI 1.05;1.79), $P = 0.023$).

–But I do know a kind of madness that lies low in the mind, half-buried in consciousness, which lives in parallel to sanity, and given the right circumstances or even just half a chance, (...)

(ABSTRACT)

OBJECTIVE

Psychotic experiences comprise auditory and visual perceptive phenomena, such as hearing or seeing things that are not there, in the absence of a psychotic disorder. Psychotic experiences commonly occur in the general pediatric population. Although the majority of psychotic experiences are transient, they are predictive of future psychotic and non-psychotic disorders. They have been associated with sleep problems, but studies with objective sleep measures are lacking. This study assessed whether psychotic experiences were associated with actigraphic sleep measures, symptoms of dyssomnia, nightmares, or other parasomnias.

METHODS

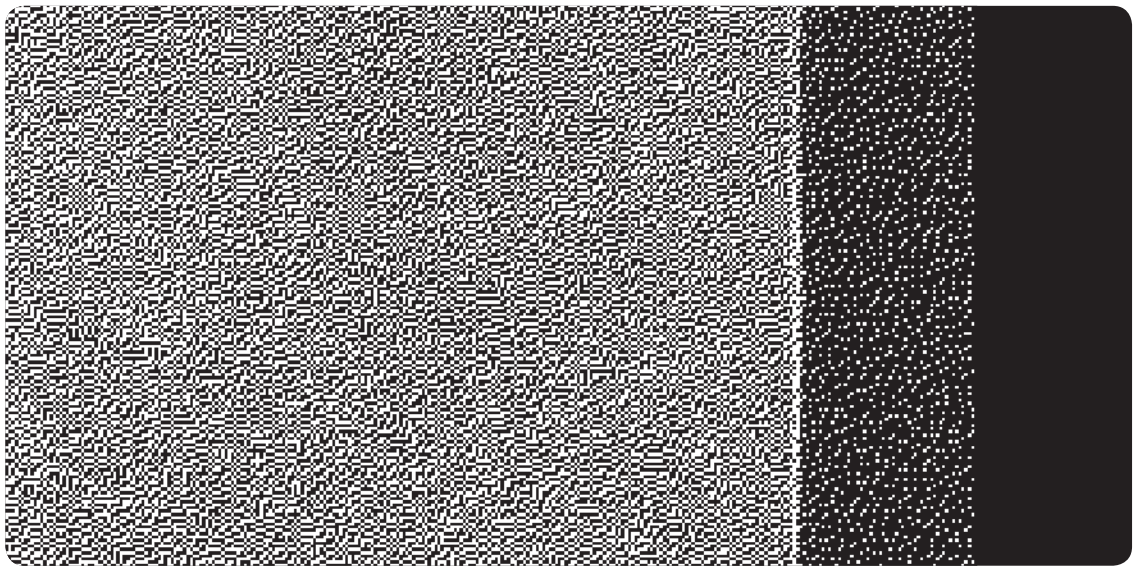
This cross-sectional population-based study comprises 4149 children from the Generation R Study. At age 10 years, psychotic experiences including hallucinatory phenomena were assessed by self-report; dyssomnia and parasomnia symptoms were assessed by mother- and child-report. Additionally, at age 11 years, objective sleep parameters were measured using a tri-axial wrist accelerometer in N = 814 children, who wore the accelerometer for five consecutive school days.

RESULTS

Psychotic experiences were not associated with objective sleep duration, sleep efficiency, arousal, or social jetlag. However, psychotic experiences were associated with self-reported dyssomnia ($B = 2.45$, 95% CI: 2.13-2.77, $p < 0.001$) and mother-reported parasomnia, specifically nightmares ($OR_{adjusted} = 3.59$, 95% CI 2.66-4.83, $p < 0.001$). Similar results were found when analyses were restricted to hallucinatory phenomena.

CONCLUSION

Childhood psychotic experiences were not associated with objective sleep measures. In contrast, psychotic experiences were associated with nightmares, which are a known risk indicator of psychopathology in pre-adolescence. More research is needed to shed light on the potential etiologic or diagnostic role of nightmares in the development of psychotic phenomena.



(...) *creeps like a lick of flame or a growing tumour up and around ordinary perception, consuming it for a while, and causing one, even when not at the movies,* (...)

CHAPTER 4

DURING DAY AND NIGHT:

CHILDHOOD PSYCHOTIC EXPERIENCES AND OBJECTIVE AND SUBJECTIVE SLEEP PROBLEMS

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Schizophrenia Research, in press.

INTRODUCTION

Psychotic experiences comprise auditory and visual perceptive phenomena, such as hearing or seeing things that are not there, or delusional thoughts, in the absence of a psychotic disorder (Kelleher, Jenner, and Cannon 2010). With a prevalence around 7%, psychotic experiences are common in the general adult population (Linscott and van Os 2013). The prevalence is particularly high in children aged 9 to12 years with rates up to 17%, whereas in adolescence the prevalence declines to 7.5 % (Kelleher, Connor, et al. 2012; Kelleher, Keeley, et al. 2012). It is important to study childhood psychotic experiences because children, who report such symptoms in late childhood or early adolescence, have a 5 to 16 times higher risk for developing psychotic disorders in adulthood (Poulton et al. 2000; Welham et al. 2009; Kelleher and Cannon 2011). Indeed, psychotic experiences share a genetic risk with psychotic disorders (Jeppesen, Larsen, et al. 2015; Zavos et al. 2014). Further, children with psychotic experiences are at increased risk for various non-psychotic psychopathologies, such as bipolar disorder, suicidal behavior, anxiety, and depressive disorders (Kelleher, Keeley, et al. 2012; Wigman et al. 2011; McGrath et al. 2016), which highlights the trans-diagnostic characteristics of psychotic experiences and supports the need to have a better understanding of their etiology and development across childhood and adolescence.

Sleep problems, such as insufficient sleep, symptoms of dyssomnia (including insomnia or excessive sleepiness), and symptoms of parasomnia (a comprehensive term for nighttime behaviors including sleep-walking, sleep-talking, and nightmares) (Fleetham and Fleming 2014; Mason and Pack 2007), are considered as possible triggers of psychotic experiences across age groups (Lee et al. 2012; Reeve, Sheaves, and Freeman 2015; Reeve et al. 2017; Thompson et al. 2015; Oshima et al. 2010; Taylor et al. 2015). In adults, sleep problems are associated with both severity and number of psychotic experiences (Reeve, Sheaves, and Freeman 2015; Andorko et al. 2017). Similarly, in high-risk adolescent populations shorter sleep duration and parasomnia have been associated with psychoses (Lunsford-Avery and Mittal 2013; Lunsford-Avery et al. 2015; Ruhrmann et al. 2010). A few studies using self- or mother-reported measures of sleep problems have been conducted in pediatric populations (Lee et al. 2012; Jeppesen, Clemmensen, et al. 2015), and found that psychotic experiences co-occur with self-reported sleep problems (Jeppesen, Clemmensen, et al. 2015). Consistent with this, others report that psychotic experiences in adolescence often are preceded by severe nightmares in childhood (Fisher et al. 2014). While there is a rising interest in the role of sleep problems in the development of psychotic experiences, so far very few clinical studies and no population-based studies used objective measures of sleep to study this association. Addressing this gap can help elucidate the developmental mechanisms behind the association between objectively assessed sleep difficulties and psychotic experiences in childhood. In this study, we investigated in a general pediatric population whether childhood psychotic experiences are associated with actigraphically measured sleep duration, sleep efficiency, and arousal. Additionally, previous literature points at the difference of week and weekend sleep in late childhood and adolescents; teenagers tend to sleep less during schooldays and make up for this during weekend days by rising later and sleeping longer (Carskadon 2011; Crowley et al. 2018). Thus, we calculated the "social jetlag". Social jetlag is the discrepancy in sleep between school days and weekend days (Wittmann et al. 2006). Third, we investigated whether childhood psychotic experiences are associated with self- or mother-reported sleep problems such as dyssomnia and parasomnia symptoms. We examined the associations between our various sleep measures and hallucinatory phenomena specifically as these have been shown to be most predictive of clinically-confirmed psychotic symptoms (Kelleher et al. 2011). Based on previous population-based studies (Lunsford-Avery et al. 2015; Jeppesen, Clemmensen, et al. 2015; Fisher et al. 2014), we expect that psychotic experiences in childhood are associated with objective shorter sleep duration and reported sleep dysfunction, such as symptoms of dyssomnia and parasomnia.

METHODS

DESIGN AND STUDY POPULATION

This cross-sectional study was embedded in Generation R Study, a prospective population-based cohort from foetal life onwards.

Women who were pregnant between April 2002 and January 2006 and living in Rotterdam were eligible for participation (61% included). This sample was largely representative of the Rotterdam female population (Jaddoe et al. 2006). The Generation R Study aims to identify genetic and environmental risk factors for the growth and development of mothers and children.

All 7393 participants who consented in the age 10 assessment wave received questionnaires and were invited at the research centre for objective behavioural assessment (Kooijman et al. 2016). Children without information on psychotic experiences or sleep problems were excluded (n=3244) yielding a sample size of 4149 children for the present study.

The subsample of 1153 children was selected based on the following criteria: first, we selected participants who had participated within the Generation R Focus Study: This includes participants with good follow-up rates (Kooijman et al. 2016). Ethnic minorities were not included in order to address genetic and epigenetic questions. Second, we oversampled children who were born premature in this study to counter the selection effects observed for children born preterm. Indeed, our subsample showed similar rates of premature children to the total cohort. Due to logistic reasons, the accelerometer data collection was conducted nearly one year after the 10 years (questionnaire) assessment. Of the invited children, 953 participants consented to participate (response rate of 82%). Children were excluded from the analyses if data on weekday sleep was not available or when data did not pass standard quality control. Data were excluded if the actigraphy wear time was under 6 hours or if sleep time was under 4 hours. Sleep time under 4 hours was often due to exceptional social activities and field trips in this population and did not reflect typical patterns or insomnia (Meltzer et al. 2012; Acebo et al. 1999). The final sample consisted of 814 children with information on psychotic experiences and good quality actigraphy measures on objective sleep (mean age 11.7 years, SD=0.20). The children participating in the subsample were more often of Dutch nationality and had mothers with higher educational levels and lower levels of psychopathology (all $p<0.001$). However there were no differences between the total sample and the actigraphy sample on mother- and self-reported exposure and outcome variables. The Medical Ethics Committee of the Erasmus Medical Center approved all study procedures, and all parents provided written informed assent.

MEASURES

PSYCHOTIC EXPERIENCES

Psychotic experiences were assessed by self-report questionnaire using three items derived from the widely used Youth Self-Report (Ivanova et al. 2018): “I hear sounds or voices that according to other people are not there”, “I see things that other people think are not there”, “I have thoughts that other people would find strange”. Responses were scored on a three-point scale, i.e. “Not at all”, “A bit” or “Clearly”. Responses from all three items were summed to calculate a total score which ranged from 0 – 6, with higher scores indicating more psychotic experiences; the correlation between the items was moderate to large (.38-.56). Scores were classified into the following categories: no symptoms (0 points), some symptoms (1-3 points), and several symptoms (4-6 points). To asses hallucinatory phenomena separately, we combined the two hallucinatory phenomena questions to a hallucinatory phenomena score categorized as: no symptoms, some symptoms (1-2 points), and several symptoms (3-4 points). These cut-offs were chosen so that the children in the upper category would have endorsed “clearly” on at least one of the items.

OBJECTIVE SLEEP MEASURES

Sleep was assessed using a tri-axial wrist accelerometer (GENEActiv; Activinsights, UK) which children wore for nine subsequent days (five school days and four weekend days) on their non-dominant wrist. The GENEActiv accelerometers record raw accelerometer data; for the current study accelerometers were set at a frequency of 50 Hz, which allowed us to use the accelerometers for 14 subsequent days without recharging and in line with another study (Ronnlund et al. 2016). The GENEActiv PC software version 2.2 was used to download the raw data as binary files. The binary files were processed

using the R-package GGIR (van Hees et al. 2014).The processing included auto calibration with gravity as reference, detection of atypical values and non-wear. The algorithm is using an accelerometer-derived arm angle averaged over 5-second epochs to detect sleep. If there is no arm-movement larger 5° for at least 5 minutes this will be classified as a period of sustained inactivity or sleep. This procedure generated the following sleep measures: sleep duration, sleep efficiency, and sleep arousal (van Hees et al. 2015). Sleep duration is the total time classified as sleep during the night, indicating the time between falling asleep and waking minus the time lying awake. Sleep efficiency is the total sleep duration divided by bed time and waking time. Arousal is the number of sleep periods during the night, the higher the number awakenings, the higher the arousal. We calculated social jetlag by taking the average midpoint sleep during the weekend subtracted by the average midpoint sleep during week (Wittmann et al. 2006). For the measures of sleep duration, sleep efficiency, and sleep arousal only school days were included in the analyses, representing the typically pattern of weekday sleep to minimize the influence of atypical weekend events.

MULTI-RATED SLEEP PROBLEMS

SELF-REPORTED DYSSOMNIA

At age 10 years, dyssomnia symptoms were assessed by self-report questionnaire asking six questions about their perceived sleep i.e. “Do you find it difficult to go to bed?”; “Do you find it difficult to fall asleep?”; “Do you think you get enough sleep?”; “If you wake up at night, do you find it difficult to fall asleep again?”; “Do you feel rested when you wake in the morning?”; “When you come out of your bed in the morning, do you feel rested?”. These questions were derived from the widely used Sleep Disturbance Scale for Children (SDSC) (Bruni et al. 1996) and slightly rephrased for our paediatric population. Similar questions can be found in other sleep scales for children such as the Sleep Self Report (Owens et al. 2000), and School Sleep Habits Survey (Wolfson and Carskadon 1998). There were three possible responses for each item: “No”, “Sometimes” or “Yes”, which were scored on a Likert scale. Responses from all six items were summed to calculate a total score with an internal consistency of $\alpha = 0.64$, higher scores indicate more dyssomnia problems.

MOTHER-REPORTED CHILD SLEEP PROBLEMS

At age 10 years, children’s sleep problems were quantified using the Child Behavior Checklist 6-18 (CBCL), a reliable and valid measure for behavioural problems (Verhulst 2013; Achenbach and Ruffle 2000). The CBCL was completed by the primary caregiver, in the majority of cases the mother, who rated various sleep problems of the child in the previous two months on a three-point Likert scale (0 = not true, 1 = somewhat true, 2 = very true).

In line with a previous study (Verhoeff et al. 2018), we selected 5 items from the CBCL/6-18 questionnaire a priori because there is no established subscale for measuring sleep problems from the CBCL/6-18. We ran a confirmatory factor analysis in order to construct a sleep problems scale at 10 years and to examine which questions loaded together. This resulted in a two-factor solution (combined internal consistency of $\alpha = 0.52$). The first factor compromised 3 questions representing dyssomnia symptoms: “Trouble with sleeping”; “Sleeps less than most kids”; “Overtired with no good reason” (internal consistency of $\alpha = 0.55$), the second factor compromised 2 questions representing parasomnia symptoms: “Nightmares” and “Talks or walks in sleep” (internal consistency of $\alpha = 0.33$).

MOTHER-REPORTED CHILD EMOTIONAL AND BEHAVIOURAL PROBLEMS

The CBCL/6-18 was also used to assess child emotional and behavioural problems at age 10 years, (Achenbach and Rescorla 2001). The CBCL/6-18 consists of 8 syndrome scales: anxious/depressed, withdrawn/depressed, somatic complaints, social problems, thought problems, attention problems, rule-breaking behaviour, and aggressive behaviour measured on a continuous severity scale. Items were scored by mothers on a three-point scale (0 = not true; 1 = somewhat true; 2 = very true), based on behaviour in the past six months. We computed a total problem scale including all items of the CBCL and excluding the items measuring sleep problems.

CONFOUNDERS

Based on previous literature we considered the following confounders (Morgan et al. 2009; Kelleher and Cannon 2011). Sleep problems and psychotic experiences are both associated with age, ethnicity, and sex of the child (Sadeh, Raviv, and Gruber 2000; Kelleher and Cannon 2011; Spauwen et al. 2003). Likewise, both sleep problems and psychotic experiences are related to maternal educational level and psychopathology, such as depressive symptoms (Kelleher and Cannon 2011; Sadeh, Raviv, and Gruber 2000). Gestational age was added as a confounder because we specifically added children born prematurely to this study. Sex and age of the children were obtained from the medical records completed by community midwives and obstetricians. Child ethnicity was considered as Dutch when both parents were born in the Netherlands, while children were classified as non-Dutch if at least one of the parents was born outside the Netherlands (further specified as 'Other Western' or 'Other Non-Western'). Information on maternal educational level was obtained by questionnaires during pregnancy. Maternal education was defined by the highest attained educational level and classified into three categories (low, middle, and high education). Finally, maternal depressive symptoms were assessed using the Brief Symptom Inventory (BSI) (De Beurs 2004) when child was at mean age 10 years.

STATISTICAL ANALYSIS

Self-reported dyssomnia was square root transformed in order to approach normality as on inspection of the data self-reported dyssomnia was not normally distributed. Because of the low prevalence of sleep problems, we categorized the scores for mother-reported dyssomnia into two categories, "no or one symptom" and "two or more symptoms" and mother-reported parasomnia into two categories, "no symptoms" and "one or more symptoms". First, we tested the association of psychotic experiences with objective sleep-duration, sleep efficiency, arousal, and social jetlag in those with accelerometer data using linear regression models. Second, we analysed the association of psychotic experiences with self-reported continuous dyssomnia symptoms using linear regression. Next, to test the association of psychotic experiences with mother-reported symptoms of dyssomnia, parasomnia, and more specifically nightmares, sleep walking and sleep talking, we conducted logistic regression analyses for these binary outcome variables. All analyses were repeated separately for hallucinatory phenomena, considered the most typical positive symptom of the psychosis continuum (Kelleher and Cannon 2011). Analyses were adjusted for the confounders, described above. To reduce bias due to missingness, missing data on the confounders were ten times imputed. All analyses were conducted in SPSS version 24 (IBM Corporation).

SENSITIVITY ANALYSES

For sensitivity analysis, models concerning objective sleep measures were rerun using combined weekend plus weekday sleep as it has been suggested that weekend sleep may better represent children's natural sleep (Snell, Adam, and Duncan 2007). In an additional step we adjusted for concurrent child psychopathology assessed with mother-reported CBCL, in order to derive specific insight into the association between psychotic experiences and sleep problems. Further, in order to obtain the estimates for the sleep duration of all weekday nights and psychotic experiences, sensitivity analyses were conducted including nights with less than 4 hours sleep duration. Finally, post-hoc Bonferroni adjustments were carried out for our 8 hypotheses, yielding more conservative alphas ($\alpha = 0.05/8 = 0.00625$).

RESULTS

Characteristics of the study population are presented in Table 1. High scores ('several symptoms') of psychotic experiences were reported by 6.0% of the children.

Table 1. Characteristics of the study population

Child characteristics	N	Total sample N = 4149	N	Accelerometer sample N = 814
Sex (% girls)	2111	50.9	814	52.6
Ethnicity			814	
Dutch %	2814	67.8	691	84.9
Other Western %	348	8.4	45	5.5
Non Western %	987	23.8	78	9.6
Psychotic experiences	4149		814	
No symptoms %	2261	54.5	404	49.6
Some symptoms %	1641	39.6	352	43.2
Several symptoms %	247	6.0	58	7.1
Hallucinatory phenomena	4149		810	
No symptoms %	2865	69.1	546	67.1
Some symptoms %	1076	25.9	221	27.3
Several symptoms %	208	5.0	43	5.3
Dyssomnia (child-reported)	4074	10.9(2.5)	802	11.0(2.5)
Dyssomnia (mother-reported)	4118		814	
Sometimes %	489	11.8	88	10.8
Not at all %	3629	87.5	694	685.3
Parasomnia	4121		814	
Sometimes %	1141	27.5	216	26.5
No or one symptom%	2980	71.8	566	69.5
Nightmares	4121		814	
Sometimes %	711	17.1	126	14.6
Not at all %	3422	82.5	668	77.2
Sleep (weekday)				
Duration (hours : minutes)	-	-	814	8:00(0:36)
Efficiency %	-	-	814	82.3(5.2)
Arousal (number awakenings)	-	-	814	24.3(3.3)
Social jetlag (hours:minutes)	-	-	813	0:45(1:01)
Maternal Characteristics				
Age at inclusion (years)	4149	31.6 (4.6)	814	32.2 (3.9)
Educational level	4149		814	
No education/ primary school %	200	4.8	14	1.8
School / lower vocational training %	1605	38.7	254	32.1
Higher or academic training %	2344	56.5	523	66.1
Depressive symptoms	4149	0.2(0.4)	814	0.2(0.3)

Data represent means (SDs) unless specified otherwise.

Table 2. The association of psychotic experiences and hallucinatory phenomena with weekday-sleep in preadolescence.

	Sleep duration, hours:minutes N= 814			Sleep efficiency, % N= 814			Arousal, no N= 814			Social Jetlag N=813		
	B	95 % CI	*p*	B	95 % CI	*p*	B	95 % CI	*p*	B	95 % CI	*p*
Psychotic experiences												
No symptoms	0	(ref.)		0	(ref.)		0	(ref.)		0	(ref.)	
Some symptom, yes	.00	-.08 – .10	.960	-.05	-.83 – .74	.903	.17	-.33 – .67	.511	-.03	-.24-.19	.820
Several symptoms, yes	-.04	-.17– .09	.551	.33	-.82 – 1.48	.588	-.29	-1.06 – .48	.452	-.20	-.52-.12	.240
Hallucinatory phenomena												
No symptoms	0	(ref.)		0	(ref.)		0	(ref.)		0	(ref.)	
Some symptom, yes	-.03	-.10 – .08	.534	.21	-.22 – .64	.621	.28	-.24 – .81	.293	-.09	-.31-.14	.454
Several symptoms, yes	.07	-.11– .26	.452	1.11	-.68 – 2.90	.192	-.62	-1.66 – .43	.255	-.26	-.71-.18	.261

Note: the associations were adjusted for sex, child's ethnicity, age at sleep assessment, gestational age, maternal education, and maternal psychopathology.

Table 3. The association of psychotic experiences and hallucinatory phenomena with multi-rated sleep problems at age 10 years in the total sample.

	Child-reported			Mother-reported								
	Dyssomnia N=4074			Dyssomnia N=4118			Parasomnia N=4121			Nightmares N=4121		
	B	95 % CI	*p*	OR	95 % CI	*p*	OR	95 % CI	*p*	OR	95 % CI	
Psychotic experiences												
No symptoms	0	(ref.)		0	(ref.)		0	(ref.)		0	(ref.)	
Some symptoms, yes	1.22	1.06–1.37	< .001	1.93	1.57–2.38	< .001	1.48	1.28–1.71	< .001	1.83	1.54–2.19	<
Several symptoms, yes	2.45	2.13–2.77	< .001	3.48	2.48–4.89	< .001	2.56	1.94–3.36	< .001	3.59	2.66–4.83	<
p for trend			< .001			< .001			< .001			< .001
Hallucinatory phenomena												
No symptoms	0	(ref.)		0	(ref.)		0	(ref.)		0	(ref.)	
Some symptom, yes	1.11	.96–1.31	< .001	1.67	1.35–2.06	< .001	1.55	1.33–1.81	< .001	1.93	1.61–2.31	<
Several symptoms, yes	2.02	1.69– 2.40	< .001	2.31	1.59–3.35	< .001	2.12	1.57–2.84	< .001	2.74	1.99–3.78	<
p for trend			< .001			< .001			< .001			< .001

Note: the associations were adjusted for sex, child's ethnicity, age at sleep assessment, gestational age, maternal education, and maternal psychopathology.

THE ASSOCIATION OF PSYCHOTIC EXPERIENCES WITH OBJECTIVE WEEKDAY-SLEEP

Psychotic experiences were not associated with objective sleep duration (B = -0.04,95%CI: -0.17-0.09), sleep efficiency (B = 0.33, 95% CI: -0.82-1.48), arousal (B = -0.29, 95% CI: -1.06-0.48), or social jetlag (B = -0.20, 95% CI: -0.52-0.12) (Table 3). Similarly, hallucinatory phenomena were not associated with sleep duration (B = 0.07, 95% CI:-0.11-0.26), sleep efficiency (B = 1.11, 95% CI: -0.68-2.90), arousal (B = -0.62, 95% CI: -1.66-0.43), and social jetlag (B = -0.26, 95% CI: -0.71-0.18) (Table 2).

THE ASSOCIATION OF PSYCHOTIC EXPERIENCES WITH SLEEP PROBLEMS SELF-REPORTED SLEEP PROBLEMS

Psychotic experiences were associated with higher levels of self-reported dyssomnia (B = 2.45, 95% CI: 2.13-2.77,). Likewise, when examined separately, hallucinatory phenomena were associated with higher levels of dyssomnia (B = 2.02, 95% CI: 1. 69-2.40)(Table 3).

MOTHER-REPORTED SLEEP PROBLEMS

Psychotic experiences were also associated with mother-reported dyssomnia ($OR_{adjusted}$ = 3.48, 95%CI:2.48-4.89). Similarly hallucinatory phenomena by itself were associated with mother-reported and dyssomnia ($OR_{adjusted}$ = 2.31, 95%CI:1.59-3.35). We observed a dose-response relationship of psychotic experiences with mother-reported dyssomnia and also of hallucinatory phenomena with mother-reported dyssomnia. For parasomnia, results indicated that more psychotic experiences were related to higher levels of mother-reported parasomnia, and specifically, more nightmares. The association between psychotic experiences of the child was not present for sleep walking or sleep talking (data not shown), indicating that the association for parasomnia was driven mainly by nightmares ($OR_{adjusted}$ = 3.59, 95% CI: 2.66-4.83). When analyzing hallucinatory phenomena specifically, the same dose-response relationship was observed; children with hallucinatory phenomena were more likely to have more mother-reported nightmares ($OR_{adjusted}$ = 2.74, 95% CI: 1.99-3.78) (Table 3).

SENSITIVITY ANALYSES

The results were essentially unchanged when we analyzed objective sleep measures including weekend sleep (Table S1). When we additionally adjusted for co-occurring child emotional and behavioural problems the null findings for objective sleep measures remained. The observed association of psychotic experiences and self-reported dyssomnia symptoms also remained but was slightly attenuated (B = 2.16, 95% CI: 1.84-2.48, $p < 0.001$). Likewise, when we tested hallucinatory phenomena separately, hallucinatory phenomena were associated with higher levels of dyssomnia (B = 1.75, 95% CI: 1.40- 2.10, $p < 0.001$). Also, the association psychotic experiences and mother-reported dyssomnia symptoms remained but was attenuated ($OR_{adjusted}$ = 2.12, 95% CI: 1.47-3.05, $p < 0.001$) when adjusted for child emotional and behavioural problems. Importantly, when analyses were restricted to hallucinatory phenomena the association between psychotic experiences and mother-reported dyssomnia disappeared. The association for psychotic experiences and mother-reported nightmares remained when adjusted for child emotional and behavioural problems, but with a smaller OR ($OR_{adjusted}$ = 2.50, 95% CI: 1.83-3.43, $p < 0.001$); similarly the association specifically for hallucinatory phenomena with nightmares was attenuated ($OR_{adjusted}$ = 2.00, 95% CI: 1.42-2.81, $p < 0.001$) (Table S2) by this adjustment. Results did not change when we reran a sensitivity analysis including the nights with a sleep duration shorter than 4 hours (B = -0.04, 95% CI: -0.17-0.10, p = 0.61; B = 0.08, 95% CI: -0.11-0.26, p = 0.41) for psychotic experiences and hallucinatory phenomena, respectively. All statistically significant results survived the multiple testing corrections.

DISCUSSION

In this population-based study we extended previous findings (Jeppesen, Clemmensen, et al. 2015; Fisher et al. 2014) by examining how psychotic experiences and hallucinatory phenomena were associated with objective measures of sleep duration. To the best of our knowledge, this study is the first to examine the association of objective and subjective sleep parameters with psychotic experiences in youth. We found no association of psychotic experiences or

hallucinatory phenomena with objective sleep duration, sleep efficiency, arousal, or social jet lag. We found that psychotic experiences were consistently associated with subjective sleep problems across raters. Consistent with this, we observed a dose-response association, whereby more child-reported psychotic experiences were associated with higher levels of mother-reported dyssomnia and parasomnia. Taken together, our findings suggest that in the general pediatric population psychotic experiences co-occur with multi-rated sleep problems and most strongly with nightmares.

Our finding that psychotic experiences were not associated with observed sleep problems is at odds with prior studies. Previous studies using actigraphic measures of sleep in young people at high risk for psychosis, reported shorter sleep duration, and more fragmented sleep during the prodromal phase prior to the onset of psychosis (Lunsford-Avery and Mittal 2013; Lunsford-Avery et al. 2015). The literature about circadian rhythm may help to clarify the seemingly conflicting results. Adolescents at high risk for psychosis often display alterations in circadian rhythm, indicating that more desynchronized day-night rhythms might result in recurrence of psychotic episodes (Lunsford-Avery et al. 2017). Important clues for the construction of the circadian rhythm are Zeitgebers. Zeitgebers are events such as exposure to light, timing of food intake, but also occupational or educational obligations (Golombek and Rosenstein 2010). Potentially, these Zeitgebers, in particular the fixed school schedule, of the relatively young children in our sample may have been protective against developing desynchronized day-night rhythms, and subsequently prevented sleep problems occurring.

We found that child self-reported psychotic experiences are associated with mother-reported parasomnia symptoms, and especially nightmares. As the phenotypical resemblance between nightmares and psychotic experiences, child self-reported psychotic experiences might be particularly susceptible to information bias and thereby over-reporting by the child (van der Steen et al. 2018). Of note, a previous study demonstrated that screening questions for psychotic experiences in the general pediatric population have a high level of accuracy for psychotic symptoms confirmed by clinical interview (Kelleher et al. 2011). The endorsement of child self-reported psychotic experiences was similar to that observed in previous work using clinical interview assessments (Polanczyk et al. 2010; Kelleher, Connor, et al. 2012). Additionally, our findings cannot be explained by shared method, i.e. reporter, bias because our observations were based on different reporters and instruments. Our finding that psychotic experiences and hallucinatory phenomena are associated with nightmares is in line with previous work (Lee et al. 2012; Jeppesen, Clemmensen, et al. 2015; Fisher et al. 2014; Thompson et al. 2015). Both psychotic experiences during the day and nightmares indicate subjective experiences produced by spontaneous neural activity (Feinberg 2011). Although, some studies report fluid passages between sleeping and waking state may result in hallucinatory phenomena (Manni and Mazzarello 2001; Arnulf et al. 2000) suggesting some sort of continuity between nightmares and hallucinatory phenomena, there is no reason to consider them part of the same phenomenon. Several other studies point out that nightmares and hallucinatory symptoms are physiologically different (Waters et al. 2016; Rek, Sheaves, and Freeman 2017). Nightmares during REM-sleep are characterized by pre-frontal area "closed-loop circuits" (Waters et al. 2016), whereas hallucinatory phenomena are characterized by abnormally modulated connections between anterior frontal areas and posterior sensory regions (Jardri et al. 2011; Hoffman and Hampson 2011). Additionally, nightmares and psychotic experiences are different in terms of parental awareness. Parents are often not aware of psychotic experiences of their children (Kelleher et al. 2011), but know of their nightmares. Potentially, in combination with other risk indicators (Polanczyk et al. 2010), mother-reported nightmares could be considered a risk indicator for psychotic experiences.

The finding that childhood dyssomnia is associated with psychotic experiences might be the result of concurrent child psychopathology. Indeed, when controlling for concurrent psychopathology, the associations of psychotic experiences with dyssomnia symptoms attenuated, but remained significant. One possibility for the attenuation of the effect is that co-occurring psychopathology may be a common cause underlying the association between psychotic experiences and dyssomnia symptoms. Indeed, from previous studies we

know that both childhood dyssomnia and psychotic experiences are known to frequently co-occur with psychopathology (Kelleher, Keeley, et al. 2012; Wigman et al. 2011; Gregory and Sadeh 2016). This could suggest that the association was partly explained by co-occurring emotional or behavioral problems, further investigation of the direction of this association is needed.

STRENGTHS AND LIMITATIONS

This study has multiple strengths. First, we made use of actigraphical measures of sleep, which is a reliable way to assess of objective sleep duration, efficiency, arousal, and social jet lag. Second, we obtained sleep measures from multiple raters, both mother and child. This enabled us to control for reporter bias and shared method variance bias as different reporters (i.e. both mother and child) and instruments (i.e. different questionnaires) were used for sleep problems. Third, because of large sample size we were able to control for various important sociodemographic confounders and co-occurring child psychopathology.

The current study also had some limitations. First, we used self-report questions to measure psychotic experiences. It would have been optimal to conduct clinical interviews to assess psychotic experiences, because self-report might inflate the prevalence of psychotic experiences (Kelleher, Connor, et al. 2012). However, self-reported psychotic experiences have been shown to be predictive of clinician-confirmed psychotic disorder (Kelleher et al. 2011), and questionnaires have been reported to increase the willingness to disclose sensitive information (Jones et al. 2008), which is particularly important for pre-adolescent children. Moreover, from previous studies we know that the genetic factors underlying psychotic experiences and clinician-confirmed psychotic disorders overlap (Zavos et al. 2014; Jeppesen, Larsen, et al. 2015). Second, questionnaires on psychotic experiences were collected at age 10 years, while actigraphical measures of sleep where at age 11 years. The literature suggests that sleep is relatively stable in school-age children (6-12 years) and typically changes with the onset of puberty only (Galland et al. 2012). Although psychotic experiences in childhood are not very persistent (Bartels-Velthuis et al. 2011), this suggests that similar sleep patterns were present when the psychotic symptoms were assessed. However, future studies should employ longitudinal designs to test the extent to which persistence or desistence of psychotic experiences in children is related to sleep difficulties. Third, our measure of nightmares was based on one item, and therefore not very detailed. However, the "nightmares" item of the CBCL is associated with well-validated sleep measures, such as the parasomnia scale and the sleep anxiety scale of the Children's Sleep Habits Questionnaire, and it is associated with parasomnia sleep disorder diagnosis (Becker, Ramsey, and Byars 2015). In the future, more in-depth information on nightmares should be assessed, including nightmare severity. Polysomnographic measures would be useful in order to map the sleep activity during nightmares. Fourth, this study was cross-sectional, which precludes the possibility of examining the direction of associations and, hence, any inferences on potential causal relations. Fifth, our study did not include a full range of maternal symptoms. However, we were able to use concurrent maternal depressive symptoms as a confounder, one of the leading causes of disability ranked in the global burden of disease scale (Ferrari et al. 2013). In future research, it will be important to apply longitudinal designs as well as a clinical follow-up to trace the associations between psychotic experiences and nightmares over the developmental course, while accounting for the dynamic course of psychotic experiences and sleep.

CONCLUSION

Our results suggest that psychotic experiences and hallucinatory phenomena are associated with subjective sleep problems, but that the association is specifically strong for nightmares. This finding can contribute to a broader understanding of the relationship between psychotic experiences and sleep. Additionally, it stresses the role of nightmares as a potential risk-indicator of psychopathology.

(...) *to quake in fear of the world and people and what they – I mean, of, we – are capable of.*
(Jenny Diski, 2002, Stranger on a Train)

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(SUPPLEMENT)

Table S1. The association of psychotic experiences and hallucinatory phenomena with (week and weekend combined) sleep in preadolescence

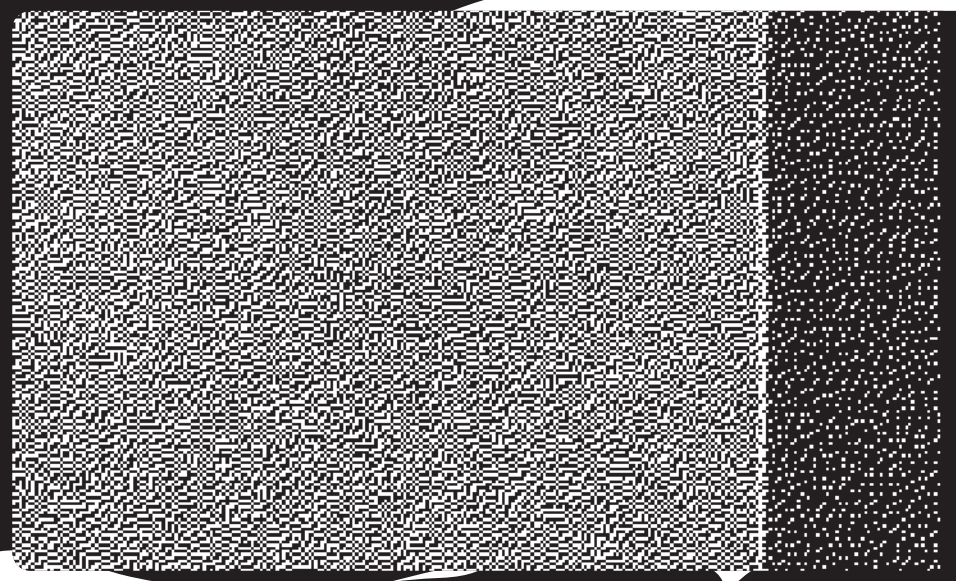
	Sleep duration, hours:minutes N= 806			Sleep efficiency, % N= 806			Arousal, no N= 806		
	B	95 % CI	*p*	B	95 % CI	*p*	B	95 % CI	*p*
Psychotic experiences									
No symptoms	0	(ref.)		0	(ref.)		0	(ref.)	
Some symptom, yes	.04	-.03 – .14	.412	.03	-.66 – .72	.927	.12	-.35 – .59	.629
Several symptoms, yes	-.06	-.20 – .06	.347	.13	-.94 – 1.21	.805	-.51	-1.23 – .22	.169
Hallucinatory phenomena									
No symptoms	0	(ref.)		0	(ref.)		0	(ref.)	
Some symptom, yes	-.03	-.12 – .06	.471	.10	-.28 – .47	.794	.07	-.43 – .57	.777
Several symptoms, yes	.07	-.11 – .25	.462	.93	-.54 – 2.40	.220	-.67	-1.68 – .35	.197

Note: tThe associations were adjusted for sex, child's ethnicity, age at sleep assessment, gestational age, maternal education, and maternal psychopathology.

Table S2. The association of psychotic experiences and hallucinatory phenomena with multi-rated sleep problems at age 10 years in the total sample adjusted for concurrent child psychopathology.

	Child-reported			Mother-reported								
	Dyssomnia N=4074			Dyssomnia N=4118			Parasomnia N=4121			Nightmares N=4121		
	B	95 % CI	*p*	OR	95 % CI	*p*	OR	95 % CI	*p*	OR	95 % CI	*p*
Psychotic experiences												
No symptoms	0	(ref.)		0	(ref.)		0	(ref.)		0	(ref.)	
Some symptom, yes	1.08	.92 – 1.23	< .001	1.49	1.20 – 1.86	< .001	1.22	1.05 – 1.42	.011	1.51	1.26 – 1.81	< .001
Several symptoms, yes	2.16	1.84 – 2.48	< .001	2.12	1.47 – 3.05	< .001	1.77	1.33 – 2.37	< .001	2.50	1.83 – 3.43	< .001
p for trend			< .001			< .001			< .001			< .001
Hallucinatory phenomena												
No symptoms	0	(ref.)		0	(ref.)		0	(ref.)		0	(ref.)	
Some symptom, yes	.96	.79 – 1.13	< .001	1.28	1.02 – 1.60	.035	1.29	1.09 – 1.51	.003	1.60	1.33 – 1.93	< .001
Several symptoms, yes	1.75	1.40 – 2.10	< .001	1.44	.96 – 2.17	.079	1.54	1.13 – 2.11	.007	2.00	1.42 – 2.81	< .001
p for trend			< .001			.013			< .001			< .001

Note: the associations were adjusted for sex, child's ethnicity, age at sleep assessment, gestational age, maternal education, and maternal psychopathology. Additionally all associations were adjusted for concurrent child psychopathology.



– Le défi auquel nous faisons face aujourd'hui, c'est d'imaginer des perspectives d'avenir centrées sur les êtres humains, (...)

(ABSTRACT)

OBJECTIVE

The etiology of schizophrenia is multi-factorial with early neurodevelopmental antecedents, likely to result from a complex interaction of genetic and environmental risk. However, few studies have examined how schizophrenia polygenic risk scores (PRS) are moderated by environmental factors in shaping neurodevelopmental brain structure, prior to the onset of psychotic symptoms. Here, we examined whether hair cortisol, a quantitative metric of chronic stress, moderated the association between genetic risk for schizophrenia and pre-adolescent brain structure.

METHODS

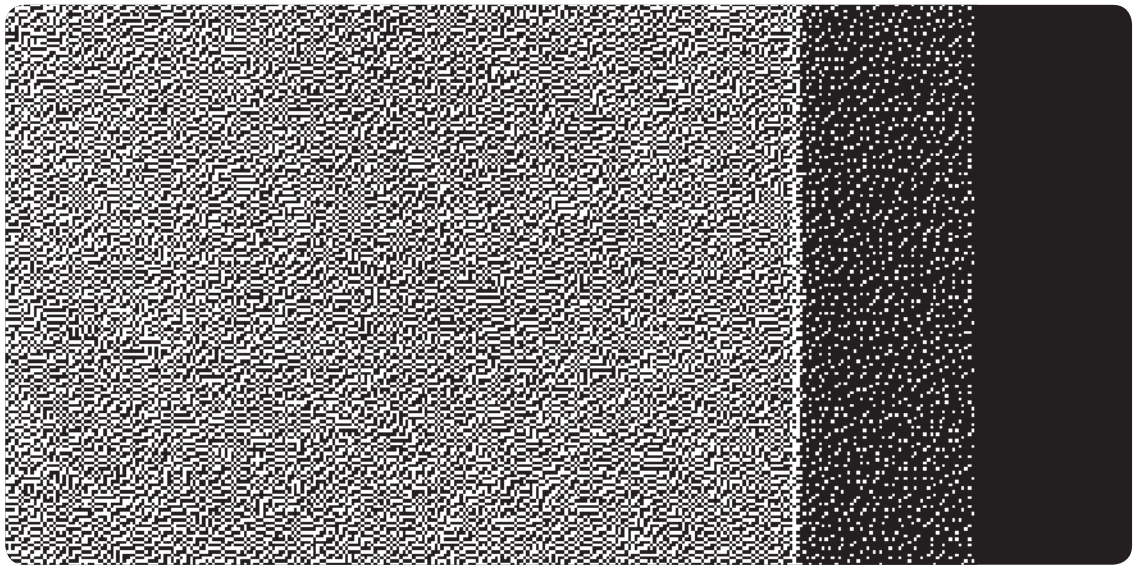
This study was embedded within the Generation R Study, involving pre-adolescents of European ancestry assessed regarding schizophrenia PRS , hair cortisol, and brain imaging (n=498 structural; n=526 diffusion tensor imaging). Linear regression was performed to determine the association between schizophrenia PRS, hair cortisol level, and brain imaging outcomes.

RESULTS

Although no single measure exceeded the multiple testing threshold, nominally significant interactions were observed for total ventricle volume ($P_{interaction} = 0.02$) and global white matter microstructure ($P_{interaction} = 0.01$) – two of the most well replicated brain structural findings in schizophrenia.

CONCLUSION

These findings provide suggestive evidence for the joint effects of schizophrenia liability and cortisol levels on brain correlates in the pediatric general population. Given the widely replicated finding of ventricular enlargement and lower white matter integrity among schizophrenia patients, our findings generate novel hypotheses for future research on gene-environment interactions affecting the neurodevelopmental pathophysiology of schizophrenia.



(...) *qui nous parlent davantage et répondent à nos attentes.*
(Aminata Traoré, 2008)

CHAPTER 5

CORTISOL BY SCHIZOPHRENIA POLYGENIC RISK MODERATION AND PRE-ADOLESCENT BRAIN STRUCTURE

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Submitted for publication.

INTRODUCTION

Schizophrenia is a highly heritable psychiatric disorder, mediated through a complex combination of common and rare genetic variants (Sullivan, Kendler, and Neale 2003). In addition to genetic risk, there is substantial epidemiological evidence for the effects of several environmental determinants on the risk of schizophrenia, including chronic cannabis use (Marconi et al. 2016), obstetric complications (Cannon, Jones, and Murray 2002), ethnic minority status (Bourque, van der Ven, and Malla 2011), urbanicity (Vassos et al. 2012), and stressful life events (Belbasis et al. 2018). Given maximum heritability estimates of ~80% based on twin heritability estimates (Hilker et al. 2018), the genetic liability of schizophrenia appears to be at least partially moderated by environmental determinants (van Os, Kenis, and Rutten 2010).

Polygenic risk scores (PRS) are derived as the weighted sum of risk alleles derived from genome-wide association studies (GWAS) (Wray et al. 2014). Several studies have employed schizophrenia PRS to investigate developmental manifestations of increased genetic liability for schizophrenia in the general population (Mistry et al. 2017). The schizophrenia PRS has been associated with early life emotional and behavioral problems (Jones et al. 2016), cognition (Riglin et al. 2017), and physical health problems(Stringer et al. 2014) in the general population. Increasingly, PRS for schizophrenia are employed in imaging genetics studies to assess underlying brain correlates of vulnerability to schizophrenia (Dima and Breen 2015; Bogdan et al. 2017). Previous studies have reported associations of schizophrenia PRS with total brain volume and gyrification indices (Terwisscha van Scheltinga et al. 2013; Caseras et al. 2015; Liu et al. 2017), though several null findings have also been published (Papiol et al. 2014; Van der Auwera et al. 2015; Van der Auwera et al. 2017). Studies that assess how genetic risk for schizophrenia is related to variations in structural brain correlates are important, given that both gray matter and white matter microstructural correlates have been consistently observed in patients with schizophrenia (Brugger and Howes 2017; van Erp et al. 2016; Kelly et al. 2018; van Erp et al. 2018), and this has also been replicated in childhood-onset schizophrenia samples (Tamnes and Agartz 2016) and first-episode psychosis patients (Fusar-Poli et al. 2014). However, no prior studies have assessed the interaction between genetic and stress-related environmental risk factors on childhood brain development, despite that schizophrenia pathophysiology is widely hypothesized to have early neurodevelopmental antecedents that are sensitive to the environmental stressors (van Os, Kenis, and Rutten 2010).

Here, in a population-based sample of ten-year-olds, an age well before the clinical onset of overt psychotic psychopathology, we used PRS as an index of genetic vulnerability to schizophrenia (Schizophrenia Working Group of the Psychiatric Genomics Consortium 2014). Cortisol levels were measured through hair analysis as a naturalistic quantitative metric of chronic physiological stress. In the recent years, hair cortisol has been increasingly studied as a biological marker of chronic physiological stress. Hair cortisol indicates long-term exposure to stress and, due to the hair's natural speed of growth, one centimeter of hair represents one month of cortisol exposure (Noppe et al. 2015). In the current study, we obtained hair samples of 3cm in length – reflecting three months of cortisol exposure. Moreover, recent studies have demonstrated that hair cortisol levels are indicative of more chronic stress exposure and exhibit high intra-subject temporal stability (Stalder et al. 2012; Stalder et al. 2017). Our primary aim was to examine whether chronic stress, as operationally defined by hair cortisol levels, moderated the relationship between schizophrenia PRS and pre-adolescent structural brain correlates. Our hypotheses focused on brain outcomes which have most consistently been associated with the underlying neurobiology of schizophrenia – cortical and subcortical gray matter (van Erp et al. 2016; van Erp et al. 2018), cerebroventricular volume (van Erp et al. 2016), and white matter microstructure (Kelly et al. 2018). We expected that, among children with an elevated genetic liability to schizophrenia, high cortisol levels would be associated with lower (sub-) cortical gray matter volume, larger ventricles and decreased white matter microstructure.

METHODS

STUDY POPULATION

The present study was embedded within the Generation R Study, a prospective population-based birth cohort from Rotterdam, the Netherlands. The aim of the Generation R Study is to identify genetic and environmental determinants that influence maternal and child development (Kooijman et al. 2016). For the current study, 2512 children of European descent (based on genetic ancestry; 53% of n = 4780 participants of European descent who were eligible for the age ten years wave) had genotype data available. Hair samples were collected in an un-selected sub-sample of the Generation R cohort at mean age six years. At mean age ten years, participants were invited for a brain magnetic resonance imaging (MRI) scan, of whom n = 593 consented for participation in the current study. After standardized quality control procedures, the final sample comprised 498 children for T1 and 526 for diffusion tensor imaging (DTI) analyses, respectively (Appendix Figure A.1 for a flowchart). Study protocols were approved by the Medical Ethics Committee of the Erasmus Medical Center. All participants and their parents provided assent and informed consent, respectively.

ATTRITION ANALYSIS

Comparisons were made between the study sample (N = 498) and participants who were genotyped but without hair cortisol or brain morphology data (N = 2512). These groups did not differ in proportion of girls (47.2% versus 49.9%, χ^2 = 1.07, df = 1, P = 0.30) or in mean schizophrenia PRS (P_t < 0.0005; 0.01 versus -0.01, t = -0.59, P = 0.56). However, children with complete data were more likely to have mothers with higher educational levels (79.5% versus 70.5%, χ^2 = 14.68, df = 1, P < 0.01).

GENOTYPING AND QUALITY CONTROL

Genotype quality control procedures for the Generation R Study were performed as previously described (Medina-Gomez et al. 2015).

Briefly, genotype data were collected from either cord blood at birth (Illumina 610K Quad Chip) or from whole venous blood collected during a follow-up visit (Illumina 660k Quad Chip). Variants were included if they passed sample (≥ 97.5%) and SNP call rates (≥ 90%), minor allele frequency ≥ 1%, and no deviation from Hardy-Weinberg disequilibrium ($P < 10^{-7}$). Participants were screened for excess heterozygosity, sex mismatch, relatedness, and missing data. Individuals of European descent were considered those within 4 standard deviations for each of the first four genetic principal components of the HapMap Phase-II Northwestern European population.

POLYGENIC RISK SCORING

Genetic risk variants associated with schizophrenia were obtained from the Psychiatric Genetics Consortium case-control GWAS meta-analysis (Schizophrenia Working Group of the Psychiatric Genomics Consortium 2014). SNPs were clumped according to linkage disequilibrium (LD) in order to obtain the most significant SNP per LD-block (kilobase pair window: 250, LD r^2<0.1). PRS were computed on imputed genotype data using PRSice (Euesden, Lewis, and O'Reilly 2015), by weighting the mean number of risk alleles against the SNP effect size. P-value thresholds (P_t) for inclusion of SNPs in the PRS varied between P_t < 0.0005 and P_t < 1. Scores were standardized to a mean of zero and standard deviation of one to facilitate interpretation.

PRS for major depressive disorder scores were derived from the most recent GWAS meta-analysis (Wray et al. 2018). If an association with the schizophrenia PRS was observed, further analyses with the major depression risk score were performed to examine specificity given the significant genetic correlations across psychiatric disorders, including schizophrenia and depression (Cross-Disorder Group of the Psychiatric Genomics et al. 2013).

HAIR CORTISOL

At the age six visit, children were invited with the consent of their mother to contribute a hair sample for steroid hormone assessment. Hair samples were cut from the posterior vertex using small surgical

scissors, as close to the scalp as possible. Parents completed a questionnaire regarding their child's use of hair products and corticosteroid medications. Steroids were extracted using LC-grade methanol at 25°C for 18 hours in the presence of deuterium-labeled steroids as an internal standard. Samples were centrifuged and cleaned using solid phase extraction, after which steroids were measured by liquid chromatography-tandem mass spectrometry, as previously described (Rippe et al. 2016). Concentrations of hair cortisol were log-transformed (Rippe et al. 2016; Noppe et al. 2015).

IMAGE ACQUISITION

Brain imaging was performed using a 3-T GE MR750w scanner with an 8-channel head coil, as previously described (White et al. 2018). Every child was invited to participate in a mock scanning session prior to the actual brain MRI scan to familiarize them with the scanning procedure. After a localizer, structural T1 was the first sequence, followed by the DTI scan. For structural T1 analyses, global metrics of volume were extracted. T1-weighted images were processed using the FreeSurfer analysis suite, version 6.0 (Fischl et al. 2004), a widely used reliable software for brain morphometry processing (Han et al. 2006; Reuter et al. 2012). Diffusion tensor imaging scanning pre-processing was conducted using the FMRIB Software Library (FSL), version 5.0.9 (Jenkinson et al. 2012). Probabilistic white matter fiber tractography was conducted on DTI images and scalar metrics (i.e. fractional anisotropy [FA], mean diffusivity [MD], axial diffusivity [AD] and radial diffusivity [RD]) were computed. Global DTI metrics were computed based on 12 well-studied white matter fiber bundles. More detail on neuroimaging acquisition and quality control is available in the Supplement.

COVARIATES

Age and sex were obtained from study records. Four ancestral principal components were used as covariates to correct for remaining population structure. Hair color, hair product use, and corticosteroid medication use were assessed through parental report.

STATISTICAL ANALYSES

All analyses were conducted using R statistical software. First, we examined main effects associations of the schizophrenia PRS with global brain correlates using linear regression. The outcomes on which we performed our analyses included cortical and subcortical gray matter volume, ventricle volume and global FA and MD. Total brain volume and white mater volume were assessed in secondary analyses. Therefore, in total we examined seven different brain outcomes in the current study. To address our main aim, whether hair cortisol levels moderated the relationship between schizophrenia PRS and brain correlates, linear regression with interaction was conducted to test deviation from additivity. The main effects of cortisol and PRS remained included in the model. All analyses were adjusted for covariates as described above. Subcortical gray matter volume and ventricular volumes were assessed as a fraction of intracranial volume to obtain estimates relative to head size. Total brain volume, cortical gray matter volume, and white matter volume were not assessed as a fraction of intracranial volume due to high correlations with the latter (r = 0.93, r = 0.87, and r = 0.89, respectively). Analyses involving schizophrenia PRS were conducted separately for each P-value threshold. For clarity of presentation, results are shown for schizophrenia risk score $P_t < 0.0005$ as this threshold has shown the strongest associations in previous work from our group (Serdarevic et al. 2018) and in the current analyses. Results from the other *P*-value thresholds are shown in the Supplement. A false discovery rate (FDR) correction was applied to adjust for multiple testing. This was based on the total number of statistical tests across polygenic scores, cortisol level, *P*-value thresholds and all brain outcomes, resulting in a stringent multiple testing correction.

Significant associations were visually explored in scatter plots. For simplicity of visualization, hair cortisol level was categorized into two levels. Interaction graphs were visualized using the *ggplot2* package, and its extension *jtools* was employed to create Johnson-Neyman plots for visualization of confidence intervals of difference between cortisol levels. Johnson-Neyman plots and intervals indicate the values of the moderator (hair cortisol) for which the slope of the

predictor (PRS) on the outcome (brain) will be statistically significant. Significant observations with the schizophrenia PRS were repeated with the major depression PRS. Finally, we assessed the relationship between schizophrenia PRS and hair cortisol level, given that an association between these variables (i.e., gene-environment correlation) would affect the estimation of gene-environment interaction models.

RESULTS

SAMPLE CHARACTERISTICS

Among the children who used corticosteroids in the 3 months prior to hair sample collection (Table 1, n = 46, 9.2%), the predominant routes of administration were cutaneous application (n = 27, 5.4%).

MAIN EFFECTS ASSOCIATIONS

No associations were observed between schizophrenia PRS and cortical gray matter volume, subcortical gray matter volume, global FA or global MD (Table 2). Schizophrenia pPRS was associated with lower ventricular volume ($P_t < 0.0005$: β = -0.13, 95% CI -0.21;-0.04, P = 0.01), but this did not survive FDR-correction for multiple testing ($P_{FDR\text{-}adjusted}$ = 0.30). Hair cortisol level was not associated with any of the global gray or white matter measures examined. Neither schizophrenia PRS nor hair cortisol were associated with any of the secondary outcome measures (Appendix Tables A.1-A.7).

INTERACTION EFFECTS ASSOCIATIONS

After adjustment for multiple testing, no statistically significant interaction effects were observed for any of the primary or secondary outcome measures. Nominally significant, i.e. uncorrected, estimates indicated that hair cortisol levels moderated the association between schizophrenia PRS and ventricle volume (Table 3; $P_t < 0.0005$: β = 0.10, 95% CI 0.01;0.18, P = 0.02), but this did not survive multiple testing correction ($P_{FDR\text{-}adjusted}$ = 0.30). Effects were most pronounced at the lower end of the distribution of schizophrenia PRS (Figure 1).

Table 1: Sample characteristics

	N	Total population (N = 498)
Child characteristics		
Age at MRI, mean (SD)	498 (0% missing)	9.91 (0.40)
Sex, girls	498 (0% missing)	47.2
Hair color	498 (0% missing)	
Red		4.8
Blond		84.7
Brown		10.4
Black		0.0
Hair cortisol concentration (pg/mg), median (IQR)	498 (0% missing)	1.31 (2.21)
Hair product use on day of hair collection	498 (0% missing)	20.7
Corticoid medication use in past 3 months	498 (0% missing)	9.2
Inhalation		2.6
Intranasal		0.6
Cutaneous		5.4
Oral		0.4
Unknown		0.2
Maternal characteristics		
Educational level	478 (4.0% missing)	
High		79.5
Medium		20.5
Low		0.0

Note: numbers represent percentages until stated otherwise.

Table 2: Main effects of schizophrenia polygenic risk score (upper panel) and hair cortisol (lower panel) on global structural volumetric and global white matter microstructural measures.

Outcome	β (95% CI)	*P*	$P_{FDR\text{-}adjusted}$
Schizophrenia polygenic risk			
Structural volumetric measures (*N*=498)			
Cortical gray matter volume	-0.04 (-0.12;0.04)	0.32	0.62
Subcortical gray matter volume	0.02 (-0.07;0.10)	0.68	0.84
Total ventricle volume	-0.13 (-0.21;-0.04)	0.01	0.30
White matter microstructural measures (*N*=526)			
Global fractional anisotropy (FA)	0.05 (-0.03;0.14)	0.24	0.53
Global mean diffusivity (MD)	-0.05 (-0.13;0.04)	0.29	0.58
Hair cortisol			
Structural volumetric measures (*N*=498)			
Cortical gray matter volume	0.00 (-0.08;0.08)	0.96	0.98
Subcortical gray matter volume	0.07 (-0.02;0.16)	0.11	0.81
Total ventricle volume	-0.06 (-0.15;0.03)	0.19	0.48
White matter microstructural measures (*N*=526)			
Global fractional anisotropy (FA)	0.04 (-0.04;0.13)	0.33	0.62
Global mean diffusivity (MD)	0.01 (-0.07;0.10)	0.73	0.85

Note: Results are shown for the P-value threshold $P_t < 0.0005$. Models were adjusted for age at MRI visit and child sex, hair color, hair product use, corticosteroid use, and 4 principal components. Subcortical grey matter volume and total ventricular volume were assessed as a fraction of intracranial volume. False discovery rate correction for multiple testing was based on the total number of statistical tests across main effects of both polygenic scores and cortisol, *P*-value thresholds and brain outcomes. Abbreviations: $P_{FDR\text{-}adjusted}$, false-discovery rate corrected *P*-value.

Table 3: Interaction effect of hair cortisol levels and schizophrenia polygenic risk score on global structural volumetric and global white matter microstructural measures.

Outcome	β (95% CI)	*P*	$P_{FDR\text{-}adjusted}$
Structural volumetric measures (*N*=498)			
Cortical gray matter volume	0.03 (-0.05;0.10)	0.52	0.76
Subcortical gray matter volume	-0.02 (-0.10;0.06)	0.62	0.81
Total ventricle volume	0.10 (0.01;0.18)	0.02	0.30
White matter microstructural measures (*N*=526)			
Global fractional anisotropy (FA)	-0.04 (-0.12;0.05)	0.37	0.64
Global mean diffusivity (MD)	0.10 (0.02;0.19)	0.01	0.30

Note: Results are shown for the P-value threshold $P_t < 0.0005$. All models all included the main effects and the interaction term. Models were adjusted for age at MRI visit and child sex, hair color, hair product use, corticosteroid use, and 4 principal components. Subcortical grey matter volume and total ventricular volume were assessed as a fraction of intracranial volume. Abbreviations: $P_{FDR\text{-}adjusted}$, false-discovery rate corrected *P*-value.

The corresponding Johnson-Neyman intervals plot demonstrated that at values of (log-transformed) cortisol below 0.36, a higher schizophrenia PRS was nominally associated with a lower ventricle volume. No significant associations were observed for cortisol levels above 0.36.

Hair cortisol moderated the association between schizophrenia risk score and global MD (Appendix Table A.7, $P_t < 0.0005$: $\beta = 0.10$, 95% CI 0.02;0.19, $P = 0.01$); again this finding did not survive correction for multiple testing ($P_{FDR\text{-}adjusted} = 0.30$). Figure 2 demonstrates nominally significant relationships at both ends of the cortisol distribution. The Neyman-Johnson plot indicates that at values of (log-transformed) hair cortisol below -0.01 and above 1.32, schizophrenia PRS was associated with global MD in a negative and a positive direction, respectively. Higher cortisol in combination with elevated polygenic risk was associated with higher global MD. Accordingly, lower cortisol in combination with higher polygenic risk was associated with lower global MD.

FOLLOW-UP ANALYSES WITH GLOBAL AD AND RD

No main effect was observed between either PRS or hair cortisol and global AD or RD (Appendix Table A.8-A.9). A nominal interaction was observed between hair cortisol and schizophrenia PRS on both global AD ($P_t < 0.0005$: $\beta = 0.10$, 95% CI 0.02;0.18, $P = 0.02$) and global RD ($P_t < 0.0005$: $\beta = 0.09$, 95% CI 0.01;0.17, $P = 0.04$). A higher schizophrenia PRS in combination with high cortisol was associated with higher global AD (Appendix Figure A.2). This was consistent with the finding for RD, which showed a different direction reflecting the reverse correlation with other white matter parameters; a lower polygenic risk in combination with lower hair cortisol was associated with higher global RD (Appendix Figure A.3).

SENSITIVITY ANALYSES WITH DEPRESSION POLYGENIC RISK

No significant interaction effect was observed between hair cortisol level and major depressive disorder polygenic risk on brain ventricle volume or global MD (Appendix Table A.10-A.11). Post-hoc power

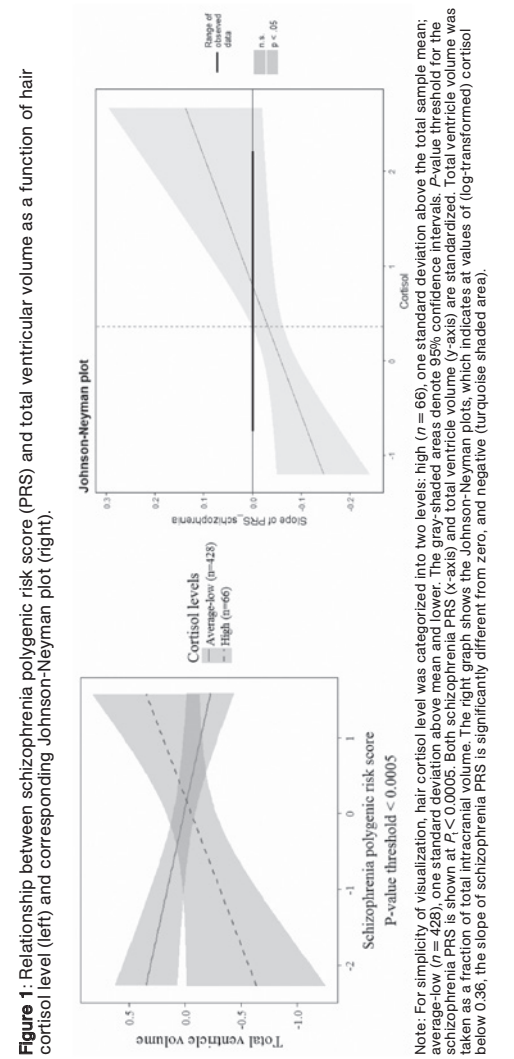


Figure 1: Relationship between schizophrenia polygenic risk score (PRS) and total ventricular volume as a function of hair cortisol level (left) and corresponding Johnson-Neyman plot (right).

Note: For simplicity of visualization, hair cortisol level was categorized into two levels: high ($n = 66$), one standard deviation above the total sample mean; average-low ($n = 428$), one standard deviation above mean and lower. The gray-shaded areas denote 95% confidence intervals. *P*-value threshold for the schizophrenia PRS is shown at $P_t < 0.0005$. Both schizophrenia PRS (x-axis) and total ventricle volume (y-axis) are standardized. Total ventricle volume was taken as a fraction of total intracranial volume. The right graph shows the Johnson-Neyman plots, which indicates at values of (log-transformed) cortisol below -0.01 and above 1.32, the slope of schizophrenia PRS is significantly different from zero, and negative (turquoise shaded area).

(Full colour image presented on page 4.)

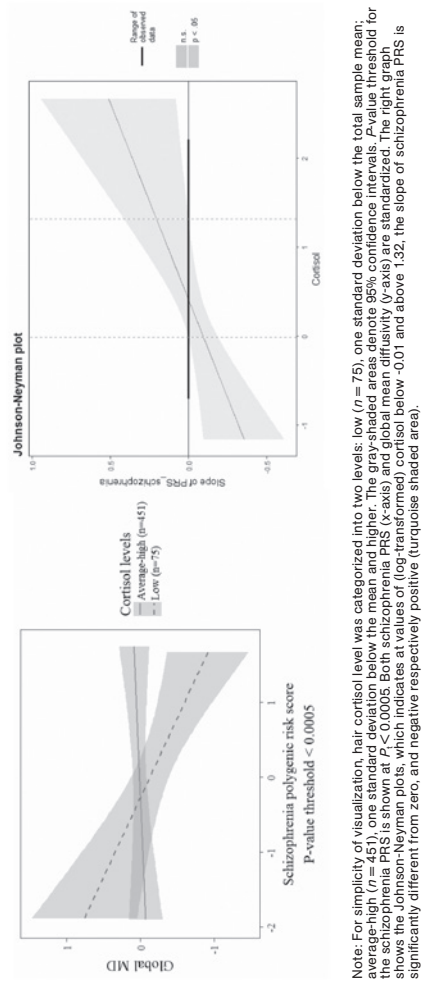


Figure 2: Relationship between schizophrenia polygenic risk score (PRS) and global mean diffusivity as a function of hair cortisol level (left) and corresponding Johnson-Neyman plot (right).

Note: For simplicity of visualization, hair cortisol level was categorized into two levels: low ($n = 75$), one standard deviation below the total sample mean; average-high ($n = 451$), one standard deviation below the mean and higher. The gray-shaded areas denote 95% confidence intervals. *P*-value threshold for the schizophrenia PRS is shown at $P_t < 0.0005$. Both schizophrenia PRS (x-axis) and global mean diffusivity (y-axis) are standardized. The right graph shows the Johnson-Neyman plots, which indicates at values of (log-transformed) cortisol below -0.01 and above 1.32, the slope of schizophrenia PRS is significantly different from zero, and negative (turquoise shaded area).

(Full colour image presented on page 7.)

Table 4: Association between schizophrenia polygenic risk scores and hair cortisol level.[a]

P-value threshold	Number of SNPs[b]	β (95% CI)	*P*
$P_t < 0.0005$	2 965	-0.03 (-0.12;0.06)	0.53
$P_t < 0.001$	4 148	-0.04 (-0.13;0.05)	0.42
$P_t < 0.005$	9 547	-0.02 (-0.11;0.07)	0.59
$P_t < 0.01$	13 916	0.01 (-0.10;0.08)	0.76
$P_t < 0.05$	34 947	-0.02 (-0.11;0.07)	0.70
$P_t < 0.1$	52 256	0.00 (-0.09;0.09)	0.96
$P_t < 0.5$	126 674	0.00 (-0.09;0.09)	1.00
$P_t < 1.0$	164 190	0.00 (-0.09;0.09)	0.97

Note: Models are adjusted for child age at hair sample collection, child sex, hair color, hair product use and 4 ancestral principal components. SNPs were clumped prior to calculation of the polygenic score. Abbreviations: P_t, *P*-value threshold; SNP, single nucleotide polymorphism.

analysis for multiple regression with total brain volume as the outcome (Soper 2018), using the following parameters: number of predictors = 11; observed $R^2 = 0.29$; *P*-value for interaction = 0.89; sample size = 498, yielded an observed statistical power of 100%. With only hair cortisol and schizophrenia PRS included in the calculation and a resulting $R^2 = 0.01$ and $P = 0.73$, resulted in a statistical power estimation of 97%.

ASSOCIATION BETWEEN SCHIZOPHRENIA VULNERABILITY AND HAIR CORTISOL

No association was observed between schizophrenia PRS and hair cortisol level (Table 4).

DISCUSSION

In this population-based neuroimaging study in pre-adolescents, we examined whether hair cortisol levels moderated the relationship of schizophrenia PRS with brain structural morphology or white matter microstructure. When stringently corrected for multiple testing across polygenic risk score *P*-value thresholds for all primary and secondary neuroimaging outcome measures, no significant main or interaction associations were observed. Analyses uncorrected for multiple testing should therefore be interpreted with caution. Uncorrected associations suggested that high cortisol in combination with elevated genetic risk for schizophrenia was related to lower white matter microstructure, and that low cortisol in combination with low schizophrenia PRS was associated with larger cerebral ventricles. These associations were was not observed for the depression PRS, possibly suggesting specificity with regard to schizophrenia genetic liability. Although our findings are suggestive, they are potentially informative in the contextualization of the genetic and environmental determinants of pre-adolescent brain structure, particularly against the background of chronic stress exposure as a moderating influence on disease-related brain pathophysiology through schizophrenia PRS.

Enlarged ventricular volume and lower white matter integrity are among the most well-established and largest effect size neurobiological findings in adult patients with schizophrenia, as confirmed by recent meta-analyses (van Erp et al. 2016; Brugger and Howes 2017; Kelly et al. 2018). However, it has been difficult to disentangle the effects of confounding factors, most notably illness course and antipsychotic medication (Van Haren et al. 2013). Against this background, our findings in pre-adolescents without a diagnosis of a psychotic disorder provide novel hypotheses into the complex interplay of genetic and environmental determinants in influencing schizophrenia pathophysiology (Belbasis et al. 2018). We observed suggestive associations in which hair cortisol moderated the association between high polygenic risk for schizophrenia and brain structure, although these associations did not survive the correction for multiple testing. These suggestive findings might also help to explain the previous null findings arising from studies examining the brain structural correlates of schizophrenia PRS (Van der Auwera et al. 2015; Van der Auwera et al. 2017; Papiol et al. 2014), as the underlying effects might have been obscured by the interacting influence of environmental determinants. Accordingly, our findings highlight the importance of integrating genetic and environmental risk factors in order to better understand the pathophysiology of psychiatric illness (van Os, Kenis, and Rutten 2010). Additional environmental determinants should also be studied in the context of (polygenic) gene by environment interaction studies. For example, recent evidence has indicated that elevated schizophrenia genetic risk is associated with a higher rate of obstetric complications (Ursini et al. 2018). In this regard, prospective longitudinal neuroimaging and follow-up assessments of multiple environmental risks would provide a powerful study design for disentangling critical windows of neurodevelopment.

Our analyses did not observe any main association between schizophrenia PRS and structural brain volumes or white matter microstructure. This is largely consistent with previous studies exploring main effects of genetic risk on brain structure (Papiol et al. 2014; Van der Auwera et al. 2015; Van der Auwera et al. 2017), although a few studies have reported neuroimaging correlates of schizophrenia genetic risk (Liu et al. 2017; Fonville et al. 2018; Terwisscha van Scheltinga et al. 2013). Moreover, the absence of significant hair cortisol by polygenic risk interaction effects might be explained by the

relatively small sample size of this study and the relatively young age of the cohort. Larger samples with more power will be needed to detect small effects in pre-adolescence. Further, the joint effects of stress hormone levels and genetic risk for schizophrenia on brain structure might only become apparent when prodromal symptoms become apparent during adolescence as opposed to earlier in childhood development prior to the symptoms onset. However, it should be noted that schizophrenia PRS has been associated with psychiatric problems in the general pediatric population from as young as 3 years of age (Jansen et al. 2018), and infant neuromotor development at 9 months of age (Serdarevic et al. 2018), suggesting that the genetic risk for schizophrenia has neurobiological manifestations from early childhood onwards. Furthermore, the schizophrenia PRS explains approximately 7% of variation in the original GWAS (Schizophrenia Working Group of the Psychiatric Genomics Consortium 2014), so it can be expected that the explained variance is much lower in an unrelated sample, much younger age group and a quite different outcome measure. In addition, functional neuroimaging modalities might be more sensitive to detect subtle neurobiological changes associated with early life genetic risk for schizophrenia (Lancaster et al. 2016). More importantly, it has been noted that interaction testing with PRS might not be ideal as risk scores assume genetic additivity and some, but not all, of the SNPs in the risk score might be involved in the interaction to various degrees and, potentially, in opposing directions (i.e. some but not all variants might follow a pattern of differential susceptibility) (Peyrot et al. 2017; Zwicker, Denovan-Wright, and Uher 2018). Another avenue for gene-environment-interaction research is to employ biologically informed multi-locus profile scores, which are composites of genetic polymorphisms from a given neural signaling pathway (Bogdan et al. 2017), and examine how these differentially associate with environmental factors (Zwicker, Denovan-Wright, and Uher 2018).

Although no significant associations were observed after correction for multiple testing, our uncorrected suggestive findings provide novel hypotheses about schizophrenia's pathophysiology. Interestingly, and somewhat paradoxically, we found that in children with low cortisol levels, reduced schizophrenia genetic liability was associated with larger ventricles. Furthermore, the combination of low genetic risk for schizophrenia and high cortisol levels was associated with lower ventricular volume. These seemingly incongruous observations should be evaluated in the context of brain development throughout the first years of life. Although larger cerebral ventricles have been strongly associated with schizophrenia in clinical samples (Brugger and Howes 2017), longitudinal cohort studies from the general population indicate that brain ventricles increase in size with advancing childhood development (Lenroot and Giedd 2006), for which relative brain ventricular enlargement has also been associated with adaptive outcomes in childhood, such as fetal growth maturation (Gilmore, Knickmeyer, and Gao 2018; Roza, Govaert, Vrooman, et al. 2008). Indeed, some studies have indicated that *lower* ventricular volume is associated with worse behavioral outcomes in childhood, such as temperamental difficulties (Roza, Govaert, Lequin, et al. 2008), and attention-deficit/hyperactivity disorder (Castellanos et al. 1996). Brain ventricles have been reported to have the highest variability of brain morphometric structures, possibly since they verge on many other brain structures (Gilmore, Knickmeyer, and Gao 2018). These observations contrast with findings from clinical samples of adults with schizophrenia, which have robustly implicated larger cerebral ventricles in the neurobiological pathophysiology of schizophrenia (van Erp et al. 2016; Brugger and Howes 2017), a finding that has also been extended to samples of childhood-onset schizophrenia (Rapoport et al. 1997). Against this background, reports of increased ventricular volume in the context of neuropsychiatric conditions should therefore be interpreted with caution. Further research across distinct sampling designs and neurodevelopmental stages are needed to examine how these seemingly contradictory observations relate to the neurodevelopmental risk of schizophrenia (Murray et al. 2017).

White matter microstructural integrity increases in normal childhood and adolescence (Di Martino et al. 2014), i.e. FA increases and diffusion indices (MD, AD and RD) decrease with age. Lower FA and higher diffusion metrics, in particular MD, have been observed across several brain regions in patients with schizophrenia (Kelly et al. 2018). This is consistent with our observation of increased MD in

children with elevated genetic risk for schizophrenia who also had high cortisol levels, even though this association did not survive correction for multiple testing. No such moderation effect was seen for FA, which we had hypothesized given the lower FA commonly observed in persons with schizophrenia (Kelly et al. 2018). However, few studies have comprehensively examined FA in conjunction with diffusion metrics, which is important for future research to disentangle neurobiological mechanisms. When viewed from the perspective of ventricle volume and white matter microstructure, it appears that the optimal level of stress exposure varies between children as a function of their genetic risk.

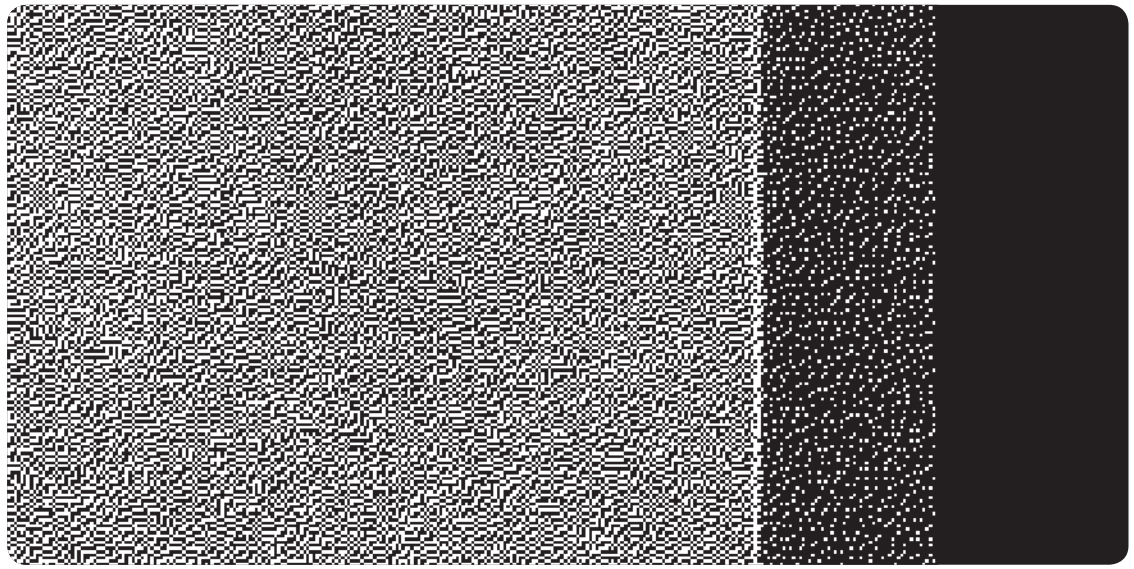
Over the past several years, hair cortisol has gained increasing attention as a biological marker of chronic physiological stress, which is interesting considering the hypothalamic-pituitary-adrenal-axis involvement in schizophrenia.HPA Consistent with prior work from our group, our current results did not identify main effect associations between hair cortisol and brain structure (Chen et al. 2016). In addition, schizophrenia PRS was not associated with hair cortisol in the current sample, consistent with previous research demonstrating a low SNP heritability (Neumann et al. 2017), and potentially reflecting the large environmental component of hair cortisol assessments. Rather, we found suggestive evidence for hair cortisol moderating the association of schizophrenia PRS with ventricle volume and global MD. Results from an earlier twin study had already hinted that the association of salivary cortisol with brain correlates might be at least partially determined by genetic factors (Kremen et al. 2010), further supporting our finding that genetic predisposition is a potential consideration in the association between environmental stressors and brain structure.

The strengths of this study included the use of exclusively biological metrics for examining determinants of psychiatric pathophysiology, its prospective design, and the population-based sample. However, our sample was not large enough to detect small effects, potentially increasing the chances of false negatives. However, the Generation R Study is the largest pre-adolescent neuroimaging study to date, so although our analyses might be underpowered, we are working with the best data available. Large collaborative efforts will be needed to further examine how increased genetic risk for psychiatric disorders affect neurodevelopment. Relatedly, the current sample size was relatively small compared with other Generation R studies using genotype data (Serdarevic et al. 2018), which might indicate a potential selective loss to follow-up. Although non-participation in population-based cohort studies has been related to higher schizophrenia PRS (Martin et al. 2016), our attrition analyses demonstrated no group differences in PRS between participants with complete data and participants lost to follow-up. Furthermore, we did not have imaging and hair cortisol data available at both ages six and ten years to study their relative prospective relationships. However, we have previously shown an absence of a statistical interaction between hair cortisol and the time interval between hair sampling and MRI scanning (Chen et al. 2016). In addition, cortisol measures, even if performed in hair, could reflect temporary states of stress. However, hair cortisol levels exhibit high intra-subject stability (Stalder et al. 2012) and have been demonstrated to reflect chronic exposure to stress (Rippe et al. 2016; Stalder et al. 2017). And, from a neurodevelopmental perspective, whether hair cortisol at age 6 years reflects stress at age 10 years is less relevant, because the neurodevelopmental process giving rise to a change in brain structure must necessarily have occurred long before age 10 years. In addition, similar to many other genetic studies, our analyses were limited to children of European descent. Future large psychiatric GWAS efforts should prioritize genetic discovery among a wide diversity of ethnic backgrounds, and not only focus on people of European ancestry. Finally, this was an observational study, which limits inferences regarding the purported causality of our observations. Long-term follow-up of this population into adolescence will provide us with the opportunity to examine how these observations relate to the onset of prodromal symptomatology.

CONCLUSIONS

In summary, following stringent correction for multiple testing, we found no significant moderating effects of hair cortisol on the association between schizophrenia PRS and pre-adolescent brain structure, precluding any firm conclusions regarding the interaction

between schizophrenia PRS and hair cortisol on neuroimaging outcomes in pre-adolescence. We obtained statistically suggestive evidence that hair cortisol levels moderated the relationship of schizophrenia PRS with brain ventricular volume and white matter microstructure. Such observations potentially attest to the importance of considering the interaction between genetic and environmental determinants in psychiatric disease pathophysiology.



–All people are not created equal. Some have real gifts and talents, and some have real problems right out of the starting block. (...)

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(SUPPLEMENT)

METHOD

IMAGE ACQUISITION

After a localizer, T1-weighted structural images were acquired with a 3D inversion recovery-prepared fast spoiled gradient recalled sequence. The following sequence parameters were used with the GE option BRAVO: TR = 8.77ms, TE = 3.4ms, TI = 600ms, Flip Angle = 10°, FOV = 220mm x 220mm, Acquisition Matrix = 220 x 220, slice thickness = 1mm, number of slices = 230, voxel size = 1mm x 1mm x 1mm, ARC Acceleration = 2. The DTI scan was acquired using an axial spin echo, echo planar imaging sequence with 3 b = 0 scans and 35 diffusion weighted images (TR = 12,500ms, TE = 72.8ms, Field of view = 240mmx240mm, Acquisition Matrix = 120x120, slice thickness = 2mm, voxel size = 2mm x 2mm x 2mm, number of slices = 65, Asset Acceleration = 2).

STRUCTURAL IMAGE PROCESSING AND QUALITY ASSURANCE

T1-weighted images were processed using the FreeSurfer analysis suite, version 6.0 (Fischl et al. 2004), a widely used reliable software for brain morphometry processing (Han et al. 2006; Reuter et al. 2012). Non-brain tissue was removed, voxel intensities were normalized for B1 inhomogeneity, whole-brain tissue segmentation was performed, and a surface-based model of the cortex was reconstructed. Freesurfer reconstructions were visually inspected using a previously described protocol (Hibar et al. 2015; Muetzel et al. 2017), and image datasets not suitable for analysis were excluded from the final sample (n = 95, Appendix Figure A.1 for a flowchart). White matter and pial surface representations were inspected for accuracy against the brain image in axial, coronal, and sagittal planes. Total ventricle volume (sum of both lateral ventricles, third and fourth ventricle) and total lateral ventricles volume were extracted, as well as other global metrics of brain structure (i.e. total brain volume, cortical and sub-cortical gray matter volume, and white matter volume). Subcortical gray matter volume and ventricular volumes were assessed as a fraction of intracranial volume to obtain estimates relative to head size. Total brain volume, cortical gray matter volume, and white matter volume were not assessed as a fraction of intracranial volume due to high correlations with the latter (r = 0.93, r = 0.87, and r = 0.89, respectively). Outliers were considered for all global metrics of brain structure, and n=4 cases with very large ventricular volumes were excluded for analyses pertaining to ventricle size. In line with previous work(Roza et al. 2008) and due to the right-skewedness of the data, values of individual ventricle volume were log-transformed to approach a normal distribution.

DTI PRE-PROCESSING

Diffusion tensor imaging scanning pre-processing was conducted using the FMRIB Software Library (FSL), version 5.0.9 (Jenkinson et al. 2012). Image processing has been described in more detail elsewhere (Muetzel et al. 2015; White et al. 2018). In short, non-brain tissue was removed and diffusion images were corrected for eddy current-induced artefacts and translations/rotations resulting from head motion. The diffusion tensor was fitted at each voxel using the RESTORE method from the Camino diffusion MRI toolkit (Cook et al. 2006), and scalar metrics (i.e. fractional anisotropy [FA], mean diffusivity [MD], axial diffusivity [AD] and radial diffusivity [RD]) were subsequently computed. FA described the directional degree of diffusion of water and ranges from 0 to 1, with 0 being completely isotropic (i.e. diffusion equal in all directions) and 1 being completely anisotropic (i.e. diffusion along only one axis). MD simply describes the average diffusion in all directions and is composed of AD (i.e. axial diffusivity) and RD (i.e. radial diffusivity). If an association with FA or MD was observed, further analyses on AD and RD were conducted in order to disentangle biological mechanisms.

WHITE MATTER PROBABILISTIC TRACTOGRAPHY

Probabilistic white matter fiber tractography was conducted on each child's DTI images using the automated FSL plugin AutoPtx (de Groot et al. 2015), to identify connectivity distributions for a number of large fiber 6 bundles such as the uncinate fasciculus and cingulum bundle. Subsequently, connectivity distributions were normalized based on the number of successful seed-to-target attempts, and then thresholded to remove voxels that were unlikely to be part of the true distribution. Average FA and MD values were computed for each white matter tract by weighting voxels based on the connectivity distribution (i.e., FA in voxels with higher probabilities received higher weight). Left and right white matter tract metric values were averaged and weighted for their respective volumes as we had no a priori hypotheses regarding the laterality of white matter tracts associated with callous-unemotional traits.

DTI QUALITY ASSURANCE

First, the DTIPrep tool (https://www.nitrc.org/projects/dtiprep/) was used to automatically examine data for slice-wise variation and characteristics of artefact in each diffusion-weighted volume. Second, the sum-of-squares error (SSE) maps from the diffusion tensor calculations were examined for structured signal that was indicative of artefact. Each SSE map was rated from 0 to 3 (0: "None", 1: "Mild", 2: "Moderate", 3: "Severe"). Cases not excluded by the automated DTIPrep tool but had a "Severe" score from the SSE rating were excluded from analyses. Further, processed tractography data were examined on their quality. Next, the registration of the DTI data to standard space was inspected for accuracy.

Table A.1: Main and interaction effects of schizophrenia polygenic risk score and hair cortisol levels on total brain volume ($N = 498$).

	Total brain volume		
P_t	β (95% CI)	P	$P_{FDR\text{-}adjusted}$
Main			
P_t<0.0005	-0.04 (-0.12;0.04)	0.23	0.53
P_t<0.001	-0.03 (-0.11;0.05)	0.49	0.76
P_t<0.005	0.01 (-0.06;0.09)	0.70	0.85
P_t<0.01	0.01 (-0.06;0.09)	0.75	0.86
P_t<0.05	0.05 (-0.03;0.13)	0.18	0.47
P_t<0.1	0.05 (-0.02;0.13)	0.18	0.47
P_t<0.5	0.05 (-0.02;0.13)	0.16	0.46
P_t<1.0	0.06 (-0.02;0.13)	0.14	0.45
Cortisol	0.02 (-0.05;0.10)	0.55	0.79
Interaction			
P_t<0.0005	0.02 (-0.05;0.09)	0.60	0.81
P_t<0.001	0.04 (-0.04;0.11)	0.34	0.62
P_t<0.005	0.02 (-0.06;0.10)	0.63	0.81
P_t<0.01	0.05 (-0.03;0.112)	0.24	0.53
P_t<0.05	0.07 (-0.01;0.14)	0.07	0.44
P_t<0.1	0.05 (-0.02;0.13)	0.17	0.46
P_t<0.5	0.07 (-0.01;0.15)	0.09	0.45
P_t<1.0	0.07 (-0.01;0.15)	0.07	0.44

Note: All models included the main effects and the interaction term. Models were adjusted for age at MRI visit and child sex, hair color, hair product use, corticosteroid use, and 4 principal components. False discovery rate correction for multiple testing was based on the total number of statistical tests across main effects of both polygenic scores and cortisol, *P*-value thresholds and brain outcomes. Abbreviations: P_t: P-value threshold.

Table A.2: Main and interaction effects of schizophrenia polygenic risk score and hair cortisol levels on cortical gray matter volume ($N = 498$).

	Cortical gray matter volume		
P_t	β (95% CI)	P	$P_{FDR\text{-}adjusted}$
Main			
P_t<0.0005	-0.04 (-0.12;0.04)	0.32	0.62
P_t<0.001	-0.03 (-0.11;0.05)	0.52	0.76
P_t<0.005	0.03 (-0.05;0.11)	0.50	0.76
P_t<0.01	0.02 (-0.06;0.10)	0.57	0.79
P_t<0.05	0.06 (-0.02;0.14)	0.13	0.45
P_t<0.1	0.06 (-0.01;0.14)	0.11	0.45
P_t<0.5	0.06 (-0.02;0.14)	0.12	0.45
P_t<1.0	0.07 (-0.01;0.14)	0.10	0.45
Cortisol	0.00 (-0.08;0.08)	0.96	0.98
Interaction			
P_t<0.0005	0.03 (-0.05;0.10)	0.52	0.76
P_t<0.001	0.03 (-0.05;0.11)	0.48	0.76
P_t<0.005	0.01 (-0.06;0.09)	0.72	0.85
P_t<0.01	0.04 (-0.04;0.12)	0.35	0.62
P_t<0.05	0.06 (-0.02;0.14)	0.14	0.45
P_t<0.1	0.05 (-0.03;0.13)	0.24	0.53
P_t<0.5	0.06 (-0.02;0.15)	0.12	0.45
P_t<1.0	0.07 (-0.01;0.15)	0.10	0.45

Note: All models included the main effects and the interaction term. Models were adjusted for age at MRI visit and child sex, hair color, hair product use, corticosteroid use, and 4 principal components. False discovery rate correction for multiple testing was based on the total number of statistical tests across main effects of both polygenic scores and cortisol, *P*-value thresholds and brain outcomes. Abbreviations: P_t: P-value threshold.

Table A.3: Main and interaction effects of schizophrenia polygenic risk score and hair cortisol levels on subcortical gray matter volume ($N = 498$).

	Subcortical gray matter volume		
P_t	β (95% CI)	P	$P_{FDR\text{-}adjusted}$
Main			
P_t<0.0005	0.02 (-0.07;0.10)	0.68	0.84
P_t<0.001	0.00 (-0.09;0.08)	0.95	0.97
P_t<0.005	0.01 (-0.08;0.09)	0.88	0.92
P_t<0.01	-0.01 (-0.10;0.07)	0.76	0.86
P_t<0.05	-0.03 (-0.11;0.06)	0.54	0.78
P_t<0.1	-0.03 (-0.11;0.06)	0.56	0.79
P_t<0.5	-0.01 (-0.10;0.08)	0.84	0.88
P_t<1.0	-0.01 (-0.10;0.07)	0.79	0.87
Cortisol	0.07 (-0.02;0.16)	0.11	0.45
Interaction			
P_t<0.0005	-0.02 (-0.10;0.06)	0.62	0.81
P_t<0.001	-0.03 (-0.12;0.06)	0.49	0.76
P_t<0.005	-0.02 (-0.10;0.07)	0.73	0.85
P_t<0.01	-0.04 (-0.13;0.05)	0.40	0.67
P_t<0.05	-0.06 (-0.15;0.02)	0.14	0.45
P_t<0.1	-0.04 (-0.13;0.05)	0.35	0.62
P_t<0.5	-0.06 (-0.15;0.03)	0.17	0.46
P_t<1.0	-0.06 (-0.15;0.03)	0.17	0.46

Note: All models included the main effects and the interaction term. Models were adjusted for age at MRI visit and child sex, hair color, hair product use, corticosteroid use, and 4 principal components. False discovery rate correction for multiple testing was based on the total number of statistical tests across main effects of both polygenic scores and cortisol, *P*-value thresholds and brain outcomes. Abbreviations: P_t: P-value threshold.

Table A.4: Main and interaction effects of schizophrenia polygenic risk score and hair cortisol levels on white matter volume ($N = 498$).

	White matter volume		
P_t	β (95% CI)	P	$P_{FDR\text{-}adjusted}$
Main			
P_t<0.0005	-0.05 (-0.13;0.03)	0.12	0.45
P_t<0.001	-0.04 (-0.11;0.04)	0.37	0.64
P_t<0.005	0.00 (-0.08;0.08)	0.95	0.97
P_t<0.01	0.00 (-0.08;0.08)	0.97	0.98
P_t<0.05	0.05 (-0.03;0.12)	0.25	0.53
P_t<0.1	0.04 (-0.04;0.12)	0.33	0.62
P_t<0.5	0.04 (-0.03;0.12)	0.26	0.53
P_t<1.0	0.05 (-0.03;0.12)	0.24	0.53
Cortisol	0.03 (-0.05;0.11)	0.45	0.73
Interaction			
P_t<0.0005	0.02 (-0.06;0.09)	0.61	0.81
P_t<0.001	0.04 (-0.03;0.12)	0.26	0.53
P_t<0.005	0.03 (-0.05;0.10)	0.52	0.76
P_t<0.01	0.06 (-0.02;0.14)	0.14	0.45
P_t<0.05	0.09 (0.01;0.16)	0.02	0.30
P_t<0.1	0.07 (-0.01;0.15)	0.09	0.45
P_t<0.5	0.09 (0.01;0.170	0.04	0.37
P_t<1.0	0.09 (0.01;0.17)	0.03	0.36

Note: All models included the main effects and the interaction term. Models were adjusted for age at MRI visit and child sex, hair color, hair product use, corticosteroid use, and 4 principal components. False discovery rate correction for multiple testing was based on the total number of statistical tests across main effects of both polygenic scores and cortisol, *P*-value thresholds and brain outcomes. Abbreviations: P_t: P-value threshold.

Table A.5: Main and interaction effects of schizophrenia polygenic risk score and hair cortisol levels on total ventricle volume ($N = 494$, no outliers).

	Total ventricle volume		
P_t	β (95% CI)	P	$P_{FDR\text{-}adjusted}$
Main			
P_t<0.0005	-0.13 (-0.21;-0.04)	0.01	0.30
P_t<0.001	-0.10 (-0.19;-0.02)	0.02	0.30
P_t<0.005	-0.10 (-0.19;-0.01)	0.03	0.36
P_t<0.01	-0.09 (-0.18;-0.01)	0.04	0.37
P_t<0.05	-0.10 (-0.19;-0.02)	0.02	0.30
P_t<0.1	-0.09 (-0.18;0.00)	0.06	0.42
P_t<0.5	-0.07 (-0.16;0.02)	0.11	0.45
P_t<1.0	-0.08 (-0.17;0.01)	0.09	0.45
Cortisol	-0.06 (-0.15;0.03)	0.19	0.48
Interaction			
P_t<0.0005	0.10 (0.01;0.18)	0.02	0.30
P_t<0.001	0.09 (0.00;0.18)	0.04	0.37
P_t<0.005	0.08 (0.00;0.17)	0.06	0.42
P_t<0.01	0.09 (0.00;0.18)	0.05	0.42
P_t<0.05	0.10 (0.01;0.19)	0.02	0.30
P_t<0.1	0.08 (-0.01;0.17)	0.08	0.45
P_t<0.5	0.07 (-0.02;0.16)	0.15	0.45
P_t<1.0	0.07 (-0.02;0.17)	0.12	0.45

Note: All models included the main effects and the interaction term. Models were adjusted for age at MRI visit and child sex, hair color, hair product use, corticosteroid use, and 4 principal components. False discovery rate correction for multiple testing was based on the total number of statistical tests across main effects of both polygenic scores and cortisol, *P*-value thresholds and brain outcomes.
Abbreviations: P_t: P-value threshold.

Table A.6: Main and interaction effects of schizophrenia polygenic risk score and hair cortisol levels on global fractional anisotropy ($N = 526$).

	Global FA		
P_t	β (95% CI)	P	$P_{FDR\text{-}adjusted}$
Main			
P_t<0.0005	0.05 (-0.03;0.14)	0.24	0.53
P_t<0.001	0.03 (-0.05;0.12)	0.45	0.73
P_t<0.005	0.01 (-0.08;0.10)	0.83	0.88
P_t<0.01	-0.02 (-0.11;0.07)	0.63	0.81
P_t<0.05	0.00 (-0.09;0.09)	1.00	1.00
P_t<0.1	-0.01 (-0.10;0.07)	0.74	0.85
P_t<0.5	-0.01 (-0.10;0.08)	0.80	0.87
P_t<1.0	-0.01 (-0.10;0.08)	0.80	0.87
Cortisol	0.04 (-0.04;0.13)	0.33	0.62
Interaction			
P_t<0.0005	-0.04 (-0.12;0.05)	0.37	0.64
P_t<0.001	-0.02 (-0.11;0.06)	0.63	0.81
P_t<0.005	0.01 (-0.08;0.10)	0.77	0.86
P_t<0.01	0.02 (-0.07;0.11)	0.63	0.81
P_t<0.05	0.02 (-0.07;0.10)	0.70	0.85
P_t<0.1	0.03 (-0.05;0.12)	0.74	0.85
P_t<0.5	0.01 (-0.08;0.10)	0.83	0.88
P_t<1.0	0.01 (-0.08;0.10)	0.81	0.88

Note: All models included the main effects and the interaction term. Models were adjusted for age at MRI visit and child sex, hair color, hair product use, corticosteroid use, and 4 principal components. False discovery rate correction for multiple testing was based on the total number of statistical tests across main effects of both polygenic scores and cortisol, *P*-value thresholds and brain outcomes.
Abbreviations: P_t: P-value threshold; FA: fractional anisotropy.

Table A.7: Main and interaction effects of schizophrenia polygenic risk score and hair cortisol levels on global mean diffusivity ($N = 526$).

	Global MD		
P_t	β (95% CI)	P	$P_{FDR\text{-}adjusted}$
Main			
P_t<0.0005	-0.05 (-0.13;0.04)	0.29	0.58
P_t<0.001	-0.02 (-0.10;0.06)	0.64	0.81
P_t<0.005	0.02 (-0.07;0.10)	0.67	0.84
P_t<0.01	0.04 (-0.04;0.12)	0.34	0.62
P_t<0.05	0.02 (-0.06;0.11)	0.57	0.79
P_t<0.1	0.03 (-0.05;0.11)	0.47	0.76
P_t<0.5	0.04 (-0.05;0.12)	0.40	0.67
P_t<1.0	0.03 (-0.05;0.12)	0.23	0.53
Cortisol	0.01 (-0.07;0.10)	0.73	0.85
Interaction			
P_t<0.0005	0.10 (0.02;0.19)	0.01	0.30
P_t<0.001	0.10 (0.02;0.18)	0.02	0.30
P_t<0.005	0.06 (-0.02;0.15)	0.15	0.45
P_t<0.01	0.08 (-0.01;0.17)	0.06	0.42
P_t<0.05	0.06 (-0.02;0.14)	0.15	0.45
P_t<0.1	0.05 (-0.04;0.13)	0.29	0.58
P_t<0.5	0.04 (-0.04;0.14)	0.26	0.53
P_t<1.0	0.05 (-0.03;0.140	0.23	0.53

Note: All models included the main effects and the interaction term. Models were adjusted for age at MRI visit and child sex, hair color, hair product use, corticosteroid use, and 4 principal components. False discovery rate correction for multiple testing was based on the total number of statistical tests across main effects of both polygenic scores and cortisol, *P*-value thresholds and brain outcomes.
Abbreviations: P_t: P-value threshold ; MD: mean diffusivity.

Table A.8: Main and interaction effects of schizophrenia polygenic risk score and hair cortisol levels on global axial diffusivity ($N = 526$).

	Global AD		
P_t	β (95% CI)	P	
Main			
P_t<0.0005	-0.03 (-0.11;0.05)	0.47	
P_t<0.001	-0.01 (-0.10;0.07)	0.77	
P_t<0.005	0.01 (-0.07;0.09)	0.80	
P_t<0.01	0.01 (-0.07;0.09)	0.80	
P_t<0.05	0.00 (-0.08;0.09)	0.94	
P_t<0.1	-0.01 (-0.09;0.08)	0.89	
P_t<0.5	0.00 (-0.08;0.08)	0.99	
P_t<1.0	0.00 (-0.09;0.08)	0.93	
Cortisol	0.06 (-0.02;0.15)	0.14	
Interaction			
P_t<0.0005	0.10 (0.02;0.18)	0.02	
P_t<0.001	0.10 (0.02;0.18)	0.01	
P_t<0.005	0.09 (0.00;0.17)	0.04	
P_t<0.01	0.11 (0.03;0.20)	0.01	
P_t<0.05	0.09 (0.01;0.17)	0.03	
P_t<0.1	0.08 (0.00;0.16)	0.06	
P_t<0.5	0.07 (-0.02;0.15)	0.13	
P_t<1.0	0.07 (-0.01;0.16)	0.10	

Note: All models included the main effects and the interaction term. Models were adjusted for age at MRI visit and child sex, hair color, hair product use, corticosteroid use, and 4 principal components.
Abbreviations: P_t: P-value threshold; AD: axial diffusivity.

Table A.9: Main and interaction effects of schizophrenia polygenic risk score and hair cortisol levels on global radial diffusivity ($N = 526$).

	Global RD	
P_t	β (95% CI)	P
Main		
P_t<0.0005	-0.06 (-0.14;0.03)	0.20
P_t<0.001	-0.03 (-0.12;0.06)	0.50
P_t<0.005	0.01 (-0.08;0.09)	0.84
P_t<0.01	0.04 (-0.05;0.12)	0.38
P_t<0.05	0.02 (-0.07;0.10)	0.66
P_t<0.1	0.03 (-0.06;0.11)	0.50
P_t<0.5	0.03 (-0.05;0.12)	0.48
P_t<1.0	0.04 (-0.06;0.11)	0.51
Cortisol	-0.01 (-0.10;0.07)	0.75
Interaction		
P_t<0.0005	0.09 (0.01;0.17)	0.04
P_t<0.001	0.07 (-0.01;0.16)	0.08
P_t<0.005	0.03 (-0.05;0.12)	0.43
P_t<0.01	0.04 (-0.04;0.13)	0.33
P_t<0.05	0.03 (-0.05;0.12)	0.42
P_t<0.1	0.02 (-0.07;0.10)	0.67
P_t<0.5	0.04 (-0.05;0.12)	0.44
P_t<1.0	0.04 (-0.05;0.13)	0.40

Note: All models included the main effects and the interaction term. Models were adjusted for age at MRI visit and child sex, hair color, hair product use, corticosteroid use, and 4 principal components.
Abbreviations: P_t: P-value threshold; RD: radial diffusivity.

Table A.10: Main and interaction effects of major depressive disorder polygenic risk score and hair cortisol levels on total ventricle volume ($N = 494$, no outliers).

	Global ventricle volume	
P_t	β (95% CI)	P
Main		
P_t<0.0005	-0.03 (-0.12;0.06)	0.56
P_t<0.001	-0.04 (-0.13;0.05)	0.34
P_t<0.005	-0.02 (-0.11;0.07)	0.67
P_t<0.01	-0.03 (-0.12;0.06)	0.56
P_t<0.05	0.00 (-0.08;0.09)	0.92
P_t<0.1	-0.01 (-0.09;0.08)	0.90
P_t<0.5	0.00 (-0.09;0.09)	0.94
P_t<1.0	0.00 (-0.09;0.09)	0.96
Cortisol	-0.07 (-0.16;0.02)	0.15
Interaction		
P_t<0.0005	0.02 (-0.07;0.11)	0.69
P_t<0.001	-0.01 (-0.10;0.08)	0.78
P_t<0.005	-0.02 (-0.11;0.06)	0.58
P_t<0.01	-0.04 (-0.13;0.05)	0.41
P_t<0.05	-0.03 (-0.12;0.07)	0.58
P_t<0.1	-0.01 (-0.10;0.08)	0.84
P_t<0.5	-0.03 (-0.12;0.07)	0.58
P_t<1.0	-0.03 (-0.12;0.06)	0.57

Note: All models included the main effects and the interaction term. Models were adjusted for age at MRI visit and child sex, hair color, hair product use, corticosteroid use, and 4 principal components. Ventricular volume was assessed as a fraction of intracranial volume. False discovery rate correction for multiple testing was based on the total number of statistical tests across main effects of both polygenic scores and cortisol, *P*-value thresholds and brain outcomes.
Abbreviations: P_t: P-value threshold.

Table A.11: Main and interaction effects of major depressive disorder polygenic risk score and hair cortisol levels on global mean diffusivity ($N = 526$).

	Global MD	
P_t	β (95% CI)	P
Main		
P_t<0.0005	0.00 (-0.09;0.08)	0.94
P_t<0.001	-0.02 (-0.10;0.06)	0.64
P_t<0.005	-0.03 (-0.11;0.06)	0.50
P_t<0.01	-0.03 (-0.11;0.06)	0.51
P_t<0.05	0.00 (-0.09;0.08)	0.98
P_t<0.1	-0.03 (-0.12;0.05)	0.43
P_t<0.5	0.00 (-0.09;0.08)	0.97
P_t<1.0	0.01 (-0.08;0.09)	0.87
Cortisol	0.01 (-0.08;0.09)	0.85
Interaction		
P_t<0.0005	0.02 (-0.06;0.11)	0.62
P_t<0.001	0.01 (-0.08;0.09)	0.90
P_t<0.005	-0.02 (-0.10;0.06)	0.61
P_t<0.01	0.00 (-0.09;0.08)	0.96
P_t<0.05	-0.03 (-0.12;0.05)	0.46
P_t<0.1	-0.04 (-0.13;0.05)	0.44
P_t<0.5	-0.04 (-0.13;0.05)	0.37
P_t<1.0	-0.04 (-0.12;0.05)	0.41

Note: All models included the main effects and the interaction term. Models were adjusted for age at MRI visit and child sex, hair color, hair product use, corticosteroid use, and 4 principal components. False discovery rate correction for multiple testing was based on the total number of statistical tests across main effects of both polygenic scores and cortisol, *P*-value thresholds and brain outcomes.
Abbreviations: P_t: P-value threshold; MD: mean diffusivity.

Figure A.1: Inclusion flow chart

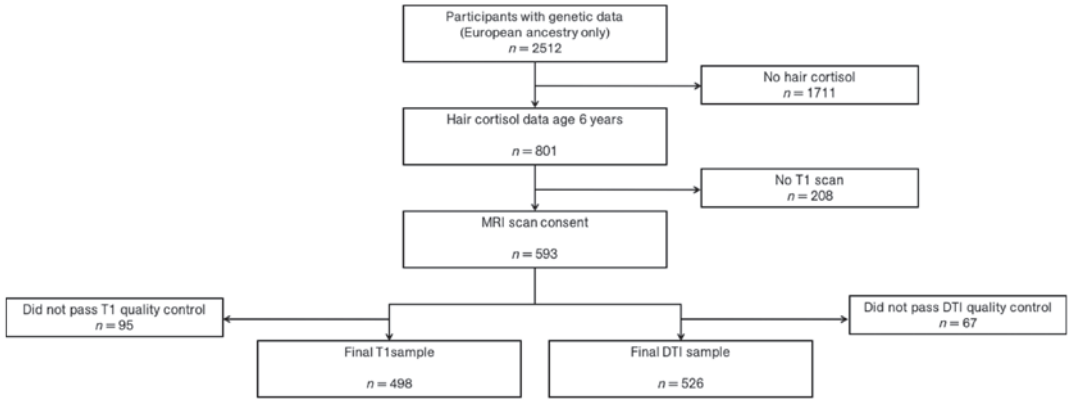
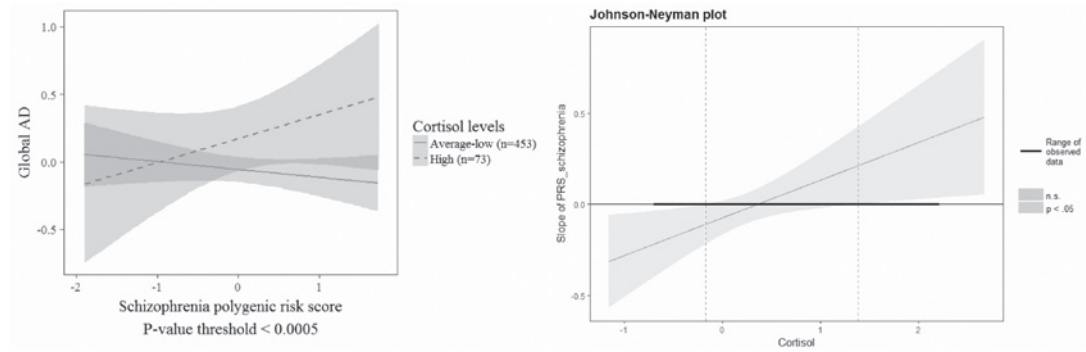
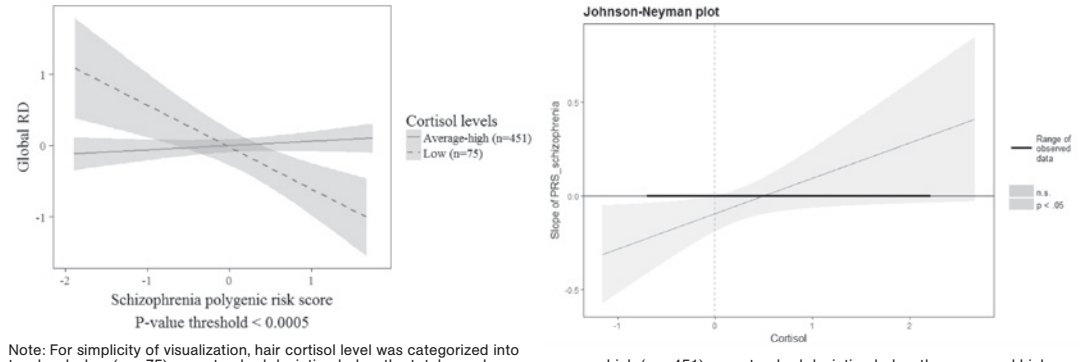


Figure A.2: Relationship between schizophrenia polygenic risk and global axial diffusivity as a function of hair cortisol level (left) and corresponding Johnson-Neyman plot (right).



Note: For simplicity of visualization, hair cortisol level was categorized into two levels: high ($n = 73$), one standard deviation above the total sample mean; average-low ($n = 453$), one standard deviation above the mean and lower. The gray-shaded areas denote 95% confidence intervals. *P*-value threshold for the schizophrenia polygenic risk score is shown at $P_t < 0.0005$. Both schizophrenia polygenic risk score (x-axis) and global mean diffusivity (y-axis) are standardized. The right graph shows the Johnson-Neyman plots, which indicates at values of (log-transformed) cortisol below -0.18 and above 1.38, the slope of schizophrenia polygenic risk score is significantly different from zero, and negative respectively positive (turquoise shaded area).

Figure A.3: Relationship between schizophrenia polygenic risk and global radial diffusivity as a function of hair cortisol level (left) and corresponding Johnson-Neyman plot (right).



Note: For simplicity of visualization, hair cortisol level was categorized into two levels: low ($n = 75$), one standard deviation below the total sample mean; average-high ($n = 451$), one standard deviation below the mean and higher. The gray-shaded areas denote 95% confidence intervals. *P*-value threshold for the schizophrenia polygenic risk score is shown at $P_t < 0.0005$. Both schizophrenia polygenic risk score (x-axis) and global mean diffusivity (y-axis) are standardized. The right graph shows the Johnson-Neyman plots, which indicates at values of (log-transformed) cortisol below -0.01, the slope of schizophrenia polygenic risk score is significantly different from zero, and negative (turquoise shaded area).

(...) *Once we accept that, we can't dodge the responsibility for social action.*
(Terrie Moffitt, 2018)

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(ABSTRACT)

OBJECTIVE

Previous studies have shown that polygenic risk scores for schizophrenia predict sub-threshold psychiatric problems in the general population. However, it is yet unclear whether environmental factors might also contribute to this association, for which a potential pathway is mediation through early life adversities. Here, we used mediation techniques to test gene-environment correlation of the association of schizophrenia polygenic risk with emotional and behavioral problems via childhood adversities.

METHODS

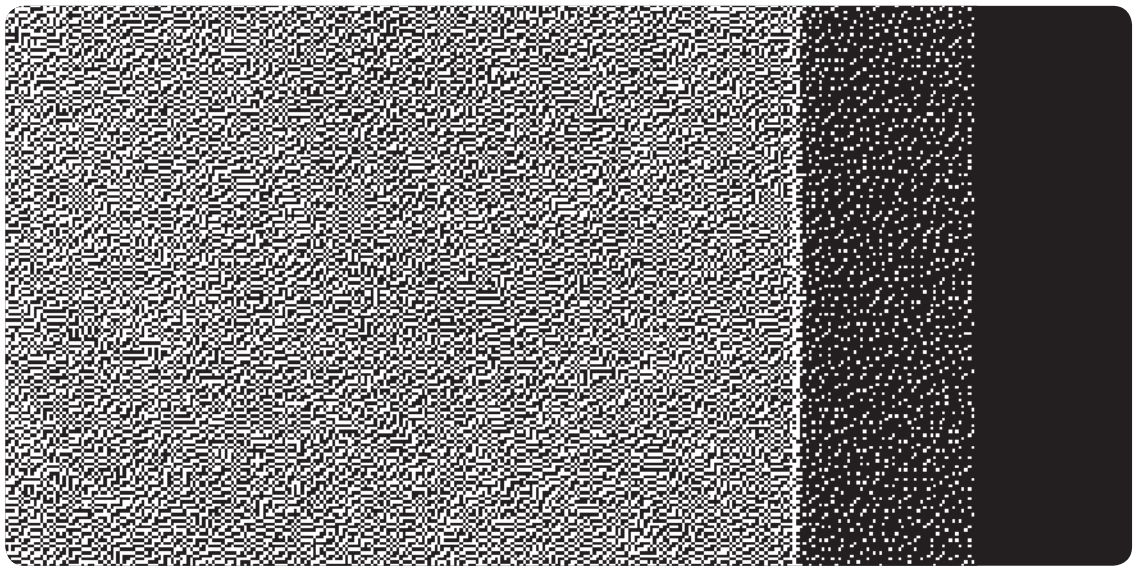
This study was embedded in the population-based Generation R Study, which included N=1901 participants of European descent with genotyping for schizophrenia polygenic risk, childhood adversity reporting, and behavioral outcomes. Childhood adversities were assessed through maternal interview and behaviour was assessed with the Child Behaviour Checklist. Associations were analyzed with mediation analysis.

RESULTS

In line with previous studies, schizophrenia polygenic risk was associated with more emotional and thought problems in children. Higher schizophrenia risk scores were associated with greater exposure to childhood adversities (P-value threshold < 0.5; OR=1.08, 95% CI 1.02–1.15), particularly for adversities occurring before 5 years of age. Childhood adversities mediated the relationship of schizophrenia polygenic risk with emotional and thought problems. Direct effects between schizophrenia polygenic risk and behaviour were also observed.

CONCLUSIONS

Genetic liability to schizophrenia increased odds for childhood adversities and emotional and thought problems. In this study, we observed that exposure to childhood adversities mediated the association of schizophrenia polygenic risk with emotional and thought problems in pre-adolescents, suggesting a potential focus for preventative interventions for children at high genetic risk.



– The slightest ambiguities,
the most inexplicable transpositions of ideas take place. (...)

CHAPTER 6

SCHIZOPHRENIA POLYGENIC RISK SCORES, CHILDHOOD ADVERSITIES, AND BEHAVIOR IN THE GENERAL PEDIATRIC POPULATION: EVIDENCE OF GENE-ENVIRONMENT CORRELATION

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Manuscript to be submitted.

INTRODUCTION

The findings of large genome-wide association (GWA) studies have substantially improved our understanding of the genetic etiology of schizophrenia (Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014). Polygenic risk scores are derived as the weighted sum of risk single nucleotide polymorphisms (SNPs) derived from GWAS, and can be utilized as a metric for additive genetic liability for schizophrenia. The schizophrenia polygenic risk score explains approximately 18% of the variance in an independent sample (Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014). Several studies have employed schizophrenia polygenic risk score to investigate developmental manifestations of increased genetic liability for schizophrenia in the general population. The schizophrenia polygenic risk score has been associated with early life emotional and behavioral problems, neuro-motor development, cognition, social and communication difficulties, and physical health problems in the general population (Jones et al., 2016; Riglin et al., 2017; St Pourcain et al., 2018; Nivard et al., 2017; Stringer, Kahn, de Witte, Ophoff and Derks, 2014; Serdarevic et al., 2018). More such studies to disentangle the prodromal pathways are needed as it is widely recognized that schizophrenia has different neurodevelopmental antecedents (van Os and Reininghaus, 2016; Serdarevic et al., 2018; Ursini et al., 2018).

We have previously reported that schizophrenia polygenic risk is associated with more emotional and thought problems at age ten years (Jansen et al., 2018). No such associations were observed for the polygenic risk scores for other psychiatric disorders such as bipolar disorder, major depressive disorder, autism spectrum disorder or attention-deficit/hyperactivity disorder (ADHD). This lends a degree of specificity to the associations of genetic liability to schizophrenia and early life emotional and thought problems. These studies complement findings from offspring studies, by showing that the genetic vulnerability for severe mental illness increases the risk for psychiatric problems (Rasic, Hajek, Alda and Uher, 2014). It is, however, unclear which mechanisms underlie this association. Genotype very likely exerts a direct effect on behavioral outcome (Wray et al., 2014; Poletti and Raballo, 2018), but this relationship may be at least partially mediated by environmental stressors, such as exposure to childhood adversity.

The notion that a child's exposure to environments partially depends on their genotype is commonly acknowledged, and twin or family studies have indicated substantial contributions of the child' genotype to factors which are typically understood as "environmental", such as parenting, social support and life events (Kendler and Baker, 2007; Vinkhuyzen, van der Sluis, de Geus, Boomsma and Posthuma, 2010). These associations are referred to as gene-environment correlation, and twin and SNP-heritability studies have suggested that childhood adversities are to some degree determined by the child's genetics (Kendler and Baker, 2007; Power et al., 2013). Further support for this phenomenon comes from population-based studies that have shown that certain environmental events are more common in people with psychiatric conditions, such as individuals with ADHD who run a greater risk of experiencing serious transport accidents (Chang, Lichtenstein, D'Onofrio, Sjolander and Larsson, 2014). The fact that most environmental exposures are heritable could indicate that their risk effect on child psychopathology is partly regulated by the child's genetics (Jaffee and Price, 2012). Only a few studies have adopted psychiatric and cognitive trait-associated polygenic risk scores to study gene-environment correlation. For example, schizophrenia polygenic risk of the child has been associated with higher paternal age at child birth, whereas lower educational achievement polygenic risk of the child predicted a variety of environmental exposures, including less breastfeeding, more smoking of the mother during pregnancy and a lower household income (Krapohl et al., 2017). Furthermore, polygenic risk for major depressive disorder has been shown to predict a lifetime history of childhood trauma (Peyrot et al., 2017), which is evidence for gene-environment correlation (Plomin, 2013; Knopik, Niederhiser, DeFries and Plomin, 2016; Jaffee and Price, 2012; Pingault et al., 2018). To our knowledge, no study has yet examined whether a child's polygenic risk for schizophrenia is associated with a greater exposure to adversities in childhood.

In this prospective population-based study, we aimed to explore the association between the child's schizophrenia polygenic risk score and the odds of experiencing childhood adversities. Various adverse

events were assessed through maternal interview, including events related to the child (person-related, e.g. high workload at school, maltreatment) and external events (environment-related, e.g. neighborhood problems, family financial problems). In addition, we sought to determine whether this association mediates the relationship of schizophrenia polygenic risk with emotional and behavioral problems. Lastly, we explored the polygenic risk score for major depression for specificity.

METHODS

STUDY POPULATION

The present study was embedded within the Generation R Study, a prospective population-based birth cohort, which included 9778 pregnant women living in Rotterdam, the Netherlands (Kooijman et al., 2016). The aim of the Generation R Study is to identify early genetic and environmental risk that influence maternal and child health and development. For this study, 2512 children of European descent (based on genetic ancestry; 53% of n = 4780 participants of European descent who were eligible for the age 10 assessment) had genotype data available which passed quality control procedures. Of these children, 1901 had information available on childhood adversities and psychiatric problems, which were assessed at mean age 10 years. See Supplemental Figure S1 for a study flowchart. Study protocols were approved by the Medical Ethics Committee of the Erasmus Medical Center. All participants and their mothers provided assent and informed consent, respectively.

ATTRITION ANALYSIS

Comparisons were made between the final study sample (N = 1901) and participants who were genotyped but without data at mean age 10 years (N = 2512). These groups did not differ in proportion of girls (50.3% versus 46.3%, χ^2=2.84, df = 1, P = 0.09) or in mean schizophrenia risk score (P_t < 0.5; -0.02 versus 0.04, t = 1.40, P = 0.16). However, children with complete data had lower mean major depression risk score (P_t < 0.5; -0.03 versus 0.01, t = 2.28, P = 0.02), and were slightly more likely to have mothers with higher educational levels (73.4% versus 68.6%, χ^2 = 6.76, df = 2, P = 0.03).

GENOTYPING AND QUALITY CONTROL

Genotype quality control procedures for the Generation R cohort have previously been described in more detail (Medina-Gomez et al., 2015). In short, genotype data were collected either from cord blood at birth (Illumina 610K Quad Chip) or via vena puncture (Illumina 660k Quad Chip) during a visit to the research center. Variants were included if they passed sample (≥97.5%) and SNP call rates (≥90%), minor allele frequency ≥1%, and no deviation from Hardy-Weinberg disequilibrium (P < 10-7). In addition, individuals were screened for excess heterozygosity, sex mismatch, relatedness, and missing data. Individuals of European descent were selected within 4 standard deviations on the first four genetic principal components of the HapMap Phase II Northwestern European (CEU) population. Principal components of ancestry used as covariates in this study were based on the European-descent sample.

CHILD POLYGENIC RISK SCORING

Common genetic risk variants associated with schizophrenia were obtained from the Psychiatric Genetics Consortium meta-analysis of case-control genome-wide association study (GWAS) of 33,640 cases and 43,456 controls (Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014), and depression polygenic risk scores were obtained from the most recent GWAS of 135,458 cases and 344,901 controls (Wray et al., 2018). SNPs were clumped according to linkage disequilibrium (LD) in order to obtain the most significant SNP per LD-block (kilobase pair window: 250, LD r2 < 0.1), in line with earlier work in Generation R (Jansen et al., 2018). Polygenic scores were computed using PRSice (Euesden, Lewis and O'Reilly, 2015), which calculates score by weighing the mean number of risk alleles by the SNP effect size. P-value thresholds for inclusion of SNPs in the score varied between P < 0.0005 and P < 1. Our primary analysis was at P < 0.5, a default cut-off used in our previous publications (Jansen et al., 2018). Polygenic risk

scores were standardized to a mean of 0 and standard deviation of 1 to facilitate interpretation.

SNP HERITABILITY OF CHILDHOOD ADVERSITIES

In the sample with genotype and childhood adversities data, the variance explained by additive effects of autosomal SNPs was estimated using GREML as implemented in GCTA 1.24.7 (Yang, Lee, Goddard and Visscher, 2011; Yang, Lee, Goddard and Visscher, 2013). The conventional genetic relatedness matrix cutoff of 0.025 was used to exclude close relatives (second-degree cousins and closer) to reduce confounding due to shared environment, as described in more detail previously (Neumann et al., 2016). This resulted in a sample is 1833 children.

CHILD EMOTIONAL AND BEHAVIORAL PROBLEMS

Emotional and behavioral problems of the child were assessed with the Child Behavior Checklist/6-18 (CBCL), an internationally validated and reliable measure of emotional and behavioral problems (Achenbach and Rescorla, 2001) at age 10 years. The CBCL/6-18 consists of an internalizing (i.e. emotional) and externalizing (i.e. behavioral) problems broadband scales. The internalizing problem scale comprised the anxious/depressed, withdrawn/depressed, and somatic complaints problems syndrome scales. The externalizing problems broadband scale comprised the rule-breaking and aggressive behavior scales. A hierarchical approach was employed here, if no association was observed for the broadband scale, no further analyses with the syndrome scales were performed. Other syndrome scales are social problems, thought problems, and attention problems. The CBCL measures emotional and behavioral problems on a continuous severity scale and has been shown to predict DSM-based psychiatric disorders in adulthood (Hofstra, van der Ende and Verhulst, 2002). Items were scored by mothers on a three-point scale (0 = not true; 1 = somewhat or sometimes true; 2 = very or often true), based on behavior of the past 6 months.

CHILDHOOD ADVERSITIES

At mean age 10 years, children and their mothers were invited to the research center, where mothers were interviewed about their offspring's childhood adversities. Mothers were asked about 24 childhood adversities, e.g. parental divorce/separation, transferring schools, and physical or sexual maltreatment (Amone-P'Olak et al., 2009; Bolhuis et al., 2018). In case of a positive response, the child's age when the event had happened was registered, and the perceived severity of each event was rated as none, a little, moderate, or a lot. Only events with at least moderate impact were coded as adversities in the present analyses. As the inclusion of adversities with moderate or a lot of impact could potentially introduce reverse causality, we performed sensitivity analyses with additional adjustment for emotional and behavior problems assessed with the CBCL at child age 3 years. We distinguished child person-related (e.g. high workload at school, maltreatment) from environment-related adversities (e.g. neighborhood problems, family financial difficulties, (Amone-P'Olak et al., 2009)), as well as adversities occurring before age 5 years versus after age 5 years. Lifetime prevalence of the examined adversities, and their categorization as person-related or environment-related adversities are listed in the Supplementary Table S1.

STATISTICAL ANALYSES

Child SNP heritability was estimated for total adversities, and specifically person-related and environment-related adversities as well as adversities occurring before versus after age 5 years. The association of schizophrenia and depression polygenic risk scores of the child with childhood adversities was performed using Poisson regression models. Models were adjusted for child age, sex, and four genetic principal components of genetic ancestry. Analyses for the other *P*-value thresholds are shown in the Supplement. Only if an association of a genetic score with a total score of childhood adversities was observed, further in-depth analyses were performed for person-related and environment-related adversities, as well as adversities that occurred before versus after age 5 years. Sensitivity analyses with additional adjustment with CBCL total problems scores at age

3 years were performed if an association was observed. All analyses were conducted using R statistical software, versions 3.2.1 (R Core Team, 2015).

Mediation analyses were conducted to examine whether the previously published association on child schizophrenia polygenic risk score with child problem behavior was mediated by childhood adversities (Figure 1 for visualization of the conceptual mediation model), according to the following procedure (Preacher and Hayes, 2008). First, separate linear regression analyses were performed for the associations between: (1) exposure (child polygenic risk scores) and outcome (child emotional and behavioral problems), i.e. the total effect; (2) exposure and mediator (childhood adversities), i.e. the gene-environment association in this case; (3) mediator and outcome controlling for exposure, i.e. the direct effect. If all three associations were statistically significant, mediation analyses were conducted in one single model to obtain the mediated effect. In case of significant mediation, the proportion to which the exposure-outcome estimate was attenuated after inclusion of the covariate was calculated. These analyses were performed using the mediation package in R (Tingley, Yamamoto, Hirose, Keele and Imai, 2014). A hierarchical approach was employed here; if no association was observed for the CBCL broadband scales of internalizing or externalizing problems, no subsequent analyses with their respective syndrome scales were performed. Associations were conducted using linear regression, which showed results similar to the main results using Poisson regression. Scores of child psychiatric problems and sum scores of childhood adversities were square root transformed in order to approach a normal distribution and improve the linear models fits.

RESULTS

SAMPLE CHARACTERISTICS

Demographic characteristics of the sample are demonstrated in Table 1. The mean age of the sample was 9.69 years (SD 0.26). The majority of children (n = 1343, 70.65%) had not encountered any childhood adversities, whereas n = 450 (23.67%) experienced one

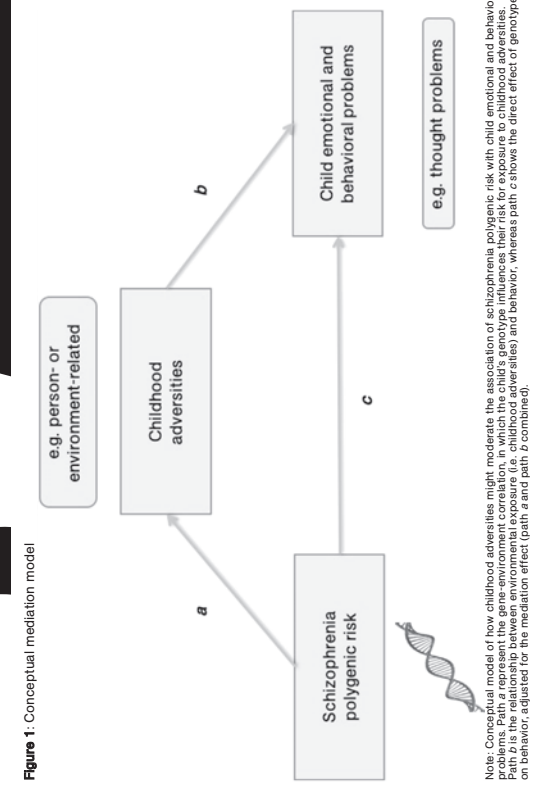


Figure 1: Conceptual mediation model of how childhood adversities might moderate the association of schizophrenia polygenic risk with child emotional and behavioral problems. Path a represent the gene-environment correlation, in which the child's genotype influences their risk for exposure to childhood adversities. Path b represents the association between exposure (i.e. childhood adversities) and behavior, whereas path c shows the direct effect of genotype on behavior, adjusted for the mediation effect (path a and path b combined).

(Full colour image presented on page 9.)

Table 2: Association of the schizophrenia and major depression polygenic risk scores with childhood adversities (N = 1901).

	Childhood adversities	
	OR (95% CI)	*P*
Schizophrenia risk score		
Total adversities	1.08 (1.02–1.15)	0.01
Person-related adversities	1.06 (0.97 – 1.16)	0.18
Environment-related adversities	1.01 (0.90 – 1.14)	0.87
Adversities before age 5 years	1.20 (1.05 – 1.36)	0.01
Adversities after age 5 years	1.05 (0.98 – 1.13)	0.13
Major depression risk score		
Total adversities	1.03 (0.97–1.10)	0.33

Note: Analyses are adjusted for age, child sex, and four principal components of genetic ancestry. Results are shown for the $P_t < 0.5$ inclusion threshold. Results for the other *P*-value thresholds are shown Supplementary Table S2-5.

Table 1: Descriptive characteristics of the study sample.

	N	Total population (N = 1901)
Child characteristics		
Age at MRI, mean (SD)	1901	9.69 (0.26)
Sex, proportion of girls	1901	50.3%
Childhood adversities, proportion		
No adversities	1343	70.7%
1 or 2 adversities	450	23.7%
3 or more adversities	108	5.7%
Adversities before age 5 years	185	9.7%
Adversities after age 5 years	496	26.1%
Total internalizing problems score, median (IQR)	1901	3.00 (5.00)
Total externalizing problems score, media (IQR)	1901	2.00 (5.00)
Maternal characteristics		
Educational level, proportion		
High	1327	73.4%
Medium	476	26.3%
Low	5	0.3%

Note: See Supplementary Table S1 for the prevalence of the individual adversities.

or two adversities, and *n* = 108 (5.68%) experienced more than two adversities. The prevalence of the individual events is listed in Supplementary Table S1; high workload at school (9.5%) and divorce/separation of parents (4.8%) were most commonly endorsed, whereas inappropriate sexual behavior (0.2%) and death of a caretaker (0.3%) were the least prevalent.

SNP-HERITABILITY OF ADVERSITIES

The following SNP heritabilities were estimated of total adversities, 23% (SE = 0.18, *P* = 0.09); person-related adversities, 34% (SE = 0.19, *P* = 0.03), environment-related adversities, 6% (SE = 0.18, *P* = 0.36), adversities before age 5 years, 20% (SE = 0.19, *P* = 0.14), and adversities after age 5 years, 9% (SE = 0.18, *P* = 0.31).

GENE-ENVIRONMENT CORRELATION BETWEEN POLYGENIC RISK SCORES AND CHILDHOOD ADVERSITIES

Child schizophrenia polygenic risk scores were associated with increased odds for total childhood adversities (Table 2). This was observed at the least stringent *P*-value thresholds (Supplementary Table S2, e.g. P_t <0.5: OR = 1.08, 95% CI 1.02 – 1.15, *P* = 0.01). Sensitivity analyses with additional adjustment for CBCL total problems scores at age 3 years yielded comparable results (Supplementary Table S3, e.g. P_t <0.5: OR = 1.06, 95% CI 1.00 – 1.14, *P* = 0.04). Depression polygenic risk scores were not associated with total childhood adversities, and, hence, therefore follow-up analyses were not conducted (Supplementary Table S4).

No association of schizophrenia polygenic scores were found with either person-related adversities or environment-related adversities (Supplementary Table S5). However, schizophrenia polygenic risk score was associated with a higher burden of childhood adversities occurring before age 5 years (Table 2 and Supplemental Table S6, P_t < 0.5: OR = 1.20, 95% CI 1.05 – 1.36, P = 0.005), which was observed at several P-value thresholds. In contrast, schizophrenia risk scores were not associated with adversities occurring after age 5 years (P_t < 0.5: OR = 1.05, 95% CI 0.98 – 1.13, *P* = 0.130).

MEDIATION EFFECT OF CHILDHOOD ADVERSITIES ON THE ASSOCIATION BETWEEN CHILD SCHIZOPHRENIA POLYGENIC RISK AND CHILD PSYCHIATRIC PROBLEMS

We assessed whether the previously published association between schizophrenia polygenic risk score and child psychiatric problems at age 10 years was mediated by childhood adversities. A significant mediation effect of childhood adversities was observed in the association of schizophrenia risk with internalizing problems, anxious depressed problems, somatic complaints, thought problems, and attention problems (Table 3). The proportion of these mediations were relatively small at 22%, 23%, 14% and 19%, respectively. The associations of schizophrenia polygenic risk with withdrawn/depressed problems, externalizing problems and aggressive behavior, rule-breaking behavior, other problems and social problems were not statistically significant in the total effects models and, hence, the mediation analyses were not conducted. Similar mediation associations were observed for adversities which specifically occurred before age 5 years (Table 4).

DISCUSSION

This is the first study to explore whether the associations between a child's genetic risk for schizophrenia and psychiatric problems in the general population are mediated by childhood adversities. Using genetic, behavioral and adversity data from almost 2000 children, we show that the child polygenic risk score for schizophrenia is associated with greater exposure to childhood adversities, a composite of various negative events, including maltreatment, school and family problems. We highlight four observations. First, we found that polygenic risk for schizophrenia predicted greater exposure to childhood adversities, and this association was mainly driven by adversities reported before child age five years. Second, the above association mediated the association of schizophrenia polygenic risk with child emotional, attention, and thought problems. Third, no association was observed for the major depression polygenic risk score, lending specificity to the genetic variants associated with schizophrenia. Fourth, we obtained suggestive evidence for SNP-heritability for

Table 3: The mediating effect of childhood adversities **before age 10 years** in the association between the schizophrenia polygenic risk score and childhood problem behavior

	Total effect		Direct effect		Mediated effect		Proportion mediated	
Outcome	β (95% CI)	*P*	β (95% CI)	*P*	β (95% CI)	*P*	β (95% CI)	*P*
Internalizing problems	0.06 (0.02;0.11)	0.01	0.05 (0.01;0.09)	0.02	0.01 (0.00;0.03)	0.05	0.22 (-0.01;0.65)	0.06
Anxious/depressed	0.06 (0.02;0.10)	0.01	0.05 (0.00;0.09)	0.03	0.01 (0.00;0.03)	0.05	0.23 (0.00;0.77)	0.05
Withdrawn/depressed	0.00 (-0.04;0.04)	0.97	NA		NA		NA	
Somatic complaints	0.05 (0.01;0.10)	0.02	0.04 (0.00;0.09)	0.06	0.01 (0.00;0.02)	0.04	0.19 (-0.02;0.83)	0.06
Externalizing problems	0.04 (0.00;0.09)	0.06	NA		NA		NA	
Other problems								
Social problems	0.04 (-0.01;0.08)	0.11	NA		NA		NA	
Thought problems	0.08 (0.04;0.12)	<0.01	0.07 (0.03;0.11)	<0.01	0.01 (0.00;0.02)	0.05	0.14 (0.00;0.34)	0.05
Attention problems	0.07 (0.02;0.11)	<0.01	0.05 (0.01;0.10)	0.01	0.01 (0.00;0.02)	0.04	0.19 (0.01;0.54)	0.04

Note: Analyses are adjusted for age, child sex, and four principal components of genetic ancestry. Results are shown for the $P_t < 0.5$ inclusion threshold.

Table 4: The mediating effect of childhood adversities **before age 5 years** in the association between the schizophrenia polygenic risk score and childhood problem behavior

	Total effect		Direct effect		Mediated effect		Proportion mediated	
Outcome	β (95% CI)	*P*	β (95% CI)	*P*	β (95% CI)	*P*	β (95% CI)	*P*
Internalizing problems	0.05 (0.01–0.10)	0.03	0.04 (0.00–0.08)	0.05	0.01 (0.00–0.02)	0.02	0.22 (0.04–0.87)	0.03
Anxious/depressed	0.06 (0.01;0.10)	0.01	0.05 (0.00;0.09)	0.04	0.01 (0.00;0.02)	0.01	0.19 (0.04;0.73)	0.02
Withdrawn/depressed	0.00 (-0.04;0.05)	0.95	NA		NA		NA	
Somatic complaints	0.05 (0.01;0.10)	0.02	0.04 (0.00;0.09)	0.05	0.01 (0.00;0.02)	0.01	0.15 (0.02;0.70)	0.03
Externalizing problems	0.03 (-0.01– 0.07)	0.17	NA		NA		NA	
Other problems								
Social problems	0.04 (-0.01;0.08)	0.10	NA		NA		NA	
Thought problems	0.07 (0.03–0.12)	<0.01	0.06 (0.02–0.11)	0.01	0.01 (0.00–0.02)	0.01	0.14 (0.03–0.40)	0.01
Attention problems	0.07 (0.02;0.11)	<0.01	0.06 (0.01;0.10)	0.01	0.01 (0.00;0.02)	0.02	0.14 (0.03;0.43)	0.02

Note: Analyses are adjusted for age, child sex, and four principal components of genetic ancestry. Results are shown for the $P_t < 0.5$ inclusion threshold.

childhood adversities, with largest effects for person-related adversities, which further supports the genetic background of early life adversity. Together, these findings contribute to a better understanding of how gene-environment interplay might be shaping behavioral development in children. Further explorations of mechanisms have the potential to identify strategies for preventative intervention.

Here, we showed that genetic variants associated with schizophrenia, as captured with a polygenic risk score, increased the odds for childhood adversities. The strongest effects were observed for adversities occurring before age 5 years. This could potentially be explained by early developmental factors, in which stronger effects of parenting style are observed at younger compared to older ages. When children are older they are out of their parents' supervision for substantially more time. This is also corroborated by our observation of a trend to genetic heritability for adversities before versus after age 5 years. SNP-heritability estimates are commonly lower than twin-based heritability estimates (Trzaskowski, Dale and Plomin, 2013; Pappa et al., 2015), which might explain our suggestive finding. No distinct associations were observed for person-related or environment-related events, which is probably due to low power, although the odds ratio for person-related adversities was larger than the estimate for environment-related adversities. In addition, we showed a 34% prediction by common genetic variants for person-related adversities, comprising maltreatment, school problems and other child behavior-related events, whereas environment-related adversities were not significantly heritable. This is more suggestive of a behaviorally-mediated gene-environment correlation, which in this case posits that a child's genetic vulnerability to schizophrenia makes the child more likely to experience adversities which are imposed on him or her through the behavior of parents or others (i.e. passive or evocative gene-environment correlation) (Plomin, 2013). Alternatively, children with an increased genetic risk for schizophrenia might experience more adversity through their own behaviors (i.e. active gene-environment correlation), although this is less likely considering the young pre-adolescent age of this sample. In the current study, we found empirical evidence for mediation, and this might indicate a mechanisms from schizophrenia genetic risk to

child emotional problems through early life adversity, which could subsequently be examined in the context of potential preventative interventions.

Gene-environment correlations have received much less attention in the developmental psychopathology literature than gene-environment interactions (e.g. (Kim-Cohen et al., 2006)), but it is now recognized that gene-environment correlations can substantially influence the estimation of risk between a genetic variant and a given outcome, as well as between an environmental factor and a given outcome (Knafo and Jaffee, 2013; Plomin, 2013; Krapohl et al., 2017). This could occur in the context of confounding, in which the risk of a certain environmental on psychopathology is partly explained by genetic effects (Pingault et al., 2018). Alternatively, the relationship between genotype and psychopathology could be mediated by environmental factors (Jaffee and Price, 2012), such as what we observed in the current study. A child's genotype might, for example, result in more certain behaviors that elicits responses from parents, resulting in a greater likelihood of adversities. More likely is that a child's genetic risk is reflective of the parents' genetic risk for behavior, which leads to more stressful adversities of the child. Such gene-environment correlations raise important issues for future studies of genetic and environmental risks.

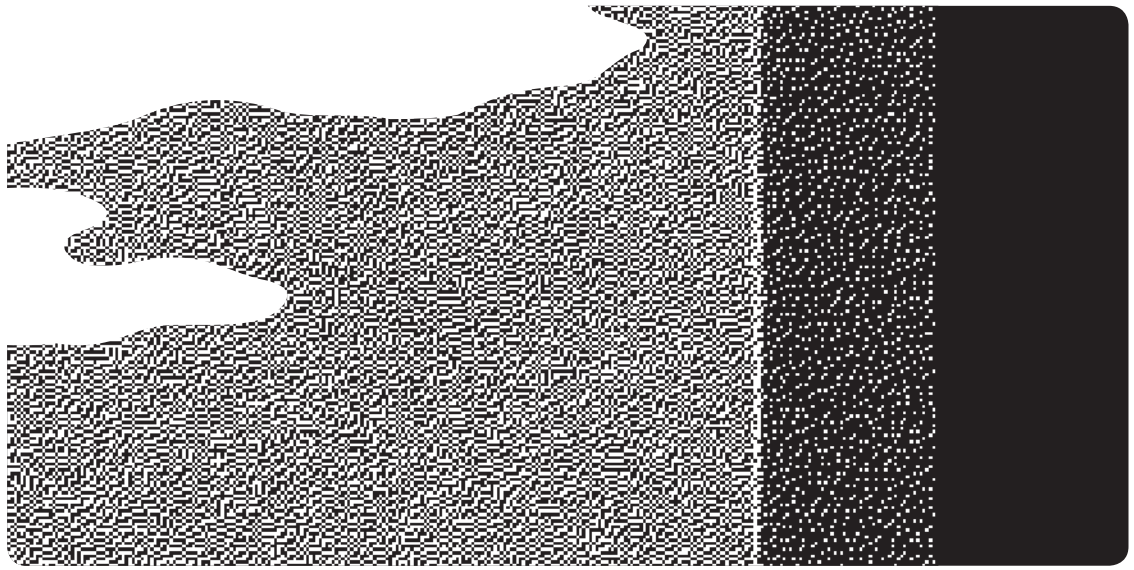
Several previous studies have employed data on familial history for schizophrenia to study the interplay between genetic and environmental risks. However, measures of familial risk are crude estimates of genetic liability as it may be poorly recorded or remembered (Do, Hinds, Francke and Eriksson, 2012) and the absence of a known family history might not adequately reflect heritable factors (McGrath, Mortensen, Visscher and Wray, 2013). In the current study, we used polygenic risk scores as an additive genetic risk metric for schizophrenia, which is a more generalizable, widely applicable and continuous measure of genetic liability than family history. This in turn increases power to examine subtle effects in general population samples (Kendler, 2016). However, it should be noted that polygenic risk scores assume additivity of the individual SNPs captured by the risk score and, potentially, it might be more biologically informative to examine the cellular mechanisms of differential gene-environment susceptibility using biologically informed scores, which capture SNPs from a particular cell-type pathways (Boyle, Li and Pritchard, 2017; Skene et al., 2018).

Sub-clinical psychiatric expression of emotional and behavioral problems is common in non-selected pediatric samples from the general population. These symptoms are more common in children with higher schizophrenia polygenic risk scores and predictive of future clinical disorder (Jones et al., 2016; Nivard et al., 2017; Riglin et al., 2017; Jansen et al., 2018), consistent with the higher prevalence of psychiatric disorder in offspring of parents with severe mental illness (Rasic et al., 2014). In line with our earlier work (Jansen et al., 2018), we only observed associations of schizophrenia polygenic risk with emotional, attention and thought problems and relationships with behavioral problems were not observed. This suggests a particular involvement of emotional problems as phenotypic expressions of elevated genetic vulnerability for schizophrenia in pre-adolescent children (Riglin et al., 2017; Bolhuis et al., 2018). This link between neuropsychiatric trait-associated genetic variants and emotional difficulties in the general population suggests a common genetic etiology.

Although the current study was population-based in design and did not include participants from clinical or high-risk samples, our findings could have important potential clinical implications. Given that the pathway from increased genetic vulnerability for schizophrenia to phenotypic manifestations of psychiatric problems was mediated through childhood adversities, counseling children with high genetic risk and their caretakers might be warranted and requires further exploration. Potentially, psychiatric problems could be prevented in children with higher genetic risk if their exposure to adversities is prevented, such as stress resulting from parental separation. For example, stable relationships between intimate partners and between mothers and their children have been associated with breaking the intergenerational transmission of abuse in families (Jaffee et al., 2013). Similar patterns could potentially be observed in children and their families with elevated genetic vulnerabilities for severe mental illness.

Our study was characterized by several strengths, including its prospective population-based design and its large sample size with both genotype and childhood adversities. In addition, we included a well-designed comprehensive interview for childhood adversities to assess timing and impact of each reported event, and sub-categorize person-related and environment-related adversities. However, several limitations need to be discussed. First, assessment of childhood adversities and child psychiatric problems relied on maternal report, which could have introduced shared reporter bias. Second, the interview of childhood adversities could have been biased by retrospective reporting as mothers might only remember adversities from longer ago because of the higher severity of these events. This underscores the need for more in-depth and multi-informant prospective assessments of stressful life events. Third, although we obtained evidence for mediation in this study, this does not necessarily infer causality (Hernan, 2018). Rather, our mediation analysis provided support for gene-environment correlation in the context of genetic vulnerability for schizophrenia. Fourth, we did not have parental genotype data available which could disentangle whether the genetic factors predicting childhood adversities were transmitted or non-transmitted to the child (Kong et al., 2018).

In conclusion, we observed that elevated genetic risk for schizophrenia, as quantified with a polygenic risk scores, is associated with higher exposure to childhood adversities. Childhood adversities mediated the relationship of schizophrenia genetic liability and it phenotypic behavioral expressions in pre-adolescence, which is evidence for gene-environment correlation. This could include passive, active, and/or evocative gene-environment correlation, and these mechanisms should be further addressed in future studies. These findings suggest that the prevention of childhood adversity in children at increased genetic risk for severe mental illness may be efficacious in reducing childhood emotional and thought problems and subsequently in adolescence and adulthood for severe psychiatric outcomes.



(...) *In sounds there is colour; in colours there is a music...*
(Charles Baudelaire, 1860, Les Paradis Artificiels)

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Supplementary Table S1: Overview of the childhood adversities ascertained through mother interview; and the corresponding prevalence rates and categorization of person-related versus environment-related adversities.

#	Item	Prevalence	Category
1	Did your child get seriously sick or did he/she have an accident?	2.9	Person
2	Did a family member get seriously sick or did someone have a serious accident?	3.3	Environment
3	Did someone else, who is important to the child, get seriously sick or did someone have a serious accident?	4.0	Environment
4	Has the father/mother or other caretaker of your child died?	0.3	Environment
5	Has someone else, who your child cared a lot about, passed away?	4.2	Environment
6	Has a pet, who you child cared a lot about, die?	2.1	Environment
7	Does or did your child have to deal with a high workload at school?	9.5	Person
8	Has your child ever repeated a grade?	1.0	Person
9	Are/were there any neighbourhood problems? E.g. vandalism or insecurity.	1.8	Environment
10	Has your family financial difficulties or had your family ever have them?	1.5	Environment
11	Does your child have ongoing conflicts with a family member (or did your child ever have them)?	2.0	Person
12	Does your child have ongoing conflicts with someone else (or did your child ever have them)?	2.7	Person
13	Do other family member have ongoing conflicts with each other (or did they ever have them)?	3.2	Environment
14	Are you and your partner divorced or separated?	4.8	Environment
15	Did one of the parents become involuntarily unemployed?	0.9	Environment
16	Did your child lose a good friend due to an argument?	0.8	Person
17	Did your child every lose something which was important to him/her? E.g. through fire, loss, or theft.	1.0	Person
18	Has someone ever used physical violence against your child? For example, beating him/her up.	0.9	Person
19	Has someone almost used physical violence against your child? So that not actually happened, but your child was frightened.	1.4	Person
20	Has someone made sexual comments or movements towards your child?	0.5	Person
21	Has your child experienced inappropriate sexual behavior?	0.2	Person
22	Has someone spread mean rumors about your child?	1.9	Person
23	Has your child moved to a different place of residence?	1.2	Environment
24	Has your child changed schools?	1.5	Environment

Supplemental Table S2: Association between the schizophrenia polygenic risk score and childhood adversities.

	Childhood adversities	
Polygenic risk score threshold	OR (95% CI)	*P*
P < 0.0005	1.00 (0.94–1.06)	1.00
P < 0.001	1.00 (0.94–1.07)	0.90
P < 0.005	1.00 (0.94–1.06)	0.89
P < 0.01	1.02 (0.96–1.08)	0.59
P < 0.05	1.04 (0.97–1.11)	0.25
P < 0.1	1.07 (1.00–1.14)	0.04
P < 0.5	1.08 (1.02–1.15)	0.01
P < 1.0	1.09 (1.02–1.16)	0.01

Note: Analyses are adjusted for age, child sex, and four principal components of genetic ancestry.

Supplemental Table S3: Association between the schizophrenia polygenic risk score and childhood adversities, with additional adjustment for CBCL total problems scores at age 3 years.

	Childhood adversities	
Polygenic risk score threshold	OR (95% CI)	*P*
P < 0.0005	1.00 (0.94–1.06)	1.00
P < 0.001	1.00 (0.94–1.07)	0.92
P < 0.005	0.99 (0.93–1.05)	0.77
P < 0.01	1.01 (0.95–1.07)	0.78
P < 0.05	1.02 (0.96–1.09)	0.47
P < 0.1	1.05 (0.99–1.12)	0.10
P < 0.5	1.07 (1.00–1.14)	0.04
P < 1.0	1.07 (1.01–1.14)	0.03

Note: Analyses are adjusted for age, child sex, four principal components of genetic ancestry, and CBCL total problems scores at age 3 years.

Supplemental Table S4: Association between the major depressive disorder polygenic risk score and childhood adversities.

	Childhood adversities	
Polygenic risk score threshold	OR (95% CI)	*P*
P < 0.0005	0.98 (0.92–1.05)	0.58
P < 0.001	0.96 (0.90–1.02)	0.23
P < 0.005	1.02 (0.96–1.09)	0.50
P < 0.01	1.01 (0.95–1.08)	0.73
P < 0.05	1.01 (0.95–1.07)	0.82
P < 0.1	1.03 (0.97–1.10)	0.37
P < 0.5	1.03 (0.97–1.10)	0.33
P < 1.0	1.04 (0.98–1.11)	0.24

Note: Analyses are adjusted for age, child sex, and four principal components of genetic ancestry.

Supplemental Table S5: Association between schizophrenia polygenic risk scores and childhood adversities, divided in person-related and environment-related events

	Childhood adversities			
	Person-related events		Environment-related events	
Polygenic risk score threshold	OR (95% CI)	*P*	OR (95% CI)	*P*
P < 0.0005	1.02 (0.93 – 1.12)	0.69	0.89 (0.79 – 1.00)	0.05
P < 0.001	1.01 (0.93 – 1.11)	0.77	0.90 (0.80 – 1.01)	0.07
P < 0.005	1.00 (0.91 – 1.09)	0.95	0.91 (0.81 – 1.02)	0.09
P < 0.01	1.01 (0.92 – 1.10)	0.87	0.94 (0.84 – 1.06)	0.32
P < 0.05	1.03 (0.94 – 1.12)	0.59	0.97 (0.86 – 1.10)	0.65
P < 0.1	1.04 (0.95 – 1.14)	0.36	1.02 (0.90 – 1.14)	0.80
P < 0.5	1.06 (0.97 – 1.16)	0.18	1.01 (0.90 – 1.14)	0.87
P < 1.0	1.06 (0.97 – 1.16)	0.18	1.02 (0.90 – 1.14)	0.80

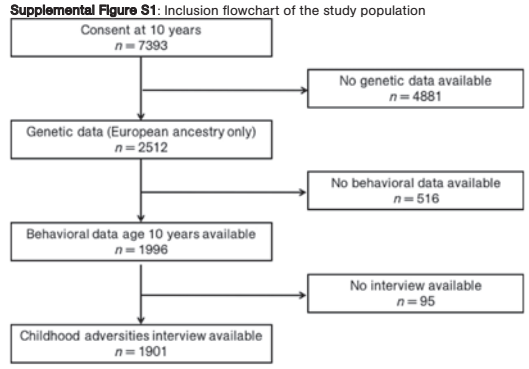
Note: Analyses are adjusted for age, child sex, and four principal components of genetic ancestry.

Supplemental Table S6: Association between schizophrenia polygenic risk scores and childhood adversities, divided in events before and after age 5 years

	Childhood adversities			
	Before age 5 years		After age 5 years	
Polygenic risk score threshold	OR (95% CI)	*P*	OR (95% CI)	*P*
P < 0.0005	1.12 (0.98 – 1.27)	0.10	0.98 (0.92 – 1.05)	0.56
P < 0.001	1.08 (0.95 – 1.23)	0.22	0.99 (0.92 – 1.06)	0.70
P < 0.005	1.15 (1.01 – 1.31)	0.03	0.96 (0.90 – 1.03)	0.26
P < 0.01	1.18 (1.04 – 1.35)	0.01	0.98 (0.91 – 1.05)	0.54
P < 0.05	1.20 (1.06 – 1.36)	0.01	1.00 (0.94 – 1.07)	0.96
P < 0.1	1.20 (1.06 – 1.37)	<0.01	1.03 (0.97 – 1.11)	0.34
P < 0.5	1.20 (1.05 – 1.36)	0.01	1.05 (0.98 – 1.13)	0.13
P < 1.0	1.20 (1.06 – 1.36)	0.01	1.06 (0.99 – 1.14)	0.10

Note: Analyses are adjusted for age, child sex, and four principal components of genetic ancestry.

Supplemental Figure S1: Inclusion flowchart of the study population



PART II:

UNRULINESS

– His anger was like a single musical phrase (...)

(ABSTRACT)

OBJECTIVE

Irritable and oppositional behaviors are increasingly considered as distinct dimensions of oppositional defiant disorder. However, few studies have explored this multidimensionality across the broader spectrum of disruptive behavior problems (DBPs). This study examined (a) the presence of dimensions and distinct subgroups of childhood DBPs, and (b) the cross-sectional and longitudinal associations between these dimensions.

METHOD

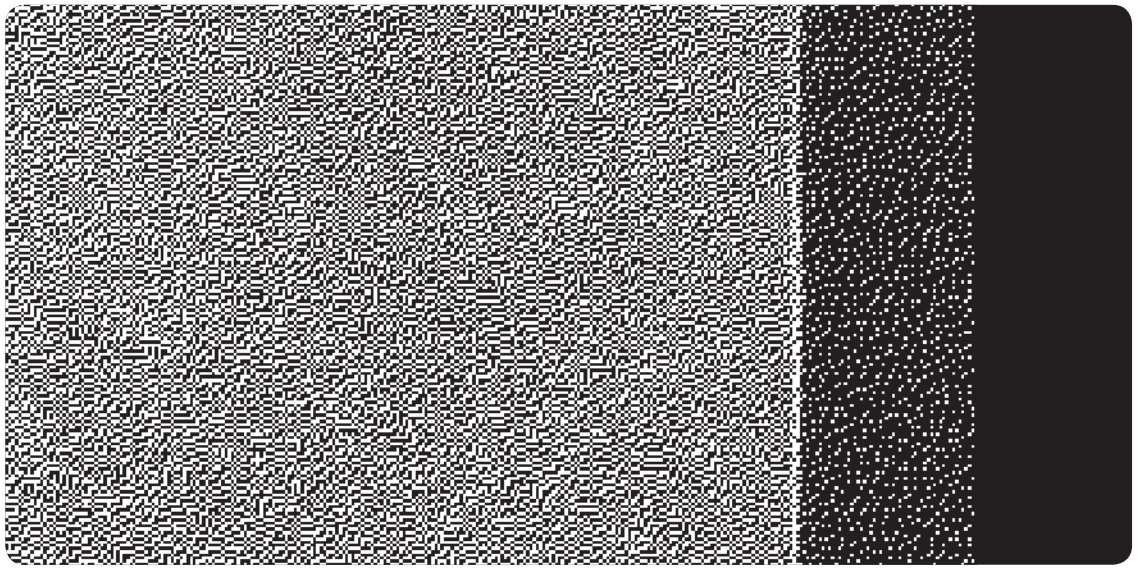
Using factor mixture models (FMMs), the presence of dimensions and subgroups of DBPs was assessed in the Generation R Study at ages 6 (N = 6209) and 10 years (N = 4724). Replications were performed in two population-based cohorts (Netherlands Twin Registry, N = 4402, and Swedish Twin study of Child and Adolescent Development, N = 1089) and a clinical sample (N = 1933). We used cross-lagged modelling in the Generation R Study to assess cross-sectional and longitudinal associations between dimensions. DBPs were assessed using mother-reported Child Behavior Checklist.

RESULTS

Empirically obtained dimensions of DBPs were oppositional behavior (age 6), disobedient behavior, rule-breaking behavior (age 10), physical aggression and irritability (both ages). FMMs suggested that one-class solutions had the best model fit for all dimensions in all three population-based cohorts. Similar results were obtained in the clinical sample. All three dimensions, including irritability, predicted subsequent physical aggression (range, 0.08-0.16).

CONCLUSION

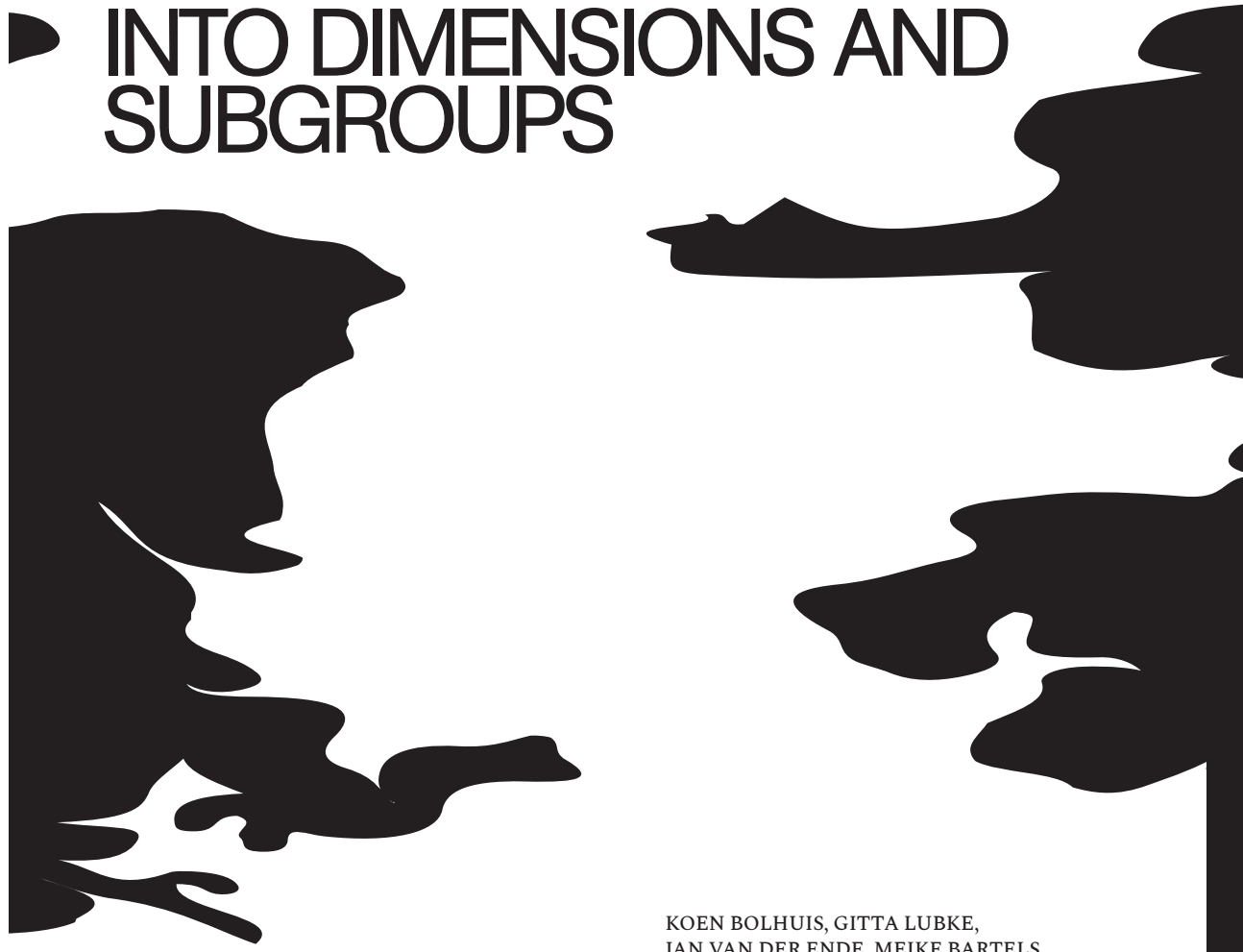
This study showed that childhood DBPs should be regarded as a multidimensional phenotype rather than comprising distinct subgroups. Incorporating multidimensionality will improve diagnostic accuracy and refine treatment. Future studies need to address the biological validity of the DBPs dimensions observed in this study; herein lies an important opportunity for neuroimaging and genetic measures.



(…) *to which in an opera several lines are sung* (…)

CHAPTER 7

DISENTANGLING HETEROGENEITY OF CHILDHOOD DISRUPTIVE BEHAVIOUR PROBLEMS INTO DIMENSIONS AND SUBGROUPS



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INTRODUCTION

The nosology of childhood disruptive behavior disorders has given rise to considerable academic debate, even since before the disorders were operationalized by the DSM in 1980 (Rutter and Shaffer 1980; Achenbach 1980; Lahey et al. 1992; Moffitt et al. 2008; Pardini, Frick, and Moffitt 2010). Many studies using different informants, instruments and study populations have addressed the heterogeneity and developmental continuities of disruptive behavior disorders (Frick et al. 1993; Bongers et al. 2004; Lahey and Waldman 2012; Odgers et al. 2008; Rowe et al. 2002; Bezdjian et al. 2011). More recently, with the development of the DSM-5, several changes in the criteria of oppositional defiant disorder (ODD) and conduct disorder (CD) were made. For example, it can now be specified whether CD had its onset before the age of 10 years, which is indicative of a poorer prognosis (Odgers et al. 2008; Moffitt 1993). Another important change is the possibility to differentiate irritable from oppositional ODD subtypes (Stringaris and Goodman 2009a; Herzhoff and Tackett 2016). However, to our knowledge these studies have not assessed whether the ODD dimensions can be discerned on a broader spectrum of disruptive behavior problems (DBPs) beyond a priori defined DSM criteria, which would strengthen our current diagnostic frameworks with an empirical basis.

Recent research established that ODD sub-types have divergent developmental courses; most notably, irritability is associated with later depression and anxiety (Stringaris and Goodman 2009a; Whelan et al. 2013; Herzhoff and Tackett 2016). Findings from behavioral genetics studies have provided further support for distinguishing irritable from oppositional symptoms (Stringaris et al. 2012), and DSM-5 now allows for better classification of ODD symptoms along these dimensions. It is still unclear how irritability is related to other DBPs. So far, significant associations of irritability with oppositionality, but less so with CD, have been described (Vidal-Ribas et al. 2016). However, earlier work from this cohort demonstrated that both irritable and headstrong dimensions predicted later ODD, CD and depression to a similar extent (Rowe et al. 2010). On the basis of these studies that emphasize the distinct developments of ODD and CD (Rowe et al. 2002; Copeland et al. 2009), DSM-5 posits CD as a disorder of physical violence and delinquency, and ODD as a disorder of oppositionality and irritability. However, comorbidity between these disorders is common (Lahey and Waldman 2012). It might well be that irritability is a distinct dimension on the broad spectrum of DBPs that influences the development of other DBPs dimensions, e.g. aggression and non-compliance. Indeed, developmental studies have provided preliminary evidence for this, as reviewed by Wakschlag *et al* (Wakschlag, Tolan, and Leventhal 2010). They discussed that problematic defiance/rule-breaking is often associated with negative affect, but most of what is known about this association is derived from small observational studies. Unfortunately, empirical studies that investigated irritability across the broad spectrum of DBPs are lacking.

Developmental scientists have stressed the importance of disentangling the heterogeneity of disruptive behavior (Pardini, Frick, and Moffitt 2010; Lahey and Waldman 2012; Wakschlag, Tolan, and Leventhal 2010; Rowe et al. 2002; Bezdjian et al. 2011; Odgers et al. 2008; Frick et al. 1993; Bongers et al. 2004), and many studies have addressed this with various approaches. One option is classifying DBPs by age of onset, as proposed by the DSM-5. However, this will in practice be less useful for clinicians, as retrospective symptoms recall is often unreliable (Moffitt et al. 2008; Tremblay 2010). Furthermore, it is still unclear whether early-onset DBPs will be limited to childhood or whether these children will continue to have problems later in life (Odgers et al. 2008). Thus, an empirically-based refinement of diagnosis based on the pattern of symptoms a child or adolescent exhibits could be more promising. Given the recent interest in the irritability sub-type of ODD it is important to examine how irritability is associated with other DBPs. A seminal meta-analysis of factor analytic studies by Frick *et al.* has demonstrated that DBPs can be classified along two principal axes, namely overt/covert, and destructive/non-destructive (Frick et al. 1993), with different developmental trajectories (Bongers et al. 2004; Tremblay 2010). So far, no study has identified a distinct irritability dimension on the broader spectrum of DBPs, even though irritability has been found to be a distinguishable dimension of ODD specifically. Additionally, it

remains to be studied more thoroughly how irritability is associated with other DBPs dimensions over time. To move towards a more developmentally sensitive nosology of DBP, which transcends current diagnostic boundaries, it is crucial to examine these symptom patterns across ages (Wakschlag, Tolan, and Leventhal 2010; Tremblay 2010; Rowe et al. 2010).

This study had two aims. First, we empirically assessed the multidimensionality across the whole spectrum of childhood DBPs, while simultaneously examining whether meaningful subgroups could be discerned. Hereby we wish to extend recent research that has focused on ODD symptoms specifically, with the goal to test whether a distinct irritability dimension can be distinguished on a broader DBPs spectrum. The majority of studies on the heterogeneity of DBPs used either dimensional (e.g. factor analysis) or categorical (e.g. latent class analysis) statistical methods (Frick et al. 1993; Herzhoff and Tackett 2016; Vidal-Ribas et al. 2016; Rowe et al. 2010; Stringaris et al. 2012; Odgers et al. 2008). We performed factor mixture models (FMMs), which allow the presence of both dimensional and categorical latent variables and are therefore appropriate for studying the heterogeneity of psychiatric problems (Lubke and Miller 2015; Miettunen et al. 2016). This is important as recent studies have examined the latent structures of DBPs without clearly characterizing the dimensional or categorical latent structures of DBPs (Vidal-Ribas et al. 2016; Stringaris and Goodman 2009a; Herzhoff and Tackett 2016; Stringaris et al. 2012; Frick et al. 1993). Second, longitudinal associations between the different dimensions of DBPs were studied using a cross-lagged model. Data from three population-based cohorts were used, as replication is important for FMMs. In addition, we explored consistency in a sample of clinically-referred children to test generalizability. Although different population subgroups might be present in clinical samples due to referral bias (Lubke and Miller 2015), these analyses will aid translation to clinical practice. Hereby our findings could be more easily interpreted by clinicians treating children with DBPs.

METHOD

STUDY POPULATIONS

This study was conducted using data from three population-based cohorts that collaborate under the FP7-ACTION consortium. Primary analyses were conducted in the Generation R Study, a prospective population-based cohort from fetal life onward, which included 9,778 pregnant women living in Rotterdam, the Netherlands. The aim of the Generation R project is to identify early environmental and genetic factors that affect health and development (Kooijman et al. 2016). For the current study, data were used from two time points. At age 6 years 6,209 children with behavioral data were included in the analyses, and comprised fewer children of ethnic minorities and lower socio-economic status than would be expected from regional demographic statistics. At age 10 years 4,724 children were included. Children who participated at follow-up were more often of Dutch nationality, had lower CBCL total problems scores ($P < 0.001$), and had older and more highly educated mothers (Kooijman et al. 2016). Study protocols were approved by the local ethics committee.

Independent replications were performed in the Netherlands Twin Registry (NTR; $N = 4402$) and the Swedish Twin Study of Child and Adolescent Development (TCHAD; $N = 1089$) cohorts. Both are twin cohorts, nationally-representative with respect to socio-economic status and ethnicities (Lichtenstein et al. 2007; van Beijsterveldt et al. 2013), which aim to explore the genetic and environmental influences on cognitive function, psychopathology, and well-being during development. From each twin pair, one twin was randomly selected.

Additional replication was conducted in a clinical sample of children aged 6-11 years of age ($N = 1933$), who were referred to one of three child and adolescent mental health services in the greater metropolitan area of Rotterdam, the Netherlands. Sampling took place in 2011 for a period of nine months. This sample is representative of the clinical population in this study base, and has previously been used for clinical validation of the Dutch CBCL (Verhulst and Van der Ende Rotterdam: ASEBA Nederland; 2013).

MEASURES

DBPs were consistently assessed with the Child Behavior Checklist (CBCL) in all samples, a widely used reliable and valid measure for behavioral problems (Achenbach and Rescorla 2000). The CBCL was completed by the primary caregiver, principally the mother (Table 1). In the Generation R sample, the CBCL/1.5-5 was used at the first time point when most children (58%) were under the age of 6 years, while the remaining children were either 6 (38%) or 7 (3%) years old. In the next examination, the CBCL/6-18 was used, which was also used in the NTR and clinical samples. TCHAD used the CBCL/4-18, an earlier version of the CBCL.

The items included in the FMM analyses were part of the Aggressive Behavior scale of the CBCL/1.5-5, and the Aggressive Behavior and Rule-Breaking Behavior scales of the CBCL/6-18. Items were selected on clinical relevance for measuring DBPs using the following three pre-defined criteria. Items were not included if (a) they did not reflect problem behavior (e.g. "prefers being with older kids"), (b) were more indicative of behavior problems or disorders other than DBPs (e.g. "can't stand waiting, wants everything now" or "demands must be met immediately", which are attention-deficit/hyperactivity disorder [ADHD] symptoms), or (c) were endorsed infrequently due to the child's young age (e.g. "drinks alcohol without parents' approval"). The items included in the analyses are listed in Table S1 (available online). They reflect the whole severity range of DBPs (e.g. "hurting animals/people" on the severe end, and "disobedience" on the less severe end of the spectrum), which is a requirement for FMMs (Lubke and Miller 2015).

At age 10 we included a validated mother-reported measure for callous traits (Pardini, Obradovic, and Loeber 2006), to incorporate into the cross-lagged model in the Generation R sample. The items are listed in Table S1 (available online).

STATISTICAL ANALYSES

In principle, two major techniques describe the variability among observed correlated symptoms in terms of unobserved (latent) variables. First, categorical latent variables explain the variability in terms of differences between two or more discrete subgroups (classes) of individuals. Second, dimensional latent variables explain the variability in terms of one or more dimensions on a linear severity scale. The first are employed in latent class analysis, and the second in factor analysis. FMMs allow for the presence of both categorical and dimensional latent variables to describe symptoms patterns. This approach is therefore most appropriate for examining whether DBPs occur as dimensional phenomena or as distinct subgroups (Lubke and Muthen 2005; Lubke and Miller 2015; Miettunen et al. 2016).

Prior to fitting the FMM, it is necessary to conduct exploratory factor analyses (EFA) to empirically assess the dimensional structures of DBPs. This was done in the Generation R sample at ages 6 and 10 years. Because of the ordinal nature of the data, the weighted least squares means and variances (WLSMV) estimator and the geomin rotation were used. To determine the number of factors, a clear and pure loading pattern had to be present, between-factor correlations mustn't indicate large overlap between factors (factor correlations larger than 0.9), and fit indices must indicate good model fit. Hence, exploratory factor solutions were compared with an increasing number of factors. Factor loading cut-offs were at 0.30 and items were assigned to the factor with the highest factor loading. In the replication samples, confirmatory factor analyses (CFA) were conducted using the factor structure found in the Generation R sample at 10 years. Model fit for all factor analyses was judged to be good when the following three fit metrics were met: Root Mean Square Error of Approximation (RMSEA) < 0.05; Comparative Fit Index (CFI) > 0.95 and Tucker Lewis Index (TLI) > 0.95. Fit was deemed acceptable when RMSEA < 0.08; CFI > 0.90 and TLI > 0.90.

Subsequently, FMMs were fitted on the item selections derived from the EFA to represent dimensions of DBPs. This was done as opposed to fitting a single FMM of all items allowing multiple factors within

Table 1: Demographic characteristics of the population-based samples.

	Generation R 6 years, *N*=6209	Generation R 10 years, *N*=4724	NTR, *N*=4402	TCHAD, *N*=1089
Child characteristics				
Gender, % girls	49.7	50.3	49.6	49.2
Age in years, mean (SD)	6.03 (0.42)	9.70 (0.23)	9.83 (0.42)	8.68 (0.47)
Ethnicity, %				
Western	70.4	74.9	96.3	97.8
Non-Western	29.9	25.1	3.8	2.1
CBCL, % completed by mother	92.3	97.5	100.0	100.0
DBPs score, median (IQR)	4.00 (6.00)	2.00 (5.00)	3.00 (7.00)	3.00 (6.00)
Maternal characteristics				
Educational level, %				
High	51.8	55.2	31.4	38.0
Medium	41.1	39.8	65.4	41.9
Low	7.1	5.0	3.3	19.5

Table 2: Factor Loadings for the Three-Factor Solution Exploratory Factor Analysis in the Generation R Sample at Age 6 Years.

Behavior	Physical aggression	Irritability	Oppositional behavior
Destroys things of others	0.41	-0.03	0.39
Fights	0.78	-0.01	0.02
Hits	0.83	0.01	0.06
Hurts animals/people	0.47	0.06	0.19
Physically attacks	0.75	0.18	-0.01
Easily frustrated	0.01	0.48	0.22
Angry moods	0.03	0.69	0.18
Screams	0.21	0.43	0.22
Stubborn or irritable	-0.07	0.62	0.34
Temper tantrums	0.17	0.73	-0.01
Defiant	0.14	0.05	0.55
Disobedient	-0.01	0.01	0.74
Doesn't feel guilty	0.13	-0.05	0.65
Punishments doesn't change behavior	0.10	0.04	0.70
Uncooperative	-0.00	0.23	0.60

Note: The shading illustrates for which particular factor an item has the highest factor loading.

Table 3: Factor Loadings for the Four-Factor Solution Exploratory Factor Analysis in the Generation R Sample at Age 10 Years.

Behavior	Physical aggression	Irritability	Disobedient behavior	Rule-breaking behavior
Argues	0.62	0.32	0.04	-0.12
Mean to other	0.87	-0.01	-0.01	-0.04
Doesn't feel guilty	0.32	0.14	0.26	0.11
Fights	0.67	0.06	0.05	0.02
Physically attacks	0.59	0.23	-0.15	0.15
Swears	0.39	0.22	0.15	0.05
Teases	0.85	-0.07	0.06	-0.01
Threatens	0.74	-0.06	0.02	0.15
Screams	0.40	0.52	0.01	-0.01
Stubborn or irritable	0.04	0.69	0.25	-0.03
Mood changes	-0.02	0.73	0.09	0.09
Temper tantrums	0.30	0.62	0.00	0.06
Disobedient home	-0.03	0.25	0.80	-0.03
Disobedient school	0.15	-0.07	0.73	0.04
Breaks rules [a]	0.10	0.10	0.69	0.09
Lying or cheating	0.19	0.06	0.36	0.25
Destroys his/her own things	0.01	0.29	-0.03	0.78
Destroys things of others	0.09	0.23	-0.02	0.78
Runs away	-0.05	0.24	0.05	0.50
Sets fires	0.02	-0.15	0.19	0.55
Steals home	0.02	-0.01	0.19	0.69
Steals outside home	-0.01	-0.13	0.14	0.68

Note: The shading illustrates for which particular factor an item has the highest factor loading
[a] This item was not part of the CBCL/4-18 (which was used in the TCHAD study), an older version of the CBCL/6-18.

each class, as mixture models with a complex multi-factor structure within class are often highly unstable (Lubke and Miller 2015). FMMs with an increasing number of classes were fitted to examine whether subgroups of DBPs could be empirically distinguished. Conversely, first fitting categorical and subsequently dimensional latent variables yields unstable results (Lubke and Miller 2015). We allowed factor variances, factor loadings, and item thresholds to vary across classes since it is unlikely that variances are equal for high-scoring and low-scoring children (Lubke and Muthen 2005). The primary fit index was the Bayesian Information Criterion (BIC), as it has been shown to be the most reliable fit index for mixture models (Nylund, Asparoutiov, and Muthen 2007). A lower BIC indicates a better model fit. The entropy statistic was given to measure the accuracy with which each individual can be classified into a latent class, with values above 0.80 indicating adequate classification (Clark and Muthén 2009). This FMM approach is conforming with methodological recommendations (Lubke and Muthen 2005; Lubke and Miller 2015) and is similar to those in other recent studies that have examined the dimensional and/or categorical nature of psychotic symptoms (Miettunen et al. 2016), autistic traits (Grove et al. 2015), and ADHD (Lubke et al. 2009).

Next, we employed a cross-lagged model in the Generation R sample. By regressing each variable in the model on all the variables that precede it in time, longitudinal associations between the DBPs dimensions could be estimated. Thus, all associations are mutually adjusted. All analyses were performed in Mplus version 7 (Muthén and Muthén Los Angeles, CA: Muthén & Muthén; 1998-2012).

RESULTS

DEMOGRAPHIC CHARACTERISTICS

All three population-based samples were comparable in terms of demographic characteristics (Table 1). However, the Generation R sample included more children of non-Western ethnic backgrounds. From the clinical sample, 1,933 children were included (mean age 8.35±1.64 years, 34.8% female).

DIMENSIONS AND SUBGROUPS OF DBPS

EFA were fitted in the Generation R sample. At age 6 years, a three-factor model provided the best solution (Table 2). Fit indices supported this model, with RMSEA = 0.031, CFI = 0.991, and TLI = 0.984. The three factors were physical aggression, irritability and oppositional behavior, respectively.

At age 10 years (using the CBCL/6-18), a four-factor solution had the clearest loading pattern (Table 3). Fit indices supported this model, with RMSEA = 0.016, CFI = 0.994 and TLI = 0.990. This wave the factors were physical aggression, irritability, disobedient behavior and rule-breaking behavior, respectively.

The dimensional structure found at age 10 in the Generation R sample was tested in the population-based and clinical samples using CFA. In all three replication samples the four-factor model fitted the data well. The fit indices for the four-factor solution in the NTR sample were RMSEA = 0.029, CFI = 0.981 and TLI = 0.978. In the TCHAD sample the fit indices were RMSEA = 0.026, CFI = 0.985 and TLI = 0.983. In the clinical sample, the fit indices for the four-factor solution were RMSEA = 0.056, CFI = 0.965, TLI = 0.961.

Next, FMM were fitted; fit indices are presented in Table S2-S6. In the Generation R sample at age 6, the one-class solution had the best fit for the physical aggression dimension (Table S2, available online). Fitting higher-class models of this dimension produced unreliable results, which was likely because resolution in the data was too low to reliably detect multiple classes in this sample. For both the irritability and oppositional behavior dimensions, two-class models had the best fit. However, in both cases the entropy statistic was low (0.42 and 0.21, respectively), which indicated substantial overlap between the two classes.

In the Generation R sample at age 10, BIC and entropy values did not the support the need of a second class, therefore the one-class solutions were selected as the preferred model for all four DBPs dimensions (Table S3, available online). The difference in BIC between one-class and two-class solutions for disobedient behavior was small, indicating that a second class was not needed to describe the data. This was consistent with the low entropy of the two-class solution (0.63), thus showing substantial overlap between the two classes. Therefore, the one-class solution was determined as the preferred model. In sensitivity analyses, we repeated our EFA and FMM analyses in the Generation R Study using all items of the CBCL Externalizing Problems scales and obtained identical results (Supplemental Material; Table S7-9, available online).

Subsequently, FMMs were fitted in the replication samples using the dimensional structure derived from CFA. Both population-based samples FMM analyses yielded similar results to those obtained in the Generation R sample. In the NTR sample, one-class solutions had the best model fit for physical aggression, disobedient behavior, and rule-breaking behavior (Table S4, available online). For irritability, a two-class solution had a better fit. However, the entropy statistic was 0.33 and the difference in BIC between one and two classes was small. In the TCHAD sample, one-class solutions had the best model fit for all four DBPs dimensions (Table S5, available online). In the clinical sample, one-class solutions fit the data best for physical aggression and rule-breaking behavior (Table S6, available online). For disobedient behavior a two-class solution would have been selected based on the lowest BIC, but the entropy was low at 0.52. For irritability, the two-class solution had the best model fit and the entropy statistic was acceptable (0.85), with one class containing 3.73% of children. This class included children with on average higher scores on all irritability items (Figure S1, available online).

CROSS-LAGGED MODEL OF DBPS DIMENSIONS

Using the Generation R sample, we examined cross-sectional and longitudinal associations between DBPs dimensions in a cross-lagged model (Figure 1). The cross-sectional standardized correlations were all substantial, ranging from 0.49-0.63 at age 6 and from 0.18-0.59 at age 10. The cross-sectional associations between rule-breaking behavior and other DBPs dimensions at 10 years were

all significantly lower (range, 0.18-0.36) than other cross-sectional associations, as can be seen form the non-overlapping confidence intervals. For example, the association between physical aggression and irritability was 0.59 (95% CI 0.57-0.62).

Longitudinally, a significant within-trait across-time association was observed for irritability (path coefficient = 0.24, 95% CI = 0.20-0.28). Similarly, age 6 oppositional behavior was associated with disobedient behavior at age 10 (path coefficient = 0.26, 95% CI = 0.22-0.30). All age 6 DBPs dimensions were significantly associated with physical aggression at 10 years (range, 0.08-0.16). Both irritability and oppositional behavior, but not physical aggression, were predictive of later irritability problems (range, 0.09-0.24). Only physical aggression and oppositional behavior, and not irritability, predicted rule-breaking behavior (range, 0.07-0.09) and callous traits (range, 0.04-0.16) at age 10.

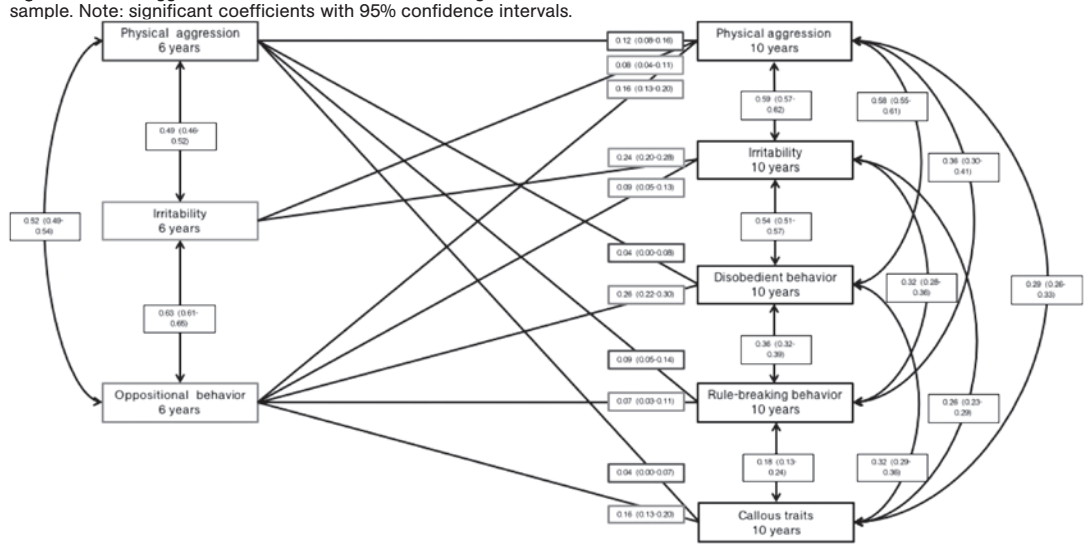
Post-hoc cross-lagged analyses separate for boys and girls demonstrated that in both sexes oppositional behavior was predictive of all DBPs dimensions at age 10 years, including callous traits (Figure S2-3, available online). Similar across both sexes, irritability did not predict rule-breaking behavior. Irritability, however, was associated with later physical aggression in both girls and boys.

DISCUSSION

The present findings suggest that childhood DBPs can best be understood as multiple correlated dimensions rather than as a mixture of distinct categorical subgroups, as indicated by the FMM results in both the population-based and clinical samples. The observed dimensions included physical aggression, irritability, oppositional/disobedient behavior, and rule-breaking behavior. All dimensions demonstrated significant developmental continuity. Interestingly, we also found longitudinal interrelations between most dimensions, e.g. all DBPs dimensions at age 6, including irritability, predicted later physical aggression symptoms.

These findings add to the existing literature on the multidimensionality of CD and ODD, where particularly the ODD sub-domains of

Figure 1: Cross-lagged model of cross-sectional and longitudinal associations between DBPs dimensions in the Generation R sample. Note: significant coefficients with 95% confidence intervals.



(Full colour image presented on page 10.)

irritability and oppositionality have received considerable recognition in the past years (Stringaris and Goodman 2009a; Herzhoff and Tackett 2016). Instead of providing another new approach to the classification of DBPs, the present study expands these investigations by assessing symptoms across the spectrum of childhood DBPs in both population-based and clinically-referred samples. Notably, we found that irritability was a distinct dimension on the broader DBPs spectrum with strong cross-sectional, and longitudinal, associations with other DBPs dimensions. We therefore argue that irritability is not only a key component of ODD, but must be defined and assessed in all children with DBPs. This is in line with some previous studies, which suggested that emotion regulation problems are involved in the development of other aspects of DBPs, such as physical aggression (Wakschlag, Tolan, and Leventhal 2010), inattention/impulsivity (Wakschlag et al. 2012; Shaw et al. 2014), and rule-breaking (Hill and Maughan 2015). Other dimensions observed in this study include physical aggression, oppositional/disobedient behavior and rule-breaking behavior, which are comparable to previous findings (Frick et al. 1993; Bongers et al. 2004). The importance of recognizing these distinct dimensions of DPBs has recently been highlighted in a review by Tremblay (Tremblay 2010), and our study offers strong empirical support for improved diagnostic accuracy of DBPs along these dimensions. It is interesting to observe that in the present study these abovementioned dimensions were all considerably correlated with the irritability dimension, underscoring the value of recognizing emotion regulation problems in youth presenting with a broad range of DBPs (Wakschlag et al. 2012; Hill and Maughan 2015).

The present cross-lagged model demonstrated significant developmental continuity for all DBPs dimensions, confirming other study findings (Bongers et al. 2004; Whelan et al. 2013; Rowe et al. 2010; Stringaris and Goodman 2009a). However, heterotypic associations (behavioral manifestations that change over time) were also found. Physical aggression at age 6 years predicted disobedience, rule-breaking behavior and callousness at age 10 years, predominantly in boys, which is well-known from previous research (Bongers et al. 2004; Odgers et al. 2008; Stringaris and Goodman 2009a). Interestingly, we found that in both girls and boys irritability

predicted later physical aggression and not rule-breaking behavior, and that oppositional behavior predicted all later DBPs dimensions, including irritability. A previous study found evidence for distinct developmental pathways of irritable and oppositional behavior (Whelan et al. 2013), while others have shown that irritability is associated with conduct problems, both cross-sectionally (Stringaris and Goodman 2009b) and longitudinally (Rowe et al. 2010). The present longitudinal results suggest that irritability is a distinct but not separate dimension in the network of DBPs (Wakschlag, Tolan, and Leventhal 2010). More longitudinal replication studies across childhood to adolescence are needed. Finally, the path coefficients from the age 6 dimensions to rule-breaking behavior at age 10 were relatively small, which was the case for both sexes. This probably reflects that rule-breaking behavior is not very prevalent at younger ages. Indeed, studies have shown that rule-breaking behavior is strongly associated with adolescent-onset disruptive behavior (Bongers et al. 2004; Tremblay 2010), and might therefore not be predicted well by childhood characteristics (Moffitt 1993; Odgers et al. 2008).

This study employed a FMM approach, which is appropriate for assessing heterogeneity of complex psychiatric traits (Miettunen et al. 2016; Lubke and Muthen 2005; Lubke and Miller 2015). Our findings suggested that one-class models were the best solution for all DBPs dimensions and this was replicated in all population-based samples, which is crucial for maximum-likelihood-based methods such as FMMs (Lubke and Miller 2015; Lubke and Muthen 2005). These results were similar in the clinical sample. However, fit indices demonstrated that a small class might be distinguished within the clinical sample, containing 3.73% of children with higher irritability scores. It is possible that irritability presents more frequently and severely in clinical than in population-based samples (Vidal-Ribas et al. 2016; Wakschlag et al. 2012). Taken together, we found no empirical support for the existence of distinct subgroups of DBPs. Instead, DBPs can be best understood as a dimensional construct on a continuum in the population (Frick et al. 1993; Lahey and Waldman 2012; Bezdjian et al. 2011; Tremblay 2010; Hill and Maughan 2015; Coghill and Sonuga-Barke 2012). This has important clinical consequences, as empirical subgroups do not seem to be a useful basis for diagnosis

of DBPs and, hence, treatment decisions will need to be made based on a conventional cut-off on the dimensional severity scale of DBPs. Thus, future studies exploring the development and heterogeneity of DBPs should move beyond categorical approaches (e.g. latent class analysis) and integrate the known multidimensionality of DBPs.

This study extends more than 30 years of research on the heterogeneity of DBPs. Important progress has been made with various approaches, such as the work on life-course persistent DBPs by Moffitt *et al.* (Odgers et al. 2008; Moffitt 1993), developmental continuities of DBPs using data from the Great Smoky Mountains Study (Rowe et al. 2010; Rowe et al. 2002), or the role of temperamental antecedents of DBPs (Wakschlag, Tolan, and Leventhal 2010). Taken together, these findings will help us to better understand the heterogeneity of DBPs and hence refine psychiatric taxonomies and improve diagnosis (Coghill and Sonuga-Barke 2012). Another important task for our diagnostic systems would be to address the etiological heterogeneity present in individuals with the same diagnosis; and efforts like the Research Domain Criteria initiative are increasingly applied to DBPs (Blair et al. 2014). For example, it would be interesting to disentangle the neurobiological etiology of DBPs based on its multidimensionality, e.g. irritability or physical aggression. This would extend recent work that demonstrated that psychopathic traits were associated with different neurodevelopmental dysfunctions compared to CD, such as poorer affect reactivity versus impaired reward-based decision-making (Alegria, Radua, and Rubia 2016). Likewise, large-scale genome-wide association projects could benefit from taking into account the multidimensionality of DBPs, as unique genetic factors might be in play for different dimensions (Viding, Frick, and Plomin 2007). Arguably, treatment studies targeting specific DBPs dimensions are even more needed. It is surprising these are lacking as irritability could be a potential target for both psychological and pharmacological treatments, which may be of high priority given that treatment success for DBPs has remained limited (Bakker et al. 2017; Hill and Maughan 2015).

This study benefitted from the use of data from three prospective population-based cohorts and a large clinical sample, and the broad and developmentally-sensitive assessment of DBPs. However, our study had several limitations. First, DBPs were maternally reported using the CBCL, which might have introduced single reporter bias and some of the findings could partly be explained by common-method variance. Second, the CBCL lacks items that measure callous-unemotional traits, which could possibly explain why a distinct dimension of these symptoms was not found. To address this limitation we included a separate measure on callous traits in the cross-lagged model to assess the longitudinal associations between DBPs dimensions and callousness. Third, there was attrition in the longitudinal cohort studies. However, as attrition might affect prevalence estimates, it is less likely to influence associations between traits (Wolke et al. 2009).

In conclusion, this study builds on a large body of literature investigating the heterogeneity of DBPs. The present findings provide little evidence for the presence of distinct subgroups, but instead support the multidimensionality of childhood DBPs. Notably, we found that irritability was a distinct dimension on the broader spectrum of DBPs, and irritability predicted later physical aggression symptoms. Future studies should investigate the shared and unique mechanisms underlying the complex multidimensionality of DBPs, and specifically the role of irritability in the development of other DBPs. Herein lies an important opportunity for genetic and neuroimaging studies.

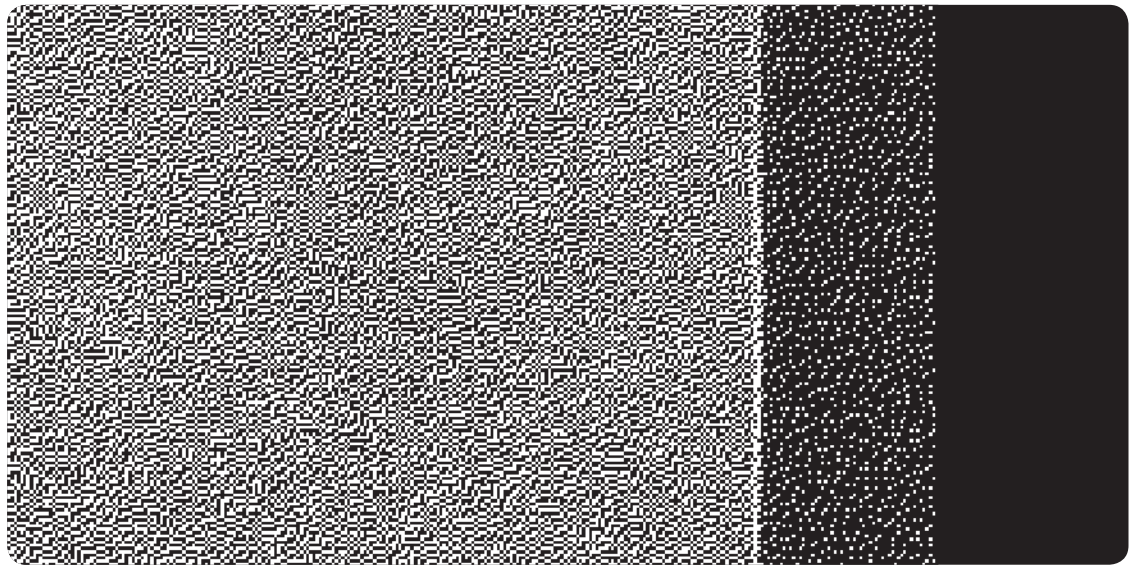
CLINICAL GUIDANCE

Childhood disruptive behavior problems can best be conceptualized as a complex multidimensional phenotype. This well-powered study provides no empirical support for the existence of distinct subgroups of children with specific patterns of disruptive behavior problems. Clinical classification of disruptive behavior problems will need

to be based on cut-offs, which are determined by (data-driven) convention, as empirical classification strategies will likely remain unsuccessful.

We have corroborated many previous studies by showing the existence of discrete dimensions of physical aggression and rule-breaking behavior.

Irritability is a distinct dimension on the broader spectrum of disruptive behavior predicting later physical aggression. This underscores the value of recognizing emotion regulation problems in youth presenting with a broad range of disruptive behavior problems.



(...) *which are entirely different from one another,* (...)

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Table S1: Overview of the Items From the Child Behavior Checklist (CBCL)/1.-5 and CBCL/6-18, Which Were Included or Excluded From the Analyses, and an Overview of the Callous Traits Items as Used in the Cross-Lagged Model.

CBCL/1.5-5	Included	CBCL/6-18	Included
		Drinks alcohol	No[c]
Can't stand waiting	Yes	Doesn't feel guilty after misbehaving	Yes
Defiant	No[b]	Breaks rules at home, school or elsewhere	Yes[d]
Demands must be met immediately	Yes	Hangs around with others who get into trouble	No[a]
Destroys things belonging to others	Yes	Lying or cheating	Yes
Disobedient	Yes	Prefers being with older kids	No[a]
Doesn't feel guilty after misbehaving	Yes	Runs away from home	Yes
Easily frustrated	Yes	Sets fires	Yes
Gets in many fights	Yes	Sexual problems	No[c]
Hits others	Yes	Steals at home	Yes
Hurts animals or people	Yes	Steals outside the home	Yes
Angry moods	Yes	Swearing or obscene language	Yes
Physically attacks people	Yes	Thinks about sex too much	No[a]
Punishment doesn't change behavior	Yes	Uses tobacco	No[c]
Screams a lot	No[a]	Truancy, skips school	No[c]
Selfish or won't share	Yes	Uses drugs for nonmedical purposes	No[c]
Stubborn, sullen or irritable	Yes	Vandalism	No[c]
Temper tantrums or hot temper	Yes	Argues a lot	Yes
Uncooperative	Yes	Cruelty, bullying or meanness to others	Yes
Wants a lot of attention	No[b]	Demands a lot of attention	No[b]
		Destroys his/her own things	Yes
		Destroys things belonging to others	Yes
		Disobedient at home	Yes
		Disobedient at school	Yes
		Gets in many fights	Yes
		Physically attacks people	Yes
		Screams a lot	Yes
		Stubborn, sullen, or irritable	Yes
Callous traits		Sudden changes in mood or feelings	Yes
Cannot be trusted with what he/she says		Sulks a lot	No[a]
Denies having done something wrong		Suspicious	No[a]
Uses or misleads other people to get what he/she wants		Teases a lot	Yes
If confronted with own behavior, can talk him-/herself out of it		Temper tantrums or hot temper	Yes
Does not keep promises		Threatens people	Yes
Does not find other people's feelings important		Unusually loud	No[b]
Is cold and indifferent			

Note: Items were excluded if: [a] they did not reflect problem behavior, e.g. "selfish" or "prefers being with older children"; [b] were more indicative of behavior problems or disorders other than disruptive behavior problems (DBPs), such as attention-deficit/hyperactivity disorder (ADHD), e.g. "can't stand waiting, wants everything now", or "demands must be met immediately"; [c] were endorsed very infrequently due to the child's young age, e.g. "drinks alcohol without parents' approval" or "uses drugs for nonmedical purposes."
[d] This item was not part of the CBCL/4-18, which was used in Swedish Twin Study of Child and Adolescent Development (TCHAD).

Table S2: Factor Mixture Model Results, Generation R Sample at Mean Age 6 Years

Number of classes	Number of parameters	Log likelihood	BIC	Entropy
Physical aggression				
1	15	-7741.078	15613.162	-
2	28	Unstable		
3	41	Unstable		
Irritability				
1	15	-18639.02	37409.05	-
2	28	-18557.38	37359.30	0.21
3	41	-18536.32	37430.73	0.41
Oppositional behavior				
1	15	-18790.76	37712.52	-
2	28	-18664.14	37572.82	0.42
3	41	-18631.47	37621.02	0.35

Note: BIC stands for Bayesian Information Criterion, with smaller values indicating a better model fit.

Table S3: Factor Mixture Model Results, Generation R Sample at Mean Age 10 Years

Number of classes	Number of parameters	Log likelihood	BIC	Entropy
Physical aggression				
1	24	-9567.57	19338.43	-
2	43	-9533.45	19431.14	0.76
3	62	-9511.78	19548.77	0.79
Irritability				
1	12	-9373.01	18847.68	-
2	Unstable			
3	34	-9321.26	18930.53	0.75
Disobedient behavior				
1	12	-8599.95	17301.55	-
2	23	-8554.26	17303.36	0.63
3	34	-8539.00	17366.01	0.67
Rule-breaking behavior				
1	18	-2181.91	4516.29	-
2	Unstable			
3	Unstable			

Note: BIC stands for Bayesian Information Criterion, with smaller values indicating a better model fit.

Table S4: Factor Mixture Model Results, Netherlands Twin Registry Sample

Number of classes	Number of parameters	Log likelihood	BIC	Entropy
Physical aggression				
1	24	-11995.16	24191.68	-
2	43	-11968.23	24297.23	0.65
3	62	-11940.19	24400.56	0.73
Irritability				
1	12	-10558.51	21217.69	-
2	23	-104699.54	21192.04	0.33
3	Unstable			
Disobedient behavior				
1	12	-8480.52	17061.72	-
2	Unstable			
3	Unstable			
Rule-breaking behavior				
1	18	-2973.972	6098.96	-
2	Unstable			
3	Unstable			

Note: BIC stands for Bayesian Information Criterion, with smaller values indicating a better model fit.

Table S5: Factor Mixture Model Results, Swedish TCHAD Sample

Number of classes	Number of parameters	Log likelihood	BIC	Entropy
Physical aggression				
1	24	-3687.35	7542.53	-
2	43	-3648.76	7598.23	0.64
3	Unstable			
Irritability				
1	12	-2581.16	5246.23	-
2	23	-2561.04	5282.91	0.67
3	34	-2550.15	5338.052	0.90
Disobedient behavior				
1	9	-1495.13	3053.20	-
2	Unstable			
3	Unstable			
Rule-breaking behavior				
1	16	-789.61	1691.11	-
2	Unstable			
3	Unstable			

Note: BIC stands for Bayesian Information Criterion, with smaller values indicating a better model fit.

Table S6: Factor Mixture Model Results, Clinical Sample

Number of classes	Number of parameters	Log likelihood	BIC	Entropy
Physical aggression				
1	24	-9655.78	19493.17	-
2	43	-9586.23	19497.84	0.40
3	62	-9541.89	19552.92	0.52
Irritability				
1	12	-7039.63	14170.07	-
2	23	-6977.75	14129.54	0.85
3	Unstable			
Disobedient behavior				
1	12	-6496.46	13083.73	-
2	23	-6432.39	13038.82	0.52
3	Unstable			
Rule-breaking behavior				
1	20	-3856.15	7863.64	-
2	Unstable			
3	Unstable			

Note: BIC stands for Bayesian Information Criterion, with smaller values indicating a better model fit.

Table S7: Factor Loadings for the 4-Factor Solutions Exploratory Factor Analysis in Generation R Study Sample at Mean Age 6 Years, Using all Child Behavior Checklist/1.5-5 Aggressive Behavior Scale Items

	Physical Aggression	Irritability	Oppositional Behavior	Attention seeking
Destroys things belonging to others	0.51	-0.07	0.18	0.18
Gets in many fights	0.83	-0.02	-0.03	-0.01
Hits others	0.83	0.03	0.14	-0.14
Hurts animals or people	0.52	0.07	0.11	0.03
Physically attacks people	0.79	0.17	-0.08	0.01
Easily frustrated	0.03	0.44	-0.01	0.30
Angry moods	-0.01	0.77	0.23	-0.11
Screams a lot	0.26	0.42	0.04	0.17
Stubborn, sullen or irritable	-0.04	0.65	0.27	0.03
Temper tantrums or hot temper	0.14	0.74	-0.06	0.06
Defiant	0.18	0.00	0.38	0.26
Disobedient	0.01	0.03	0.79	-0.01
Doesn't feel guilty after misbehaving	0.33	-0.01	0.34	0.14
Punishment doesn't change behavior	0.28	0.11	0.43	0.08
Uncooperative	0.11	0.27	0.43	0.06
Can't stand waiting	0.20	0.01	-0.02	0.78
Demands must be met immediately	-0.05	0.04	0.14	0.79
Wants a lot of attention	0.09	0.21	0.04	0.48
Selfish or won't share	0.15	0.17	0.15	0.27

Note: Fit indices: root mean square error of approximation = 0.031 (95% CI = 0.029-0.033); comparative fit index = 0.990; Tucker Lewis Index = 0.984.

Table S8: Factor Loadings for the 5-Factor Solutions Exploratory Factor Analysis in the Generation R Sample at Mean Age 10 Years, Using all Child Behavior Checklist/6-18 Externalizing Problems Scales Items

	Physical aggression	Irritability	Disobedient Behavior	Rule-Breaking Behavior	
Argues a lot	0.54	0.23	0.05	-0.11	0.20
Cruelty, bullying or meanness to others	0.83	0.09	-0.03	0.00	-0.04
Doesn't feel guilty after misbehaving	0.34	0.07	0.25	0.11	0.10
Gets into fights	0.70	-0.02	0.03	0.02	0.10
Hangs around with others who get into trouble	0.42	0.05	0.07	0.14	-0.10
Physically attacks people	0.51	-0.03	-0.10	0.11	0.41
Prefers being with older kids	0.35	0.06	0.04	-0.02	-0.02
Swearing or obscene language	0.36	0.06	0.17	0.03	0.21
Teases a lot	0.84	-0.02	0.04	0.01	-0.02
Threatens people	0.70	-0.06	0.01	0.14	0.12
Stubborn, sullen, or irritable	-0.01	0.69	0.18	-0.02	0.16
Sudden changes in mood or feelings	0.00	0.62	0.00	0.09	0.29
Sulks a lot	0.04	0.77	0.02	0.06	0.02
Suspicious	0.24	0.63	-0.04	0.03	-0.02
Disobedient at home	-0.06	0.09	0.86	-0.03	0.10
Disobedient at school	0.22	-0.13	0.75	0.05	-0.06
Lying or cheating	0.21	0.17	0.30	0.28	-0.08
Breaks rules at home, school or elsewhere	0.14	0.00	0.69	0.09	0.05
Destroys his/her own things	-0.03	0.01	-0.02	0.71	0.52
Destroys things belonging to others	0.05	-0.04	0.00	0.74	0.49
Runs away from home	-0.06	0.08	0.03	0.48	0.32
Sets fires	0.03	-0.16	0.17	0.55	0.09
Steals at home	0.02	0.22	0.08	0.71	-0.03
Steals outside the home	0.04	0.17	0.00	0.72	-0.20
Demands a lot of attention	0.21	0.13	0.23	-0.01	0.38
Screams a lot	0.32	0.23	0.04	-0.07	0.47
Temper tantrums or hot temper	0.23	0.36	0.02	0.04	0.43
Unusually loud	0.27	0.06	0.21	-0.05	0.45

Note: Fit indices: root mean square error of approximation = 0.015 (95% CI = 0.013 – 0.017); comparative fit index = 0.994; Tucker Lewis Index = 0.990.

Table S9: Factor Mixture Model Results, Child Behavior Checklist Sensitivity Analyses, Generation R Sample Mean Age 10 Years.

Number of classes	Number of parameters	Log likelihood	BIC	Entropy
Physical aggression				
1	30	-13565.27	27384.66	-
2	53	-13525.31	27499.58	0.67
3	76	-13491.71	27627.58	0.77
Irritable mood				
1	12	-8150.40	16402.46	-
2	23	-8105.53	16405.89	0.98
3	Unstable			

Note: BIC = Bayesian Information Criterion, with smaller values indicating a better model fit.

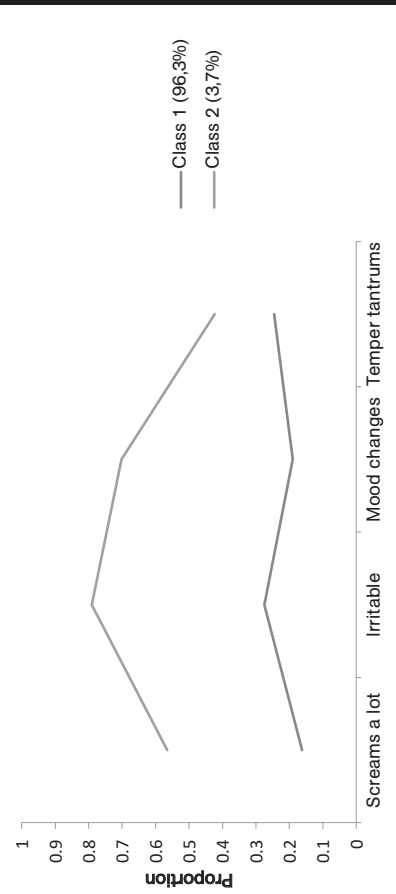


Figure S1: Irritability item endorsement proportions for two irritability classes in the clinical sample (N = 1,933). Note: Shown are the proportions of children endorsing the highest category of the ordinal trichotomous items (2 points).

Figure S2: Crossed-lagged model of cross-sectional and longitudinal associations between dimensions of disruptive behavior problems (DBPs) in boys from the Generation R Sample. Note: Displayed are the significant coefficients with 95% confidence intervals.

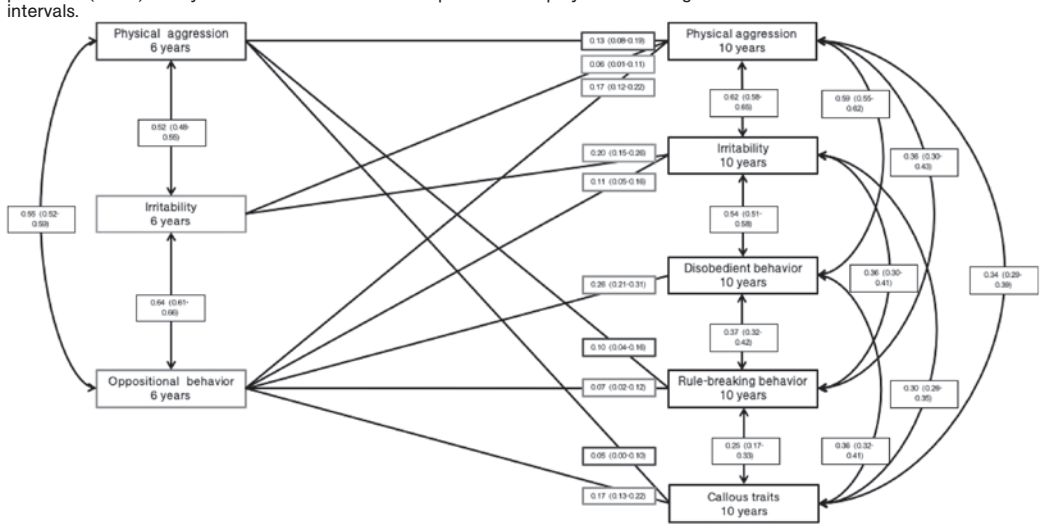


Figure S3: Crossed-lagged model of cross-sectional and longitudinal associations between dimensions of disruptive behavior problems (DBPs) in girls from the Generation R Sample. Note: Displayed are the significant coefficients with 95% confidence intervals.

(ABSTRACT)

OBJECTIVE

Studies of white matter connectivity in children with disruptive behavior have yielded inconsistent results, possibly due to the trait's heterogeneity, which comprises diverse symptoms like physical aggression, irritability, and delinquency. This study examined associations of global and specific white matter connectivity with childhood disruptive behavior problems, while accounting for their complex multidimensionality.

METHODS

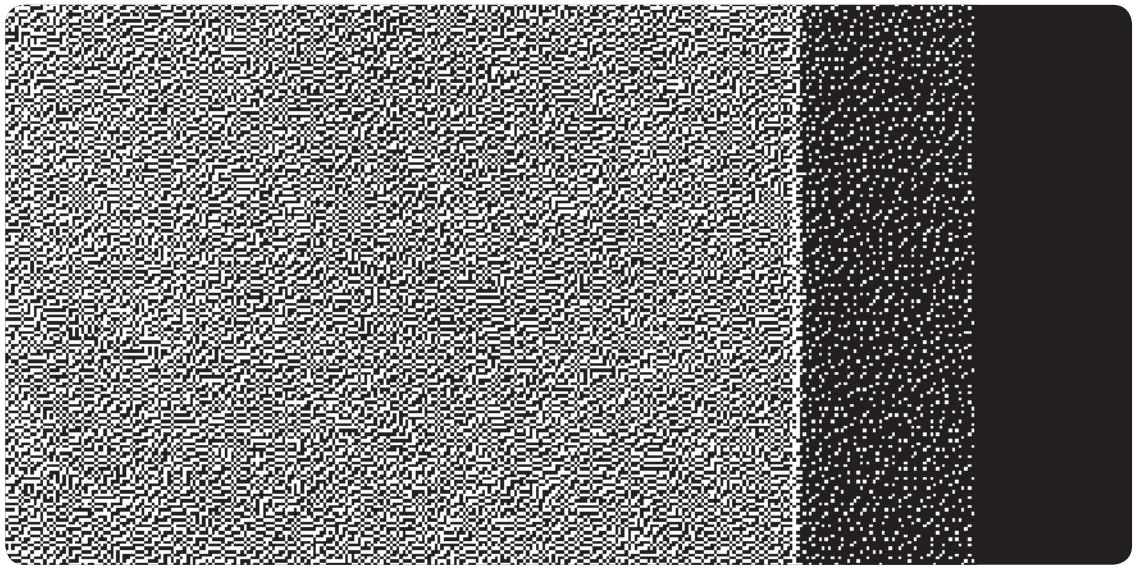
In a large cross-sectional population-based study of 10-year-old preadolescents (N=2567), we assessed four previously described empirically derived dimensions of disruptive behavior problems using the Child Behavior Checklist: physical aggression, irritability, disobedient behavior, and delinquent behavior. Global and specific white matter microstructure was assessed by diffusion tensor imaging.

RESULTS

Global fractional anisotropy and mean diffusivity were not associated with broad measures of disruptive behavior, e.g. Child Behavior Checklist externalizing problems. Global fractional anisotropy was negatively associated with delinquent behavior ($\beta = -.123$, PFDR-adjusted $= 0.028$), and global mean diffusivity was positively associated with delinquent behavior ($\beta = .205$, PFDR-adjusted < 0.001), suggesting reduced white matter microstructure in preadolescents with higher levels of delinquent behavior. Lower white matter microstructure in the inferior longitudinal fasciculus, superior longitudinal fasciculus, cingulum and uncinate underlie these associations. Global white matter microstructure was not associated with physical aggression, irritability, or disobedient behavior.

CONCLUSION

Delinquent behavior, a severe manifestation of childhood disruptive behavior, was associated with lower white matter microstructure in tracts connecting frontal and temporal lobes. These brain regions are involved in decision-making, reward-processing and emotion regulation. This study demonstrated that incorporating the multi-dimensional nature of childhood disruptive behavior traits shows promise in advancing the search for elucidating neurobiological correlates of disruptive behavior.



(...) *if one studies the words, in meaning and character,* (...)

CHAPTER 8

STRUCTURAL BRAIN CONNECTIVITY IN CHILDHOOD DISRUPTIVE BEHAVIOUR PROBLEMS: A MULTI-DIMENSIONAL APPROACH

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INTRODUCTION

Disruptive behavior problems, including aggression, irritability, and delinquency, are among the commonest reasons for referral to child and adolescent psychiatric services, and they greatly impact society in terms of costs, criminal convictions and service utilization (Scott et al. 2001; Peterson et al. 1996; Rivenbark et al. 2017). Several studies have addressed risk factors for childhood disruptive behaviors, and some progress has been made with regards to their neurobiological background (Blair et al. 2014). Heterogeneity among childhood disruptive behavior disorders has been thought to play a role in inconclusive findings regarding their neurobiological background, particularly with respect to white matter connectivity (Blair et al. 2014; Waller et al. 2017). Surprisingly, few studies have disentangled the neurobiological underpinnings of childhood disruptive behavior problems by taking into account their well-known heterogeneous presentation (Tremblay 2010; Moffitt et al. 2008; Blair et al. 2014; Lahey and Waldman 2012).

Etiological studies of childhood disruptive behavior problems have not often investigated structural white matter networks. White matter tracts provide high-speed communication of neuronal signals between gray matter regions in the brain. White matter microstructure, which is thought to reflect white matter integrity, can be measured via diffusion tensor imaging (DTI). The few existing studies using DTI to assess the white matter networks associated with childhood disruptive behavior have yielded inconsistent results (Waller et al. 2017). Some studies observed no differences in white matter microstructure between youth with disruptive behavior and controls (Finger et al. 2012; Zhang, Gao, et al. 2014; Hummer et al. 2015), whereas others showed that disruptive behavior was associated with both decreases (Haney-Caron, Caprihan, and Stevens 2014; Breeden et al. 2015; Peper et al. 2015) and increases (Passamonti et al. 2012; Sarkar et al. 2013; Zhang, Zhu, et al. 2014; Decety, Yoder, and Lahey 2015; Puzzo et al. 2017; Menks et al. 2017; Sethi et al. 2018) in connectivity. Findings have been observed across various white matter tracts in the brain, e.g. association tracts (including tracts connecting frontal and limbic regions), commissural tracts, and projection tracts in both hemispheres (Waller et al. 2017). It has been proposed that the small clinically-referred samples, and the large age range of the young people included in these studies might explain the seemingly conflicting mixed findings (Waller et al. 2017). Moreover, the presence of varying levels of distinct disruptive behaviors (e.g. physical aggression, disobedient behavior, irritability), and comorbid conditions (e.g. lower intelligence) may have contributed to the contradictory results (Haney-Caron, Caprihan, and Stevens 2014). Further, comprehensive assessments of disruptive behavior problems, tailored to developmental stage of the child, are required (Lahey and Waldman 2012; Moffitt et al. 2008; Blair et al. 2014).

Using factor-mixture analyses in three unreffered samples and one clinical sample of youth, we previously demonstrated four correlated dimensions of disruptive behavior problems, i.e. physical aggression, irritability, disobedient behavior and delinquency (Bolhuis et al. 2017). Potentially, disentangling the multidimensionality of disruptive behavior problems would aid the search for their correlates, which is particularly pertinent given that few neuroimaging studies have benefitted from taking into account this multidimensionality (Blair et al. 2014; Waller et al. 2017). Therefore, in the current population-based neuroimaging study, we assessed the relationship between white matter microstructure and these dimensions. First, we examined the relationship between global white matter microstructure and observed broadband externalizing problems sum scores. Using structural equation modelling, we then tested the hypothesis that reduced global white matter microstructure was associated with disruptive behavior dimensions, e.g. physical aggression and delinquent behavior. Structural equation modelling allows for examination of both latent dimensions of disruptive behavior problems, which - akin to factor analysis - weighs observed items and allows uncertainty in the model using latent behavioral variables, and observed white matter microstructural indices. It is therefore optimal for addressing our aims. Such refinement of behavioral phenotyping can potentially help identify underlying neurobiological mechanisms for childhood disruptive behavior problems. Moreover, simultaneously studying these dimensions in one neuroimaging study could lend neurobiological support for a dimension's validity (Wakschlag

et al. 2017). The specific tracts underlying these associations were expected to include structural connections between frontal, temporal and subcortical structures (Alegria, Radua, and Rubia 2016; Rogers and De Brito 2016; Brotman et al. 2017; Waller et al. 2017), with stronger global effects expected for more severe dimensions (e.g. delinquent behavior) and weaker associations of irritability with frontal-limbic connections (Leibenluft 2017).

METHODS AND MATERIALS

STUDY POPULATION

This cross-sectional population-based neuroimaging study was embedded in the Generation R Study, a prospective birth cohort, which included pregnant women living in Rotterdam, the Netherlands between 2002-2006 (Kooijman et al. 2016). The aim of the Generation R Study is to identify environmental and genetic factors influencing health, disease and development from prenatal life onwards. Study protocols were approved by the local ethics committee and written informed consent and assent was obtained for all participants.

For the current study, data from the age 10-years-of-age data collection wave were used. This examination included a research center visit, questionnaires, and a magnetic resonance imaging (MRI) assessment (Kooijman et al. 2016). Participants were included if data on the mother-reported Child Behavior Checklist (CBCL) and a DTI scan were available. Lastly, one random twin was excluded from each twin pair (n = 33), leaving a final sample of 2567 (see Supplemental Figure S1 for the inclusion flowchart). In a subsample of 2242, data was available on non-verbal intelligence.

DISRUPTIVE BEHAVIOR PROBLEMS

Disruptive behavior problems were assessed with the CBCL school-age version,(CBCL/6-18) a reliable and valid instrument to measure child externalizing problems over the previous 6 months (Achenbach and Rescorla 2001). Individual items were summed to obtain total scores. The CBCL is generalizable across many different nationalities and societies (Ivanova et al. 2007) and has previously been shown to adequately identify and screen for disruptive behavior disorders (Hudziak et al. 2004).

To assess disruptive behavior problems, items were selected from the CBCL externalizing problems scale, and the multidimensionality of these disruptive behavior problems scores was modelled along four inter-correlated dimensions, as is described in more detail elsewhere (Bolhuis et al. 2017). Based on clinical relevance for measuring disruptive behavior problems in preadolescent children, items were excluded following three pre-defined criteria: 1) did not reflect problem behavior; 2) were more indicative of behavior problems/disorders other than disruptive behavior disorders; or 3) were endorsed infrequently due to the child's young age. The following dimensions of disruptive behavior problems were modelled: 1) physical aggression, which captures problem behaviors such as fighting and physically attacking; 2) irritability, which includes items such as temper tantrums and frequent mood changes; 3) disobedient behavior, which is characterized by disobedience and lying/cheating; and 4) delinquent behavior, which comprises behaviors such as destroying things belonging to others and stealing. An overview of the individual items which loaded on these dimensions is presented in Supplemental Figure S2.

INTELLIGENCE

Child intelligence (IQ) was measured using the Snijders-Oomen nonverbal intelligence test when the children were on average 6 years old (Tellegen et al. 2005), as data on IQ were not available at later ages. Considering the developmental stage of the children, this test was selected due to its demonstrated reliability as a measure for nonverbal IQ in toddlerhood (Basten et al. 2014). These data were available in a subsample of 2274 children.

IMAGE ACQUISITION

Before undergoing brain MRI, children were invited to participate in a mock scanning session to familiarize them with the procedure

(White et al. 2017). If the child was at any point too anxious about the procedure, he or she did not progress to the actual MRI scanning.

Images were acquired on a 3T GE MR750W Discovery scanner (GE Healthcare, Milwaukee, WI) using an eight-channel head coil. The DTI scan was acquired using an axial spin echo, echo-planar imaging sequence with 3 b = 0 scans and 35 diffusion directions (repetition time = 12,500 ms, echo time = 72.8 ms, field of view = 240 mm x 240 mm, acquisition matrix = 120 x 120, slice thickness = 2 mm, voxel size = 2 mm x 2 mm x 2 mm, number of slices = 65, asset acceleration = 2).

IMAGE PREPROCESSING

DTI image preprocessing was conducted using the FMRIB Software Library (FSL), version 5.0.9 (Jenkinson et al. 2012), as described in more detail elsewhere (Muetzel et al. 2015). In short, nonbrain tissue was removed and diffusion images were corrected for eddy current-induced artifacts and translations/rotations resulting from head motion. The diffusion tensor was fitted at each voxel using the RESTORE method from the Camino diffusion MRI toolkit (Cook et al. 2006), and scalar metrics (e.g. fractional anisotropy [FA], mean diffusivity [MD]) were computed.

WHITE MATTER PROBABILISTIC TRACTOGRAPHY

Probabilistic white matter fiber tractography was conducted on each child's diffusion-weighted images in native space using the automated FSL plugin "AutoPtx" (de Groot et al. 2015), to identify connectivity distributions for a number of large, commonly reported fiber bundles. Connectivity distributions were then normalized based on the number of successful seed-to-target attempts, and thresholded to remove voxels that were unlikely to be part of the true distribution. Average FA and MD values were computed for each white matter tract by weighting voxels based on the connectivity distribution (i.e. FA in voxels with higher probabilities received higher weight). For the tract-specific analyses, left and right white matter tract FA and MD values were averaged and weighted for their respective volumes as we had no a priori hypotheses regarding the laterality of white matter tracts associated with disruptive behavior problems.

IMAGE QUALITY ASSURANCE

Diffusion image processing was conducted using DTIPrep tool (https://www.nitrc.org/projects/dtiprep/), by inspecting a combination of manual and automated checks, including examining slice-wise variation in the diffusion signal, examining the sum-of-squares error of the tensor calculation, and inspecting intersubject registration accuracy.

COVARIATES

Statistical models were adjusted for a number of potential confounding factors. First, child age at MRI (date of birth) and sex were obtained from medical records. Child ethnicity was classified based on parental birth place and dichotomized into European (mostly Dutch, European and North American) or Non-European descent. Last, maternal education was defined by the highest attained educational level and classified into low/medium (lower & intermediate vocational training, primary school and lower) or high (higher vocational education, and university). Covariates were dichotomized because of the low percentages in separate categories as this facilitated the structural equation modelling.

STATISTICAL ANALYSIS

All analyses were conducted in R statistical software, version 3.2.2 (R Core Team 2015), using the Lavaan package for structural equation modelling (Rosseel 2012). The structural equation model was constructed as follows (Figure 1). Separately for FA and MD, multiple white matter tracts were set to load on a single latent factor that represents the global DTI measure (green dashed arrows in Figure 1). Left and right hemisphere DTI metrics (e.g. left and right uncinate FA) were allowed to co-vary (not shown Figure 1) as this significantly improved model fit, in line with previous work using a similar approach (Muetzel et al. 2015). All CBCL items loaded on separate dimensions (latent factors) of disruptive behavior problems (purple

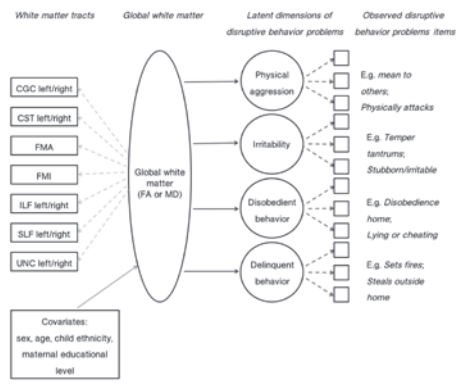
dashed arrows in Figure 1). All CBCL raw item scores were dichotomized (i.e., scores of 1 or 2 were collapsed) to reduce the number of empty cells in bivariate frequency tables and, hence, to facilitate polychoric correlations. First, we examined the relationship between global FA and MD and each dimension of disruptive behavior problems separately (red solid arrows in Figure 1). Next, all four dimensions of disruptive behavior problems were allowed to correlate with one another in order to accommodate the multidimensional nature of disruptive behavior problems (not shown Figure 1).

Initial analyses of the relationship between white matter connectivity and behavior examined whether latent constructs of global FA and MD were associated with the observed (i.e. not latent) raw scores of the CBCL scales externalizing problems, and its syndrome scales aggressive behavior and rule-breaking behavior. Next, we examined the association between global FA and MD and the dimensions of disruptive behavior problems, e.g. physical aggression and delinquent behavior. As the disruptive behavior problems dimensions were allowed to correlate with one another, the estimates reflect the effect of global DTI indices on each distinct dimension over and above general effects on disruptive behavior problems, i.e. estimates were mutually adjusted for each distinct dimension of disruptive behavior problems. Subsequently, separate association analyses were performed between specific white matter tracts (e.g. uncinate) and dimensions of disruptive behavior problems. Finally, structural equation regression paths were adjusted for confounders (blue solid arrow in Figure 1).

In sensitivity analyses, the association between global white matter connectivity and dimensions of disruptive behavior problems were additionally adjusted for nonverbal IQ scores. Also, we repeated our analyses with the additional irritability item "sulks a lot" in order to tap into irritability's more tonic, persistent elements. Furthermore, the main analyses on the association between global FA and MD and dimensions of disruptive behavior problems were repeated in those children who scored in the highest decile of externalizing problems.

All structural equation models were estimated using the weighted least squares means and variances estimator because of the binary

Figure 1: Outline of the structural equation model.



Note: Factor loading paths are depicted with dashed lines and structural equation regression paths are depicted with solid lines. For the sake of simplicity of the figure the interhemispheric correlations between white matter tracts (e.g. left and right uncinate) are not shown. In addition, all four disruptive behavior problems dimensions were allowed to correlate with one another, this is also not shown in the figure. CGC, cingulate gyrus part of cingulum bundle; CST, corticospinal tract; FMA, forceps major; FMI, forceps minor; ILF, inferior longitudinal fasciculus; SLF, superior longitudinal fasciculus; UNC, uncinate fasciculus; FA, fractional anisotropy; MD, mean diffusivity.

(Full colour image presented on page 10.)

Table 1: Descriptive sociodemographic characteristics of the study sample (n = 2567)

	Mean ± SD or %
Child characteristics	
Age at MRI, mean (SD)	10.12 ± 0.58
Sex, % girls	50.68
Ethnicity, %	
European descent	74.96
Non-European descent	25.03
Non-verbal IQ, mean (SD)	104.29 ± 14.53
Maternal characteristics	
Educational level, %	
High	65.11
Medium and Low	34.89

Table 2: Associations between global white matter measures and CBCL scales.

White matter metric	Outcome	*B*	95% CI	β	*p*	RMSEA	CFI	TLI
FA	Externalizing problems	-0.115	-0.536;0.306	-.012	.593	0.055	0.936	0.923
	Aggressive problems	-0.035	-0.358;0.288	-.005	.830	0.055	0.937	0.924
	Rule-breaking problems	-0.073	-0.204;0.058	-.024	.272	0.056	0.934	0.921
MD	Externalizing problems	0.000	-0.261;0.261	.000	1.000	0.072	0.916	0.899
	Aggressive problems	-0.017	-0.217;0.183	-.003	.871	0.072	0.917	0.900
	Rule-breaking problems	0.019	-0.061;0.099	.010	.640	0.073	0.914	0.897

Note: Analyses are corrected for child age, child sex, child ethnicity, and maternal educational level. CBCL, Child Behavior Checklist; CFI, comparative fit index; CI, confidence interval; FA, fractional anisotropy; MD, mean diffusivity; RMSEA, root mean square error of approximation; TLI, Tucker Lewis index.

Table 3: Associations between global white matter measures and dimensions of disruptive behavior problems

		Model 1 (Unidimensional)				Model 2 (Multidimensional)			
White matter metric	Outcome	*B*	95% CI	β	*p*	95% CI	β	*p*	p_{adj}
FA	Physical aggression	-0.027	-0.132;0.079	-.016	.626	-0.139;0.089	-.013	.693	.924
	Irritability	-0.003	-0.105;0.099	-.002	.960	-0.114;0.106	-.002	.945	.945
	Disobedient behavior	-0.073	-0.193;0.047	-.037	.231	-0.190;0.050	-.038	.215	.573
	Delinquent behavior	-0.309	-0.458;-0.160	-.174	<.001	-0.382;-0.060	-.123	.007	.028
MD	Physical aggression	-0.014	-0.067;0.039	-.014	.604	-0.078;0.039	-.019	.498	.924
	Irritability	-0.012	-0.069;0.045	-.012	.667	-0.074;0.048	-.012	.673	.924
	Disobedient behavior	0.003	-0.064;0.070	.002	.932	-0.064;0.070	.003	.924	.945
	Delinquent behavior	0.224	0.140;0.308	.211	<.001	0.129;0.309	.205	<.001	<.001

Note: All analyses are corrected for child age, child sex, child ethnicity, and maternal educational level. Model 1 includes separate structural regression analyses for each dimension. In Model 2 all dimensions are correlated in a multidimensional fashion. Fit indices for global fractional anisotropy (FA) are the following: root mean square error of approximation (RMSEA) = 0.023; comparative fit index (CFI) = 0.954; Tucker-Lewis index (TLI) = 0.950. Fit indices for global mean diffusivity (MD)were the following: RMSEA = 0.029; CFI = 0.979; TLI = 0.977.
CI, confidence interval; p_{adj}: false discovery rate-adjusted p value.

nature of the CBCL items and to account for missingness on covariates (Muthén and Muthén 1998-2002). Model fit was judged to be good when the following three fit metrics were satisfied: root mean square of approximation < 0.05; Comparative Fit Index > 0.95; and Tucker Lewis Index > 0.95. In the primary analysis, a false discovery rate (FDR) correction was applied to control for the number of dimensions of disruptive behavior in each statistical model performed in R, as this might increase type I error due to multiple testing (Benjamini and Hochberg 1995).

RESULTS

ATTRITION ANALYSIS

Within the group of children with CBCL data (n = 4920), we compared demographic covariates between the study population (n = 2567) and participants who did not have usable DTI data available (n = 2353). An overview of the flowchart and how many participants were excluded at different steps is presented in Supplemental Figure S1. The children did not differ in age (9.71 vs. 9.72 years of age, t = 1.43, p = .152), sex (50.68% vs. 50.23% girls, χ^2 = 0.08, p = .775), and proportion of participants of non-European ethnicity (25.04% vs. 26.60%, χ^2 = 1.45, p = .228). Children who had DTI data available were more likely to have mothers with higher educational levels (65.11% vs. 59.27%, χ^2=16.90, p < .001) and had lower CBCL externalizing problems scores (3.66 vs. 4.17 points, t = 3.62, p < .001).

DEMOGRAPHIC CHARACTERISTICS

Descriptive characteristics of the study population are shown in Table 1. Supplemental Figure S2 demonstrates the endorsement of the CBCL items pertaining to the dimensions of disruptive behavior problems. Symptoms of the irritability dimension and disobedient behavior dimension were most commonly endorsed. Physical aggression symptoms were less frequently reported, and delinquent behavior symptoms were relatively rarely endorsed.

In contrast, global FA was negatively associated with delinquent behavior (Table 3) (β = -.123; $p_{\text{FDR adjusted}}$ = .028), but not with other disruptive behavior problems dimensions. Similarly, delinquent behavior was associated with global MD, in a consistent direction as reflected by a positive association (β = .205, $P_{\text{FDR adjusted}}$ < .001). No other disruptive behavior problems dimensions were associated with global MD. Model fit of the structural equation models on the association between global DTI measures and multi-dimensional disruptive behavior problems were good (Table 3, footnote). Sensitivity analyses with the additional irritability item "Sulks a lot" led to similar results (Supplemental Table S1).

Considering the low endorsement of delinquent behavior items (range 0.4-3.4% of the sample had high delinquent behavior items scores) (Supplemental Figure S2), we considered the possibility of outliers driving this association. Hence, a scatterplot of the latent global FA/MD score and the latent score of delinquent behavior is presented in Supplemental Figure S3. No outliers were observed that would give disproportionate weight to certain values in the associations between delinquent behavior latent score and global FA/MD latent scores.

ASSOCIATIONS BETWEEN INDIVIDUAL WHITE MATTER TRACTS AND DISRUPTIVE BEHAVIOR PROBLEMS

Supplemental Table S2 shows the results of the secondary analyses between FA metrics of white matter tracts and dimensions of disruptive behavior problems, and Table S3 shows the post-hoc results of the models involving MD. Several DTI tracts were associated with delinquent behavior. In terms of FA metrics, negative associations were observed with the inferior longitudinal fasciculus (β = -.111, p = .007), superior longitudinal fasciculus (β = -.102, p = .011), and uncinate (β = -.093, p = .021). In terms of MD metrics, positive associations were observed with the cingulum (β = .118, p = .006), forceps minor (β = 0.094, p = .046), inferior longitudinal fasciculus (β = .148, p = .001), superior longitudinal fasciculus (β = 0.174, p < .001), and uncinate (β = .180, p < .001). Physical aggression was only associated with higher cingulum FA (β = .056, p = .045), and lower inferior longitudinal fasciculus FA (β = -.065, p = .023). Disobedient behavior was negatively associated with inferior longitudinal fasciculus FA (β =

-.073, p = .006). Irritability was not associated with any DTI tract FA or MD score. The latter findings should be interpreted cautiously as no association was observed between global FA or MD and physical aggression, disobedient behavior or irritability. The strengths of the associations between each individual tract FA and dimensions of disruptive behavior problems are depicted in Figure 2.

SENSITITIVITY ANALYSIS WITH INTELLIGENCE

The correlation between the delinquent behavior latent score and non-verbal IQ was low but statistically significant (r = -.078, p < 0.001). Thus, the main analyses involving global FA and MD were repeated with additional adjustment for non-verbal intelligence, and comparable results were obtained (Supplemental Table S4). Specifically, global white matter FA and MD were associated with delinquent behavior (global FA [β = -.140, p = .002], global MD [β = .244, p < .001]), but not with any other dimension of disruptive behavior problems.

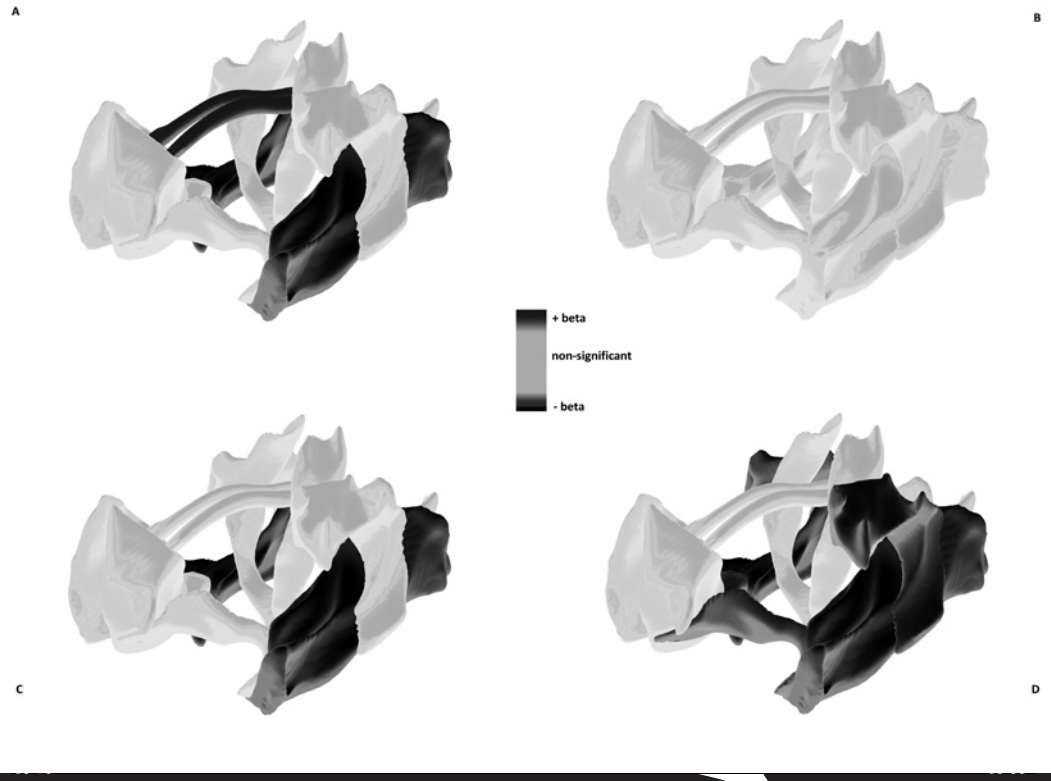
SENSITIVITY ANALYSIS IN A SUBSET OF CHILDREN AT INCREASED CLINICAL RISK

We repeated our main analyses in a group of children who scored in the highest decile of the CBCL externalizing problems scale (n = 478) (Supplemental Table S5). Results in this subsample were similar to those in the full sample, where lower FA and higher MD were associated with delinquent behavior (global FA [β = -.287, $p_{\text{FDR adjusted}}$ = .056], global MD [β = .359, $p_{\text{FDR adjusted}}$ < .001]), but not with any other dimension.

DISCUSSION

In this population-based neuroimaging study we demonstrated that lower global FA and higher global MD, indicators of less developed white matter integrity, were uniquely associated with delinquent

Figure 2: Associations between individual white matter tracts fractional anisotropy and dimensions of disruptive behavior problems: **(A)** physical aggression, **(B)** irritability, **(C)** disobedient behavior, **(D)** delinquent behavior. Nonsignificant associations are depicted in red, positive associations are depicted in yellow, and negative associations are depicted in blue.



(Full colour image presented on page 15.)

behavior, and not with other dimensions of childhood disruptive behavior problems, including physical aggression, irritability and disobedient behavior. The individual white matter tracts underlying the association between reduced global white matter integrity and delinquent behavior comprised the inferior longitudinal fasciculus, superior longitudinal fasciculus, cingulum and uncinate. These observations are in line with some previous DTI studies (Haney-Caron, Caprihan, and Stevens 2014; Breeden et al. 2015; Peper et al. 2015), but not with others (Passamonti et al. 2012; Sarkar et al. 2013; Zhang, Zhu, et al. 2014; Decety, Yoder, and Lahey 2015; Puzzo et al. 2017; Menks et al. 2017; Sethi et al. 2018). The current results should be evaluated in the context of mixed findings of prior studies (Waller et al. 2017). Against this background, these results provide empirical neurodevelopmental support for the multi-dimensional approach of childhood disruptive behavior problems, and underscore the importance of acknowledging the heterogeneity of child psychiatric phenotypes in the search for their (neurobiological) characteristics.

To our knowledge, this is the first study that examined whether the well-known heterogeneity of childhood disruptive behavior problems can be explained through differential structural neurobiological correlates. In contrast, we were not able to demonstrate an association between white matter microstructure and established measures of conduct problems, i.e. CBCL broadband externalizing problems, or aggressive behavior and rule-breaking problems syndrome scores. Furthermore, our main findings extended to a subset of children with high externalizing problems scores, representing a group of children at higher risk of clinical disorder. This further supports the dimensional specificity of white matter microstructure correlates associated with delinquent behavior.

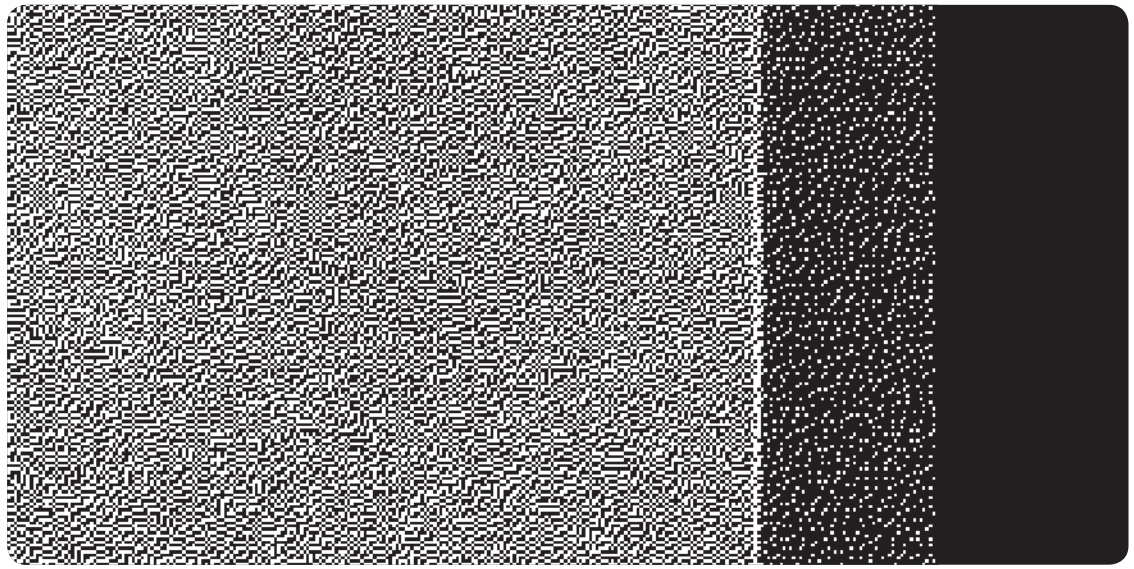
Less developed global white matter microstructure was uniquely associated with delinquent behavior in this study, and not with any other dimension of disruptive behavior problems. Delinquent behavior, which consisted of behaviors such as stealing and destroying property, was relatively rarely endorsed in this sample and is indeed quite rare in preadolescent youth (Bongers et al. 2004; Tremblay 2010; Odgers et al. 2008). This low prevalence notwithstanding, delinquent behavior was robustly associated with reduced global white matter microstructure, also in analyses additionally adjusted for nonverbal IQ. It could be argued that the presence of delinquent behavior at young ages is indicative of greater psychiatric problems severity, which might affect normal childhood neurodevelopment. For example, severe delinquent behaviors are associated with social learning and behavioral inhibition deficits (Tremblay 2010), which might potentially disrupt normative white matter development. In line with this, previous work from our group showed that higher rates of early childhood externalizing symptoms at baseline were associated with smaller increases in subcortical gray matter volume and global FA over time (Muetzel et al. 2017). Although the current study is cross-sectional, it might be possible that the severe nature of delinquent behavior in early childhood has significant effects on childhood brain development. Against this background, as children with high scores of delinquent behaviors are at increased risk for continued antisocial behavior, substance use, and more service use in adulthood (Tremblay 2010; Odgers et al. 2008; Rivenbark et al. 2017; Moffitt 1993), those children who also exhibit less developed white matter microstructure could be at highest risk for these problems in adulthood. Further neurodevelopmental assessments of these children are necessary in order to more accurately examine the direction of associations between behavioral and neurodevelopmental trajectories.

The white matter tracts associated with delinquent behavior included fronto-limbic connections involving the anterior cingulate cortex and orbitofrontal cortex, structures that have previously been implicated in reward processing and affect regulation (Alegria, Radua, and Rubia 2016). These observations are compatible with results from meta-analyses of functional and structural MRI studies (Alegria, Radua, and Rubia 2016; Rogers and De Brito 2016), and extend observations from DTI studies using smaller samples of clinically referred youth (Haney-Caron, Caprihan, and Stevens 2014; Breeden et al. 2015; Waller et al. 2017), by demonstrating the involvement of various white matter tracts in delinquent behavior in preadolescents from the general population. It is surprising that in the current study irritability was not associated with variation in any white matter

tract. This might be due to the population-based as opposed to clinically referred sample of this study, possibly reflecting less severe phenotypic presentations of irritability. It is has become increasingly clear that childhood irritability co-occurs with both internalizing and externalizing problems (Humphreys et al. 2018; Brotman et al. 2017). In this study, irritability was modelled in the context of disruptive behavior problems, and potentially the neural correlates of irritability are best studied in conjunction with internalizing problems such as depression and anxiety (Pagliaccio et al. 2018; Kircanski et al. 2018). However, several developmental and genetic studies support the substantial association of irritability with disruptive behavior problems (Bolhuis et al. 2017; Wakschlag, Tolan, and Leventhal 2010; Riglin et al. 2017; Stringaris, Zavos, et al. 2012). Alternatively, the neurobiological correlates of irritability might not lie with structural white matter. Recent reviews of functional neuroimaging studies suggest that irritability is characterized by heightened external threat orientation as well as deficits in reward processing and affect regulation networks (Brotman et al. 2017; Leibenluft 2017; Wakschlag et al. 2017), but to our knowledge no study has yet examined structural white matter connectivity in association with childhood irritability. Our group and others have previously described the correlation between irritability and other dimensions of disruptive behaviors (Wakschlag, Tolan, and Leventhal 2010; Bolhuis et al. 2017; Rowe et al. 2010; Wakschlag et al. 2017; Riglin et al. 2017; Stringaris, Zavos, et al. 2012). It is possible that the observed associations between delinquent behavior and fronto-limbic tracts, e.g. the uncinate and cingulum, reflect a combination of dysfunctions in affect regulation and reward processing (Murray 2007), salience processing, and associative learning deficits (Aghajani et al. 2017). These inter-related processes have similarly been associated with other dimensions of disruptive behavior, including, but not limited to, delinquent behavior and irritability (Alegria, Radua, and Rubia 2016; Tremblay 2010; Blair et al. 2014; Rogers and De Brito 2016; Brotman et al. 2017; Cohn et al. 2015; Waller et al. 2017; Muetzel et al. 2017). This study's strengths included its large population-based sample, advanced structural equation modelling strategy to accommodate the multi-dimensional characteristics of disruptive behavior, and prospective assessments of behavior problems and intelligence. However, several limitations should be noted. First, this was a cross-sectional study and is therefore not possible to infer causality from the associations found between white matter microstructure and dimensions of disruptive behavior problems. Although it is generally assumed that neurobiological abnormalities underlie psychiatric problems or disorders, recent research shows that the relationship between these might actually be bidirectional (Muetzel et al. 2017). Second, children with higher scores on CBCL externalizing problems were less likely to have useable DTI data, which might have affected the current findings. However, although attrition influences prevalence, it seems that attrition typically only marginally affects the validity of association analyses in population-based studies (Wolke et al. 2009). Furthermore, our results extended to a subsample of children with high scores of disruptive behavior problems. Third, it would have been optimal to have concurrent assessments of IQ, disruptive behavior and DTI, but IQ was only examined at age 6 years in this study population. However, intelligence is moderately stable during childhood (Trzaskowski et al. 2014), supporting our analyses with additional adjustment for IQ at 6 years. Last, relying on the CBCL to generate an irritability domain might not have adequately captured both irritability's tonic and episodic elements. However, sensitivity analyses with additional irritability items led to similar results and these items are comparable to those used previously (Stringaris, Zavos, et al. 2012; Aebi et al. 2013; Althoff et al. 2014; Savage et al. 2015), and to those included in more detailed assessments such as the Affective Reactivity Index (Leibenluft et al. 2003; Stringaris, Goodman, et al. 2012).

These limitations notwithstanding, our findings demonstrate that employing a multi-dimensional approach is advantageous in the search for neurobiological correlates of childhood disruptive behavior problems. We observed a negative association between global white matter microstructure and delinquent behavior, a relatively severe presentation of disruptive behavior at this developmental stage. The individual white matter tracts underlying this association included structural connections between frontal and limbic brain regions, which reciprocally connect the amygdala to prefrontal cortices. These findings provide novel clues on behavior-specific neurobiological characteristics of childhood disruptive behavior problems in

the general population. These findings might aid the development of future etiologic research of early life disruptive behavior. This is needed considering the relatively strong associations between white matter microstructure and delinquent behavior observed at such a young age. This also suggests that early screening for delinquent behavior in high-risk populations might be necessary to prevent the development of severe antisocial behavior later in adolescence. Investigations of strategies that have shown increases in daily-life functioning and brain structure, such as daily physical training (Hernandez et al. 2016), music training (Hudziak et al. 2014), mindfulness meditation (Johnson et al. 2014), and other related interventions, should all be further investigated as candidate therapies for improving neurodevelopmental outcomes in at-risk children.



(...) *but which the music assimilates by a common sentiment.*
(Marcel Proust, 1920, The Guermantes Way)

(SUPPLEMENT)

Table S1: Associations between global white matter measures and disruptive behavior problems dimensions, with the item "Sulks a lot" added to the irritability dimension.

		Disruptive behavior problems dimensions			
White matter metric	Outcome	B	95% CI	β	P
FA	Physical aggression	-0.023	-0.137;0.091	-0.013	0.691
	Irritability	0.012	-0.094;0.118	0.007	0.822
	Disobedient behavior	-0.076	-0.196;0.044	-0.038	0.214
	Delinquent behavior	-0.220	-0.381;-0.059	-0.123	0.007
MD	Physical aggression	-0.020	-0.079;0.039	-0.019	0.497
	Irritability	-0.021	-0.080;0.038	-0.020	0.480
	Disobedient behavior	0.003	-0.066;0.072	0.003	0.924
	Delinquent behavior	0.218	0.128;0.308	0.203	<0.001

Note: Analyses are corrected for child age, child sex, child ethnicity, and maternal educational level. Fit indices for global FA: RMSEA (root mean square error of approximation) = 0.023; CFI (comparative fit index) = 0.955; TLI (Tucker Lewis index) = 0.952. Fit indices for global MD: RMSEA = 0.029; CFI = 0.991; TLI = 0.977. FA: fractional anisotropy; MD: mean diffusivity.

Table S2: Associations between white matter tract fractional anisotropy (FA) and disruptive behavior problems dimensions.

		Disruptive behavior problems dimensions						
Tract	Outcome	B	95% CI	β	P	RMSEA	CFI	TLI
CGC	Physical aggression	0.048	0.001;0.095	0.056	0.045	0.026	0.961	0.957
	Irritability	0.020	-0.027;0.067	0.023	0.409			
	Disobedient behavior	0.016	-0.033;0.065	0.016	0.532			
	Delinquent behavior	-0.057	-0.124;0.010	-0.064	0.095			
CST	Physical aggression	0.017	-0.026;0.060	0.020	0.442	0.025	0.963	0.959
	Irritability	0.012	-0.031;0.055	0.015	0.575			
	Disobedient behavior	0.002	-0.047;0.051	0.003	0.923			
	Delinquent behavior	0.014	-0.049;0.077	0.017	0.661			
FMA	Physical aggression	-0.007	-0.046;0.032	-0.009	0.725	0.025	0.964	0.959
	Irritability	-0.017	-0.058;0.024	-0.021	0.430			
	Disobedient behavior	-0.020	-0.067;0.027	-0.023	0.393			
	Delinquent behavior	-0.058	-0.127;0.011	-0.071	0.095			
FMI	Physical aggression	-0.009	-0.054;0.036	-0.012	0.676	0.025	0.964	0.959
	Irritability	-0.018	-0.061;0.025	-0.022	0.417			
	Disobedient behavior	-0.024	-0.069;0.021	-0.027	0.295			
	Delinquent behavior	-0.040	-0.112;0.033	-0.049	0.275			
ILF	Physical aggression	-0.057	-0.106;-0.008	-0.065	0.023	0.024	0.965	0.960
	Irritability	-0.023	-0.070;0.024	-0.026	0.338			
	Disobedient behavior	-0.072	-0.123;-0.021	-0.073	0.006			
	Delinquent behavior	-0.100	-0.173;-0.027	-0.111	0.007			
SLF	Physical aggression	-0.021	-0.070;0.028	-0.024	0.395	0.025	0.963	0.959
	Irritability	-0.011	-0.058;0.036	-0.012	0.655			
	Disobedient behavior	-0.022	-0.075;0.031	-0.022	0.425			
	Delinquent behavior	-0.092	-0.163;-0.021	-0.102	0.011			
UNC	Physical aggression	-0.014	-0.057;0.029	-0.016	0.521	0.026	0.962	0.957
	Irritability	0.011	-0.036;0.058	0.012	0.655			
	Disobedient behavior	0.008	-0.045;0.061	0.009	0.750			
	Delinquent behavior	-0.084	-0.155;-0.013	-0.093	0.021			

Note: left and right tracts were averaged and weighted for their respective volumes. Analyses are corrected for child age, child sex, child ethnicity, and maternal educational level. CGC: cingulate gyrus part of cingulum bundle; CST: corticospinal tract; FMA: forceps major; FMI: forceps minor; ILF: inferior longitudinal fasciculus; SLF: superior longitudinal fasciculus; UNC: uncinate fasciculus; RMSEA: root mean square error of approximation; CFI: comparative fit index; TLI: Tucker Lewis index.

Table S3: Associations between white matter tract mean diffusivity (MD) and disruptive behavior problems dimensions.

		Disruptive behavior problems dimensions						
Tract	Outcome	B	95% CI	β	P	RMSEA	CFI	TLI
CGC	Physical aggression	-0.013	-0.062;0.036	-0.015	0.601	0.026	0.960	0.956
	Irritability	-0.015	-0.062;0.032	-0.017	0.538			
	Disobedient behavior	0.005	-0.046;0.056	0.005	0.839			
	Delinquent behavior	0.105	0.029;0.181	0.118	0.006			
CST	Physical aggression	-0.009	-0.062;0.044	-0.010	0.730	0.025	0.964	0.960
	Irritability	-0.015	-0.052;0.022	-0.015	0.429			
	Disobedient behavior	0.019	-0.026;0.064	0.018	0.410			
	Delinquent behavior	0.038	-0.052;0.128	0.039	0.412			
FMA	Physical aggression	0.008	-0.037;0.053	0.010	0.727	0.025	0.964	0.960
	Irritability	0.003	-0.039;0.043	0.004	0.878			
	Disobedient behavior	0.021	-0.026;0.068	0.024	0.377			
	Delinquent behavior	0.037	-0.036;0.110	0.045	0.318			
FMI	Physical aggression	0.000	-0.039;0.039	0.001	0.985	0.025	0.964	0.960
	Irritability	0.005	-0.038;0.048	0.007	0.802			
	Disobedient behavior	0.014	-0.033;0.061	0.016	0.547			
	Delinquent behavior	0.076	0.002;0.150	0.094	0.046			
ILF	Physical aggression	0.022	-0.019;0.063	0.026	0.280	0.025	0.963	0.959
	Irritability	0.003	-0.042;0.048	0.003	0.906			
	Disobedient behavior	0.037	-0.012;0.086	0.039	0.136			
	Delinquent behavior	0.130	0.056;0.204	0.148	0.001			
SLF	Physical aggression	0.016	-0.025;0.057	0.020	0.438	0.026	0.962	0.957
	Irritability	0.009	-0.034;0.052	0.011	0.673			
	Disobedient behavior	0.037	-0.012;0.086	0.040	0.140			
	Delinquent behavior	0.149	0.082;0.216	0.174	<0.001			
UNC	Physical aggression	-0.031	-0.078;0.016	-0.036	0.200	0.026	0.961	0.956
	Irritability	-0.010	-0.055;0.035	-0.011	0.666			
	Disobedient behavior	-0.013	-0.062;0.036	-0.013	0.601			
	Delinquent behavior	0.159	0.090; 0.228	0.180	<0.001			

Note: left and right tracts were averaged and weighted for their respective volumes. Analyses are corrected for child age, child sex, child ethnicity, and maternal educational level. CGC: cingulate gyrus part of cingulum bundle; CST: corticospinal tract; FMA: forceps major; FMI: forceps minor; ILF: inferior longitudinal fasciculus; SLF: superior longitudinal fasciculus; UNC: uncinate fasciculus; RMSEA: root mean square error of approximation; CFI: comparative fit index; TLI: Tucker Lewis index.

Table S4: Associations between global white matter measures and disruptive behavior problems dimensions, additionally adjusted for child IQ ($n = 2242$).

		Disruptive behavior problems dimensions			
White matter metric	Outcome	B	95% CI	β	P
FA	Physical aggression	-0.043	-0.157;0.071	-0.025	0.454
	Irritability	0.012	-0.100;0.124	0.007	0.841
	Disobedient behavior	-0.067	-0.189;0.055	-0.035	0.286
	Delinquent behavior	-0.240	-0.393;-0.087	-0.140	0.002
MD	Physical aggression	0.007	-0.056;0.070	0.006	0.830
	Irritability	-0.016	-0.083;0.051	-0.015	0.625
	Disobedient behavior	0.006	-0.067;0.079	0.005	0.878
	Delinquent behavior	0.262	0.174;0.350	0.244	<0.001

Note: Analyses are corrected for child age, child sex, child ethnicity, maternal educational level, and child non-verbal IQ. Fit indices for global FA: RMSEA (root mean square error of approximation) = 0.026; CFI (comparative fit index) = 0.94; TLI (Tucker Lewis index) = 0.936. Fit indices for global MD: RMSEA = 0.032; CFI = 0.971; TLI = 0.968. FA: fractional anisotropy; MD: mean diffusivity.

Table S5: Associations between global white matter measures and disruptive behavior problems dimensions in top 10% of CBCL Externalizing problems scale ($n = 478$).

		Disruptive behavior problems dimensions								
		Model 1 (uni-dimensional)				Model 2 (multi-dimensional)				
White matter metric	Outcome	B	95% CI	β	P	$P_{adj.}$	B	95% CI	β	P	$P_{adj.}$
FA	Physical aggression	-0.183	-0.401;0.035	-0.160	0.099	0.264	-0.057	-0.302;0.188	-0.044	0.647	0.714
	Irritability	0.092	-0.126;0.310	0.079	0.404	0.539	0.087	-0.168;0.342	0.066	0.504	0.714
	Disobedient behavior	0.046	-0.366;0.458	0.024	0.828	0.828	0.084	-0.367;0.535	0.040	0.714	0.714
	Delinquent behavior	-0.358	-0.640;-0.076	-0.286	0.013	0.052	-0.478	-0.860;-0.096	-0.287	0.014	0.056
MD	Physical aggression	0.083	-0.039;0.205	0.121	0.181	0.362	0.074	-0.044;0.192	0.108	0.221	0.381
	Irritability	-0.036	-0.150;0.078	-0.053	0.535	0.611	-0.018	-0.138;0.102	-0.026	0.764	0.764
	Disobedient behavior	-0.091	-0.295;0.113	-0.074	0.381	0.539	-0.113	-0.321;0.095	-0.094	0.286	0.381
	Delinquent behavior	0.356	0.152;0.560	0.385	0.001	0.008	0.349	-0.155;0.543	0.359	<0.001	<0.001

Note: All analyses are corrected for child age, child sex, child ethnicity, and maternal educational level. Model 1 includes separate structural regression analyses for each dimension. In Model 2 all dimensions are correlated in a multi-dimensional fashion. Fit indices for global FA: RMSEA (root mean square of approximation) =0.046; CFI (comparative fit index) =0.680. Fit indices for global MD: RMSEA=0.043; CFI=0.851; TLI=0.838. DTI: diffusion tensor imaging; FA: fractional anisotropy; MD: mean diffusivity; P_{adj}: false discovery rate adjusted P-value.

Figure S1: Inclusion flowchart

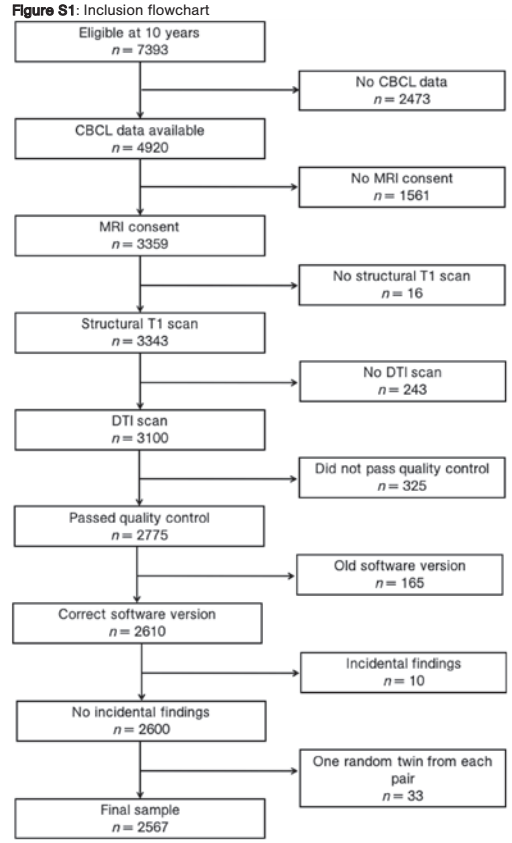
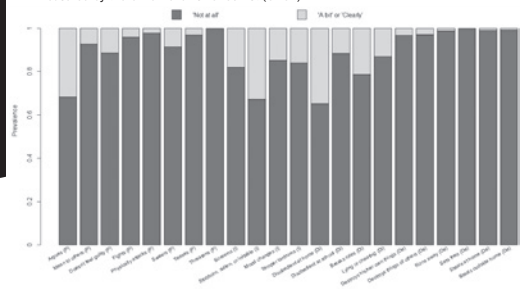
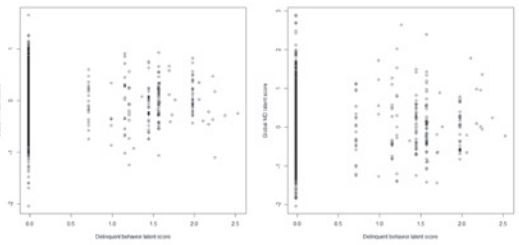


Figure S2: Endorsement of the individual disruptive behavior problems items as measured by the Child Behavior Checklist (CBCL).



Note: each item is dichotomized into a binary variable. In between brackets it is specified in which dimension the item was included: P: Physical aggression, I: Irritability, Di: Disobedient behavior, De: Delinquent behavior.

Figure S3: Scatterplots of the delinquent behavior latent score and global fractional anisotropy (FA; left panel) and global mean diffusivity (MD; right panel) latent scores.



(ABSTRACT)

OBJECTIVE

Studies on the long-term consequences of maternal cannabis use on child development beyond the neonatal period are sparse. In the current study, we use a multi-information approach to assess the association of prenatal cannabis exposure and child behavioural and emotional functioning. To explore the possible causal nature of the association, we investigated whether maternal tobacco and paternal cannabis use during pregnancy were also associated with child problems.

METHODS

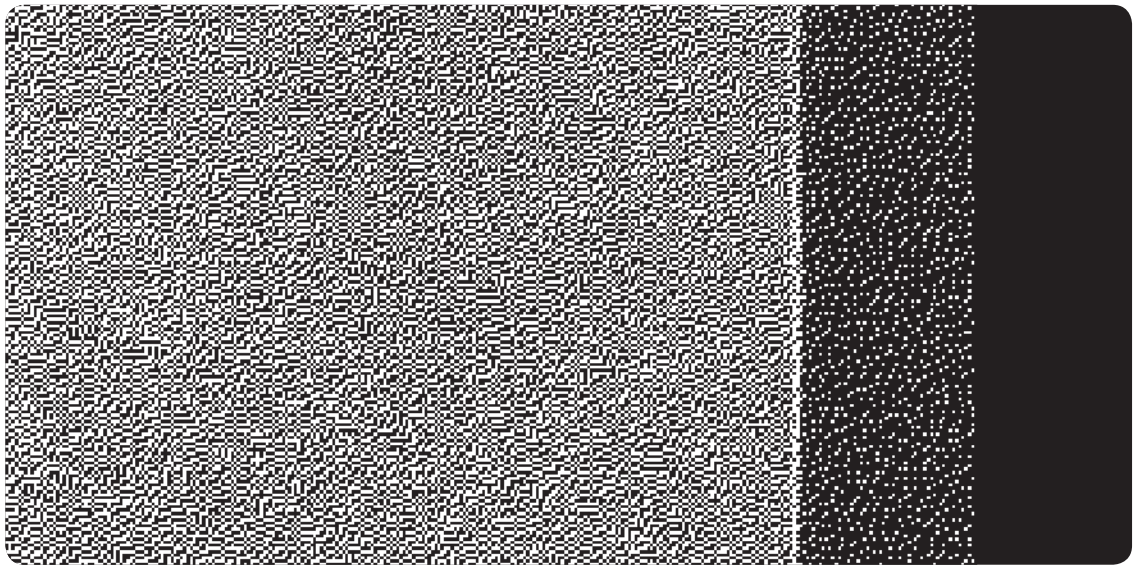
The study population included children of a population-based birth cohort in the Netherlands (n = 5903). Information on parental cannabis use was collected using questionnaires; urine of mothers was analysed for the presence of cannabis metabolites. Child behavioural and emotional problems at approximately 7-10 years were measured using validated teacher-, child- and mother-reports.

RESULTS

Our findings show associations of maternal cannabis use during pregnancy with offspring externalising problems (B = 0.53; 95% CI: 0.29 - 0.77), but not with internalising problems (B = -0.10; 95% CI: -0.31 - 0.11). However, maternal cannabis use before pregnancy was also associated with offspring externalising problems (B = 0.27; 95% CI: 0.02 - 0.52). Further, cannabis use by the father was associated to externalising problems (B = 0.36; 95% CI: 0.22 - 0.49), but not internalising problems.

CONCLUSIONS

Prenatal exposure to maternal cannabis use is specifically associated with offspring behavioural problems, but not emotional problems. This association is probably not due to an intrauterine cannabis exposure on fetal development, because both maternal and paternal cannabis exposure during pregnancy were related to offspring externalising problems. Our findings suggest that the association can be explained through residual confounding, most likely through shared genetic vulnerabilities for parental cannabis use and offspring behavioural problems.



– Yes, my dear Wilhelm,
nothing on earth is closer to my heart than children. (...)

CHAPTER 9

PRECONCEPTION AND PRENATAL CANNABIS USE AND THE RISK OF BEHAVIOURAL AND EMOTIONAL PROBLEMS IN THE OFFSPRING;

A MULTI-INFORMANT PROSPECTIVE LONGITUDINAL STUDY

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INTRODUCTION

Increasingly, pregnant women use cannabis, with reported prevalence of 2-10% (Fergusson, Horwood, and Northstone 2002; Lozano et al. 2007; Williamson et al. 2006; El Marroun, Tiemeier, et al. 2011). The psychoactive ingredients of cannabis, including Δ-9-tetrahydrocannabinol (THC), can cross the placenta and reach the fetus (Little and VanBeveren 1996). Prenatal cannabis exposure has been associated with adverse birth outcomes (Hayatbakhsh et al. 2012; Hatch and Bracken 1986; Sherwood et al. 1999; Day et al. 1991; Gunn et al. 2016). However, studies on long-term consequences of maternal cannabis use and child development are sparse. Only two longitudinal studies have addressed this (Fried, Watkinson, and Gray 2003; Goldschmidt et al. 2008), and these demonstrate childhood cognitive deficits, impairments in inhibitory control, hyperactivity, impulsivity, attention problems, conduct problems, adolescent delinquency and increased risk of drugs abuse later in life (Day, Leech, and Goldschmidt 2011; Day et al. 1994; Fried 2002; Goldschmidt et al. 2012; Willford et al. 2010; Day, Goldschmidt, and Thomas 2006; Goldschmidt, Day, and Richardson 2000; O'Connell and Fried 1991). A recent review emphasizes the importance of genetic factors, gene-environment interactions, and comorbidity as they may confound the association between gestational substance use and offspring behaviour problems (Ruisch et al. 2018). Previously, in a population-based prospective cohort, we showed that prenatal cannabis exposure was related to fetal growth retardation, decreased fetal blood flow (El Marroun et al. 2009; El Marroun et al. 2010), and behaviour problems in toddlerhood (El Marroun, Hudziak, et al. 2011). Furthermore, using neuroimaging we reported that prenatal cannabis exposure was associated with cortical thickness differences (El Marroun et al. 2016).

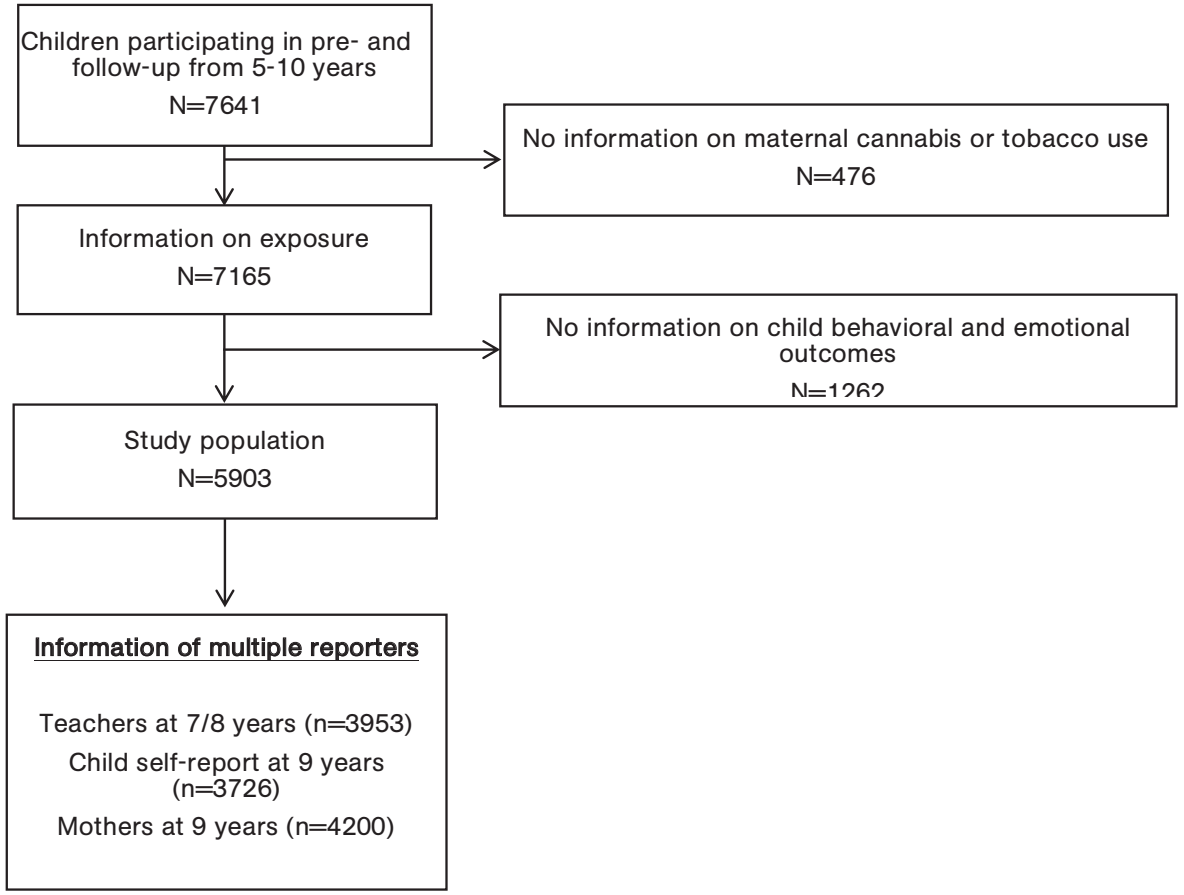
Assuring that associations between maternal substance use and child psychopathology are not confounded by other factors is difficult. A method that supports causal inference includes comparing the associations for maternal and paternal substance use during pregnancy (Smith 2008; Davey Smith et al. 2009). Yet, to date very few studies on prenatal substance use and offspring development have applied this approach. Moreover, it is also important to explore the effects of cannabis use exclusively preceding pregnancy. Disentangling the effects of prenatal cannabis exposure from tobacco exposure is challenging as prenatal exposure to tobacco can also negatively influence child development. Therefore, in the current study, we compared several non-overlapping groups, (i) pregnant women who used cannabis, (ii) women who used cannabis before pregnancy only, (iii) pregnant women who smoked tobacco only, and (iv) non-using pregnant women (reference). Finally, the importance of multiple informants for assessing child behavioural and emotional problems has long been emphasized (Achenbach, McConaughy, and Howell 1987). Correlations between different informants are generally low and may reflect variations in children's behaviours across diverse settings and relational circumstances (Stanger and Lewis 1993; De Los Reyes et al. ; Achenbach, McConaughy, and Howell 1987) or informant error (Grietens et al. 2004). Summarized, we aim to investigate the association of prenatal cannabis exposure with child behavioural and emotional functioning using a multi-information approach.

METHODS

DESIGN AND SETTING

The present study is embedded in the Generation R Study, a population-based birth cohort in Rotterdam, the Netherlands (Kooijman et al. 2016). All children were born between April 2002 and January 2006 in the city of Rotterdam, and follow-up is ongoing. The Medical Ethics Committee of the Erasmus Medical Centre, Rotterdam has given ethical approval of the study. Written informed consent was obtained from all participants. Only children participating in the prenatal assessment and the follow-up examination between 5 to 10 years of age (n = 7641) were considered (Figure 1). We excluded 476 children, as information on maternal cannabis was unavailable. Information on behavioural and emotional functioning was obtained in n = 5903 (77.3%) children; this information was reported by teachers (n = 3953) at 7/8 years, mothers (n = 4200) and children (n = 3726) at 9 years.

Figure 1: Flowchart of the study population



MATERNAL CANNABIS USE

To optimize the cannabis assessment, two information sources were used (i) maternal self-reports of cannabis use and (ii) maternal urinary THC-levels. In the first trimester, mothers indicated whether they used cannabis before or during pregnancy, and whether they were still using cannabis (El Marroun, Tiemeier, et al. 2011). Information about the product used and frequency of use (daily, weekly, monthly) was available. Urine samples were collected in early, mid and late pregnancy; the first available sample was used for urinalysis in a subset of the cohort (El Marroun, Tiemeier, et al. 2011). Urine samples were tested for 11-nor-Δ9-THC-9-COOH using the DRI® Cannabinoid Assay (Microgenics) with a cutoff value of 50 µg/l as recommended. Agreement between self-reports and THC-levels (Yule's Y=0.77) was substantial (El Marroun, Tiemeier, et al. 2011).

MATERNAL TOBACCO SMOKING

Maternal tobacco smoking was assessed by postal questionnaires in each trimester (Roza et al. 2007). Maternal smoking was categorized, using these questionnaires, into 'no smoking during pregnancy', 'until pregnancy was known' and 'continued during pregnancy'. Women that used tobacco until the pregnancy was known were included in the reference group, because previously we could not document an association with adverse neurodevelopmental outcomes (El Marroun et al. 2014; Roza et al. 2007).

PATERNAL SMOKING AND CANNABIS USE

In the first trimester, mothers provided information on paternal tobacco smoking and cannabis use. We used maternal reports of tobacco smoking and cannabis use of the father of the child, because not all fathers completed a questionnaire. Maternal report of paternal tobacco or cannabis use was highly correlated to paternal self-reports ($r_{\text{tobacco use}}$ = 0 .85 and $r_{\text{cannabis use}}$ = 0.80; $P < 0.001$).

Table 1: Descriptive statistics of the study population

	Reference group # (n=4804)	Cannabis use during pregnancy (n=169)		Cannabis use before pregnancy only(n=158)		Tobacco use only throughout pregnancy, (n=772)	
Maternal characteristics							
Maternal age at intake, yrs.	30.7 ± 0.07	27.2 ± 0.44	<0.001	28.9 ± 0.47	<0.001	29.5 ± 0.20	<0.001
Prenatal psychopathology	0.28 ± 0.01	0.54 ± 0.04	<0.001	0.39 ± 0.04	<0.001	0.41 ± 0.02	<0.001
Maternal body mass index	24.7 ± 0.06	24.1 ± 0.36	0.07	23.8 ± 0.30	0.01	25.4 ± 0.17	<0.001
Educational level							
Primary education (%)	8.1	15.6	<0.001	9.6	<0.001	15.7	<0.001
Secondary education (%)	41.8	61.6		52.1		61.3	
Higher education (%)	50.3	22.8		38.3		23.0	
Maternal ethnicity							
Dutch (%)	54.8	50.6	0.26	63.9	0.01	50.9	0.05
Non-Dutch Western (%)	8.3	9.6		10.8		7.6	
Non-Dutch non-Western (%)	36.9	39.8		25.3		41.5	
Tobacco use							
Never smoked in pregnancy (%)	90.3	9.9	n.a.	51.0	n.a.	-	n.a.
Smoked in early pregnancy (%)	9.7	16.8		18.1		-	
Smoked throughout pregnancy (%)	-	73.3		31.0		100	
Alcohol use							
Never drank in pregnancy (%)	45.1	30.5	<0.001	27.9	<0.001	42.0	0.10
Drinking until pregnancy was known (%)	15.1	20.2		12.3		15.3	
Continued drinking, occasionally (%)	32.3	38.1		47.0		32.7	
Continued drinking, regularly (%)	7.5	11.2		12.8		10.0	
Paternal substance use							
Cannabis use during pregnancy (%)	5.1	79.3	<0.001	63.8	<0.001	15.5	<0.001
Tobacco smoking during pregnancy (%)	37.3	79.9	<0.001	65.0	<0.001	74.4	<0.001
Drinking alcohol during pregnancy (%)	76.7	82.1	0.14	89.8	<0.001	76.0	0.73
Child characteristics							
Gender of the child (% boys)	49.3	55.6	0.13	52.5	0.34	52.8	0.07
Birth weight, grams	3446 ± 8.1	3211 ± 40.5	<0.001	3427 ± 38.1	0.68	3291 ± 19.8	<0.001
Gestational age at birth, weeks	39.9 ± 0.03	39.8 ± 0.12	0.48	40.1 ± 0.10	0.08	39.7 ± 0.07	0.007

Note: Groups are categorized on maternal cannabis and/or tobacco use during pregnancy and are non-overlapping. All continues variables are presented as means ± standard errors; all categorical variables are presented as percentages. There were no missing data on these variables as they were imputed using multiple imputation methods. P-values are derived from ANOVAs for parametric continuous variables, Kruskal-Wallis tests for non-parametric continuous variables and $\chi 2$-tests for categorical variables with reference group as the comparison group.

CHILD BEHAVIOURAL AND EMOTIONAL FUNCTIONING

At age 9 years, children were asked to complete the validated Brief Problem Monitor (BPM), to obtain the child's self-report of behavioural and emotional problems (Achenbach and Rescorla 2001a). The BPM contained 19 items and was rated on a 3-point scale (0 = not true, 1 = somewhat or sometimes true and 2 = very or often true). Mothers filled out the school-age version of the Child Behavior Checklist (CBCL/6-18) when their child was approximately 9 years. The CBCL measures the degree of children's behavior problems (Achenbach and Rescorla 2001a). We collected teacher ratings of child behavioural and emotional problems at 7/8 years using the Teacher Report Form (TRF/6-18) (Achenbach and Rescorla 2001a). Both CBCL and the TRF contain 118 problem items based on the preceding 2 months rated on the same 3-point scale.

A broadband internalizing/emotional problems (i.e. anxious/depressed, withdrawn and somatic complaints) and externalizing/behavioural problems (aggressive behavior and rule-breaking behavior) scales were computed. Additionally, we used clinical cut-off scores (80^{th} percentile) to classify children as having problems in the clinical range. Good reliability and validity have been reported for the CBCL, TRF and BPM (Achenbach and Rescorla 2001b). In line with literature on cross-informant agreement (Rescorla et al. 2014), correlations between parent, teacher and child self-reports were modest ($r_{CBCL\text{-}TRF}$ = 0.30, $r_{CBCL\text{-}BPM}$ = 0.45 and $r_{TRF\text{-}BPM}$ = 0.17 for the total problems score). Further, we found moderate correlations between the internalizing and externalizing scales in the same informant ($r_{TEACHER\ REPORT}$ = 0.36, $r_{SELF\ REPORT}$ = 0.33 and $r_{MATERNAL\ REPORT}$ = 0.53).

COVARIATES

We considered several maternal and child characteristics as potential confounders (Fergusson et al. 2002). Maternal characteristics were: maternal age at intake, ethnicity, educational level, pre-pregnancy body mass index, and alcohol use. Child characteristics were: age at assessment, gender, gestational age at birth, and birth weight. Maternal ethnicity was defined according to the classification of

Statistics Netherlands. Maternal educational level was categorized into: primary (no or primary education), secondary (lower and intermediate vocational training), and higher (higher vocational education and university) education. Information on maternal alcohol use was collected using questionnaires in each trimester as described above for smoking. Maternal psychopathology during pregnancy was assessed using the validated Brief Symptom Inventory in mid-pregnancy (de Beurs 2004; Derogatis and Melisaratos 1983). Gestational age at birth, weight at birth and gender of the child were extracted from medical records.

STATISTICAL ANALYSES

Descriptive statistics of the four groups were compared using analyses of variance (ANOVA) for parametric data, Kruskal-Wallis tests for non-parametric data and Chi-square tests for categorical data. Supplementarily, we provide descriptive information on the frequency of tobacco and alcohol use per trimester (Supplementary Table 1). To test the associations of maternal and paternal cannabis use and childhood behavioural and emotional problems, both linear and logistic (for problems in the clinical range) regressions were performed. For the continuous outcomes were square root transformed to approximate a normal distribution. Covariates were selected based on prior literature and on the 5%-change-in-estimate criterion (Mickey and Greenland 1989). We performed additional adjustment for internalizing problems if outcome was externalizing problems as recommended by Achenbach and colleagues, as internalizing and externalizing problems are not fully independent of one another (Achenbach et al. 2016). The results using cut-offs for behavioural and emotional problems are only presented in the Supplementary Material. Additionally, we tested the association of maternal alcohol use during pregnancy and childhood behavioural and emotional problems.

Missing information on the covariates was between 0 and 5%, with the exception of maternal psychopathology (18.1%). To avoid the bias of complete case analysis, we accounted for missing information on the covariates by using a multiple imputation technique, and generated 10 imputed datasets.

Table 2. Maternal cannabis and/or tobacco use during pregnancy in relation to child behavioural and emotional problems reported by teachers and self-report

		Child Behavioural and emotional Problems							
		Externalizing Problems				Internalizing Problems			
Maternal exposure		Teacher report at 7/8 years [d]				Teacher report at 7/8 years [d]			
		Model I [a]		Model II [b]		Model I [a]		Model II [b]	
	N	B (95% CI) [c]	P	B (95% CI) [c]	P	B (95% CI) [c]	P	B (95% CI) [c]	P
No use	3151	Reference		Reference					
Cannabis during	120	0.55 (0.30 to 0.81)	<0.001	0.53 (0.29 to 0.77)	<0.001	0.06 (-0.16 to 0.28)	0.60	-0.10 (-0.31 to 0.11)	0.3
Cannabis before	106	0.26 (-0.00 to 0.52)	0.05	0.27 (0.02 to 0.52)	0.03	-0.02 (-0.26 to 0.21)	0.84	-0.10 (-0.32 to 0.12)	0.3
Continued smoking	575	0.25 (0.12 to 0.37)	<0.001	0.19 (0.07 to 0.31)	0.001	0.14 (0.03 to 0.25)	0.01	0.07 (-0.03 to 0.18)	0.1
		Self-report at 9 years				Self-report at 9 years			
		Model I [a]		Model II [b]		Model I [a]		Model II [b]	
	N	B (95% CI) [c]	P	B (95% CI) [c]	P	B (95% CI) [c]	P	B (95% CI) [c]	P
No use	3143	Reference		Reference		Reference		Reference	
Cannabis during	85	0.61 (0.19 to 1.03)	0.005	0.48 (0.09 to 0.87)	0.02	0.39 (-0.07 to 0.84)	0.10	0.16 (-0.27 to 0.59)	0.4
Cannabis before	96	0.42 (0.03 to 0.81)	0.03	0.37 (0.01 to 0.74)	0.05	0.16 (-0.26 to 0.59)	0.45	0.01 (-0.39 to 0.40)	0.9
Continued smoking	385	0.29 (0.08 to 0.50)	0.006	0.26 (0.06 to 0.45)	0.009	0.09 (-0.13 to 0.32)	0.40	-0.01 (-0.23 to 0.20)	0.9

[a] Model I, adjusted for age at assessment and gender of the child, birth weight, maternal age, maternal body mass index, educational level, ethnicity, psychopathology during pregnancy, and alcohol consumption during pregnancy.
[b] Model II, additionally adjusted for externalizing problems when the outcome is internalizing problems, and vice versa.
[c] All reported regression coefficients are B-values and quantify the difference in behavioural and emotional problems scores as compared to the reference group
[d] Data were square root transformed to approximate a normal distribution.

Table 3. Cannabis use of the father in relation to child behavioural and emotional problems reported by teachers and self-report

		Child behavioural and emotional problems							
		Externalizing Problems				Internalizing Problems			
Paternal exposure		Teacher report at 7/8 years [d]				Teacher report at 7/8 years [d]			
		Model I [a]		Model II [b]		Model I [a]		Model II [b]	
	N	B (95% CI) [c]	P	B (95% CI) [c]	P	B (95% CI) [c]	P	B (95% CI) [c]	
No use	3132	Reference		Reference		Reference		Reference	
Cannabis use	378	0.37 (0.23 to 0.52)	<0.001	0.36 (0.22 to 0.49)	<0.001	0.04 (-0.09 to 0.17)	0.55	-0.07 (-0.19 to 0.06)	
		Self-report at 9 years				Self-report at 9 years			
		Model I [a]		Model II [b]		Model I [a]		Model II [b]	
	N	B (95% CI) [c]	P	B (95% CI) [c]	P	B (95% CI) [c]	P	B (95% CI) [c]	
No use	3083	Reference		Reference		Reference		Reference	
Cannabis use	298	0.29 (-0.06 to 0.52)	0.01	0.21 (-0.01 to 0.43)	0.06	0.26 (0.01 to 0.51)	0.045	0.15 (-0.09 to 0.39)	

[a] Model I, adjusted for age at assessment and gender of the child, birth weight, maternal age, maternal body mass index, educational level, ethnicity, psychopathology during pregnancy, and alcohol consumption during pregnancy.
[b] Model II, additionally adjusted for externalizing problems when the outcome is internalizing problems, and vice versa.
[c] All reported regression coefficients are B-values and quantify the difference in behavioural and emotional problems scores as compared to the reference group.
[d] Data were square root transformed to approximate a normal distribution.

RESULTS

DESCRIPTIVE INFORMATION

Women using cannabis during pregnancy were younger, had higher psychopathology scores, had lower educational levels, and more often used alcohol during pregnancy (Table 1). Children exposed to cannabis during pregnancy had a lower birth weight (Table 1).

CANNABIS USE AND SMOKING

Table 1 demonstrates that of the women who used cannabis during pregnancy, only 9.9% never smoked any tobacco during pregnancy. Pregnant women using cannabis consumed it regularly: 51 (30.2%) women reported daily consumption, 48 (28.4%) women reported weekly, and 21 (12.4%) women reported monthly cannabis consumption. 49 (29.0%) cannabis users did not report frequency of use. Pregnant women consumed either marijuana (*n* = 86) or hashish (*n* = 55); in 28 women the cannabis product was unknown. Further, 118 women consumed cannabis until pregnancy was known and 23 women continued cannabis use even after knowing to be pregnant.

SUBSTANCE USE OF THE FATHER

Most partners of cannabis consuming pregnant women also used cannabis (79.3%, see Table 1), and tobacco (79.9%). Paternal alcohol consumption was highest (89.8%) in the group of mothers who used cannabis before pregnancy.

MATERNAL CANNABIS AND TOBACCO USE IN RELATION TO CHILD BEHAVIOURAL AND EMOTIONAL PROBLEMS

Table 2 demonstrates that maternal cannabis use during pregnancy was associated with teacher-reported externalizing problems in children (Model I: B = 0.55, 95% CI: 0.30 to 0.81), but not with internalizing problems (Model I: B = -0.06, 95% CI: -0.16 to 0.28). Maternal cannabis use prior to pregnancy and tobacco smoking during pregnancy were related to externalizing problems in offspring (Model I: $B_{cannabis\ before}$ = 0.26, 95% CI: -0.00 to 0.52, $B_{tobacco}$ = 0.25, 95% CI: 0.12 to 0.37). These associations remained when additionally adjusting for internalizing problems (Model II). The effect estimates (Model II) of cannabis use during pregnancy and externalizing problems were higher than the effect estimates of tobacco use during pregnancy and child externalizing problems ($B_{difference}$ = 0.34, 95% CI: 0.07 to 0.60).

Next, we examined the associations of maternal cannabis and tobacco smoking and child self-reported behavioural and emotional problems. Consistent with the results based on the teacher reports, prenatal cannabis exposure during pregnancy was related to higher scores of externalizing problems (Model II: B = 0.48, 95% CI: 0.09 to 0.87), but not with internalizing problems. Again, maternal cannabis use prior to pregnancy (Model II: B = 0.37, 95% CI: 0.01 to 0.74) and tobacco smoking during pregnancy (Model II: B = 0.26, 95% CI: 0.06 to 0.45) were related to externalizing problems in offspring. There was no association between maternal tobacco smoking and internalizing problems. Self-reported externalizing problems did not differ between tobacco-exposed or cannabis-exposed children (Model II: $B_{difference}$= 0.22 , 95% CI: -0.22 to 0.66). The results of the logistic regression models were similar (Supplementary Table 2).

PATERNAL CANNABIS USE AND CHILD BEHAVIOURAL AND EMOTIONAL PROBLEMS

Paternal cannabis use was associated with offspring teacher-reported externalizing problems (Table 3; Model II: B = 0.36, 95% CI: 0.22 to 0.49), but not with internalizing problems (Model II: B = -0.07, 95% CI:-0.19 to 0.06). When analyses were based on child self-reports, we found that paternal cannabis use was associated with neither externalizing problems (Model II: B = -0.21, 95% CI: -0.01 to 0.43) nor internalizing problems (Model II: B = 0.15, 95% CI: -0.09 to 0.39).

In sensitivity analyses of paternal cannabis use, we excluded the mothers who used cannabis before or during pregnancy. Results were very similar; paternal cannabis use was associated with teacher-reported offspring externalizing problems (Model II: B = 0.31, 95% CI: 0.14 to 0.48), but again not to internalizing problems (Model II: B = -0.07, 95% CI: -0.22 to 0.08).

MATERNAL REPORTS OF CHILD BEHAVIOURAL AND EMOTIONAL PROBLEMS

Supplementary analyses were performed using the maternal reports of child behavioural and emotional problems (Supplementary Table 3). In these analyses, the mother reported on both the exposure and outcome. We found no associations with maternal cannabis use during pregnancy or tobacco smoking and child behavioural and emotional problems when taking into account the confounders although the sample size for this analysis was larger than in other analyses.

MATERNAL ALCOHOL USE AND CHILD BEHAVIOURAL AND EMOTIONAL PROBLEMS

We found that prenatal exposure to alcohol use was associated with less teacher-reported internalizing problems (B = -0.20; 95% CI: -0.29 to -0.12, $P < 0.001$), whereas we found no association with teacher-reported externalizing problems (B = 0.08; 95% CI: -0.02 to 0.18, $P = 0.10$). Additionally, when children's self-report was used, prenatal alcohol exposure was not related to internalizing (B = -0.03; 95% CI: -0.19 to 0.12, $P = 0.68$) or externalizing problems ((B = -0.04; 95% CI: -0.17 to 0.10, $P = 0.62$).

DISCUSSION

MAIN FINDINGS

The aim of this study was to investigate the association between prenatal cannabis exposure and child behavioural and emotional problems, using a multi-informant approach. To study causality of this association, different contrasting or negative exposures, including tobacco exposure, cannabis use before pregnancy and paternal cannabis use, were used. Overall, our findings consistently show that maternal cannabis use during pregnancy is associated with child externalizing problems. However, maternal cannabis use prior to pregnancy and tobacco smoking during pregnancy were similarly related to an increased risk of offspring externalizing problems. Moreover, cannabis use of the father was to a comparable extent associated with more child externalizing problems.

INTERPRETATION

Our findings suggest that cannabis exposure is specifically associated with offspring externalizing problems, but not internalizing problems. Importantly, both maternal and paternal cannabis exposure during pregnancy were related to externalizing problems. This was also observed for paternal cannabis use without any maternal cannabis use, and thus the association is most certainly partly due to shared familial or genetic confounding factors . Shared familial confounding factors, such as socioeconomic position and parental behaviours (e.g. poor diet) associated with both parental smoking and offspring behavioural problems, could confound the association of prenatal cannabis use and offspring behaviour (Bradley and Corwyn 2002). In our analyses, we adjusted for several socioeconomic indicators, and thus it is more likely that the association is due to common genetic factors. Smoking cannabis may be a marker for underlying psychiatric problems in parents (e.g. conduct disorder), and risk factors that predispose to smoking cannabis could be the same that predispose offspring to behavioural problems. For example, externalizing behavior, smoking and substance use initiation have been shown to have common genetic and environmental origins (Korhonen et al. 2012).

Further, the estimate of maternal cannabis use during pregnancy and offspring externalizing problems is somewhat higher than the estimate of paternal cannabis use and offspring externalizing behaviour. This may reflect an accumulated genetic predisposition, because 80% of the fathers use cannabis when mothers do so. Assortative mating in substance use disorders and other psychiatric disorders could be a plausible mechanism that results in an increased risk for externalizing problems in offspring of cannabis-using parents (Nordsletten et al. 2016). Also, maternal cannabis use prior to pregnancy was associated with offspring externalizing problems, which further supports that these susceptibilities co-occur in parents and their offspring. This may also implicate involvement of preconception effects; including epigenetic transgenerational inheritance of co-occurring substance use and psychiatric disorders (Yohn, Bartolomei, and Blendy 2015).

Second, the associations of maternal cannabis use and externalizing behavior were more prominent when the teacher reported on child

problems. The teachers' perspective is valuable and additive since teachers observe child behaviour in task-oriented situations requiring concentration. Moreover, teachers can compare a particular child's behaviour with that of a large group of classmates of the same developmental level. Lastly, teachers are unaware of any parental substance use during pregnancy. Importantly, the associations of prenatal cannabis use and externalizing problems were consistent across teacher- and child-reports, despite their low correlations .

Finally, residual confounding factors is certainly possible. It is known that mothers who use cannabis during pregnancy have different characteristics from those who do not (El Marroun et al. 2008), and these could be related to increased risk for offspring psychiatric problems. Although we have adjusted for multiple confounding factors, residual confounding may still be present. Indeed, the analyses contrasting the findings of prenatal cannabis exposure to other exposure groups with similar confounding patterns (e.g. maternal tobacco smoking; maternal cannabis use exclusively before pregnancy) are compatible with the possibility of residual confounding.

PREVIOUS STUDIES

Previous studies have demonstrated that maternal cannabis use during pregnancy was related with childhood externalizing problems, impulsivity and delinquency, assessed through mother-report, teacher-report and neuropsychological tasks (Goldschmidt, Day, and Richardson 2000; Fried, Watkinson, and Gray 1992). Although these studies were able to take into account many confounding factors, these studies were not able to contrast the findings to the potential effects of paternal cannabis use. This is important as this contrasting exposure can give insight in whether the observed effect is a direct biological intrauterine effect or whether the effect could be due to shared confounding or genetic factors (Smith 2008; Davey Smith et al. 2009).

The seemingly contradictory finding that maternal alcohol use was related to less internalizing problems and not externalizing problems is a known phenomenon, as light alcohol consumption is a marker of relative socio-economic advantage (Kelly et al. 2009; Kelly et al. 2012), also in the current population (Bakker et al. 2010).

STRENGTHS AND WEAKNESSES

Despite several strengths , such as the prospective nature of the study, the multiple informants of the outcome, information on various relevant confounders and the availability of contrasting exposures, some limitations should be discussed. First, it was not feasible to obtain clinical diagnoses. However, the instruments used are suitable for the general population as they map a continuum of psychopathology with good reliability and validity (Achenbach and Rescorla 2001b). Moreover, cannabis use was assessed only once during pregnancy and information about postnatal cannabis use was not available. Nevertheless, self-reports were in agreement with urinary THC-levels assessed in a subsample (El Marroun, Tiemeier, et al. 2011), and the cannabis use prevalence in our sample was similar to national prevalence of cannabis use among young women (Rodenburg et al. 2007). We could not investigate maternal cannabis use in the absence of paternal cannabis use as approximately 80% of the mothers using cannabis during pregnancy had cannabis-using partners. Last, we were unable to study the directionality of the associations between parental cannabis use and parental psychopathology; whether cannabis use mediated the association between parental psychopathology and offspring emotional and behavior problems or contributed to the occurrence of parental psychiatric symptoms. Future studies with repeated assessments of parental psychopathology and substance use before, during and after pregnancy will be needed to address this.

IMPLICATIONS

The current study shows that maternal cannabis use was associated with offspring externalizing problems. Nonetheless, as offspring externalizing problems were also associated with maternal cannabis use before pregnancy, maternal tobacco smoking during pregnancy, and paternal cannabis use, it is unlikely that intrauterine exposure to cannabis causally increases the risk for child externalizing problems.

(...) *When I watch them and see in the smallest of creatures the seeds of all the virtues and strengths they will one day need so badly;* (...)

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(SUPPLEMENT)

Supplemental Table 1: Descriptive statistics of tobacco use and alcohol use in each trimester in the study population

	Reference group # (n=4804)	Cannabis use during pregnancy (n=169)	Cannabis use before pregnancy (n=158)	Tobacco use throughout pregnancy, (n=772)
Tobacco use first trimester				
None (%)	90.5	12.5	51.3	6.8
<5 per day (%)	5.6	48.7	26.0	47.1
5-10 per day (%)	1.9	21.7	14.3	27.6
>10 per day (%)	2.0	17.1	8.4	18.5
Tobacco use second trimester				
None (%)	100	31.1	72.2	9.3
<5 per day (%)	-	43.4	13.5	48.0
5-10 per day (%)	-	19.7	7.5	26.3
>10 per day (%)	-	5.7	6.8	16.4
Tobacco use third trimester				
None (%)	100	33.1	73.1	9.6
<5 per day (%)	-	41.1	12.3	44.2
5-10 per day (%)	-	16.9	9.2	28.3
>10 per day (%)	-	8.9	5.4	17.8
Alcohol use first trimester				
None (%)	54.3	35.3	41.8	53.0
<1 per week (%)	26.5	32.1	29.4	25.5
1-6 per week (%)	16.8	27.6	22.2	17.8
>1 per day (%)	2.4	5.1	6.5	3.7
Alcohol use second trimester				
None (%)	65.3	54.0	44.4	62.9
<1 per week (%)	26.3	34.1	39.1	25.0
1-6 per week (%)	8.0	11.1	15.0	10.2
>1 per day (%)	0.4	0.8	1.5	2.0
Alcohol use third trimester				
None (%)	62.0	56.5	51.6	62.7
<1 per week (%)	27.7	32.3	34.4	25.8
1-6 per week (%)	10.0	10.5	12.5	10.4
>1 per day (%)	0.3	0.8	1.6	1.1

Supplemental Table 2: Maternal cannabis and/or tobacco use during pregnancy in relation to the risk of child behavioural and emotional problems reported teachers and self-report

	Child behavioural and emotional problems							
	Externalizing Problems				Internalizing Problems			
Maternal exposure	Teacher report at 7/8 years				Teacher report at 7/8 years			
	Model I [a]		Model II [b]		Model I [a]		Model II [b]	
Exposure groups	N	OR (95% CI) [c]	P	OR (95% CI) [c]	P	OR (95% CI) [c]	P	OR (95% CI) [c]	P
No use	3151	1.0 (ref)		1.0 (ref)		1.0 (ref)		1.0 (ref)	
Cannabis during	120	1.54 (1.02 to 2.32)	0.04	1.59 (1.04 to 2.42)	0.03	0.99 (0.65 to 1.53)	0.98	0.89 (0.57 to 1.38)	0.62
Cannabis before	106	1.28 (0.80 to 2.04)	0.30	1.27 (0.78 to 2.06)	0.33	0.97 (0.61 to 1.56)	0.91	0.92 (0.57 to 1.49)	0.74
Continued smoking	575	1.37 (1.11 to 1.70)	0.005	1.31 (1.05 to 1.64)	0.02	1.39 (1.13 to 1.70)	0.002	1.31 (1.06 to 1.62)	0.01
	Self-report at 9 years				Self-report at 9 years			
	Model I [a]		Model II [b]		Model I [a]		Model II [b]	
Exposure groups	N	OR (95% CI) [c]	P	OR (95% CI) [c]	P	OR (95% CI) [c]	P	OR (95% CI) [c]	P
No use	3143	1.0 (ref)		1.0 (ref)		1.0 (ref)		1.0 (ref)	
Cannabis during	85	2.06 (1.32 to 3.22)	0.001	1.98 (1.25 to 3.14)	0.003	1.46 (0.90 to 2.38)	0.13	1.22 (0.73 to 2.01)	0.44
Cannabis before	96	1.34 (0.88 to 2.06)	0.17	1.34 (0.87 to 2.08)	0.17	1.09 (0.68 to 1.76)	0.72	1.02 (0.62 to 1.67)	0.94
Continued smoking	385	1.45 (1.15 to 1.82)	0.001	1.42 (1.13 to 1.80)	0.003	1.20 (0.93 to 1.55)	0.15	1.10 (0.85 to 1.43)	0.47

[a] Model I, adjusted for age at assessment and gender of the child, birth weight, maternal age, maternal body mass index, educational level, ethnicity, psychopathology during pregnancy, and alcohol consumption during pregnancy.
[b] Model II, additionally adjusted for externalizing problems when the outcome is internalizing problems, and vice versa.
[c] The OR represents the odds that behavioural and emotional problems (above the 80th percentile) will occur given the exposures as compared to the odds of the outcome occurring in the absence of that exposure (non-exposed group is the reference).

Supplemental Table 3: Maternal and paternal cannabis use in relation to child behavioural and emotional problems reported by mothers

	Child behavioural and emotional problems							
	Externalizing Problems				Internalizing Problems			
Maternal cannabis use	Maternal report at 9 years [d]				Maternal report at 9 years [d]			
	Model I [a]		Model II [b]		Model I [a]		Model II [b]	
	N	B (95% CI) [c]	P	B (95% CI) [c]	P	B (95% CI) [c]	P	B (95% CI) [c]
No use	3536	Reference		Reference				
Cannabis during	94	0.18 (-0.08 to 0.43)	0.18	0.16 (-0.05 to 0.38)	0.15	0.03 (-0.21 to 0.27)	0.83	-0.06 (-0.21 to 0.26)
Cannabis before	112	0.04 (-0.19 to 0.27)	0.74	-0.09 (-0.29 to 0.10)	0.36	0.23 (0.01 to 0.45)	0.04	0.21 (0.01 to 0.45)
Continued smoking	454	0.08 (-0.04 to 0.21)	0.18	0.07 (-0.03 to 0.17)	0.18	0.02 (-0.09 to 0.14)	0.68	-0.02 (-0.09 to 0.14)
Cannabis use of the biological father [e]	Maternal report at 9 years [d]				Maternal report at 9 years [d]			
	Model I [a]		Model II [b]		Model I [a]		Model II [b]	
	N	B (95% CI) [c]	P	B (95% CI) [c]	P	B (95% CI) [c]	P	B (95% CI) [c]
No use	3427	Reference		Reference		Reference		Reference
Cannabis use	206	0.36 (0.19 to 0.53)	0.001	0.19 (0.04 to 0.33)	0.01	0.29 (0.14 to 0.46)	<0.001	0.12 (-0.02 to 0.26)

[a] Model I, adjusted for age at assessment and gender of the child, birth weight, maternal age, maternal body mass index, educational level, ethnicity, psychopathology during pregnancy, and alcohol consumption during pregnancy.
[b] Model II, additionally adjusted for externalizing problems when the outcome is internalizing problems, and vice versa.
[c] All reported regression coefficients are B-values and quantify the difference in behavioural and emotional problems scores as compared to the reference group
[d] Data were square root transformed to approximate a normal distribution.
[e] Children of mothers who used cannabis before or during pregnancy were excluded from the analyses.

(ABSTRACT)

BACKGROUND

Callous traits during childhood, e.g. lack of remorse and shallow affect, are a key risk marker for antisocial behavior. Although callous traits have been found to associate with structural and functional brain alterations, evidence to date has been almost exclusively limited to small, high-risk samples of boys. Here, we characterized gray and white matter brain correlates of callous traits in over 2000 children from the general population.

METHODS

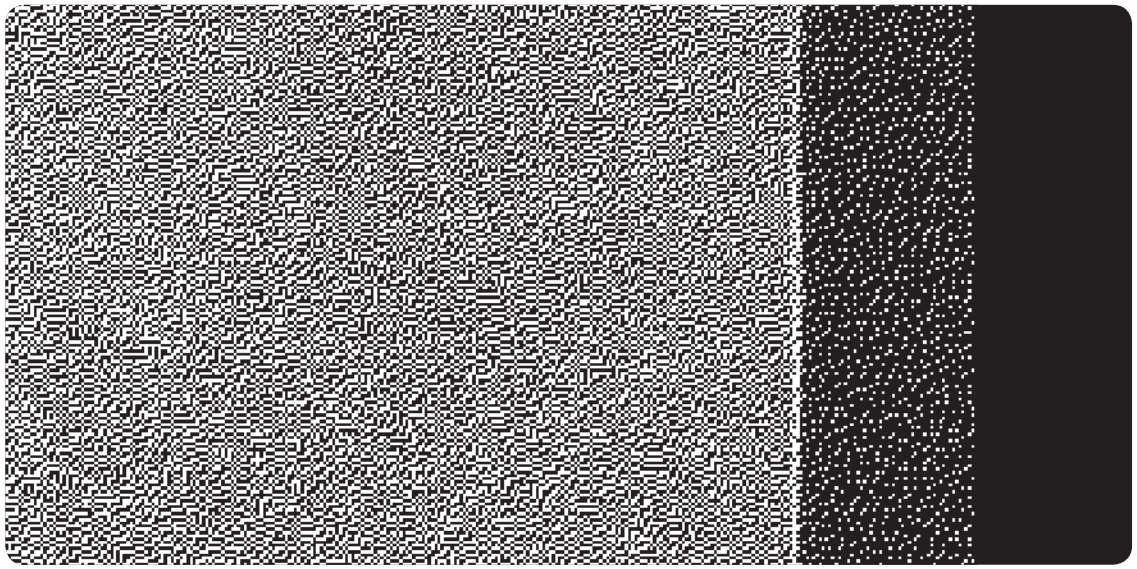
Data on mother-reported callous traits and brain imaging were collected at age 10 years from the Generation R Study. Structural MRI was used to investigate brain morphology using volumetric indices and whole-brain analyses (n = 2146); diffusion tensor imaging (DTI) was used to assess global and specific white matter microstructure (n = 2059).

RESULTS

Callous traits were associated with lower global brain (e.g. total brain) volumes as well as decreased cortical surface area in frontal and temporal regions. Global mean diffusivity was negatively associated with callous traits, suggesting higher white matter microstructural integrity in children with elevated callous traits. Multiple individual tracts contributed to this global association, including the uncinate and cingulum. While no sex differences were observed for global volumetric indices, white matter associations were present only in girls .

CONCLUSIONS

This is the first study to provide a systematic characterization of the structural neural profile of callous traits in the general pediatric population. These findings extend previous work based on selected samples by demonstrating that childhood callous traits in the general population are characterized by widespread macro- and microstructural differences across the brain.



(...) *when I see their obstinacy as future resolution and firmness of character, and their caprice as good humour* (...)

CHAPTER 10

NEURAL PROFILE OF CALLOUS TRAITS IN CHILDREN:

A POPULATION-BASED NEUROIMAGING STUDY

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INTRODUCTION

Callous traits, including shallow affect, remorselessness, and a callous lack of empathy, are a key risk marker for antisocial behavior (Hare and Neumann 2008). In childhood, callous traits are part of a broader set of callous-unemotional/psychopathic traits used to identify a particularly problematic subgroup of children with conduct problems, as operationalized by the DSM-5 specifier of "low prosocial emotions" (American Psychiatric Association 2013), distinguished by more severe and chronic antisocial behavior and at least partially distinct etiology to their presentation (Viding and McCrory 2018). Effects extend well beyond childhood, as callous traits independently predict a wide range of negative outcomes across the lifecourse, including adult psychopathy, antisocial personality disorder, criminality, and substance abuse (Blair et al. 2014). Consequently, youth callous traits are an important target for etiologic research, prevention and intervention (Wakschlag et al. 2018).

A growing number of studies have been conducted to characterize the neurodevelopment of callous and related traits. Several different measures, varying in their coverage of specific behaviors, have been used to study callous-unemotional traits (Salekin 2017; Wakschlag et al. 2018), and this needs to be considered when interpreting the existing literature and, in particular, findings that have not replicated across studies. The majority of these have employed task-based functional magnetic resonance imaging (fMRI) in clinical/extreme samples of males (Baker et al. 2015; Alegria, Radua, and Rubia 2016). Based on a recent meta-analysis of fMRI studies, which included 108 cases and 115 controls from 9 studies, youth with elevated psychopathic traits demonstrated decreased activity in ventromedial prefrontal cortex and the limbic system, and increased activation in fronto-striatal regions (Alegria, Radua, and Rubia 2016). These regions are known to be involved in reward processing and affect regulation (Murray 2007), and partly converge with findings from structural MRI (sMRI) studies. Indeed, a recent meta-analysis including 188 cases and 122 controls pooled from 5 studies reported gray matter volume reductions of the putamen in youth with elevated callous-unemotional traits (Rogers and De Brito 2016). However, findings from structural studies regarding other brain regions have been inconsistent, likely due to heterogeneity in samples, analytic methods and participant age (Baker et al. 2015; Waller et al. 2017). Finally, very few studies have employed diffusion tensor imaging (DTI) to characterize the microstructural properties of white matter associated with youth callous traits. Several studies have been published on externalizing behavior more broadly (Waller et al. 2017), and we have recently demonstrated that lower whole-brain white matter connectivity was associated with more delinquent behavior in children (Bolhuis et al. 2018). Publications on callous traits specifically are more sparse with small samples, and these have reported mixed findings, with both increased and decreased white matter integrity observed in various tracts (Waller et al. 2017). Most of these studies have uniquely focused on the uncinate fasciculus, a fiber bundle that connects prefrontal and subcortical structures, although recent work supports the involvement of a wider set of tracts (Pape et al. 2015).

Overall, the above evidence points to neurobiological alterations associated with callous traits (and related phenotypes). However, knowledge on the neurodevelopmental underpinnings of callous traits is limited in four key ways. First, findings have been primarily based on small, selected samples, so that it remains unclear to what extent structural brain differences are associated with callous traits in the general pediatric population. This is a notable gap, given compelling evidence that callous traits exist along a continuum in the general population (Viding et al. 2005). Second, no neuroimaging study has examined both sMRI and DTI data to assess both gray and white brain structural correlates of youth callous traits–an important step towards integrating mixed findings in the literature. Third, existing studies have focused primarily on males due to the higher prevalence of conduct problems. As such, little is known about neuroanatomical correlates of callous traits in girls, and whether these differ from boys. Fourth, while previous imaging studies have largely focused on specific brain regions, it is important to employ a whole-brain approach to study callous traits as it is well-known from the wider neuroimaging literature that the brain functions in networks (Di Martino et al. 2014).

Here we examined the relationship between brain structure and callous traits in over 2000 children, the largest neuroimaging study on pediatric callous traits to date, using data from a population-based cohort. Our aims were to assess both (i) structural brain morphology and (ii) white matter microstructure in relation to child callous traits. Both aims were addressed following a hierarchical approach, i.e. first global metrics were analyzed, followed by more detailed regional analysis if an association with global measures was observed. Based on existing literature, we expected global gray matter reductions as well as regional reductions in sub-cortical structure volumes. Regarding DTI analyses, we had no specific hypotheses given the mixed findings in the literature. Potential sex differences were also explored. However, because most prior neuroimaging studies have been based on males, we had no specific hypothesis.

METHODS AND MATERIALS

STUDY POPULATION

This cross-sectional study was embedded in the Generation R Study, a prospective population-based cohort from Rotterdam, the Netherlands (Kooijman et al. 2016). Study protocols were approved by the local ethics committee, and written informed consent and assent was obtained from all parents and children. At mean age ten years (range 8-11), mothers completed a questionnaire about their child's callous traits and children were invited to participate in a neuroimaging assessment (White et al. 2017). For the current study, participants were included if they had data on callous traits and a sMRI scan or DTI scan available (N = 2146, and N = 2059, respectively, see Figure S1).

MEASURES

CALLOUS TRAITS

Callous traits were assessed through maternal report when the child was on average ten years old, using a brief validated questionnaire adapted from the Youth Self-Report and the Inventory for Callous-Unemotional Traits (Pardini, Obradovic, and Loeber 2006). The questionnaire comprises 7 items on mainly interpersonal callous traits, which were scored on a 4-point scale (range 0–21, see Figure S2), including "Does not find other people's feelings important", and "Is cold and indifferent". Although this measure does not comprehensively capture the full spectrum of unemotional or psychopathic traits, it has been shown to adequately capture childhood callous traits on a dimensional scale (Pardini, Obradovic, and Loeber 2006), correlates strongly with other measures of youth psychopathy and is predictive of adult antisocial traits (Pardini and Loeber 2008). Endorsement of the seven items is shown in Table S1. The Cronbach's α in the current sample was 0.73.

OTHER BEHAVIORAL DATA

At age 10 years, co-occurring emotional and behavioral problems at were assessed through mother-report and child-report using the well-validated Child Behavior Checklist and Brief Problem Monitor, respectively (Achenbach and Rescorla 2001; Achenbach et al. 2011), and mothers and children also completed the Strengths and Difficulties Questionnaire Prosocial scale (Goodman 2001). Concurrently, maternal psychopathology was assessed through four subscales of the self-reported Brief Symptom Inventory (Derogatis and Melisaratos 1983). Child intelligence (IQ) was measured at six years with the Snijders-Oomen non-verbal intelligence test (Tellegen et al. 2005). See Supplement 1for more detailed information.

BRAIN IMAGING

An overview of the imaging procedure, sequences, and quality assessment has been described previously (White et al. 2017), and can be found in Supplement 2. Every child was invited to participate in a mock scanning session prior to the MRI scan to familiarize them with the procedure. If at any point, she/he was too anxious about the procedure, they did not progress to the MRI scan. All images were acquired on a 3T GE MR750W Discovery scanner using an 8-channel head-coil.

COVARIATES

All analyses were adjusted for the following covariates. Child sex and date of birth were retrieved from birth records. Child ethnicity was defined according to the classification of Statistics Netherlands, i.e. Dutch, Other Western, and Other Non-Western. Maternal educational level was categorized into primary (no or primary education), secondary (lower and intermediate vocational training), and higher (higher vocational training and university) educational attainment.

STATISTICAL ANALYSES

Prior to the main analyses, we validated our measure of callous traits by examining whether correlations with mother- and child-reported emotional and behavioral problems, prosocial behavior, and IQ were in line with the previous literature. We then proceeded to examine neural correlates of callous traits, specifically (i) structural brain morphology and (ii) white matter microstructure, using separate linear regressions. All sMRI and DTI analyses were adjusted for covariates as described above. A hierarchical step-wise approach was conducted to limit the number of comparisons.

With respect to sMRI measures, first total global and sub-cortical volumetric indices were assessed in association with callous traits. Analyses pertaining to sub-cortical volumes were corrected for intracranial volume. A false-discovery rate (FDR) correction was applied to these analyses to address multiple testing (Benjamini and Hochberg 1995; Nichols et al. 2017). If an association with any global measure was observed, subsequent vertex-wise analyses were conducted to investigate local differences in cortical morphology associated with callous traits.

With respect to DTI, initial analyses were performed with global FA and MD in association with callous traits. Next, if an association between global FA or MD and callous traits was observed, (1) subsequent analyses were conducted on individual white matter tracts and (2) associations with AD and RD (which are composites of MD, see Supplement 2) were explored. For these analyses, multiple-testing was addressed using an FDR-adjustment.

In sensitivity analyses, our models were additionally adjusted for co-occurring emotional and behavioral problems, non-verbal IQ and maternal psychiatric problems, in line with recent recommendations based on developmental studies (Viding and McCrory 2018; Achenbach et al. 2016). In addition, sex differences of observed associations were explored using interaction analyses. Similarly, we investigated whether CBCL conduct problems moderated the association of callous traits with global volumetric and white matter outcomes. We also explored non-linear relationships by adding quadratic terms.

Because of skewness (Figure S2), callous traits sum scores were square root transformed to approach a normal distribution. Standardized coefficients are presented throughout. All analyses were conducted in R statistical software (R Core Team 2015). Missing values on covariates were dealt with using multiple imputations in MICE 2.25 (van Buuren and Groothuis-Oudshoorn 2011); estimates from analyses of 100 imputed datasets were pooled.

RESULTS

BEHAVIORAL VALIDATION OF CALLOUS TRAITS

As expected, callous traits showed high positive correlations with mother-reported conduct problems, followed by ODD and ADHD symptoms. In contrast, we observed significantly lower correlations for affective, anxiety and somatic symptoms (Table 1, difference in correlations, all Z-score > 4.9, $P < 0.001$). Similarly, child-reported externalizing and attention problems correlated more strongly with callous traits than internalizing problems (all Z-score > 5.3, $P < 0.001$). Mother-reported and child-reported prosocial behavior were negatively correlated with callous traits.

STRUCTURAL BRAIN MORPHOLOGY

Total brain, cortical gray matter, and white matter volumes were all negatively associated with callous traits (Table 2). Right amygdala volume was negatively associated with callous traits, which did not

Table 1: Sample characteristics.

	N (% missing data)	Descriptive statistics	Correlation with callous traits, *r*
Child characteristics			
Age at MRI, mean (SD)	2146 (0% missing)	10.10 (0.58)	-
Sex, proportion girls	2146 (0% missing)	49.9%	-
Ethnicity	2132 (0.7% missing)		
Dutch		68.6	-
Other, Western		8.3	-
Other, non-Western		22.8	-
Callous traits, median (IQR)	2146 (0% missing)	2.00 (3.00)	-
Non-verbal IQ at age 6 years, mean (SD)	1904 (11.3% missing)	104.4 (14.64)	-0.08**
Mother-reported CBCL, median (IQR)			
Affective Problems	2078 (3.3% missing)	1.00 (2.00)	0.23**
Anxiety Problems	2074 (3.5% missing)	0.00 (2.00)	0.15**
Somatic Complaints	2062 (3.9% missing)	0.00 (2.00)	0.09**
ADHD Problems	2073 (3.4% missing)	2.00 (4.00)	0.36**
ODD Problems	2071 (3.6% missing)	1.00 (2.00)	0.39**
CD Problems	2078 (3.3% missing)	0.00 (1.00)	0.47**
Child-reported BPM, median (IQR)			
Internalizing problems	2044 (4.8% missing)	2.00 (3.00)	0.07**
Externalizing problems	2042 (4.8% missing)	2.00 (3.00)	0.22**
Attention problems	2042 (4.8% missing)	3.00 (3.00)	0.20**
SDQ – Prosocial scale, median (IQR)			
Mother-reported	2098 (2.2% missing)	9.00 (2.00)	-0.22**
Child-reported	2056 (4.2% missing)	9.00 (2.00)	-0.12**
Maternal characteristics			
Educational level, %	2020 (6.0% missing)		
High		66.7	
Medium		31.7	
Low		1.6	

Note: ** $P < 0.01$
Abbreviations: SD, standard deviation; IQR, interquartile range; IQ, intelligence quotient; CBCL, Child Behavior Checklist; ADHD, attention deficit/hyperactivity disorder; ODD, oppositional defiant disorder; CD, conduct disorder; BPM, Brief Problem Monitor; SDQ, Strengths and Difficulties Questionnaire

Table 2: Association of global structural volumetric and global white matter microstructural measures with callous traits.

	Callous traits		
	β (95% CI)	*P*	FDR-adjusted *P*
Structural volumetric measures (*N* = 2146)			
Total brain volume	-0.10 (-0.15;-0.05)	<0.001	-
Cortical grey matter volume	-0.10 (-0.15;-0.05)	<0.001	<0.001
White matter volume	-0.08 (-0.13;-0.03)	0.001	0.003
Subcortical structures			
Left amygdala	-0.03 (-0.08;0.02)	0.194	0.652
Right amygdala	-0.06 (-0.11;-0.01)	0.030	0.420
Left hippocampus	-0.03 (-0.08;0.02)	0.233	0.652
Right hippocampus	-0.02 (-0.07;0.04)	0.559	0.862
Left thalamus	0.00 (-0.06;0.06)	0.950	0.987
Right thalamus	-0.03 (-0.09;0.03)	0.361	0.760
Left caudate	-0.03 (-0.08;0.02)	0.223	0.652
Right caudate	-0.04 (-0.09;0.01)	0.132	0.652
Left putamen	-0.01 (-0.06;0.04)	0.616	0.862
Right putamen	0.00 (-0.05;0.05)	0.987	0.987
Left globus pallidus	0.00 (-0.05;0.05)	0.956	0.987
Right globus pallidus	-0.01 (-0.06;0.03)	0.562	0.862
Left nucleus accumbens	0.02 (-0.03;0.07)	0.380	0.760
Right nucleus accumbens	0.01 (-0.04;0.05)	0.751	0.956
White matter microstructural measures (*N* = 2059)			
Global fractional anisotropy (FA)	0.01 (-0.03;0.06)	0.633	-
Global mean diffusivity (MD)	-0.06 (-0.11;-0.02)	0.006	-

Note: All analyses are corrected for child sex, child age at MRI visit, child ethnicity, and maternal educational level. Sub-cortical volumes are additionally adjusted for intracranial volume. Estimates reflect standardized coefficients.

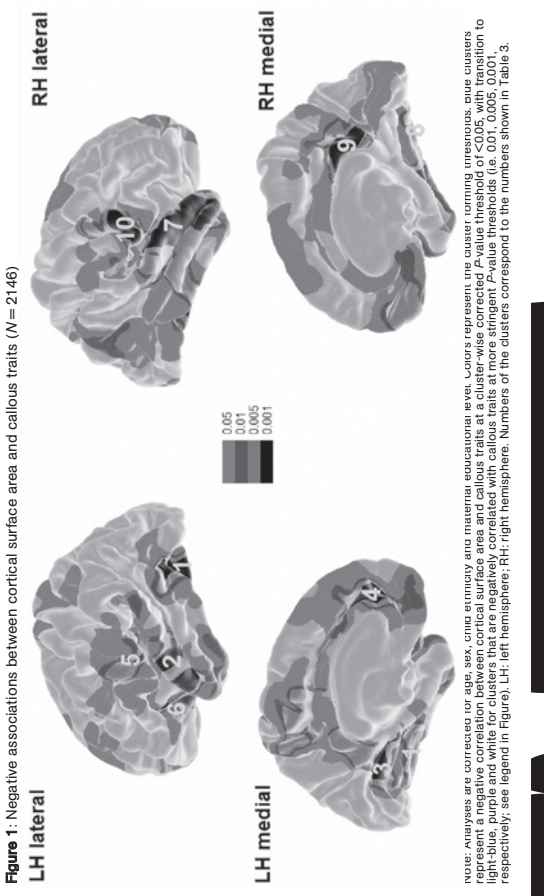

Figure 1: Negative associations between cortical surface area and callous traits ($N = 2146$)

Note: Analyses are corrected for age, sex, child ethnicity and maternal educational level. Colors represent the cluster forming thresholds. Blue clusters represent a negative correlation between cortical surface area and callous traits at a cluster-wise corrected *P*-value threshold of <0.05, with transition to light-blue, purple and white for clusters that are negatively correlated with callous traits at more stringent *P*-value thresholds (i.e. 0.01, 0.005, 0.001, respectively; see legend in Figure). LH: left hemisphere; RH: right hemisphere. Numbers of the clusters correspond to the numbers shown in Table 3.

(Full colour image presented on page 16.)

Table 3: Vertex-wise analyses of cortical surface area and callous traits ($N = 2146$).

Hemisphere and region	Cluster size (mm^2)	Talairach Coordinates (x, y, z)	Number of Vertices within Cluster	β (average across cluster)	Cluster-wise *P*-values
Left					
1. Fusiform	1476.88	-41.8, -47.3, -14.0	2445	-0.08	0.0001
2. Superior temporal	918.28	-51.8, 8.0, -18.0	1718	-0.07	0.0001
3. Lingual	681.36	-20.7, -54.0, -2.9	1422	-0.08	0.0003
4. Superior frontal	522.48	-13.7, 45.9, 3.5	973	-0.08	0.0016
5. Post-central	457.15	-51.7, -12.5, 15.9	1209	-0.06	0.0036
6. Lateral orbitofrontal	357.56	-32.2, 26.6, -10.3	727	-0.07	0.0127
Right					
7. Middle temporal	1446.11	49.3, 7.5, -32.9	2541	-0.08	0.0001
8. Fusiform	545.16	41.6, -46.3, -16.6	1066	-0.06	0.0012
9. Isthmus of cingulate	411.04	6.0, -20.1, 20.9	1036	-0.07	0.0065
10. Post-central	396.81	60.0, -8.2, 15.9	872	-0.07	0.0077

Note: Analyses are corrected for age, sex, child ethnicity and maternal educational level. Numbers of the clusters correspond to the numbers shown in Figure 1. Cluster forming threshold of 0.001.

survive FDR-correction. No associations were found between sub-cortical volumes and callous traits. Similar results were observed in analyses with additional adjustment for co-occurring psychiatric problems, non-verbal IQ, and maternal psychopathology (Table S2-S4). In vertex-wise analyses, ten brain regions showed negative correlations between cortical surface area and callous traits (Table 3, Figure 1), which were localized in the frontal and temporal lobes of both hemispheres. No vertex-wise associations were found between cortical thickness and callous traits. Three gyrification clusters in the temporal lobe were negatively associated with callous traits (Table S5). Additional adjustment for IQ and maternal psychopathology did not considerably alter these observations (Table S7-S8), but after adjustment for co-occurring psychiatric problems only the superior frontal gyrus was associated with callous traits (Table S6).

WHITE MATTER MICROSTRUCTURE

Global MD, but not global FA, was negatively associated with callous traits (Table 2). Similarly, global AD and RD were negatively associated with callous traits (Table S9). Several white matter tracts contributed to this global association (Table 4), including the superior longitudinal fasciculus, corticospinal tract, uncinate and cingulum. These associations all survived FDR-correction. Comparable results were observed in analyses with additional adjustment for co-occurring psychiatric problems, non-verbal IQ, and maternal psychopathology (Table S2-4; S10-S12). Callous traits were negatively associated with AD of the inferior and superior longitudinal fasciculi and corticospinal tract, and with uncinate and cingulum RD (Table S13). A visualization of the associated white matter tracts is presented in Figure S2.

SEX INTERACTION ANALYSES

Callous traits were significantly higher in boys than in girls (2.33 versus 1.85, $t = 5.1$, $P < 0.001$). Boys scored higher on almost all callousness items (Table S14-15); correlations between behavioral problems and callous traits were similar across sexes. Non-verbal IQ negatively correlated with callous traits in boys but not in girls

Table 4: Associations between mean diffusivity (MD) in individual white matter tracts and callous traits.

	β (95% CI)	*P*	FDR-adjusted *P*
Inferior longitudinal fasciculus	-0.04 (-0.09;0.00)	0.072	0.089
Superior longitudinal fasciculus	-0.06 (-0.11;-0.01)	0.010	0.021
Forceps minor	-0.04 (-0.08;0.00)	0.076	0.089
Forceps major	-0.01 (-0.06;0.03)	0.528	0.528
Corticospinal tract	-0.15 (-0.26;-0.04)	0.008	0.021
Uncinate fasciculus	-0.06 (-0.11;-0.02)	0.002	0.014
Cingulum bundle	-0.06 (-0.10;-0.01)	0.012	0.021

Note: All analyses are corrected for child sex, child age at MRI visit, child ethnicity, and maternal educational level. Microstructural properties of left and right tracts were combined and weighted for their respective volumes, except for forceps minor and major. Estimates reflect standardized coefficients.

(Table S16). No interaction was observed for structural volumetric measures (Table S17). A significant gender-by-brain interaction was observed for the associations of MD with callous traits ($P = 0.005$). Stratified analyses demonstrated that our findings in the full sample were driven by the associations in girls, and these effects were observed in several tracts across the brain (Table S18-S19). No such associations were found in boys.

SENSITIVITY ANALYSES

Conduct problems did not moderate the associations of callous traits with global volumetric and white matter outcomes (Table S20). Associations with quadratic terms were all non-significant (Table S21).

DISCUSSION

This is the first study to characterize the structural neural profile of callous traits in the general pediatric population. Based on sMRI and DTI data from over 2000 children, we demonstrate that callous traits at age 10 are characterized by widespread macrostructural and microstructural differences across the brain. We highlight three key findings. First, childhood callous traits were associated with reduced global gray matter, and decreases in cortical surface area and gyrification across several frontal and temporal areas. These observations are consistent with prior research using high-risk samples. Second, we observed increased global white matter microstructure in children with elevated callous traits, suggesting increased white matter integrity across various white matter tracts. Third, we found that white matter – but not gray matter – associations differed by sex, with associations only observed in girls. Together, the present findings contribute to a more complete understanding of the relationship between brain structure and callous traits, and may be used as a guiding framework for future research to uncover causal neurodevelopmental pathways.

Findings from the sMRI analyses indicated that callous traits are associated with lower global brain volumes. More specifically, decreased cortical surface area and reduced gyrification was observed

in various brain regions, including the temporal gyri and several (pre-)frontal gyri. These regions have previously been associated with behavioral inhibition, social cognition, and emotion regulation (Aron, Robbins, and Poldrack 2004; Ochsner and Gross 2005; Aoki et al. 2014; Fairchild et al. 2014), which have been implicated in the development of callousness (Viding and McCrory 2018; Salekin 2017). Our findings corroborate studies that observed gray matter volume reductions in orbitofrontal, cingulate, and temporal cortices in older youths with callous traits in the clinical range (Rogers and De Brito 2016), and support others that observed reduced cortical surface or gyrification across similar regions (Hyatt, Haney-Caron, and Stevens 2012; Wallace et al. 2014; Fairchild et al. 2015; Sarkar et al. 2015). We identified a nominally significant association between callous traits and lower right amygdala volume, which did not survive multiple-testing correction when accounting for other subcortical regions. While aberrant amygdala function has been robustly associated with callous-unemotional traits (Alegria, Radua, and Rubia 2016), structural volumetric differences of the amygdala are rarely observed (Rogers and De Brito 2016; De Brito et al. 2009; Fairchild et al. 2011; Fairchild et al. 2013; Sebastian et al. 2016). This inconsistency between structural and functional neuroimaging findings could partly be explained by the use of different significance thresholds in studies taking a region-of-interest vs. whole-brain approach. Our findings suggest the involvement of many regions with small effects. By extending these clinical MRI studies, our findings corroborate the notion that callous traits exist along a continuum in the general population, which has also been evidenced in genetic studies (Viding and McCrory 2018; Wakschlag et al. 2018; Viding et al. 2005). Moreover, associations remained consistent after additional adjustment for co-occurring emotional, behavioral and attention problems, IQ, and maternal psychopathology. In other words, while callous traits were significantly associated with other psychiatric symptoms (including conduct and ADHD problems) and IQ–consistent with the extant literature–these comorbid symptoms did not explain our global neuroimaging findings. Co-occurring emotional and behavioral problems did, however, account for a large portion of the explained variance in vertex-wise cortical surface area analyses, supporting the presence of at least some shared neural alterations in callous traits

and comorbid psychiatric problems (Viding and McCrory 2018; Blair et al. 2014). Of interest, unique variance for callous traits was observed in the superior frontal gyrus, which has been linked to callous traits in clinical cohorts (Fairchild et al. 2015; De Brito et al. 2009).

Whereas structural brain connectivity has been examined in the context of externalizing problems more generally (Waller et al. 2017; Bolhuis et al. 2018), few studies to date have examined the white matter microstructure profile of callous traits. This work has mainly focused on the uncinate fasciculus in older, selected samples, and produced mixed results, reporting both lower and higher microstructure in adolescents with elevated callous traits (Waller et al. 2017; Sethi et al. 2018). Two studies employing a whole-brain approach – both of which are based on data from adolescent (primarily males) arrestee cohorts – reported that callous traits were associated with higher white matter integrity in many tracts across the brain, including the corticospinal tract, superior longitudinal fasciculus and uncinate (Pape et al. 2015; Puzzo et al. 2017). These findings are consistent with the higher microstructural integrity in various tracts observed in the current study, e.g. uncinate and cingulum, which connect frontal with temporal/parietal brain regions (Hoppenbrouwers et al. 2013; Breeden et al. 2015; Finger et al. 2012). This is noteworthy considering the substantial differences in design and sample characteristics between these studies and ours, including the focus on different developmental periods, proportion of boys/girls, and the use of a high-risk versus general population sample. The decreases in MD identified across these studies suggest *higher* white matter microstructure, possibly indicating accelerated or precocious white matter development in children with elevated callous traits (Di Martino et al. 2014). Importantly, decreased integrity has also been observed within high-risk samples (Hoppenbrouwers et al. 2013; Breeden et al. 2015; Finger et al. 2012). The reason for such discrepancy is unclear, potential reasons include different sampling strategies, varying levels of exposure to adversities and comorbid psychiatric problems, case-control versus dimensional perspectives, and different definitions of the callousness phenotypes. Our current findings are in contrast with our previous publication where we showed *lower* white matter microstructure in pre-adolescent children

with elevated levels of delinquent behavior (Bolhuis et al. 2018), suggesting that callous traits and other externalizing behaviors are associated with differential neural correlates even though these behaviors are correlated. This is consistent with fMRI studies showing, for example, amygdala reactivity to fearful faces to be negatively associated with callous traits and positively associated with conduct problems across multiple independent samples, despite these psychiatric phenotypes being positively correlated with one another (Viding et al. 2012; Marsh et al. 2008; White et al. 2012). Findings from sMRI and DTI have been much less consistent (Rogers and De Brito 2016; Waller et al. 2017), although differential amygdala volume reductions have been observed for callous-unemotional versus conduct problems (Cohn et al. 2016; Cardinale et al. 2018). In this study, conduct problems were not found to moderate associations between callous traits and global brain measures. Importantly, in sensitivity analyses we adjusted for all co-occurring problems, which left our sMRI and DTI findings unchanged even though callous traits were substantially correlated with externalizing behaviors. This, together with our previous observations (Bolhuis et al. 2018), suggests specific brain-callousness correlates independent of other types of psychopathology, indicating that there is added value in screening for callous traits in children at elevated risk for antisocial behavior.

This is the first study to examine neural correlates of callous traits using both sMRI and DTI. Overall, our findings corroborate (1) previous high-risk sMRI studies reporting associations between callous traits and *lower* brain volume across frontal and temporal regions; and (2) previous high-risk DTI studies indicating *higher* microstructural integrity of the white matter tracts connecting these areas (De Brito et al. 2009; Wallace et al. 2014; Fairchild et al. 2015; Pape et al. 2015; Puzzo et al. 2017; Sethi et al. 2018). As such, our findings support these seemingly discrepant associations, and suggest that these are not simply the result of methodological differences between studies. The inverse relationship between the sMRI and DTI findings could potentially indicate decreased cortical functioning and consequently more dysregulated white matter connectivity, or vice versa (Di Martino et al. 2014). Multi-modal neuroimaging approaches incorporating fMRI assessments are required to disentangle the origins of these observations.

While boys and girls are known to differ considerably in prevalence of callous traits and trajectories of brain development (Clayden et al. 2012; Viding and McCrory 2018), it is unclear whether there are sex differences in the neural profile of callous traits, as existing studies have primarily focused on males. The equal distribution of boys and girls in our sample offered a unique opportunity to address this gap. We found no sex differences in global volumetric measures. However, we did find that the relation of global white matter microstructure with callous traits was only significant in girls. Given that white matter has been shown to develop more quickly in girls compared with boys (Clayden et al. 2012), it is possible that our findings reflect advanced white matter maturation in girls with elevated callous traits and thus potential residual (brain) age confounding. In *post-hoc* analyses, we found that age did not moderate the association of global MD, AD or RD with callous traits in girls (all $P > 0.100$). However, potentially chronological age does not adequately capture differences in neurobiological maturation (Cole et al. 2018). Recent smaller studies have observed more pronounced cortical differences for callous traits in adolescent boys versus girls (Raschle et al. 2018), which is not what we observed here. These findings could potentially signify that callous traits and their associated neural profile reflect differential development in girls compared with boys. Repeated neuroimaging assessments at later ages – in combination with pubertal development measures – will be particularly valuable for clarifying whether these sex differences persist across brain development or whether the developmental trajectories are similar for boys and girls, with possibly different onsets.

Our study had several strengths, including the use of a large sample of non-selected children from the community and the analysis of both sMRI and DTI data. Our hierarchical analytical approach allowed us to investigate both global and specific brain metrics without substantially increasing the risk of type II error. Stringent sensitivity analyses further enabled us to ascertain that our findings were robust to additional adjustment for co-occurring psychiatric problems, IQ, and maternal psychopathology. Finally, our study was the first to examine neuroanatomical correlates of callous traits in a sample with an equal distribution of boys and girls. Despite these

strengths, several limitations should be noted. First, our measure of callous traits did not adequately cover unemotional/affective aspects, which are important features of callous-unemotional and broader psychopathic traits, and which have been studied in the wider literature in clinical samples (Wakschlag et al. 2018). Future work will need to take this limitation into account by exploring associations across a broader spectrum of traits (Salekin 2017) and, additionally, employ a multi-informant approach to childhood callous traits. Second, our findings were cross-sectional and, hence, should be interpreted as a neurobiological characterization of callous traits, rather than an underlying biological mechanism. Furthermore, we were unable to assess whether observed brain-behavior associations predicted functional outcomes, both concurrently and longitudinally, such as academic performance. Furthermore, the participants are still too young (i.e. do not have enough variability in behavior) for examining other relevant functional domains, such as substance use, risk-taking, and contact with law enforcement. In future, it will be important to draw on longitudinal designs with repeated measures of neuroimaging and callous traits in order to trace neurodevelopmental trajectories of callous traits and their utility for predicting clinically relevant outcomes in later life. Third, a growing body of literature points to the existence of distinct developmental pathways to youth callous traits (Viding and McCrory 2018), with groups being differentially related to exposure to early adversity in childhood and accompanied anxiety symptoms, and the other group who develops similarly severe callous traits through inherited vulnerabilities (Viding and McCrory 2018). Our current population-based cross-sectional design did not allow us to study these differential developmental pathways. Repeated assessments of both neuroimaging and callous traits across childhood are needed, particularly with regards to differential developmental pathways (Cecil et al. 2014; Tremblay 2010). Nevertheless, we adjusted for behavioral as well as emotional problems in sensitivity analyses, which did not alter our main findings. Fourth, non-verbal IQ was assessed 4 years prior to callous traits and MRI assessments; it would have been better to have concurrent assessments of each. Despite this, intelligence is moderately stable during childhood (Trzaskowski et al. 2014), which supports the reliability of our analysis with adjustment for IQ at 6 years. Fifth, while our hierarchical analysis approach reduces the likelihood of false positives, it also increases chances of false negatives, i.e. very focal findings might have been obscured if global associations were not found. Sixth, while the Generation R Study is an ethnically diverse study, most participants are of European descent. More research needs to be conducted in non-white populations, which is a considerable gap in the literature. Finally, more research should employ multi-modal approaches, for example integrating fMRI data to further characterize the neural profile of callous traits .

In conclusion, we found evidence for widespread macro- and microstructural brain alterations in callous traits based on a large community sample of children . These results underscore that youth callous traits are not uniquely associated with brain differences in frontal-limbic or frontal-striatal connections; rather, structural brain differences were observed in a wide range of areas across the brain. Our study provides further support for the value of conceptualizing pediatric callous traits as a neurodevelopmental condition. Priority should be given to prospective developmentally-sensitive research, which will enable to examine early environmental and neurobiological pathways to callous traits, potential sex differences, and their utility in predicting clinically relevant functional domains in later life. Finally, the current results might indicate that children with elevated callous traits show differences in brain development, which holds promise for etiologic research for a better understanding of the development of severe antisocial behavior later in life.

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(SUPPLEMENT)

SUPPLEMENT 1

DESCRIPTION OTHER
BEHAVIORAL DATA

CO-OCCURRING EMOTIONAL AND
BEHAVIORAL PROBLEMS(AGE 10)

At the same time point, i.e. child age 10 years, mothers completed the school-age version of Child Behavior Checklist (CBCL/6-18) to rate emotional and behavioral problems of the child (Achenbach and Rescorla 2001). The CBCL covers a broad range of emotional and behavioral problems which were rated on a 3-point scale (0 = not true, 1 = sometimes/somewhat true, 2 = very/often true). The CBCL is a widely used and validated measure for child and adolescent behaviour problems on a continuous scale, and has been shown to predict clinical psychiatric diagnoses in adulthood (Hofstra, van der Ende, and Verhulst 2002; Roza et al. 2003). Individual items can be summed to obtain a total problems score, and items load on 6 independent DSM-based scales of internalizing and externalizing problems, i.e. affective problems, anxiety problems, somatic problems, attention-deficit/hyperactivity problems, oppositional defiant problems, and conduct problems. Similarly, child self-reported problems were assessed at age 10 years, for which the Brief Problem Monitor (BPM) was used (Achenbach et al. 2011). The BPM encompasses three scales of internalizing, externalizing and attention problems and is a validated abbreviated child-reported version of the CBCL. In the current study, mother-reported and child-reported problems were correlated with the callous traits sum score to obtain external behavioral validation of our measure of callous traits. In addition, CBCL total scores were used in a sensitivity step in our main analyses in order to examine the extent to which our observed associations were explained by co-occurring emotional and behavioral problems.

PROSOCIAL BEHAVIOR (AGE 10)

At mean age ten years both mothers and children completed the prosocial scale of the Strengths and Difficulties Questionnaire (SDQ; (Goodman 2001)). This scale comprises five items, such as “[My child / I often spontaneously offers to help others”, which are rated on a three-point scale (1 = not true, 2 = somewhat/sometimes true, 3 = certainly/always true). The prosocial scale of the SDQ has been shown to have acceptable test-retest reliability and construct validity. Together with the mother- and child-reported problems described above, prosocial behavior was used in the current study as an external behavioral validation of our measure of callous traits.

NON-VERBAL INTELLIGENCE (AGE 6)

Child intelligence (IQ) was measured at age 6 using the Snijders-Oomen nonverbal intelligence test (Tellegen et al. 2005). At this developmental stage, a nonverbal IQ assessment is the preferred method and this measure has been shown to reliably determine non-verbal cognitive ability in early childhood (Basten et al. 2014).

MATERNAL PSYCHIATRIC
PROBLEMS (AGE 10)

Psychiatric problems of the mother were assessed with the Brief Symptom Inventory (BSI), when the children were at mean age 10 years (Derogatis and Melisaratos 1983). Four validated subscales were used, i.e. interpersonal sensitivity, depression, anxiety and hostility, of which a sum score was calculated. The BSI is a validated, reliable measure to continuously examine adult psychiatric problems along a continuum.

SUPPLEMENT 2

BRAIN IMAGING METHOD

IMAGE ACQUISITION

After a localizer, a structural T1 scan was the first sequence to be performed, followed by the DTI. Structural images were processed through the FreeSurfer analysis suite, version 6.0 (Fischl et al. 2004). DTI pre-processing was conducted using the FMRIB Software Library (FSL), version 5.0.9 (Jenkinson et al. 2012). For structural MRI analyses, global metrics of volume were extracted. In addition, separate whole-brain vertex-wise analyses were performed to examine cortical thickness, cortical surface area, and gyrification in association with callous traits. With respect to DTI, probabilistic white matter fiber tractography was conducted on each child’s DTI images and fractional anisotropy (FA) mean diffusivity (MD), axial diffusivity (AD) and radial diffusivity (RD) were computed for each tract. Global brain FA, MD, AD and RD were computed based on 12 well-studied white matter tracts, as described previously (Muetzel et al. 2015). Head movement was addressed by accommodating the child as much as possible, through detailed explanation and reassurance before the MRI scanning session, the option to watch a movie during scanning, and cushions were put next to the child’s head to limit further head motion. After a localizer, T1-weighted structural images were acquired with an inversion recovery-prepared fast spoiled gradient recalled sequence. The following sequence parameters were used with the GE option BRAVO: TR = 8.77ms, TE = 3.4ms, TI = 600ms, Flip Angle = 10°, FOV = 220mm x 220mm, Acquisition Matrix = 220 x 220, slice thickness = 1mm, number of slices = 230, voxel size = 1mm x 1mm x 1mm, ARC Acceleration = 2. The DTI scan was acquired using an axial spin echo, echo planar imaging sequence with 3 b = 0 scans and 35 diffusion weighted images (TR = 12,500ms, TE = 72.8ms, Field of view = 240mm x 240mm, Acquisition Matrix = 120 x 120, slice thickness = 2mm, voxel size = 2mm x 2mm x 2mm, number of slices = 65, Asset Acceleration = 2). Children with an incidental structural brain abnormality were excluded from both the sMRI and DTI analyses (see Figure S1)

STRUCTURAL IMAGE PROCESSING AND
QUALITY ASSURANCE

Structural images were processed through the FreeSurfer analysis suite, version 6.0 (Fischl et al. 2004). Freesurfer morphometry has demonstrated good test-retest reliability across scanner manufacturers and field strengths (Han et al. 2006; Reuter et al. 2012). In summary, non-brain tissue was removed, voxel intensities were normalized for B1 inhomogeneity, whole-brain tissue segmentation was performed, and a surface-based model of the cortex was reconstructed. Global metrics of volume (i.e. total brain volume, total cortical grey matter volume, white matter volume, amygdala and hippocampus volumes) were extracted. Freesurfer reconstructions were visually inspected using a previously described protocol (Hibar et al. 2015; Muetzel et al. 2017), and image datasets not suitable for analysis were excluded from the final sample. In brief, the white and pial surface representations for all subjects were inspected for accuracy against the brain image at a number of slices in different plains, i.e. axial, coronal and sagittal. Additional inspection with a metric of automated structural neuroimaging quality assessment (White et al. 2018) revealed that this index was not associated with callous traits (r = -0.01, 95% CI -0.06-0.02, P = 0.409).

VERTEX-WISE ANALYSES

Whole-brain cortical morphological analyses were performed to obtain more detailed information of morphological correlates of callous traits. These vertex-wise analyses were conducted using in house R package (https://github.com/muet0005/QdecR) that allows for inter-subject/group averaging and inference using the general linear model on the morphometric data produced by the FreeSurfer pre-processing stream. Thickness, surface area, and local gyrification maps from each subject were co-registered to a common stereotaxic space, and subsequently smoothed with either a 10mm (thickness and surface area) or 5mm (local gyrification) Gaussian kernel. Analyses were corrected for multiple comparisons using the built-in Monte Carlo simulation at thresholds varying from P < 0.05 to 0.001, a cluster-wise correction that controls for the rate of false positive clusters. Further, cluster-wise p-values were adjusted for running analyses in both left and right hemispheres. Results were shown for all clusters to demonstrate the magnitude of association with callous trait for each cluster.

DTI PRE-PROCESSING

Diffusion tensor imaging scanning pre-processing was conducted using the FMRIB Software Library (FSL), version 5.0.9 (Jenkinson et al. 2012). Image processing has been described in more detail elsewhere (White et al. 2017). In short, non-brain tissue was removed and diffusion images were corrected for eddy current-induced artefacts and translations/rotations resulting from head motion. The diffusion tensor was fitted at each voxel using the RESTORE method from the Camino diffusion MRI toolkit (Cook et al. 2006), and scalar metrics (i.e. fractional anisotropy [FA], mean diffusivity [MD], axial diffusivity [AD] and radial diffusivity [RD]) were subsequently computed. FA described the directional degree of diffusion of water and ranges from 0 to 1, with 0 being completely isotropic (i.e. diffusion equal in all directions) and 1 being completely anisotropic (i.e. diffusion along only one axis). MD simply describes the average diffusion in all directions and is composed of AD (i.e. axial diffusivity) and RD (i.e. radial diffusivity).

WHITE MATTER PROBABILISTIC
TRACTOGRAPHY

Probabilistic white matter fiber tractography was conducted on each child’s DTI images using the automated FSL plugin AutoPtx (de Groot et al. 2015), to identify connectivity distributions for a number of large fibre bundles such as the uncinate fasciculus and cingulum bundle. Subsequently, connectivity distributions were normalised based on the number of successful seed-to-target attempts, and then thresholded to remove voxels that were unlikely to be part of the true distribution. Average FA and MD values were computed for each white matter tract by weighting voxels based on the connectivity distribution (i.e., FA in voxels with higher probabilities received higher weight). Left and right white matter tract metric values were averaged and weighted for their respective volumes as we had no a priori hypotheses regarding the laterality of white matter tracts associated with callous-unemotional traits.

DTI QUALITY ASSURANCE

First, the DTIPrep tool (https://www.nitrc.org/projects/dtiprep/) was used to automatically examine data for slice-wise variation and characteristics of artefact in each diffusion-weighted volume. Second, the sum-of-squares error (SSE) maps from the diffusion tensor calculations were examined for structured signal that was indicative of artefact. Each SSE map was rated from 0 to 3 (0: “None”, 1: “Mild”, 2: “Moderate”, 3: “Severe”). Cases not excluded by the automated DTIPrep tool but had a “Severe” score from the SSE rating were excluded from analyses. Further, processed tractography data were examined on their quality. Next, the registration of the DTI data to standard space was inspected for accuracy. Additional quality assessment inspection with the number of slices or volumes affected by motion, cardiac pulsation or other artefacts(Muetzel et al. 2015) demonstrated that these QA indices were not correlated with callous traits (number of total affected slices: r = -0.01, 95% CI -0.05;0.03, P = 0.687; number of total affected volumes: r = 0.00, 95%CI -0.04;0.05, P = 0.869).

Supplementary Table S1: Endorsement of the mother-reported callous traits items in the current sample ($N = 2159$).

	Does not apply at all, *n* (%)	Does apply slightly, *n* (%)	Does apply very much, *n* (%)	Does apply completely, *n* (%)
Cannot be trusted with regard to what he/she says	1721 (80.5)	382 (17.9)	21 (1.0)	13 (0.6)
Denies having done something wrong, even though it is certain he/she did do something wrong	1146 (53.5)	922 (43.1)	58 (2.7)	15 (0.7)
Uses or misleads other people in order to get what he/she wants	1784 (83.3)	330 (15.4)	20 (0.9)	8 (0.4)
If confronted about his/her behaviour, he/she is able to talk him/herself out of it easily	1174 (55.1)	745 (35.0)	166 (7.8)	45 (2.1)
Does not keep any promises	1428 (66.9)	647 (30.3)	46 (2.2)	13 (0.6)
Does not find other people's feelings important	1801 (84.2)	286 (13.4)	40 (1.9)	13 (0.6)
Is cold and indifferent	2017 (94.4)	105 (4.9)	9 (0.4)	5 (0.2)

Supplementary Table S3: Association of global structural volumetric and global white matter microstructural measures with callous traits, with additional adjustment non-verbal IQ.

	Callous traits	
	β (95% CI)	*P*
Structural volumetric measures ($N = 2146$)		
Total brain volume	-0.10 (-0.15;-0.05)	<0.001
Cortical grey matter volume	-0.10 (-0.15;-0.05)	<0.001
White matter volume	-0.08 (-0.12;-0.03)	0.003
Subcortical structures		
Left amygdala	-0.03 (-0.08;0.02)	0.217
Right amygdala	-0.05 (-0.11;0.00)	0.038
Left hippocampus	-0.03 (-0.08;0.02)	0.247
Right hippocampus	-0.01 (-0.07;0.04)	0.585
Left thalamus	0.00 (-0.06;0.06)	0.982
Right thalamus	-0.03 (-0.09;0.03)	0.371
Left caudate	-0.03 (-0.08;0.02)	0.261
Right caudate	-0.04 (-0.09;0.01)	0.155
Left putamen	-0.01 (-0.06;0.04)	0.598
Right putamen	0.00 (-0.05;0.05)	0.988
Left globus pallidus	0.00 (-0.05;0.05)	0.976
Right globus pallidus	-0.01 (-0.06;0.04)	0.600
Left nucleus accumbens	0.02 (-0.03;0.07)	0.427
Right nucleus accumbens	0.01 (-0.04;0.05)	0.748
White matter microstructural measures ($N = 2059$)		
Global fractional anisotropy (FA)	0.02 (-0.03;0.06)	0.438
Global mean diffusivity (MD)	-0.07 (-0.11;-0.02)	0.006

Note: All analyses are corrected for child sex, child age at MRI visit, child ethnicity, maternal educational level, and non-verbal IQ of the child. Sub-cortical volumes are additionally adjusted for intracranial volume. Estimates reflect standardized coefficients.

Supplementary Table S2: Association of global structural volumetric and global white matter microstructural measures with callous traits, with additional adjustment for co-occurring emotional and behavioral problems.

	Callous traits	
	β (95% CI)	*P*
Structural volumetric measures ($N = 2146$)		
Total brain volume	-0.07 (-0.12;-0.03)	0.002
Cortical grey matter volume	-0.07 (-0.12;-0.03)	0.002
White matter volume	-0.06 (-0.10;-0.01)	0.009
Subcortical structures		
Left amygdala	-0.03 (-0.08;0.01)	0.159
Right amygdala	-0.05 (-0.10;-0.01)	0.026
Left hippocampus	-0.02 (-0.07;0.02)	0.329
Right hippocampus	-0.01 (-0.05;0.04)	0.741
Left thalamus	0.01 (-0.05;0.06)	0.849
Right thalamus	-0.02 (-0.08;0.04)	0.471
Left caudate	-0.02 (-0.06;0.03)	0.482
Right caudate	-0.02 (-0.07;0.03)	0.392
Left putamen	-0.02 (-0.07;0.02)	0.349
Right putamen	0.00 (-0.04;0.05)	0.935
Left globus pallidus	-0.01 (-0.05;0.04)	0.746
Right globus pallidus	-0.02 (-0.07;0.02)	0.348
Left nucleus accumbens	0.03 (-0.01;0.07)	0.173
Right nucleus accumbens	0.01 (-0.03;0.05)	0.615
White matter microstructural measures ($N = 2059$)		
Global fractional anisotropy (FA)	0.02 (-0.02;0.06)	0.236
Global mean diffusivity (MD)	-0.06 (-0.10;-0.02)	0.005

Note: All analyses are corrected for child sex, child age at MRI visit, child ethnicity, maternal educational level, and CBCL total problems scores. Sub-cortical volumes are additionally adjusted for intracranial volume. Estimates reflect standardized coefficients.

Supplementary Table S4: Association of global structural volumetric and global white matter microstructural measures with callous traits, with additional adjustment maternal psychiatric problems.

	Callous traits	
	β (95% CI)	*P*
Structural volumetric measures ($N = 2146$)		
Total brain volume	-0.10 (-0.15;-0.05)	<0.001
Cortical grey matter volume	-0.10 (-0.15;-0.05)	<0.001
White matter volume	-0.08 (-0.12;-0.03)	0.002
Subcortical structures		
Left amygdala	-0.03 (-0.08;0.02)	0.205
Right amygdala	-0.06 (-0.11;-0.01)	0.028
Left hippocampus	-0.04 (-0.09;0.02)	0.183
Right hippocampus	-0.02 (-0.07;0.03)	0.438
Left thalamus	0.00 (-0.07;0.06)	0.885
Right thalamus	-0.03 (-0.10;0.03)	0.281
Left caudate	-0.03 (-0.08;0.02)	0.194
Right caudate	-0.04 (-0.09;0.01)	0.104
Left putamen	-0.02 (-0.07;0.03)	0.452
Right putamen	-0.04 (-0.05;0.05)	0.860
Left globus pallidus	-0.01 (-0.06;0.04)	0.674
Right globus pallidus	-0.02 (-0.07;0.02)	0.332
Left nucleus accumbens	0.03 (-0.02;0.07)	0.295
Right nucleus accumbens	0.01 (-0.04;0.06)	0.628
White matter microstructural measures ($N = 2059$)		
Global fractional anisotropy (FA)	0.01 (-0.03;0.05)	0.663
Global mean diffusivity (MD)	-0.07 (-0.11;-0.02)	0.007

Note: All analyses are corrected for child sex, child age at MRI visit, child ethnicity, maternal educational level, and maternal psychopathology scores. Subcortical volumes are additionally adjusted for intracranial volume. Estimates reflect standardized coefficients.

Supplementary Table S5: Vertex-wise analyses of gyrification and callous traits ($N = 2146$)

Hemisphere and region	Cluster size (mm^2)	Talairach Coordinates (x, y, z)	Number of Vertices within Cluster	Cluster-wise *P*-values
Left				
Superior temporal	608.64	-51.8, -6.8, -4.9	1312	0.0018
Middle temporal	540.78	-54.9, -27.2, -12.5	1068	0.0034
Right				
Middle temporal	1377.31	46.2, -59.9, 6.7	2978	0.0001

Note: Analyses are corrected for age, sex, child ethnicity and maternal educational level.

Supplementary Table S6: Vertex-wise analyses of cortical surface area and callous traits, with additional adjustment co-occurring emotional and behavioral problems ($N = 2146$).

Hemisphere and region	Cluster size (mm^2)	Talairach Coordinates (x, y, z)	Number of Vertices within Cluster	β (average across cluster)	Cluster-wise *P*-values
Left					
1. Superior frontal	604.95	-13.5, 46.5, 4.4	1096	-0.07	0.0006

Note: Analyses are corrected for age, sex, child ethnicity and maternal educational level. Numbers of the clusters correspond to the numbers shown in Figure 1. Cluster forming threshold of 0.001.

Supplementary Table S7: Vertex-wise analyses of cortical surface area and callous traits, with additional adjustment for non-verbal IQ ($N = 2146$).

Hemisphere and region	Cluster size (mm^2)	Talairach Coordinates (x, y, z)	Number of Vertices within Cluster	β (average across cluster)	Cluster-wise *P*-values
Left					
1. Fusiform	1334.24	-41.8, -47.3, -14.0	2218	-0.08	0.0001
2. Superior temporal	684.41	-51.6, 8.7, -18.0	1278	-0.06	0.0003
3. Lingual	543.88	-20.3, -53.9, -3.1	1150	-0.06	0.0012
4. Superior frontal	445.83	-13.4, 46.0, 4.0	836	-0.05	0.0040
Right					
5. Middle temporal	1197.06	49.7, 7.2, -32.3	2061	-0.06	0.0001
6. Isthmus of cingulate	326.15	6.0, -50.1, 20.9	818	-0.07	0.0188
7. Post-central	310.58	60.0, -8.2, 15.9	671	-0.06	0.0218

Note: Analyses are corrected for age, sex, child ethnicity and maternal educational level. Numbers of the clusters correspond to the numbers shown in Figure 1. Cluster forming threshold of 0.001.

Supplementary Table S8: Vertex-wise analyses of cortical surface area and callous traits, with additional adjustment maternal psychiatric problems ($N = 2146$).

Hemisphere and region	Cluster size (mm^2)	Talairach Coordinates (x, y, z)	Number of Vertices within Cluster	β (average across cluster)	Cluster-wise *P*-values
Left					
1. Inferior temporal	1176.29	-45.6, -62.3, -6.0	1976	-0.05	0.0001
2. Superior frontal	524.18	-13.7, 45.9, 3.5	995	-0.04	0.0011
3. Lingual	482.47	-20.3, -53.9, -3.1	1025	-0.07	0.0032
4. Superior temporal	477.69	-51.8, 8.0, -18.0	907	-0.06	0.0032
5. Lateral orbitofrontal	369.63	-31.7, 26.3, -10.6	753	-0.05	0.0109
Right					
6. Middle temporal	1163.62	50.2, 7.3, -32.8	2017	-0.06	0.0001

Note: Analyses are corrected for age, sex, child ethnicity and maternal educational level. Numbers of the clusters correspond to the numbers shown in Figure 1. Cluster forming threshold of 0.001.

Supplementary Table S9: Association of global white matter axial diffusivity (AD) and radial diffusivity (RD) with callous traits.

	Callous traits	
	β (95% CI)	*P*
White matter microstructural measures ($N = 2059$)		
Global axial diffusivity (AD)	-0.06 (-0.12;-0.02)	0.003
Global radial diffusivity (RD)	-0.05 (-0.09;0.00)	0.043

Note: All analyses are corrected for child sex, child age at MRI visit, child ethnicity, maternal educational level, and CBCL total problems scores. Estimates reflect standardized coefficients.

Supplementary Table S10: Associations between mean diffusivity (MD) in individual white matter tracts and callous traits, with additional adjustment for co-occurring emotional and behavioral problems.

	Interpersonal callousness traits	
	β (95% CI)	*P*
Inferior longitudinal fasciculus	-0.03 (-0.07;0.01)	0.150
Superior longitudinal fasciculus	-0.06 (-0.10;-0.02)	0.005
Forceps minor	-0.05 (-0.09;-0.01)	0.026
Forceps major	-0.02 (-0.06;0.02)	0.393
Corticospinal tract	-0.14 (-0.24;-0.04)	0.008
Uncinate fasciculus	-0.06 (-0.10;-0.02)	0.004
Cingulum bundle	-0.05 (-0.09;-0.01)	0.011

Note: All analyses are corrected for child sex, child age at MRI visit, child ethnicity, maternal educational level, and CBCL total problems scores. Microstructural properties of left and right tracts were combined and weighted for their respective volumes, except for forceps minor and major. Estimates reflect standardized coefficients.

Supplementary Table S11: Associations between mean diffusivity (MD) in individual white matter tracts and callous traits, with additional adjustment for non-verbal IQ.

	Interpersonal callousness traits	
	β (95% CI)	*P*
Inferior longitudinal fasciculus	-0.04 (-0.09;0.00)	0.066
Superior longitudinal fasciculus	-0.06 (-0.11;-0.02)	0.007
Forceps minor	-0.04 (-0.08;0.00)	0.072
Forceps major	-0.02 (-0.06;0.03)	0.500
Corticospinal tract	-0.15 (-0.27;-0.04)	0.008
Uncinate fasciculus	-0.07 (-0.11;-0.03)	0.002
Cingulum bundle	-0.06 (-0.10;-0.01)	0.013

Note: All analyses are corrected for child sex, child age at MRI visit, child ethnicity, maternal educational level, and non-verbal IQ of the child. Microstructural properties of left and right tracts were combined and weighted for their respective volumes, except for forceps minor and major. Estimates reflect standardized coefficients.

Supplementary Table S12: Associations between mean diffusivity (MD) in individual white matter tracts and callous traits, with additional adjustment for maternal psychiatric problems.

	Interpersonal callousness traits	
	β (95% CI)	*P*
Inferior longitudinal fasciculus	-0.04 (-0.09;0.01)	0.102
Superior longitudinal fasciculus	-0.06 (-0.10;-0.01)	0.012
Forceps minor	-0.04 (-0.08;0.00)	0.066
Forceps major	-0.02 (-0.06;0.03)	0.392
Corticospinal tract	-0.15 (-0.26;-0.04)	0.007
Uncinate fasciculus	-0.07 (-0.11;-0.02)	0.003
Cingulum bundle	-0.05 (-0.10;-0.01)	0.014

Note: All analyses are corrected for child sex, child age at MRI visit, child ethnicity, maternal educational level, and non-verbal IQ of the child. Microstructural properties of left and right tracts were combined and weighted for their respective volumes, except for forceps minor and major. Estimates reflect standardised coefficients.

Supplementary Table S13: Associations between axial diffusivity (AD) and radial diffusivity (RD) in individual white matter tracts and callous traits.

	β (95% CI)	P	FDR-adjusted P
Inferior longitudinal fasciculus			
Axial diffusivity (AD)	-0.07 (-0.12;-0.03)	0.002	0.007
Radial diffusivity (RD)	-0.01 (-0.05;0.04)	0.693	0.795
Superior longitudinal fasciculus			
Axial diffusivity (AD)	-0.07 (-0.12;-0.03)	0.002	0.007
Radial diffusivity (RD)	-0.04 (-0.09;0.00)	0.066	0.116
Forceps minor			
Axial diffusivity (AD)	-0.01 (-0.05;0.03)	0.655	0.764
Radial diffusivity (RD)	-0.04 (-0.08;0.00)	0.031	0.072
Forceps major			
Axial diffusivity (AD)	-0.01 (-0.06;0.03)	0.609	0.764
Radial diffusivity (RD)	-0.01 (-0.06;0.04)	0.795	0.795
Corticospinal tract			
Axial diffusivity (AD)	-0.23 (-0.41;-0.05)	0.011	0.026
Radial diffusivity (RD)	-0.02 (-0.07;0.02)	0.310	0.434
Uncinate fasciculus			
Axial diffusivity (AD)	-0.04 (-0.08;0.01)	0.116	0.203
Radial diffusivity (RD)	-0.06 (-0.10;-0.02)	0.007	0.025
Cingulum bundle			
Axial diffusivity (AD)	0.00 (-0.04;0.04)	0.987	0.987
Radial diffusivity (RD)	-0.06 (-0.11;-0.02)	0.004	0.025

Note: All analyses are corrected for child sex, child age at MRI visit, child ethnicity, and maternal educational level. Microstructural properties of left and right tracts were combined and weighted for their respective volumes, except for forceps minor and major. Estimates reflect standardized coefficients.

Supplementary Table S14: Endorsement of the mother-reported callous traits items in boys only ($n = 1071$).

	Does not apply at all, *n* (%)	Does apply slightly, *n* (%)	Does apply very much, *n* (%)	Does apply completely, *n* (%)
Cannot be trusted with regard to what he/she says*	825 (77.1)	222 (20.7)	15 (1.4)	8 (0.7)
Denies having done something wrong, even though it is certain he/she did do something wrong*	521 (48.6)	505 (47.1)	37 (3.4)	10 (0.9)
Uses or misleads other people in order to get what he/she wants	894 (83.2)	168 (15.6)	7 (0.7)	6 (0.6)
If confronted about his/her behaviour, he/she is able to talk him/herself out of it easily*	549 (51.4)	407 (38.1)	93 (8.7)	20 (1.9)
Does not keep any promises*	672 (63.0)	362 (34.0)	24 (2.3)	8 (0.8)
Does not find other people's feelings important*	865 (80.8)	174 (16.2)	24 (2.2)	8 (0.7)
Is cold and indifferent	1001 (93.6)	61 (5.7)	6 (0.6)	2 (0.2)

* Significantly higher endorsement frequencies in boys compared with girls as assessed with chi-square difference tests ($P < 0.05$)

Supplementary Table S16: Correlations between callous traits and behavioral traits, stratified by sex

	Boys	Girls
Non-verbal IQ at age 6 years, mean (SD)	-0.11**	-0.04
Mother-reported CBCL, median (IQR)		
Affective Problems	0.23**	0.22**
Anxiety Problems	0.16**	0.14**
Somatic Complaints	0.11**	0.07**
ADHD Problems	0.38**	0.31**
ODD Problems	0.41**	0.36**
CD Problems	0.51**	0.39**
Child-reported BPM, median (IQR)		
Internalizing problems	0.09**	0.08**
Externalizing problems	0.24**	0.19**
Attention problems	0.19**	0.20**
SDQ – Prosocial scale, median (IQR)		
Mother-reported	-0.24**	-0.18**
Child-reported	-0.09**	-0.12**

** $P < 0.001$

Supplementary Table S17: Association of interaction of child sex with global structural volumetric and global white matter microstructural measures on callous traits.

	Callous traits	
	β (95% CI)	*P*
Structural volumetric measures ($N = 2148$)		
Total brain volume	0.02 (-0.08;0.12)	0.700
Cortical grey matter volume	0.03 (-0.08;0.12)	0.577
White matter volume	0.00 (-0.10;0.09)	0.951
Subcortical structures		
Right amygdala	-0.04 (-0.13;0.05)	0.382
White matter microstructural measures ($N = 2059$)		
Global mean diffusivity (MD)	-0.13 (-0.22;-0.04)	0.005

Note: Sex interaction was only explored for those indices for which a main association was observed. All analyses are corrected for child sex, child age at MRI visit, child ethnicity, maternal educational level. Amygdala volume is additionally adjusted for intracranial volume. Estimates reflect standardized coefficients for the interaction term.

Supplementary Table S18: Associations between global white matter microstructural measures and callous traits, stratified by sex.

	Callous traits	
	β (95% CI)	*P*
Boys ($n = 1028$)		
Global mean diffusivity (MD)	0.00 (-0.07;0.06)	0.878
Girls ($n = 1031$)		
Global mean diffusivity (MD)	-0.13 (-0.20;-0.07)	<0.001
Global axial diffusivity (AD)	-0.10 (-0.16;-0.03)	0.003
Global radial diffusivity (RD)	-0.12 (-0.18;-0.05)	<0.001

Note: All analyses are corrected for child sex, child age at MRI visit, child ethnicity, maternal educational level. Estimates reflect standardized coefficients.

Supplementary Table S15: Endorsement of the mother-reported callous traits items in girl only ($n = 1075$).

	Does not apply at all, *n* (%)	Does apply slightly, *n* (%)	Does apply very much, *n* (%)	Does apply completely, *n* (%)
Cannot be trusted with regard to what he/she says*	896 (84.0)	160 (15.0)	6 (0.6)	5 (0.5)
Denies having done something wrong, even though it is certain he/she did do something wrong*	625 (58.5)	417 (39.0)	21 (2.0)	5 (0.5)
Uses or misleads other people in order to get what he/she wants	890 (83.4)	162 (15.2)	13 (1.2)	2 (0.2)
If confronted about his/her behaviour, he/she is able to talk him/herself out of it easily*	625 (58.9)	338 (31.9)	73 (6.9)	25 (2.4)
Does not keep any promises*	756 (70.8)	285 (26.7)	22 (2.1)	5 (0.5)
Does not find other people's feelings important*	936 (87.6)	112 (10.5)	16 (1.5)	5 (0.5)
Is cold and indifferent	1016 (95.3)	44 (4.1)	3 (0.3)	3 (0.3)

* Significantly lower endorsement frequencies in boys compared with girls as assessed with chi-square difference tests ($P < 0.05$)

Supplementary Table S19: Associations of individual white matter mean diffusivity, axial diffusivity and radial diffusivity with callous traits, shown for girls only ($n = 1031$).

	Callous traits β (95% CI)	*P*
Inferior longitudinal fasciculus		
Mean diffusivity	-0.09 (-0.16;-0.02)	0.009
Axial diffusivity	-0.08 (-0.14;-0.01)	0.017
Radial diffusivity	-0.07 (-0.014;-0.01)	0.033
Superior longitudinal fasciculus		
Mean diffusivity	-0.15 (-0.21;-0.08)	<0.001
Axial diffusivity	-0.12 (-0.19;-0.06)	<0.001
Radial diffusivity	-0.12 (-0.18;-0.05)	<0.001
Forceps minor		
Mean diffusivity	-0.08 (-0.14;-0.02)	0.011
Axial diffusivity	0.00 (-0.05;0.06)	0.921
Radial diffusivity	-0.09 (-0.15;-0.03)	0.002
Forceps major		
Mean diffusivity	-0.04 (-0.11;0.02)	0.174
Axial diffusivity	-0.04 (-0.11;0.02)	0.170
Radial diffusivity	-0.03 (-0.10;0.03)	0.281
Corticospinal tract		
Mean diffusivity	-0.30 (-0.46;-0.14)	<0.001
Axial diffusivity	-0.30 (-0.56;-0.05)	0.020
Radial diffusivity	-0.07 (-0.13;-0.01)	0.029
Uncinate fasciculus		
Mean diffusivity	-0.10 (-0.16;-0.04)	0.002
Axial diffusivity	-0.06 (-0.12;0.01)	0.080
Radial diffusivity	-0.08 (-0.14;-0.02)	0.011
Cingulum bundle		
Mean diffusivity	-0.11 (-0.17;-0.05)	<0.001
Axial diffusivity	-0.02 (-0.08;0.04)	0.547
Radial diffusivity	-0.11 (-0.17;-0.05)	<0.001

Note: All analyses are corrected for child age at MRI visit, child ethnicity, and maternal educational level. Microstructural properties of left and right tracts were combined and weighted for their respective volumes, except for forceps minor and major. Estimates reflect standardized coefficients.

Supplementary Table S20: Associations of child conduct problems by callous traits interaction on global structural volumetric and global white matter microstructural measures.

	Callous traits β (95% CI)	*P*
Structural volumetric measures ($N = 2146$)		
Total brain volume	-0.01 (-0.04;0.03)	0.726
Cortical grey matter volume	0.00 (-0.04;0.04)	0.976
White matter volume	0.00 (-0.04;0.03)	0.806
Subcortical structures		
Right amygdala	0.03 (-0.01;0.07)	0.111
White matter microstructural measures ($N = 2059$)		
Global mean diffusivity (MD)	0.02 (-0.02;0.07)	0.204

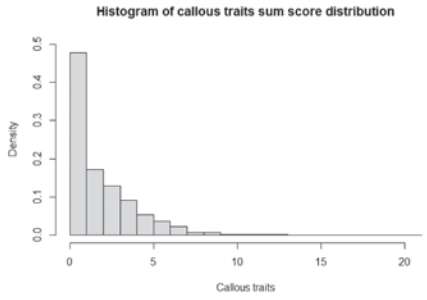
Note: Sex interaction was only explored for those indices for which a main association was observed. All analyses are corrected for child sex, child age at MRI visit, child ethnicity, and maternal educational level. Amygdala volume is additionally adjusted for intracranial volume. Estimates reflect standardized coefficients for the interaction term.

Supplementary Table S21: Association of global structural volumetric and global white matter microstructural measures with callous traits, testing for non-linear (quadratic) associations.

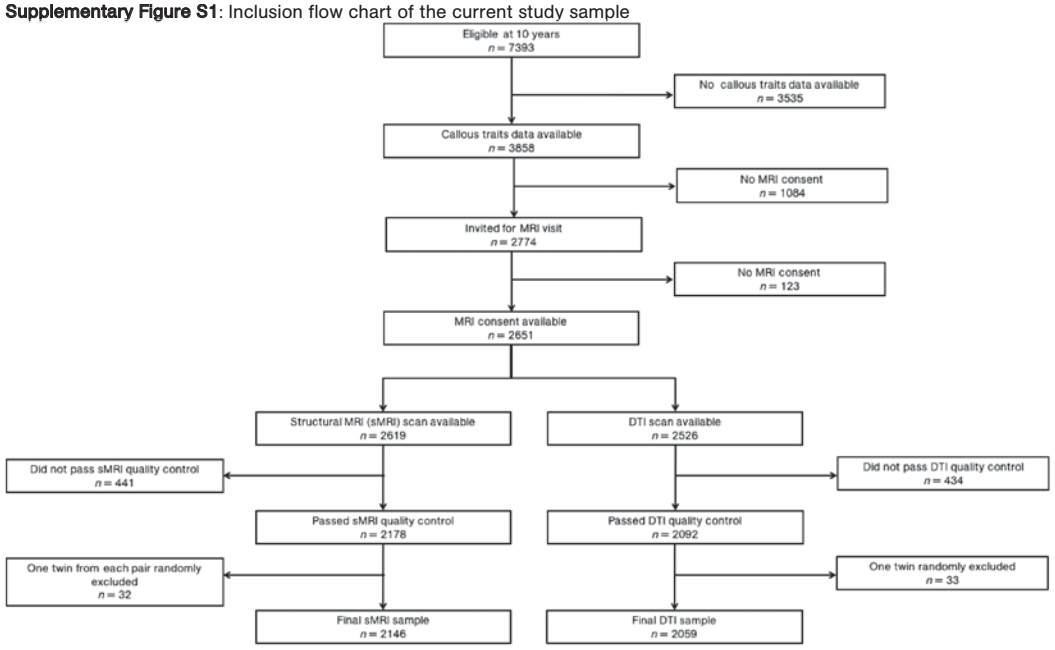
	Callous traits β (95% CI)	*P*
Structural volumetric measures ($N = 2146$)		
Total brain volume	-0.02 (-0.05;0.01)	0.189
Cortical grey matter volume	-0.02 (-0.05;0.01)	0.187
White matter volume	-0.02 (-0.05;0.01)	0.285
Subcortical structures		
Left amygdala	-0.01 (-0.04;0.02)	0.358
Right amygdala	0.00 (-0.03;0.02)	0.741
Left hippocampus	-0.02 (-0.05;0.04)	0.093
Right hippocampus	-0.01 (-0.04;0.01)	0.251
Left thalamus	-0.01 (-0.04;0.02)	0.503
Right thalamus	0.00 (-0.03;0.03)	0.921
Left caudate	0.00 (-0.03;0.02)	0.763
Right caudate	-0.02 (-0.04;0.01)	0.269
Left putamen	0.00 (-0.03;0.03)	0.941
Right putamen	-0.01 (-0.04;0.01)	0.329
Left globus pallidus	-0.02 (-0.04;0.01)	0.240
Right globus pallidus	-0.01 (-0.04;0.01)	0.395
Left nucleus accumbens	0.00 (-0.02;0.03)	0.840
Right nucleus accumbens	-0.01 (-0.04;0.02)	0.458
White matter microstructural measures ($N = 2059$)		
Global fractional anisotropy (FA)	0.02 (-0.02;0.05)	0.320
Global mean diffusivity (MD)	0.02 (-0.01;0.05)	0.183

Note: All analyses are corrected for child sex, child age at MRI visit, child ethnicity, maternal educational level, and non-verbal IQ of the child. Sub-cortical volumes are additionally adjusted for intracranial volume. Estimates reflect standardized coefficients.

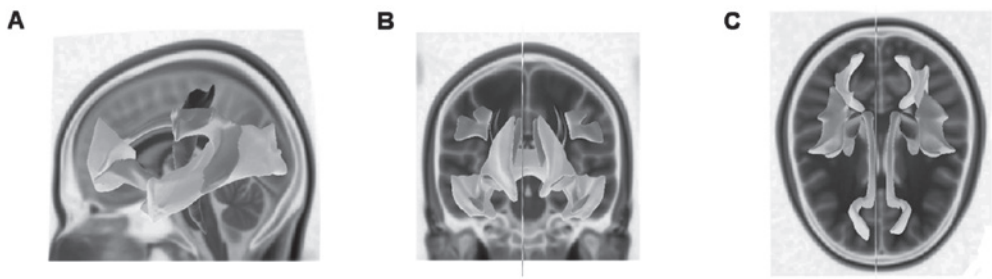
Supplementary Figure S2: Histogram of the endorsement distribution of callous traits ($N = 2146$)



Supplementary Figure S1: Inclusion flow chart of the current study sample



Supplementary Figure S3: Associations between individual white matter tracts mean diffusivity (MD) and callous traits ($N = 2059$).



Note: Panel A shows the sagittal view, panel B the coronal view, and panel C the transversal view. Non-significant associations are depicted in red, positive associations are depicted in yellow, and negative associations are depicted in blue. The brighter the color, the stronger the association.

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(ABSTRACT)

OBJECTIVE

We examined the prospective, potentially bi-directional, association of aggressive behavior with body mass index (BMI) and body composition across childhood in three population-based cohorts.

METHOD

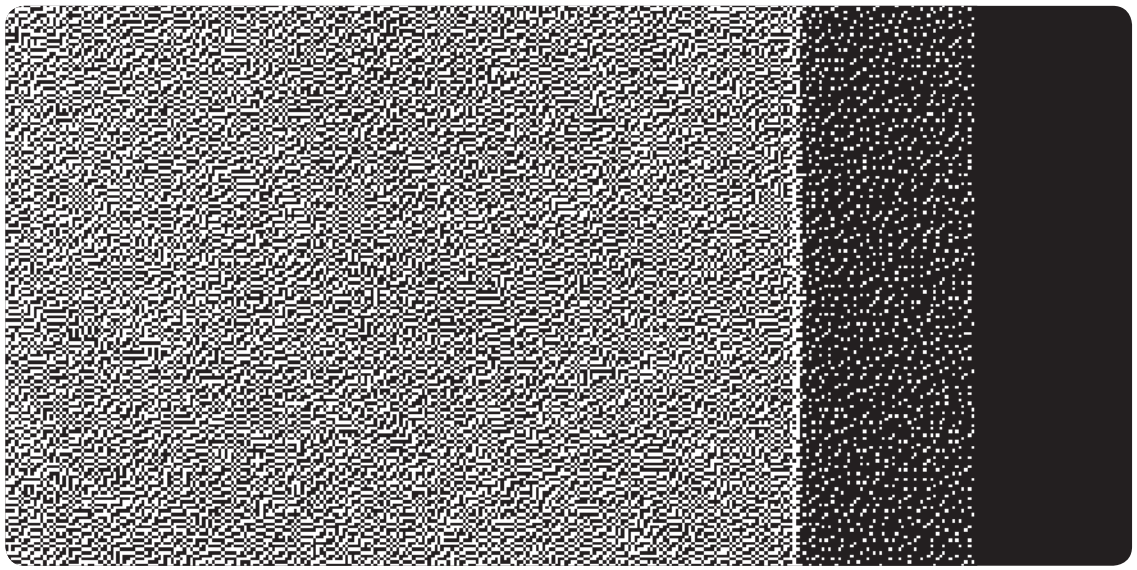
Repeated measures of aggression and BMI were available from the Generation R Study between 6 and 10 years (N=3,974), the Netherlands Twin Register between 7 and 10 years (NTR, N=10,328) and the Swedish Twin Study of Child and Adolescent Development between 9 and 14 years (TCHAD, N=1,462). In all samples aggression was assessed with the Child Behavior Checklist. Fat mass and fat-free mass were available in the Generation R Study. Associations were examined with cross-lagged modeling.

RESULTS

Aggressive behavior at 6/7 years was associated with higher BMI at 10 years in the Generation R Study (β=0.02, 95%CI 0.01;0.04) in NTR (β=0.04, 95%CI 0.02;0.06), and in TCHAD (β=0.03, 95%CI -0.02;0.07). Aggressive behavior was prospectively associated with higher fat mass (β=0.03, 95%CI 0.02;0.05), but not fat-free mass. There was no evidence that BMI or body composition preceded aggressive behavior.

CONCLUSION

More aggressive behavior was prospectively associated with higher BMI and fat mass. This suggests that aggression contributes to the obesity problem, and future research should study whether these behavioral pathways to childhood obesity are modifiable.



(...) *and that light touch which makes it so easy to negotiate the troubles of life,* (...)

CHAPTER 11

ASSOCIATION BETWEEN CHILDHOOD AGGRESSION AND BMI: RESULTS FROM THREE POPULATION-BASED COHORTS

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*CONTRIBUTED EQUALLY.

Conditionally accepted in *Obesity*.

INTRODUCTION

Childhood obesity is a worldwide public health problem, with an increasing prevalence expected to reach 9% of children in 2020 (de Onis, Blossner, and Borghi 2010). Although accumulating evidence suggests that children with overweight show more externalizing behavioral problems – e.g. aggressive, oppositional behaviors - than children with normal weight (Sawyer et al. 2006; Datar and Sturm 2004), the directionality of the association between high Body Mass Index (BMI) and aggressive behavioral problems in childhood remains unclear.

Four longitudinal population-based studies have previously examined the potential bi-directional association between externalizing behavior problems – consisting of aggressive behavior and/or attention-deficit/hyperactivity (ADHD) problems (Lahey and Waldman 2012) – and high BMI in childhood (Bradley et al. 2008; Anderson et al. 2010; Garthus-Niegel, Hagtvet, and Vollrath 2010; Camfferman et al. 2016). These studies yielded mixed results, raising questions about the directionality of effects. Two prospective studies reported that early externalizing behavior problems predicted higher BMI in early childhood (Camfferman et al. 2016), and early adolescence (Anderson et al. 2010), while no association between childhood BMI and subsequent increases in externalizing behavior was found. Conversely, two other prospective population-based studies observed no longitudinal associations between externalizing behavior and BMI in children aged 2 years at baseline and 12 years at follow-up (Bradley et al. 2008), or in toddlers aged 18 months at baseline and 36 months at follow-up (Garthus-Niegel, Hagtvet, and Vollrath 2010). These conflicting findings call for further investigations into the potential bi-directionality of this association. While it is well-known that ADHD poses a risk for developing obesity (Cortese et al. 2016), there is a particular need to elucidate the association between aggressive behavior and BMI in childhood. Aggressive behaviors are the most common reasons for referral to child and adolescent mental health services, and they are substantially predictive to poorer long-term functioning and high societal costs (Rivenbark et al. 2017). And although ADHD is often comorbid with aggression, no study has yet examined their independent prospective associations with BMI, which could provide further insight into the specific behavioral mechanisms of the development of obesity in childhood. Furthermore, previous studies have focused on BMI only, while adequately distinguishing fat mass from fat-free mass may benefit determining specificity of associations (Derks et al. 2017).

The aim of the current study was to examine the prospective, potentially bi-directional, associations between aggressive behavior and BMI across childhood. Insights obtained from this study will contribute to a better understanding of the behavioral – and potentially modifiable – pathways to obesity risk in childhood. Here, we assessed the direction of association between aggressive behavior and BMI in three large population-based cohorts from early childhood (ages 6-7 to 10 years) to early adolescence (ages 9 to 14 years). Furthermore, in one cohort we also assessed the directionality of associations of aggressive behavior with fat mass and lean mass in order to examine more in-depth weight-related obesity indicators. Sensitivity analyses were conducted with additional adjustment for co-occurring attention and internalizing problems at baseline. We hypothesized that aggressive behavior would be associated with higher BMI and fat mass at later ages rather than vice versa, as behavioral inhibition deficits associated with aggression might increase the risk for unhealthy life styles and, therefore, higher BMI (Graziano, Calkins, and Keane 2010).

METHOD

STUDY DESIGN AND POPULATION

Three population-based cohort samples were included in the current study that collaborate under the FP7-ACTION consortium (Bartels et al. 2018). First, primary analyses were conducted in the Generation R Study, a prospective cohort from fetal life onwards in Rotterdam, The Netherlands. The study has been designed to investigate early environmental and genetic pathways leading to normal and abnormal growth, development and health (Kooijman et al. 2016). The study was approved by the Medical Ethical Committee of the

Erasmus Medical Center, Rotterdam. For the current study, children with data on BMI, body composition, and aggressive behavior at both ages 6 and 10 years were included, resulting in a study sample of 3,974 children.

Independent replication of the relationship between BMI and aggressive behavior was performed in the Netherlands Twin Register (NTR; *N* = 10,328, assessed at ages 7 and 10 years) (van Beijsterveldt et al. 2013; Estourgie-van Burk et al. 2010) and the Swedish Twin Study of Child and Adolescent Development (TCHAD; *N* = 1,462, assessed at ages 9 and 14 years) (Lichtenstein et al. 2007). Both twin cohorts are designed to investigate the genetic and environmental effects on children's cognitive functioning, health, and emotional and behavioral problems during development. For the current analyses, both twins from each twin pair were included in the analyses, which were adjusted for family relatedness. Written informed consent and assent was obtained for all participants from all cohorts. Previous research in both twin cohorts has shown higher twin correlations for aggressive behavior in monozygotic (range, *r* = 0.48-0.84) versus dizygotic (range, *r* = 0.35-0.78) twins (Tuvblad, Eley, and Lichtenstein 2005; Porsch et al. 2016), indicating moderate-to-high twin heritability. Similarly, the twin heritability for BMI has been estimated to be moderately high in childhood (Silventoinen et al. 2016).

MEASUREMENTS

AGGRESSIVE BEHAVIOR

The subscale Aggressive behavior of the Child Behavior Checklist (CBCL) was employed in all three studies. The CBCL was completed by the mothers and rated on a three-point Likert scale (0 = not true, 1 = somewhat true, sometimes true, 2 = very true, often true). The CBCL is a reliable, valid measurement of emotional and behavior problems (Achenbach and Rescorla 2001), including affective, anxiety, attention and aggression problems, and generalizable across societies worldwide (Ivanova et al. 2007). Moreover, the CBCL has been shown to be a valid screening instrument for DSM-IV externalizing disorders (Hudziak et al. 2004). In the Generation R Study, the CBCL/1.5-5 was used to measure aggressive behavior at mean age 6 years (Achenbach and Rescorla 2000). This version of the CBCL was chosen as it was expected most children would be younger than 6 years at assessment. Indeed, 57.4% of the children were 5 years old at the assessment wave, the remainder were 6 years (37.7%) or 7 years or older (4.9%), and we used the CBCL 1½–5 version for all children during this assessment wave to enhance comparability across all children, as recommended in the ASEBA manual (Achenbach and Rescorla 2000). The items of the aggressive behavior problems scale largely overlap with the CBCL/6-18 (e.g. "physically attacks" and "stubborn, sullen or irritable"). Earlier work from the Generation R Study has demonstrated that the internal consistency (Cronbach alpha) was similar for all syndrome scales for children aged 5 years versus children older than 5 years (Basten et al. 2013), indicating that the CBCL/1.5-5 assesses aggressive behavior problems similarly in 5-year-old children and 6-7-year-old children. At the age of 10 years, aggressive behavior was measured with the CBCL/6-18, which was also used in the NTR cohort at both waves. In the TCHAD sample, the CBCL/4-18 was used to assess aggressive behavior, which is an earlier version of the CBCL but with identical items that assess aggressive behavior problems.

CHILD BMI AND BODY COMPOSITION

At the age of 6 and 10 years, child weight and height were measured at the Generation R research center. The obtained BMI (kg/m^2) was standardized into Body Mass Index Standard Deviation Scores (BMI-SDS) by correcting for sex and age, using the Dutch national reference in the Growth Analyzer program (http://www.growthanalyser.org). In the NTR and TCHAD cohorts, height (m) and weight (kg) data were based on mother-reports, which were used to calculate BMI.

In the Generation R Study, body composition at both ages was measured with Dual-energy X-ray absorptiometry (DXA) scanner (iDXA, GE-Lunar, 2008, Madison, WI, USA). Fat Mass Index (FMI) and Fat-Free Mass Index (FFMI) were converted into sex- and age-adjusted standardized scores.

COVARIATES

Based on prior studies (Sawyer et al. 2006; Datar and Sturm 2004), the following sociodemographic covariates were included in the analyses. With respect to Generation R, sex and birth weight were determined using data from medical records. Maternal age, maternal education and ethnicity of the child were assessed using questionnaires. Ethnicity of the child was categorized as Western and non-Western national origin. Highest attained maternal educational level was categorized into low, medium and high educational level. Maternal psychopathology symptoms were determined through a self-reported questionnaire using the reliable and validated Brief Symptom Inventory (BSI) (Derogatis and Melisaratos 1983), which includes 53 items encompassing a spectrum of psychiatric symptoms, comprising all subscales such as depression, anxiety and hostility. Maternal BMI was measured at the research center when children were 6 years old.

In the NTR and TCHAD samples, similar covariates were available for adjustment of the models, namely gender, age at baseline, ethnicity of the child, birth weight, maternal educational level, maternal BMI (NTR only), and gestational age (NTR only). These data were derived from parent-reported questionnaires.

STATISTICAL ANALYSES

Cross-lagged structural equation modeling in Mplus 7.0 was used to examine the bi-directional relation between aggressive behavior and BMI / body composition over time. The cross-lagged model consisted of stability paths across two consecutive time points for each variable, cross-sectional paths between aggressive behavior and BMI, and cross-lagged paths between behavior and BMI over two timepoints. The cross-lagged paths indicated the extent to which aggressive behavior or BMI/body composition at time point 1 predicted scores on the other measure at time point 2, while accounting for stability and cross-sectional paths. In all three cohorts, separate sensitivity analyses were conducted with additional adjustment for attention and internalizing problems at baseline. Furthermore, we repeated our analyses in the Generation R Study after excluding all twin participants (n = 114) to ensure there was no overlap between this sample and the Netherlands Twin Registry. Finally, to increase the interpretability of results, the cross-lagged model of aggression with BMI in the Generation R study was repeated using weight status categories (underweight/normal weight vs. overweight/obese) instead of BMI continuously at 6 and 10 years. For the twin cohorts, the 'complex option' of Mplus (clustering corrected robust maximum likelihood estimation) was used to take family dependency of the observations into account. To determine the model fit, Root Mean Square Error of Approximation (RMSEA ≤ 0.08), Comparative Fit Index (CFI ≥ 0.95), and Tucker Lewis Index (TLI ≥ 0.95) were used as indices to determine good model fit.(Hooper, Coughlan, and Mullen 2008) The above analyses were repeated in the replication studies. In all samples, Full Information Maximum Likelihood was used to account for missing data. Standardized estimates are presented throughout.

RESULTS

STUDY SAMPLE DEMOGRAPHICS

The baseline characteristics of the three samples were comparable (Table 1). Of note, the Generation R Study included more children with a non-Western ethnic background than the replication samples, reflecting the urban population base of the former. As expected, averages for gestational age and birth weight were lower for the twin cohorts than in the Generation R Study.

BI-DIRECTIONAL ASSOCIATION OF AGGRESSIVE BEHAVIOR WITH BMI

Figure 1 shows the results of the cross-lagged model of aggressive behavior and BMI in the three cohorts. In the Generation R Study, BMI was highly stable over time (β = 0.80, 95%CI 0.79-0.81), while aggressive behavior was moderately stable across the two time points (β = 0.56, 95%CI 0.54-0.59). No cross-sectional associations were observed between aggressive behavior and BMI. With regards to the longitudinal relationships, aggressive behavior at age 6 years was associated with higher BMI at age 10 years (β = 0.02, 95%CI

0.01-0.04). No such association was observed in the opposite direction, i.e. BMI at 6 years was not predictive of subsequent increases or decreases in aggressive behavior problems at 10 years. In the NTR sample, aggressive behavior was associated with BMI at both time points in cross sectional analysis (β = 0.03, 95%CI 0.01-0.06 and β = 0.03, 95%CI 0.01-0.05, respectively). Even following adjustment for this cross-sectional relationship, aggressive behavior at 7 years was prospectively associated with subsequent higher BMI at 10 years (β = 0.04, 95%CI 0.02-0.06), but, conversely, BMI at 7 years was not associated with subsequent more aggressive behavior at age 10 years. With respect to the TCHAD sample, no cross-sectional association between aggressive behavior and BMI was observed at age 10 years, but only at age 14 years (β = 0.06, 95%CI 0.01-0.11). Prospectively, aggressive behavior at 10 years was not significantly associated with subsequent higher BMI at age 14 years, although the effect estimate was of similar magnitude (β = 0.03, 95%CI -0.02-0.07) as in the other two cohorts. An association in the reversed direction was not observed. Fit statistics of the cross-lagged models indicated good model fit in all three samples.

BI-DIRECTIONAL ASSOCIATION OF AGGRESSIVE BEHAVIOR WITH FAT MASS AND FAT-FREE MASS

In the Generation R Study, aggressive behavior at 6 years was associated with a subsequent higher fat mass index at 10 years (Figure 2, β=0.03, 95%CI 0.02-0.05). Again, this association was not seen in the opposite direction. No associations were observed between aggressive behavior and fat-free mass index in either direction. Fit indices for the cross-lagged models pertaining to body composition both indicated good model fit.

SENSITIVITY ANALYSES

Further analyses with additional adjustment with co-occurring attention problems and internalizing problems yielded similar results as the main findings (Supplementary Figure S1-S4). This was the case for the analyses pertaining to BMI, fat mass index as well as fat-free mass index. Analyses excluding twin participants from the Generation R Study yielded results comparable to the main findings (data not shown). Finally, in analyses using categories of normal weight vs. overweight/obesity, i.e. a 1 unit increase in aggressive behavior scores was associated with a greater odds of overweight/obesity (OR = 1.03, 95% CI 1.01-1.05, data not shown).

DISCUSSION

We observed that early aggressive behavior problems was associated with subsequent higher BMI later in childhood in three independent population-based cohorts. We also demonstrated that aggressive behavior was specifically associated with subsequent higher fat mass and not fat-free mass. No associations were observed in the opposite direction, i.e. higher BMI or fat mass at baseline did not predict more aggressive behavior problems at follow-up. These observations were robust to additional adjustment for comorbid attention and internalizing problems.

Whilst several studies have examined ADHD symptoms or the broader concept of externalizing problems in relation to BMI, to our knowledge, this is the first study focusing on the prospective relation between aggressive behavior and children's BMI. The estimates of the association between aggressive behavior – a component of externalizing problems – and increase in BMI were all relatively small in magnitude but consistent. Small effect estimates were expected given the relationship studied and the use of cross-lagged model analyses, which are adjusted for cross-sectional and longitudinal stability paths as well as covariates. Nonetheless, estimates were small to modest, indicating that higher levels of aggressive behavior are only marginally predictive of higher BMI at follow-up, which is not surprising for composite complex phenotypes with many risk determinants such as BMI (VanderWeele 2016). Our findings in the Generation R sample of aggressive behavior predicting a higher BMI were replicated in the NTR sample of similar ages. Effect estimates in the older and smaller TCHAD sample were comparable in size to those observed in the other cohorts albeit non-significant. Our findings extend previous studies showing associations between externalizing behavior and subsequent increases in BMI in children of

Table 1: Demographic characteristics of the included study populations.

	Generation R (*n* = 3,974)		NTR (*n* = 10,328)		TCHAD (*n* = 1,462)	
	6 years	10 years	7 years	10 years	9 years	14 years
Child characteristics						
Sex, % female	50.4		51.2		51.6	
Age, y, mean (SD)	6.08 (0.40)	9.75 (0.28)	7.42 (0.40)	10.05 (0.37)	8.67 (0.47)	13.67 (0.47)
Ethnicity, %						
Dutch/Swedish	67.0		94.3		92.2	
Other Western	8.8		2.9		5.9	
Other Non-Western	24.2		2.9		1.9	
Birth weight, gram, mean (SD)	3425.83 (567.20)		2508.97 (538.97)		2615.75 (526.39)	
Gestational age, mean (SD)	39.80 (1.85)		36.73 (2.47)		N/A	
BMI, kg/m^2, mean (SD)	15.99 (1.62)	17.34 (2.52)	15.35 (1.73)	16.40 (2.16)	16.25 (2.06)	19.06 (2.74)
FMI, kg/m^2, mean (SD)	3.89 (1.20)	4.68 (1.91)	N/A	N/A	N/A	N/A
FFMI, kg/m^2, mean (SD)	11.93 (0.89)	12.55 (1.05)	N/A	N/A	N/A	N/A
Aggressive behavior score, median (IQR)	4.00 (6.00)	2.00 (4.00)	4.00 (6.00)	3.00 (5.00)	3.00 (6.00)	2.00 (5.00)
Maternal characteristics						
Age mother at baseline, mean (SD)	37.98 (4.50)		N/A		N/A	
BMI mother, (kg/m^2), mean (SD)	25.27 (4.67)		23.59 (3.66)		N/A	
Maternal psychopathology symptoms, median (IQR)	0.10 (0.19)		N/A		N/A	
Maternal educational level, %						
Low	8.9		2.6		10.6	
Medium	29.2		51.2		56.1	
High	61.6		46.2		33.3	

Note: Values are based on original, unimputed data. Abbreviations, SD: standard deviation; IQR: interquartile range; BMI: body mass index; FMI: fat mass index; FFMI: fat-free mass index. Missingness was highest for maternal psychopathology symptoms in Generation R (24.3%), for maternal BMI in NTR (22.6%) and for ethnicity in TCHAD (7.5%).

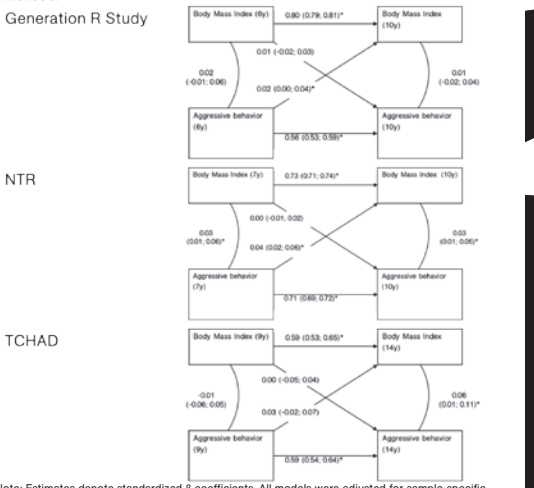


Figure 1: Cross-lagged association model for the association between aggressive behavior and body mass index in three population-based samples across childhood.

Note: Estimates denote standardized β coefficients. All models were adjusted for sample-specific covariates. Fit indices: Generation R sample (*N* =3,974), Root Mean Square Error of Approximation (RMSEA) = 0.06, Comparative Fit Index (CFI) = 0.95, and Tucker Lewis Index (TLI) = 0.90; NTR sample (*N* = 10,328), RMSEA = 0.03, CFI = 0.98, and TLI = 0.95; TCHAD sample (*N* = 1,462), RMSEA = 0.02, CFI = 0.99, and TLI = 1.00. * Significant at *P* < 0.05; numbers in between brackets denote 95% confidence intervals.

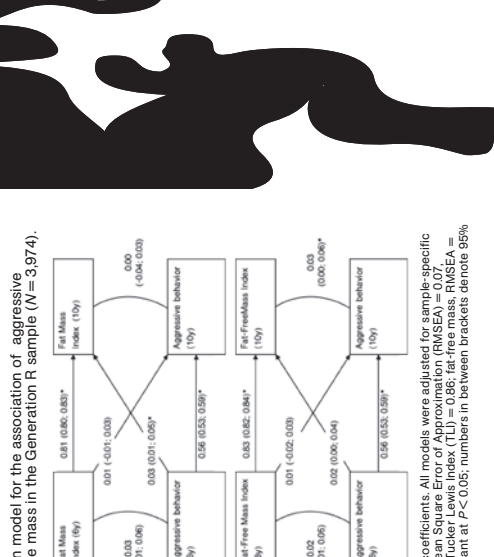


Figure 2: Cross-lagged association model for the association of aggressive behavior with fat mass and fat-free mass in the Generation R sample (*N* = 3,974).

Note: Estimates denote standardized β coefficients. All models were adjusted for sample-specific covariates Fit indices: fatt mass, Root Mean Square Error of Approximation (RMSEA) = 0.07, Comparative Fit Index (CFI) = 0.94, and Tucker Lewis Index (TLI) = 0.86; fat-free mass, RMSEA = 0.05, CFI = 0.96, andTLI = 0.92. * Significant at *P* < 0.05; numbers in between brackets denote 95% confidence intervals.

younger and older ages (Bradley et al. 2008; Anderson et al. 2010; Garthus-Niegel, Hagtvet, and Vollrath 2010; Camfferman et al. 2016), but similar to our study did not find an association in the direction from BMI to subsequent higher externalizing problems. Our present study extends these investigations by providing a specific focus on aggressive behavior, which is important given that aggressive behaviors predict substantial societal costs in terms of health and social service use (Rivenbark et al. 2017). The results from the aforementioned studies which examined externalizing problems more generally, could have been clouded by the well-established association between ADHD symptoms and obesity (Cortese et al. 2016). Importantly, our findings remained in sensitivity analyses with additional adjustment for baseline attention problems, further suggesting a specific prospective link between aggressive behavior and higher BMI.

Thus, our findings suggest that aggressive behavior problems constitute one of the many contributing components of obesity in childhood. Although small in magnitude, the relatively modest effects obtained in the current study are of interest as these associations might be indicative of one of the many likely pathways to increased weight and obesity later in life. Of note, the association between aggressive behavior and subsequently higher BMI was not statistically significant in the TCHAD sample of young adolescents. This lack of replication might potentially be due to the smaller sample size of the TCHAD cohort, which is not impossible considering the small estimates observed in the Generation R and NTR samples. These studies were larger in sample size, with corresponding lower standard errors and, hence, narrower confidence intervals around regression estimates. Future examinations from childhood and adolescence into adulthood are required in order to determine specific developmentally sensitive periods, which is needed considering the stability of weight status across the life course (de Onis, Blossner, and Borghi 2010).

We observed an association of aggressive behavior with subsequent higher fat mass and not fat-free mass, indicating that aggressive behavior is specifically associated with weight-related physical health. This more in-depth finding of body composition adds to the existing

literature, which typically has focused on BMI more generally (Bradley et al. 2008; Anderson et al. 2010; Garthus-Niegel, Hagtvet, and Vollrath 2010; Camfferman et al. 2016). No associations were observed from baseline fat mass or lean mass to future changes in aggressive behavior problems, which further supported our main findings with regards to BMI. These findings lend support to the observation that aggressive behavior might also be considered in the multidisciplinary assessment of childhood obesity. Future research should focus on other behavioral indices of cardiometabolic health and the potential modifiability of these purported risk indicators.

A potential mechanism underlying the observed association might be that children who exhibit more aggressive behavior could also have more problems with behavioral self-regulation and inhibitory control (Eiden, Edwards, and Leonard 2007). Deficits in self-regulation, specifically emotion regulation and behavioral inhibition, arise from executive functioning deficits (Bridgett et al. 2013). Children with deficits in self-regulation potentially do not have the ability to respond adequately to their internal feelings of hunger or satiety cues, which in turn leads to overeating (Graziano, Calkins, and Keane 2010), resulting in weight gain and potentially obesity in the long term. Indeed, studies indicated that the neural pathways controlling appetite and behavioral inhibition are interrelated (McEwen 2008). In addition, temperament, behavior traits, taste preferences, and appetite are regulated by the dopaminergic system (Pecina and Berridge 2000; Keskitalo et al. 2007; Krueger et al. 2007). Moreover, evidence suggested that traits such as eating behavior (Leibowitz and Alexander 1998; Kuikka et al. 2001), and aggressive behavior (Stanley et al. 2000) are both regulated via the same neurotransmitter pathways. Furthermore, aggressive behavior and BMI might share genetic vulnerabilities, which possibly explains the phenotypic associations identified in this study. Recent work has demonstrated common pathophysiological mechanisms for depressive symptoms and obesity (Milaneschi et al. 2017), and this could potentially also apply to other psychiatric problems such as aggressive behavior, which are (genetically) related to depressed mood (Rowe et al. 2008; Wertz et al. 2015).

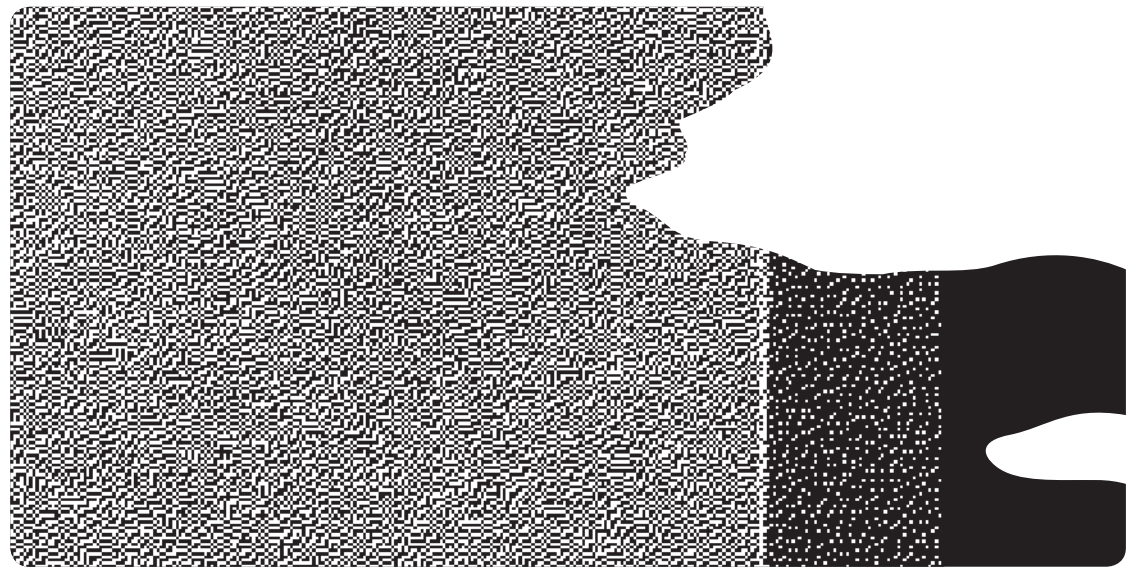
Another potential mechanism that could explain the association between aggressive behavior and increased BMI and fat mass, comprises inadequate coping mechanisms of parents in response to the challenging aggressive behavior of their child. Parents may allow their child to consume more sweets or unhealthy food, they might accept more easily their child's refusal of healthy food, and parents may agree that their children exhibit sedentary activities such as watching television to avoid difficult behavior of their children (Mamun et al. 2009). These actions may eventually, if performed regularly, result in a relatively high weight gain of children (Hughes et al. 2008), and could thus mediate the relationship between aggressiveness and subsequent high BMI.

Strengths of the present study include the prospective study design of all three pediatric community cohorts with an identical instrument of aggressive behavior. Moreover, we were able to analyze body composition in addition to BMI. However, several limitations should be noted. First, our analyses relied solely on mother reports of the CBCL, which could be subject to reporter bias. Repeated multiple informant assessments of aggressive behavior would be preferential, but this was not achievable in the current population-based design. Moreover, the CBCL is a valid and reliable measurement for aggressive behavior (Ivanova et al. 2007), and has good diagnostic accuracy for clinical disruptive behavior disorders (Hudziak et al. 2004). In addition, our analyses in the Generation R Study were adjusted for maternal BMI. Second, as all population-based studies, the included cohorts experienced attrition. However, albeit affecting prevalence, selective loss to drop-out often does not influence the strength of association (Wolke et al. 2009). Third, it is well-established that not only obesity is important for determining physical health. Other factors, such as physical activity, also pose a significant risk for poorer physical health. Here, we were not able to examine the directionality and possible mediation mechanisms between aggressive behavior, physical activity, and obesity and further research is required to address these, potentially modifiable, pathways of risk. Fourth, measures of maternal psychopathology were unavailable in the NTR and TCHAD cohorts. However, adjustment for psychiatric symptoms of the mother only marginally affected our estimates in the Generation

R Study. Fifth, the CBCL/1.5-5 was also used for children aged 6-7 years in the Generation R Study, which might not be appropriate for this age. However, internal consistency of the aggressive behavior scale was similar for 5-year-olds versus 6/7-year-olds (Basten et al. 2013), and the multi-dimensional factor structure of the aggression behavior scale is also comparable with the CBCL/6-18 (Bolhuis et al. 2017). Finally, we suggested a causal association from aggressive behavior to subsequent higher BMI and fat mass instead of vice versa. However, actual causal inference of composite phenotypes such as obesity is complex (VanderWeele 2016), and is restricted in this observational study.

CONCLUSION

The present study showed a small association between aggressive behavior and subsequent increased BMI and fat mass in childhood. This association indicates that aggressive behavior problems observed by mothers is part of one of the many composite risks for obesity in childhood. Hence, it might be helpful to carefully screen for aggressive behavior problems in children with increased risk of obesity. Moreover, our findings signal a need for conducting trials assessing the extent to which treating aggressive behavior in children improves their physical health and well-being (Lumeng et al. 2017). In general, professionals as well as parents and other people involved in the care for children with weight difficulties should be aware of the possible behavioral mechanisms associated with higher BMI and fat mass. Most importantly, future research should examine the extent to which these behavioral pathways to childhood obesity are modifiable, although bearing in mind that effects might be small as observed in the current study.



(...) *and all of it so unspoilt, so intact!*
(Johann Wolfgang Goethe, 1787, The Sorrows of Young Werther)

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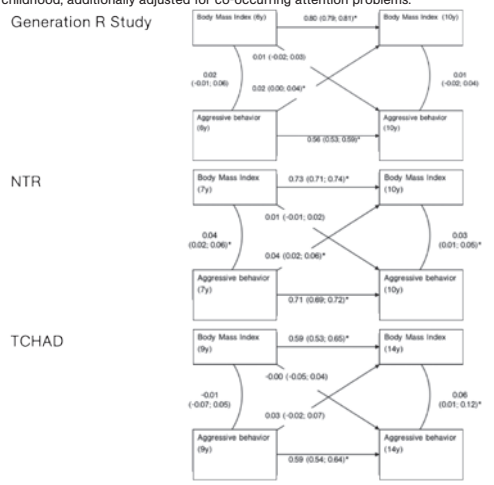
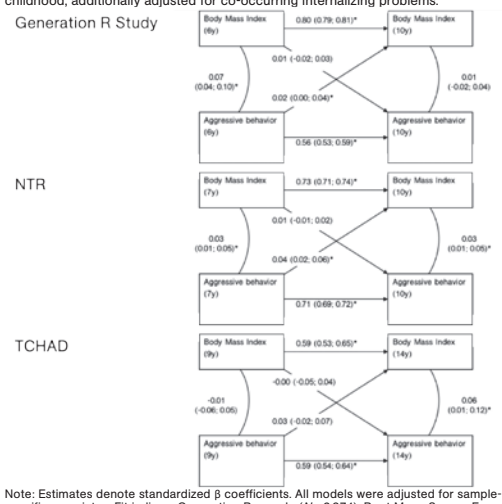
(SUPPLEMENT)

Figure S1: Cross-lagged association model for the association between aggressive behavior and body mass index in three population-based samples across childhood, additionally adjusted for co-occurring attention problems.



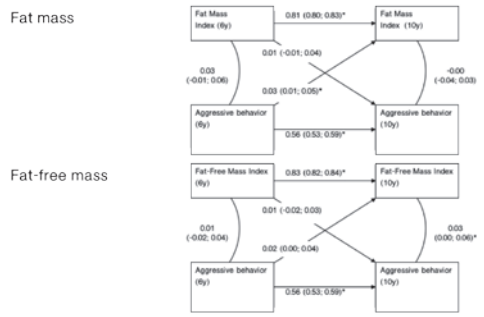
Note: Estimates denote standardized β coefficients. All models were adjusted for sample-specific covariates. Fit indices: Generation R sample (N =3,974), Root Mean Square Error of Approximation (RMSEA) = 0.06, Comparative Fit Index (CFI) = 0.96, and Tucker Lewis Index (TLI) = 0.91; NTR sample (N = 10,328), RMSEA = 0.03, CFI = 0.98, and TLI = 0.96; TCHAD sample (N = 1,462), RMSEA = 0.02, CFI = 0.99, and TLI = 0.99. * Significant at $P <$ 0.05; numbers in between brackets denote 95% confidence intervals.

Figure S2: Cross-lagged association model for the association between aggressive behavior and body mass index in three population-based samples across childhood, additionally adjusted for co-occurring internalizing problems.



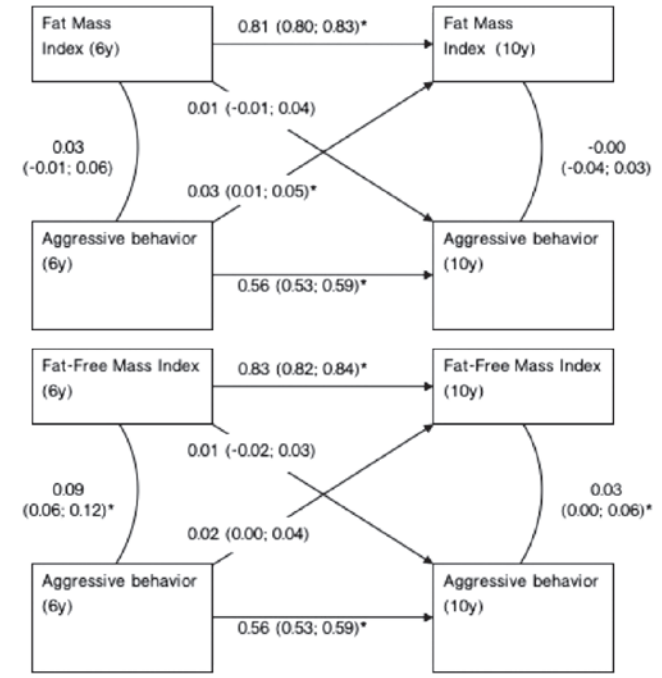
Note: Estimates denote standardized β coefficients. All models were adjusted for sample-specific covariates. Fit indices: Generation R sample (N =3,974), Root Mean Square Error of Approximation (RMSEA) = 0.06, Comparative Fit Index (CFI) = 0.97, and Tucker Lewis Index (TLI) = 0.92; NTR sample (N = 10,328), RMSEA = 0.03, CFI = 0.98, and TLI = 0.96; TCHAD sample (N = 1,462), RMSEA = 0.01, CFI = 0.99, and TLI = 0.99. * Significant at $P <$ 0.05; numbers in between brackets denote 95% confidence intervals.

Figure S3: Cross-lagged association model for the association of aggressive behavior with fat mass and fat-free mass in the Generation R sample (N = 3,974), additionally adjusted for co-occurring attention problems.



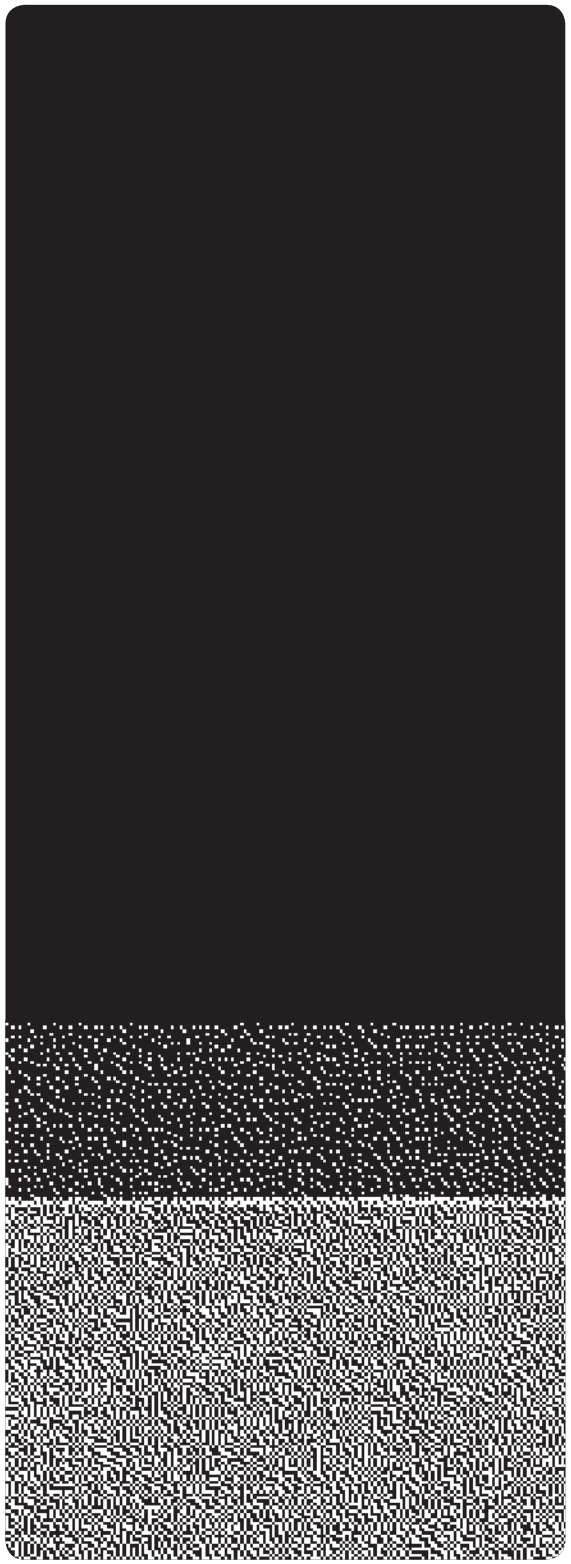
Note: Estimates denote standardized β coefficients. All models were adjusted for sample-specific covariates Fit indices: fatt mass, Root Mean Square Error of Approximation (RMSEA) = 0.06, Comparative Fit Index (CFI) = 0.95, and Tucker Lewis Index (TLI) = 0.88; fat-free mass, RMSEA = 0.05, CFI = 0.97, andTLI = 0.93. * Significant at $P <$ 0.05; numbers in between brackets denote 95% confidence intervals.

Figure S4: Cross-lagged association model for the association of aggressive behavior with fat mass and fat-free mass in the Generation R sample (N = 3,974), additionally adjusted for co-occurring internalizing problems.



Note: Estimates denote standardized β coefficients. All models were adjusted for sample-specific covariates Fit indices: fatt mass, Root Mean Square Error of Approximation (RMSEA) = 0.06, Comparative Fit Index (CFI) = 0.96, and Tucker Lewis Index (TLI) = 0.90; fat-free mass, RMSEA = 0.05, CFI = 0.97, andTLI = 0.94. * Significant at $P <$ 0.05; numbers in between brackets denote 95% confidence intervals.

– Your senses become extraordinarily keen and acute. Your sight is infinite. (...)



(...) *Your ear can discern the slightest perceptible sound, even through the shrillest of noises.* (...)

CHAPTER 12

GENERAL DISCUSSION

RATIONALE

Severe mental illness in adulthood is commonly preceded by psychiatric problems in childhood or adolescence. However, much of the early childhood antecedents of these problems remains underexplored, in particular their heterogeneous presentation, and their neurobiological risks factors. In this thesis, we examined the neurodevelopmental pathways of children at increased risk for severe mental illness. Our focus was on psychotic phenomena and disruptive behaviour problems with the aim of extending existing concepts of the neurodevelopmental models. We approached these phenotypes from a dimensional perspective instead of the classic case-control perspective based on (historically defined) clinical categories, in order to leverage the full spectrum of severity of psychiatric problems. In part I, we addressed the developmental correlates of the extended psychosis phenotype, first by studying the longitudinal associations of psychotic experiences with early behaviour, sleep, and parental cannabis use and, second, by studying the phenotypic expressions of polygenic risk scores of schizophrenia. In part II, we studied the heterogeneity of disruptive behaviour problems and callous traits in children, which we further examined in association with structural brain parameters. In this chapter, I will discuss the findings of this thesis in the broader context of the literature. In addition, I will provide an overview of several methodological considerations and will touch upon the relevance of our observations for future research and clinical practice.

NEURODEVELOPMENTAL MODEL OF PSYCHOSIS

The neurodevelopmental model of psychosis is not new. Early, neurodevelopmental antecedents have been associated with a subsequent onset of psychosis since the late 19th century, as it was first postulated by the Scottish psychiatrist Thomas Clouston in 1891 (Clouston 1891; Murray et al. 2017). This was however soon displaced by the neurodegenerative model of *dementia praecox* as postulated by Emil Kraepelin and later Eugen Bleuler. Psychotic disorders, and in particular schizophrenia, were regarded as adult-onset disorders in which a decay of cognitive functions were considered the key pathophysiological mechanisms. The Kraepelian views held a central place in our thinking about psychosis aetiology for a long time, until the interest in the neurodevelopmental origins of schizophrenia began to re-emerge. Soon, studies were able to show that lower birthweight, obstetric complications, maternal infections and nutritional deficiencies during pregnancy were associated with an increased risk of later psychosis (Murray 2017; Murray et al. 2017). Findings from the Dutch Hunger Winter study, for example, suggest that severe malnutrition during the first trimester of pregnancy increases the risk for schizophrenia-related disorders in women (Susser and Lin 1992), which was replicated in a study on the massive 1959-1961 famine in China (St Clair et al. 2005). These and other studies underscore the importance of considering the early environment in the causal pathways leading to severe mental illness, suggesting that tightly-regulated mechanisms of neurodevelopment can be affected by certain environmental stressors.

Since the late 1980s and early 1990s, the neurodevelopmental theory of psychosis has again been very influential. According to this theory, the early impact on childhood brain development leads to subtle developmental deficits before the onset of overt adverse symptomatology in late adolescence or early adulthood. Findings from the Dunedin birth cohort from New Zealand have been very important in this regard. This population-based cohort is well-known for its high retention of study participants and its comprehensive phenotypic characterisation from birth to adulthood. A study by Cannon and colleagues (2000) demonstrated that people from the Dunedin study with a schizophrenia-spectrum disorder at age 26 years were characterized by more neuro-motor problems, cognitive deficits and lower language skills in early childhood (Cannon et al. 2002). These observations were specific to schizophreniform disorder, as emotional and interpersonal problems were only related to the subsequent development of depression and mania. Moreover, these neurodevelopmental antecedents of schizophreniform disorder were to a similar extent associated with subclinical psychotic experiences at age 11 years, suggesting that these experiences might constitute a developmental intermediate in the pathway from early risk to schizophrenia in adulthood (Poulton et al. 2000). Further support for this

comes from the Generation R Study, which has recently shown that genetic liability for schizophrenia, as quantified by a polygenic risk score, was associated with poorer infant neuro-motor development (Serdarevic et al. 2018).

It is thought that a better understanding of these neurodevelopmental processes will hold the key to the aetiology of psychosis. Psychotic experiences in childhood or adolescence were shown to predict later psychosis, but later work suggested that they conveyed a more general non-specific risk for psychopathology, supporting a trans-diagnostic approach to the extended psychosis phenotype (Kelleher and Cannon 2014; van Os and Reininghaus 2016). Similarly, studies on clinical or familial high risk populations have shifted their focus from narrowly defined psychosis outcomes to a broader focus on psychiatric at-risk phenotypes across diagnoses. The idea behind this approach is that a broad definition would improve specificity – while reducing sensitivity – of a broader trans-diagnostic range of outcome syndromes (van Os and Guloksuz 2017; McGorry et al. 2018). This raises questions about the validity of the search for causal risk factors if the focus is on psychosis specifically. It is, for example, well established that genetic risk alleles for schizophrenia overlap with those for other mental disorders (Cross-Disorder Group of the Psychiatric Genomics et al. 2013; Jones et al. 2018; Brainstorm et al. 2018), in particular major depressive disorder and bipolar disorder, even though there seems to be evidence for independent genetic risk for distinct disorders.

Further support for a developmental, trans-diagnostic approach of psychosis comes from studies which employ the polygenic risk score of schizophrenia variants (Schizophrenia Working Group of the Psychiatric Genomics Consortium 2014). Polygenic risk scores are weighted sum scores of genetic risk variants derived from large genome-wide association studies (GWAS). Common genetic variants, single-nucleotide polymorphisms (SNPs), assessed in this discovery GWAS are examined across different *P*-value thresholds of significance. The more stringent this threshold used for the risk score, the more the included SNPs discriminated schizophrenia cases from controls in the original GWAS. For these SNPs, their risk alleles and its effect sizes are assessed, which are summed and weighted across the genome. The polygenic risk score for schizophrenia explains approximately 7% of schizophrenia case-control variation (Schizophrenia Working Group of the Psychiatric Genomics Consortium 2014). Importantly, only common genetic variants are included in the risk score and, hence, such information from GWAS studies does not directly consider rare SNPs or structural variants. The polygenic risk score of schizophrenia has been used in population-based samples to examine associations with various traits, which has been observed for neurodevelopmental deficits in childhood, social communication difficulties, anxiety, negative symptoms in adolescence, and cognition (Jones et al. 2016; Riglin et al. 2017). These studies are important as they show the developmental antecedents of increased genetic vulnerability for severe mental illness, such as schizophrenia, and how these may manifest in non-selected samples from the community.

In this thesis, we employed the schizophrenia polygenic risk score metric. We provide suggestive evidence for joint effects of the genetic risk for schizophrenia and chronic stress exposure on brain structure in pre-adolescence, in particular ventricle volume and white matter integrity. These observations, albeit of suggestive significance, fit with seminal work from Robin Murray's group using neuroimaging data from monozygotic twins discordant for schizophrenia (Reveley et al. 1982). Monozygotic twins with schizophrenia had larger ventricles than their unaffected co-twins, suggesting that environmental factors, possibly perinatal damage, played a substantial role. The authors stressed the importance of assessing the interplay of genetic and environmental risk in shaping brain development, but also highlight the approach of elevated risk for severe mental illness from a (neuro)developmental perspective (van Os, Kenis, and Rutten 2010). Also in this thesis, we demonstrated that the schizophrenia polygenic risk is associated with a child's greater likelihood to experience adverse life events, and that this association mediates the relationship of schizophrenia polygenic risk with phenotypic expressions of emotional, attention and thought problems in childhood. These gene-environment correlations, which refer to the notions that a person's exposure to environment (e.g. adverse life events) partially

depends on their genetic make-up, are important factors to take into consideration in developmental psychopathology. These findings stress the complex interplay of genetic, behavioural, environmental, and biological factors. Potentially, multi-exposome in combination with multiple trans-diagnostic outcomes will hold the future for research risk and development of psychiatric disorders (Guloksuz, van Os, and Rutten 2018; Brainstorm et al. 2018).

NEURODEVELOPMENTAL MODEL OF DISRUPTIVE BEHAVIOUR

Disruptive behaviours are highly prevalent in young people; these behaviours represent one of the most common reasons for referral to child and adolescent mental health services (Peterson et al. 1996; NICE Guidelines 2013; Hill and Maughan 2015). Some children and adolescents exhibit high levels of more persistent disruptive behaviours along a spectrum of severity, and these behaviours can take different expressions such as temper outburst, fighting, not complying with rules or a combination. Such phenotypic diversity (or, rather, heterogeneity) has been a subject of many studies and was one of the core themes of this thesis. In chapter 7 we aimed to disentangle the heterogeneity of disruptive behaviour problems in children from community and clinical samples. We observed that childhood disruptive behaviour problems can best be conceptualised as a complex multi-dimensional phenotype, comprising the dimensions physical aggression, irritability, disobedient behaviour and delinquent behaviour. Notably, we found that irritability was a distinct dimension on the broader spectrum of disruptive behaviours predicting later physical aggression, underscoring the value of recognising emotion dysregulation in youths presenting with a broad range of disruptive behaviour problems. Importantly, in this well-powered study, we were unable to obtain any empirical support for the existence of distinct subgroups of children with specific patterns of disruptive behaviour problems.

Our work extends the many other studies spanning a period of more than 30 years of research on the heterogeneity of disruptive behaviour problems in youth. One of the most influential papers on this topic has been published by Moffitt in 1993, which described the developmental taxonomy of antisocial behaviour based on age of onset and persistence/transience of behaviours (Moffitt 1993). Adolescence-limited disruptive behaviour was postulated to be distinct from early onset life-course-persistent disruptive behaviour, where the latter is characterized by substantially more neuropsychological and criminogenic environmental risks than the former. Children on these early onset, persistent trajectories show declining mental health, poorer physical health and more economic insecurity in adulthood, and they were more likely to engage in violent offending than youth on adolescence-limited trajectories (Odgers et al. 2008). These and other studies on the early developmental origins of antisocial or disruptive behaviour have been pivotal for our understanding of certain developmental risk mechanisms. Studies on genetic and neurobiological/neurocognitive mechanisms have been less conclusive about the differentiation between early-onset versus adolescence-onset disruptive behaviour (Tremblay 2010).

Twin studies have indicated that aggressive behaviours in children are considerably heritable (i.e. approximately 50%). High heritability estimates have been found for early-onset aggression (Silberg et al. 2007; Silberg, Moore, and Rutter 2015), and for aggression with elevated callous-unemotional traits (Viding et al. 2005). The search for candidate genes for disruptive behaviour in children has been rather disappointing. Meta-analyses of candidate gene studies have shown that the monoamine oxidase A polymorphism moderates the effect of environmental stress on the development of antisocial behaviour in children (Kim-Cohen et al. 2006; Byrd and Manuck 2014), one of the very few candidate gene by environment interaction findings that has received some meta-analytic support. Genome-wide association studies on disruptive behaviour in children have not been very successful (Pappa et al. 2016). A recent GWA study combining adult and paediatric samples (N = 16,400) indicated that antisocial behaviour is heritable, polygenic and that part of the genetic architecture is sex-specific (Tielbeek et al. 2017), but was underpowered to consistently find any gene loci associated with antisocial behaviours. Large collaborative efforts working together in the ACTION (Aggression in Children: unravelling gene-environment interplay to inform Treatment and InterventION

strategies) consortium are underway to unravel the genetic background of aggressive behaviours in young people.

A growing number of studies have been conducted to characterize the neurobiology of disruptive behaviour problems in young people. The majority of these studies employed task-based functional magnetic resonance imaging (fMRI) techniques in clinical/high-risk samples of (mostly) males. A meta-analysis of these individual studies has demonstrated that youth with disruptive behaviour disorders show decreased activation of the dorso-rostral anterior cingulate cortex, medial prefrontal cortex, and ventral striatum, which are involved in top-down regulation of motivation, affect and reward-related decision-making (Alegria, Radua, and Rubia 2016). These findings partially converge with a recent meta-analysis of structural MRI studies using voxel-based morphometry, which has shown that youth with conduct problems have grey matter volume reductions in the amygdala, insula, and (pre-)frontal and temporal regions (Rogers and De Brito 2016). Only a few studies have employed diffusion tensor imaging (DTI) to examine the integrity of white matter tracts connecting cortical brain regions associated with youth disruptive behaviour problems. A review of DTI studies in young people with disruptive behaviours problems concluded that the inconsistent findings can be likely explained by heterogeneity of the samples and (co-morbid) behaviours of the included individuals, different analytic methods and wide age ranges (Waller et al. 2017).

In chapter 8 we proposed that disentangling the multidimensionality of disruptive behavior problems would aid the search for their neurobiological correlates. This is particularly pertinent given that few neuroimaging studies have benefited from considering this multidimensionality (Blair et al. 2014). We studied the association of global and tract-specific white matter integrity with dimensions of disruptive behaviour problems (i.e. physical aggression, irritability, disobedient behaviour and delinquent behaviour), which we described earlier in chapter 7. We observed that less developed global white matter microstructure was uniquely associated with delinquent behavior, and not with other dimensions of childhood disruptive behavior problems. The individual white matter tracts underlying this global association comprised the inferior longitudinal fasciculus, superior longitudinal fasciculus, cingulum, and uncinate. We concluded that acknowledging the heterogeneity of disruptive behaviour problems is important in the search for their neurobiological aetiologies and might be promising for elucidating causal pathways. Such methodologies might also be worthwhile for psychiatric phenotypes other than disruptive behaviour problems, such as depression with or without irritable features, although this needs to be further explored.

Similarly, we aimed to assess the neurobiological correlates of callous traits in children. Callous traits, such as shallow affect, remorselessness, and a callous lack of empathy, are used to identify a particularly problematic subgroup of children with disruptive behaviour problems, distinguished by more severe and chronic antisocial behavior and a poor long-term prognosis (American Psychiatric Association 2013). Research using fMRI has identified a differential pattern for conduct problems youth between those with or without psychopathic traits (Alegria, Radua, and Rubia 2016), supporting the need of a specifier in the diagnosis. However, research on the structural (neuroanatomical) correlates of callous traits in children has been less consistent (Rogers and De Brito 2016; Waller et al. 2017) as it is limited by its focus on small clinical/high-risk samples of males and its lack of whole-brain approaches including both structural MRI and DTI data. Our findings from chapter 10 suggest that callous traits at age 10 years are characterized by widespread macrostructural and microstructural grey and white matter differences across the brain, which provide further support for the value of conceptualizing paediatric callous traits as a neurodevelopmental condition (Wakschlag et al. 2018; Raine 2018).

The neurobiological findings that we discussed in chapters 8 and 10 should be appreciated as descriptive cross-sectional observations in a general population sample of children. It is interesting that our findings overlap with those from studies using samples of young people from clinical or childhood arrestee cohorts, who are usually from poor socioeconomic backgrounds, have higher psychiatric co-morbidity rates, and a greater likelihood of exposure to violence or adversity (Viding and McCrory 2018). Future research on causal

mechanisms for the development of severe disruptive/antisocial behaviour should adopt a prospective designs to examine risk across the life course (e.g. the prenatal phase or early adolescence), and incorporate multiple biological and psychosocial methodologies, such as neuroimaging and childhood adversity data (Tremblay 2010).

METHODOLOGICAL CONSIDERATIONS

ANALYTICAL METHODS OF BEHAVIOURAL HETEROGENEITY IN PSYCHIATRY

Phenotypic heterogeneity among child and adolescent psychiatric disorders is the rule rather than the exception. Two children with the same psychiatric diagnosis often markedly differ in their phenotypic expression of emotional and behavioural problems. This has been an on-going debate in child psychiatric academia as this has important consequences for nosology (i.e. the branch of medicine dealing with the classification of disease), diagnosis, treatment, prognosis and aetiological research (Caron and Rutter 1991). In this section we will focus on phenotypic heterogeneity in terms of nosology and diagnosis, and we will draw on the example of the heterogeneity of disruptive behaviour problems, which we have described in chapter 7. Finally, we will touch upon novel approaches which have recently been used to disentangle the etiologic heterogeneity of psychiatric diagnosis, such presented in chapter 8.

We have described in chapter 7 that in the past many studies have attempted to disentangle the heterogeneity of disruptive behavior problems in children, using various informants, instruments, and study populations. This has influenced the modification of the criteria for oppositional defiant disorder (ODD) and conduct disorder (CD) in the classification schemes such as the *Diagnostic and Statistical Manual of Mental Disorders, 5th edition (DSM-5)* and the *International Classification of Diseases 11th revision (ICD-11)*. For example, it is now possible to diagnostically distinguish irritable from oppositional ODD sub-types, and to specify whether CD co-occurs with low prosocial emotions or not. Although these classifications of children into distinct sub-groups is relatively straightforward and therefore appealing for clinical practice, it has some caveats. Most importantly, the majority of these classifications and sub-typing have not been empirically investigated beyond *a priori* defined symptoms already aligned with classification criteria. Data-driven efforts on a broader spectrum of behavioural problems have been lacking, but are needed to strengthen the current psychiatric diagnostic frameworks with an empirical basis.

Latent variable modelling is a useful analytical approach that allows the study of patterns of observations in cross-sectional data and to draw conclusions about unobserved (i.e. latent) sources of population heterogeneity (Lubke and Miller 2015). In principle, two major techniques describe the variability among observed correlated symptoms in terms of latent (i.e. unobserved) variables. First, dimensional latent variables explain the variability in terms of one or more dimensions on a linear severity scale. Second, categorical latent variables explain the variability in terms of differences between two or more discrete subgroups (classes) of individuals. The first are used in factor analysis, and the second in latent class analysis (LCA) (Figure 1, left and middle panels).

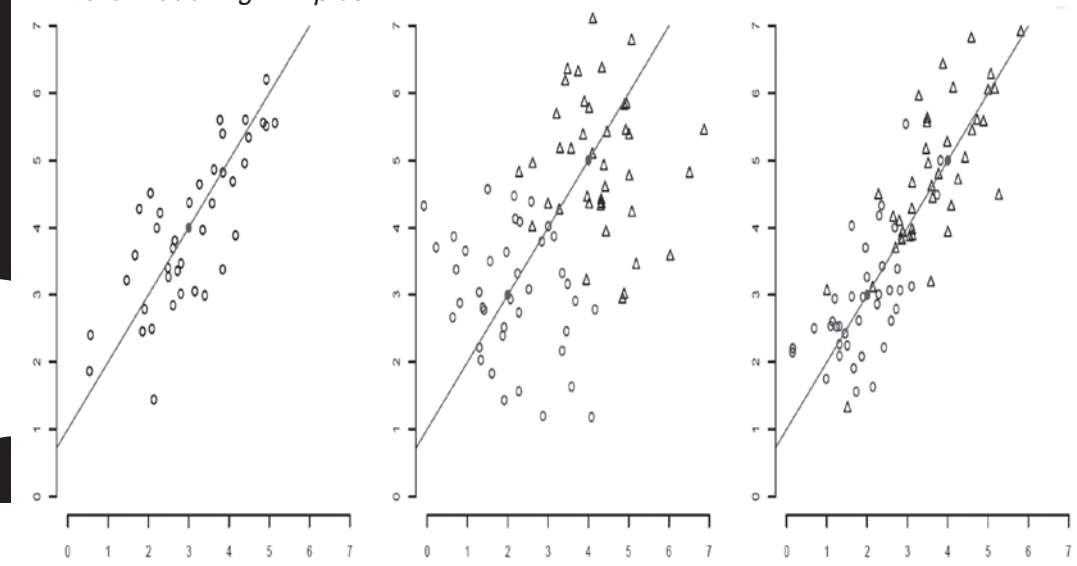
Importantly, LCA classifies individuals into homogenous subgroups or latent classes and makes a strong assumption of local independence. This means that the latent class variable accounts for all observed variation patterns observed in the data of a given population and that within individual classes, there is no correlation among the observed variables. In practice, this assumption of local independence might be unrealistic for many psychiatric traits. For example, in both clinical and general population samples, related symptoms of disruptive behaviour such as rule-breaking and physical aggression are often (moderately to highly) correlated and may thus violate the local independence assumption of traditional LCA (Lubke and Miller 2015; Miettunen et al. 2016).

Models that allow for more variation among observed variables might be more suitable for psychiatric data. Factor mixture models (FMMs) allow for the additional presence of dimensional latent variables, aside from categorical latent variables, to describe symptoms patterns. Dimensional latent variables explain the variability in terms of one or more dimensions on a linear severity scale. FMMs are different from LCA because they do not assume local independence, as within classes

one or more factors are allowed, which results in covariation among the observed variables within class. Furthermore, compared with LCA, FMMs extract fewer classes to explain the observed associations, considering that covariation among observed variables is attributed to mean differences between classes. FMMs are thus more parsimonious, and therefore most appropriate, for examining whether a psychiatric trait occurs as a dimensional phenomenon or as distinct subgroups (Lubke and Muthen 2005; Lubke and Miller 2015; Miettunen et al. 2016).

In chapter 7, we have employed factor mixture modelling to examine the presence of dimensions or subgroups of disruptive behaviour problems. Using data from three population-based and one clinical cohort, we concluded that disruptive behaviour problems in children should be regarded as a multi-dimensional phenotype, rather than comprising distinct subgroups. Incorporating such multi-dimensionality will improve the diagnostic accuracy of children with disruptive behaviour problems and will potentially aid aetiological research in the search for causes of disruptive behaviours. We showed the benefits of this approach in a neuroimaging setting in chapter 8. Another important novel field of research is genomic structural equation modelling, a complex latent variable model which incorporates path relations between genetic variants and latent variables (Grotzinger et al. 2018). Genomic structural equation models a common genetic architecture across different phenotypes with factors that may be considered general genetic liabilities, which allow for the identification of genetic variants which are influential across traits (similar to genetic pleiotropy), but also to identify SNPs that confer specific risk for individual specific traits (Cross-Disorder Group of the Psychiatric Genomics 2013). In addition, polygenic risk scores based on a genomic structural equation model of five inter-correlated psychiatric traits were consistently shown to have more predictive power than polygenic risk scores based on individual traits. To conclude, incorporating the multi-dimensional nature of psychiatric traits shows promise in advancing the search for their aetiology. We have shown this for the neurobiological correlates of childhood disruptive behaviour problems.

Figure 1: Covariation due to a continuous latent factor (left panel), due to mean differences between latent classes (middle panel), or due to both continuous factors and latent class differences (right panel). Adapted from Lubke, G. 2012. *Mixture Modelling in Mplus*.



(Full colour image presented on page 16.)

CONTROVERSY OF ASSESSING PSYCHOTIC EXPERIENCES IN CHILDREN

It is now well-documented that psychotic experiences, such as hallucinations and delusions, can commonly occur outside the context of clinical psychotic disorders (Linscott and van Os 2013). Within the general population, psychotic experiences have a prevalence of 7-8% in adults (Linscott and van Os 2013), which is substantially higher than the 1-3% prevalence of psychotic disorders. Epidemiological research in this field from the past few years has resulted in what is now known as a *continuum of psychosis* (van Os and Reininghaus 2016), which posits that psychotic experiences can occur along a spectrum of reality testing, and that these experiences do not necessarily meet the full psychotic severity of impairment.

Early work has focused on the relationship between psychotic experiences and clinical psychotic disorder and, indeed, a prospective link was established between psychotic experiences in adolescence and subsequent psychosis meeting clinical diagnostic thresholds (Poulton et al. 2000; Welham et al. 2009; Zammit et al. 2013). This finding supports the developmental ontology of psychotic symptomatology across the lifespan. Furthermore, childhood sub-clinical psychotic experiences are strongly predictive of various non-psychotic psychiatric disorders, such as anxiety and depression. Remarkably, only 10% of children with psychotic symptoms in childhood or early adolescence do not develop a psychiatric or substance use related disorder later in life (Fisher et al. 2013). Of even more compelling importance, studies in the last couple of years have consistently shown that childhood sub-clinical psychotic experiences index an increased liability for future suicidal behaviour (including suicidal ideation and suicide attempts), increased mental health service use, and a particularly poor prognosis (Kelleher et al. 2013; Bhavsar et al. 2017; Dhossche et al. 2002). This suggests that, although childhood sub-clinical psychotic experiences are very heterogeneous and do not specifically increase the future risk for psychosis, they are a substantial risk indicator of children in need of mental health support.

A common critique on the concept of sub-clinical psychotic experiences in children is that of measurement error. Are we indeed assessing "true" psychotic symptomatology when we ask 9 to 12 year old children whether they hear voices or sounds that are not there, or if they see things that other people cannot see? Are we not actually enquiring about an imaginary friend or about a scary ghost under the child's bed? This critique is supported by cross-sectional observations of 5-to-7-year-olds, in whom hallucinations have been linked to a greater likelihood of having an imaginary companion and larger deficits in theory of mind, i.e. a person's ability to attribute mental states to oneself and to others and to understand the mental states of other people (Pignon et al. 2018). The study suggests that clinical evaluation of psychotic experiences in such contexts might result in over-medicalisation of behaviours which actually constitute normative childhood behaviour. Similarly, in clinical samples of children seeking help for auditory hallucinations, only 11.6% suffers from a psychotic disorder (Maijer, Palmen, and Sommer 2017). The high prevalence of psychotic experiences in pre-adolescent children, namely approximately 17%, which implies that not nearly every child with a psychotic experience meets the diagnostic threshold for substantial psychiatric impairment further fuelled the worries of over-medicalisation (Kelleher, Connor, et al. 2012). The majority of psychotic experiences in childhood tends to remit with age (Thapar et al. 2012; Bartels-Velthuis et al. 2016), although it is not clear from these studies whether children with transitory psychotic experiences are at minimal risk for other types of psychopathology, such as mood problems.

Should we treat psychotic experiences in children as a mere benign, unharmful phenomenon? Notwithstanding the fact that most children tend to no longer exhibit psychotic experiences after some time, these symptoms do index a risk for future psychiatric illness, including, but not exclusively limited to, psychosis (Fisher et al. 2013). Studies have indicated that child self-reported psychotic experiences predict clinician-confirmed symptoms of psychotic disorders (Kelleher et al. 2011). And even self-reported experiences that are unconfirmed by clinical interview, i.e. 'false-positive' psychotic experiences, index an increased risk for clinically relevant psychotic symptoms (van der Steen et al. 2018; Rimvall et al. 2018). Moreover, children or adolescents with psychotic experiences are characterised by psychiatric multi-morbidity, i.e. they are more likely to suffer from more than one psychiatric disorder than to have only one or no

disorder (Kelleher, Keeley, et al. 2012). In this thesis, we described in chapter 2 that pre-adolescents who reported psychotic experiences were more likely to have emotional and behavioural problems from as early as age 3 years. This implies that their psychiatric problems started early in life. In other words, we can observe temporal continuities from common psychiatric problems in early development to psychotic experiences in pre-adolescence, which might in turn denote an increased risk for severe mental illness in adolescence and early adulthood.

The liability to severe psychiatric illness might present differently across the lifespan, as emotional difficulties in childhood, as psychotic symptoms in adolescence, and finally as clinical psychotic illness in early adulthood (Jones et al. 2018). It is not yet entirely clear what constitutes this common vulnerability, but it is likely that heritable factors play an important role. For example, parents of children who report psychotic experiences more often have psychiatric diagnoses than parents of offspring without psychotic experiences. Some studies report a particularly strong association between parental psychotic disorders and offspring psychotic experiences, suggesting a specific intergenerational transmission of psychotic symptomatology (Jeppesen et al. 2015). Other studies have demonstrated that parental depression and general parental psychopathology also increase the odds for offspring psychotic experiences (Zammit et al. 2008; Wigman et al. 2012).

These findings are corroborated by a recent study that has investigated polygenic risk scores of severe mental illness, i.e. schizophrenia, bipolar disorder and major depressive disorder, in relation to psychotic experiences. Although no study to date has identified specific genetic variants for psychotic experiences, this particular study has shown that the polygenic risk scores for schizophrenia and depression predicted psychotic experiences in adolescence (Pain et al. 2018), providing evidence for biological pathways which are shared between psychotic experiences and clinical psychiatric disorders. Similar to other work relying on polygenic risk scores for the prediction of behavioural traits, the explained variance was quite low at ~0.10%, indicating that the polygenic risk scores for psychiatric disorders are not (yet) appropriate for clinical utility. Twin studies have provided additional information on genetic influences on psychotic experiences by establishing that the same genetic and environmental factors influence both the more frequent and severe as well as the less frequent and milder psychotic experiences in adolescence (Zavos et al. 2014). Twin heritability estimates for psychotic experiences are moderate, and psychotic experiences are genetically correlated with emotional and behavioural problems (Shakoor et al. 2018). Nevertheless, there is evidence for independent genetic effects on psychotic experiences, over and above the genetic effects on psychopathology more generally. This suggest that they are not a mere (aetiological) extension of other psychopathology, but a distinct phenotype against a complex background of aetiologies, which are both unique but also shared with other psychiatric phenotypes.

In summary, psychotic experiences can be conceptualised as existing on the psychosis continuum, both in terms of severity from subclinical psychotic experiences to clinical psychosis, as well as in the developmental ontology of psychotic symptoms across the lifespan. Furthermore, psychotic experiences signal an increased risk for subsequent severe mental health outcomes, such as suicidality. As psychotic phenomena are relatively easily assessed, and preliminary evidence indicates they are responsive to psychotherapeutic interventions (Maijer, Palmen, and Sommer 2017), we see no barriers to more clinical assessments of psychotic experiences in young people. This will result in a better understanding of these symptoms in both research and clinical settings, which on the long-term need be evaluated in treatment and prevention trials for severe mental illness.

CANNABIS USE AND PSYCHOPATHOLOGY

There is a wealth of studies exploring the links between substance use and psychiatric disorders. It is overwhelmingly clear that the prevalence of substance use (including alcohol, tobacco and cannabis use) is higher in people with psychiatric disorders. This raises the question as to whether substance use causes psychopathology, or whether this relationship is in the opposite direction or bi-directional. This has been a particularly pertinent discussion in the context of

cannabis use and psychosis, as the effects of hashish on perception and thought control have been noted since the mid-19th century (Moreau 1845). For example, Charles Baudelaire wrote in "Les paradis artificiels" in 1860 that due to the effects of hashish "your senses become extraordinarily keen and acute. Your sight is infinite. Your ear can discern the slightest perceptible sound, even through the shrillest of noises. The slightest ambiguities, the most inexplicable transpositions of ideas take place. In sounds there is colour; in colours there is a music...".

Various reviews and meta-analyses of prospective epidemiological studies have concluded that the use of cannabis increases the risk for subsequent psychotic outcomes beyond the transient intoxication effects, including psychotic experiences and clinical psychotic disorders (Arseneault et al. 2004; Moore et al. 2007; Gage, Hickman, and Zammit 2016; Belbasis et al. 2018). Furthermore, a dose-response relationship has been observed, i.e. among the most frequent users of cannabis the increased odds for psychosis-related outcomes ranged from 2.09 to 3.90 (Moore et al. 2007; Marconi et al. 2016). These studies provide reasonable evidence that cannabis use can increase the risk of psychotic outcomes. Each of these studies has argued that cannabis use should be discouraged among youth vulnerable to psychiatric disorders/high risk youth. Even though there substantial observational evidence for a relationship between cannabis consumption and subsequent psychotic symptoms, a causal link cannot be unambiguously established. More specifically, cannabis use does not appear to be a necessary or a sufficient cause of psychosis. It is not a necessary cause as not all adults with psychosis have used cannabis prior to the onset of their symptoms. Also, cannabis use is not a sufficient cause of psychosis as the majority of adolescent cannabis users do not develop a subsequent psychotic disorder. Rather, cannabis use appears to constitute part of a constellation of risks, including heritable factors and early life adversities (Arseneault et al. 2004; Belbasis et al. 2018).

However, this purported causality of cannabis use for psychosis should be evaluated in the context of stringent criteria for causality. Important factors such as confounding and bias have been insufficiently considered. For example, in the context of the link between cannabis use and psychosis, it could be argued that there are common aetiological factors that explain their overlap. In chapters 4 and 9 we have demonstrated the familial co-aggregation of parental cannabis use with offspring psychotic experiences and externalizing behaviours at age ten years, well before the risk period of adolescent cannabis use initiation. This is can be explained by common risk factors, most likely shared genetic vulnerabilities. Indeed, twin studies as well as molecular genetic studies indicate there is a moderate genetic correlation between cannabis use and psychosis (Power et al. 2014; Nesvag et al. 2017; Verweij et al. 2017). Such a genetic correlation between "environmental" determinants and a given outcome is important to consider when estimating the impact of purported environmental risks for psychosis.

Novel approaches such as Mendelian randomisation (MR), where genetic information is used as an instrumental variable to test the causality of an environmental determinant of interest, can be used to test the assumption of no unmeasured confounding. When the assumptions required for MR hold, genetic variants associated with an exposure (e.g. cannabis use) will only be associated with an outcome of interest (e.g. schizophrenia), when the exposure causes the outcome. The most recent genome-wide association study of 184,765 individuals has identified four independent single nucleotide polymorphisms for lifetime cannabis use (Pasman et al. 2018), which have been further examined in bi-directional two-sample MR analysis to establish whether cannabis use causes schizophrenia and/or vice versa. No clear evidence was obtained for lifetime cannabis use causally influencing schizophrenia whereas, conversely, schizophrenia was positively associated with lifetime cannabis use. This is in line with an earlier MR study, which employed similar genetic instruments (Gage et al. 2017) but not with another which only tested causality in one direction (Vaucher et al. 2018). The authors noted that the lack of causal effect on schizophrenia might be due to the low explained variance of the genetic instrumental variables (i.e. 0.15-1.12% as opposed to the higher explained variance of schizophrenia genetic instruments, i.e. 3.38%), which is indeed a clear limitation (Gage et al. 2013). In addition, the genetic variants were based on lifetime and

not regular cannabis, which introduces heterogeneity and reduces power for genetic association analyses. Other important issues that need to be taken into account is polygenicity and genetic heterogeneity (Swanson et al. 2017; Gage et al. 2013), as it unlikely that cannabis use is caused by only a few genes from independent biological pathways (Pasman et al. 2018), and it is possible that these genes overlap with the genes involved in schizophrenia, as demonstrated by their positive genetic correlation (Nesvag et al. 2017; Pasman et al. 2018).

There is a clear need to know the causal direction of the association between cannabis use and psychosis, in order to subsequently explore the potential mechanisms which might underlie this relationship. This would aid the development of preventative interventions. It has been suggested that individuals with a high risk for psychosis (e.g. through genetic or familial vulnerabilities) experience prodromal symptoms which would make them more likely to initiate cannabis consumption. This is not unlikely given that risk-taking behaviours and lower conscientiousness have been associated with both cannabis use and psychotic outcomes (Pasman et al. 2018). Future research with assessments of early behavioural development prior to cannabis use initiation (i.e. in pre-adolescence) is needed to examine the pathway from prodromal functioning and familial circumstances to later substance use to subsequent severe mental health outcomes in late adolescence. In this thesis we described the familial co-aggregation of parental cannabis use and offspring (prodromal) psychotic experiences at an age well before cannabis use initiation, which provides preliminary support to this above suggested pathway, although follow-up assessments on substance use of the child are needed. These mechanisms will preferably need to be investigated including information on genetic risk, for example, in (familial or clinical) high risk populations and large community samples with repeated measures of behaviour and substance use across development. Additional comparisons with use of other substances (e.g. alcohol, tobacco) is necessary to address to causal pathways of addictive behaviours more generally or toxic/psychostimulant aspects of a substance more specifically (Gage et al. 2013), which can help inform prevention interventions. Furthermore, as the consumption of cannabis is increasing worldwide and also among pregnant women, it is paramount to further investigate the mechanisms behind the familial co-aggregation of parental cannabis use and offspring psychopathology, such as described in this thesis. This would provide further insight into the question of whether cannabis use during pregnancy has any adverse effects on the developing foetus. Such endeavours would include observational twin/sibling designs as well as experimental lab study designs by taking into account genetic and other familial confounds and detailed assessments of substance use.

CLINICAL IMPLICATIONS

From the work discussed in this thesis we can highlight several implications for mental health clinical practice. First, we propose that the clinical assessment of psychotic experiences should become routine practice in child and adolescent mental health services. Hearing sounds or voices that are not there, or seeing things that other people cannot see, or other hallucinatory phenomena can potentially be very frightening for children. The presence of these symptoms can also be very distressing for parents, who might think that their child is severely ill or at risk for psychosis. It is, hence, not surprising that specialist clinics for children who seek help for hearing voices observe high levels of stress and reduced functioning in these families (Maijer, Palmen, and Sommer 2017). Even when these children did not meet diagnostic criteria for psychosis or any other psychiatric disorder, they were characterised by elevated levels of perceived stress and impairment, and they thus required clinical attention (Maijer, Palmen, and Sommer 2017; Maijer et al. 2018). Importantly, a clinical evaluation of a young person with psychotic experiences would include a careful diagnostic assessment for psychiatric diagnoses; which is particularly relevant for older youth with psychotic experiences, who are more likely to suffer from one or more psychiatric disorders (Kelleher, Keeley, et al. 2012). Further trials on screening of psychotic experiences and subsequent treatment or prevention interventions are needed to shed more light on this issue. Psychoeducation, teaching of coping skills, and other cognitive behaviour therapy elements will be useful for these children and their

families. Finally, but most importantly, a clinical assessment of a young person seeking help for mental health problems should always include an evaluation for the presence of psychotic experiences and risk of suicidality. Even though the majority of these psychotic experiences remit with age, in the present mental state they can be extremely distressing for the young person. As described in this thesis, psychotic phenomena tend to co-occur with other emotional and behavioural problems, and these developmental associations from early emotional and behavioural problems to subsequent psychotic experiences were observed from as early as age 3 years. Furthermore, psychotic experiences index an increased risk for future (psychotic and non-psychotic) psychopathology, including severe outcomes such as suicidal behaviour. Hence, the possibility of a young person exhibiting these symptoms should be taken seriously, and the clinical assessment of these symptoms should be done with the utmost scrutiny.

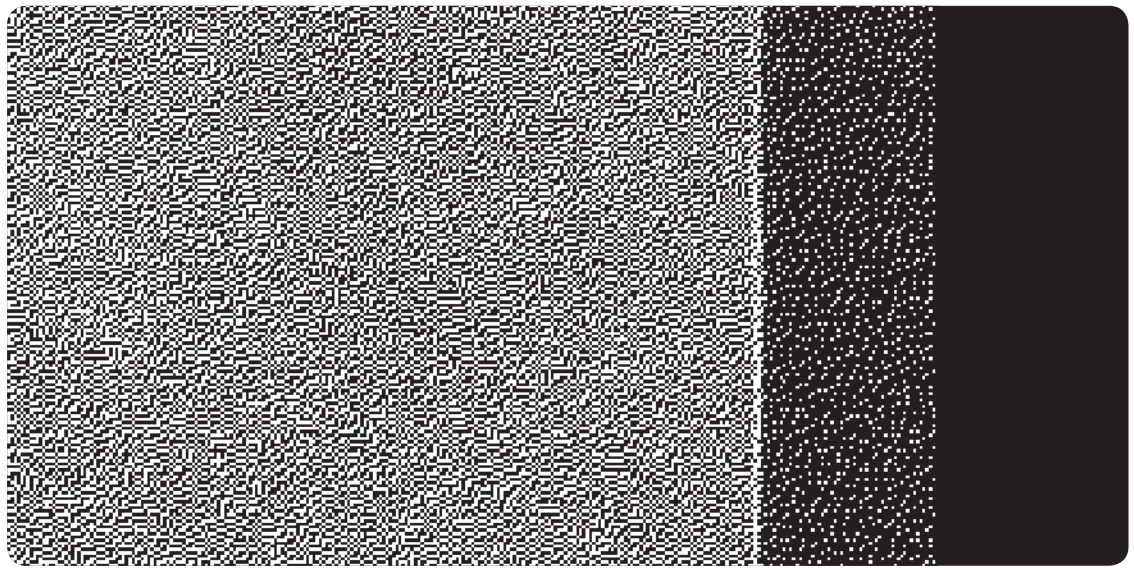
Second, disruptive behaviour problems are best conceptualised as a multi-dimensional phenotype rather than comprising distinct subgroups, as we described in chapter 7 of this thesis. Even though clinical cut-offs are important and many studies have tried (and will try in future efforts!) to base these cut-offs on data-driven results, our finding of the lack of distinct sub-groups suggests that empirical classification strategies will likely remain unsuccessful. The multidimensional phenotype of disruptive behaviour problems is just too complex to be based on a single narrow cut-off of classification and diagnosis. This classification could – if needed – rather be based on cut-offs determined by clinical convention, also considering other factors such as psychosocial impairment, heritability, previous exposure to adversity, and neurobiology. In chapter 8, we observed reduced global white matter microstructural integrity in children with elevated levels of delinquent behaviour. Interestingly, and in contrast with several previous studies, we also observed that irritability (characterised by temper tantrums, sullenness and irritable mood) is at cross-sectional and longitudinal levels associated with phenotypically severe dimensions of disruptive behaviour problems such as physical aggression and delinquent behaviour. This underscores the value of recognizing emotion regulation problems in youth presenting with a broad range of disruptive behaviour problems (Wakschlag et al. 2018). Another specific constellation of symptoms on the broad spectrum of disruptive behaviour problems that merits specific consideration is that of callous traits. Youth with elevated levels of these symptoms are at substantially increased risk for antisocial behaviour and criminal offending in adulthood (Pardini et al. 2018), for which treatments and interventions are urgently needed. Work described in this thesis has shown developmental links of early physical aggression and oppositional behaviour with future callous traits (chapter 7), and that callous traits are characterised by widespread macro- and microstructural brain differences (chapter 10). As it is too early for clinical recommendations for risk stratification based on behaviour and neurodevelopment, our observations might inspire future longitudinal research on developmental windows that are sensitive to intervention.

RECOMMENDATIONS FOR FUTURE RESEARCH

It is beyond dispute that the aetiology of psychiatric disorders is very complex, both for disorders occurring in childhood as well as for disorders occurring in adulthood. Further, it is difficult to deny that psychiatric problems cross the arbitrary boundaries of developmental stages, i.e. paediatric psychiatric problems can develop early in life and persist well into adulthood, and psychiatric problems of adulthood usually also have antecedents in childhood or adolescence. Therefore, taking a developmental life-course perspective approach would be more advantageous for exploring sensitive periods of development, i.e. when from pre-conception until adulthood a person is the most vulnerable for a certain risk factors (Insel et al. 2010; Raine 2018; McGorry et al. 2018; Cannon et al. 2002). In this thesis it was our aim to study childhood risk for (adult) severe mental illness from a developmental viewpoint, which also resonates with other studies cited in this thesis. Interestingly, although much effort has been put into getting recognition for dimensional and trans-diagnostic conceptualisations of severe mental illness, a greater understanding is needed of (neuro-) developmental aspects. Here, the central questions remain: how do healthy children develop into healthy adults; what happens if things go awry; and how can we make sure individuals remain on a stable and nurturing trajectory?

Therefore, more studies with prospective assessments across childhood, adolescence and adulthood are needed. Ideally, these studies would include data from general population samples, high-risk samples (based on family history or clinical examination), and clinical samples. These different designs can complement each other in the development of credible causal inference about determinants of psychopathology. For example, gene by environment interplay research is needed to disentangle different causal pathways for psychotic disorders. This work needs to be undertaken in prospective general population samples to maximise power and generalisability, and to infer causality using genetic information (Pingault et al. 2018). Additional replication in clinical or high-risk samples of patients would facilitate clinical decision-making. In addition, smaller samples (from either clinical/high-risk cohorts or sub-samples from larger population-based cohorts) with more detailed and comprehensive assessments can be complementary in this regard. By providing in-depth information on, for example, executive functioning, epigenetic alterations, or (semi-experimental) response to certain stressors, we can more precisely further disentangle pathways of risk to outcome. By combining such designs, the development of credible causal inference about determinants of psychopathology can be improved; if findings are consistent across designs.

With regard to improving outcomes of clinical populations, we need stronger collaborations between epidemiological and clinical research in child mental health. For example, we have shown in chapter 7 that irritable mood difficulties constitute an integral component of the broad spectrum of disruptive behaviour problems in children, even predicting later physical aggression. Treatment studies targeting specific dimensions of disruptive behaviour problems are clearly lacking, which is particularly surprising considering irritability could be a primary target for pharmacological as well as psychological interventions. This should be of high priority, considering treatment success for disruptive behaviour disorders has remained quite limited (Hill and Maughan 2015; Bakker et al. 2017). Addressing irritability symptoms could potentially also be effective for treating other disruptive behaviour problems, such as physical aggression or rule-breaking behaviour. Similarly, epidemiological observations from this thesis (but also from other studies) on the prevalence of psychotic experiences in childhood and their association with distress, sleep difficulties and other emotional and behavioural problems could encourage more work on treatment and prevention of childhood adversity and psychotic experiences, and how their mitigation might reduce the likelihood of subsequent severe mental illness. Cognitive behavioural therapy (CBT) has shown promise for treating psychotic experiences in help-seeking youth, but needs to be assessed in randomised trials (Maijer, Palmen, and Sommer 2017). In addition, CBT for insomnia can significantly reduce hallucinations and paranoia in young adult populations, although its effects in child or adolescent populations remain unexplored (Freeman et al. 2017). More specialised work needs to be done, but trans-disciplinary collaborations are definitely the way forward.



(...) *The slightest ambiguities,*
the most inexplicable transpositions of ideas take place. (...)

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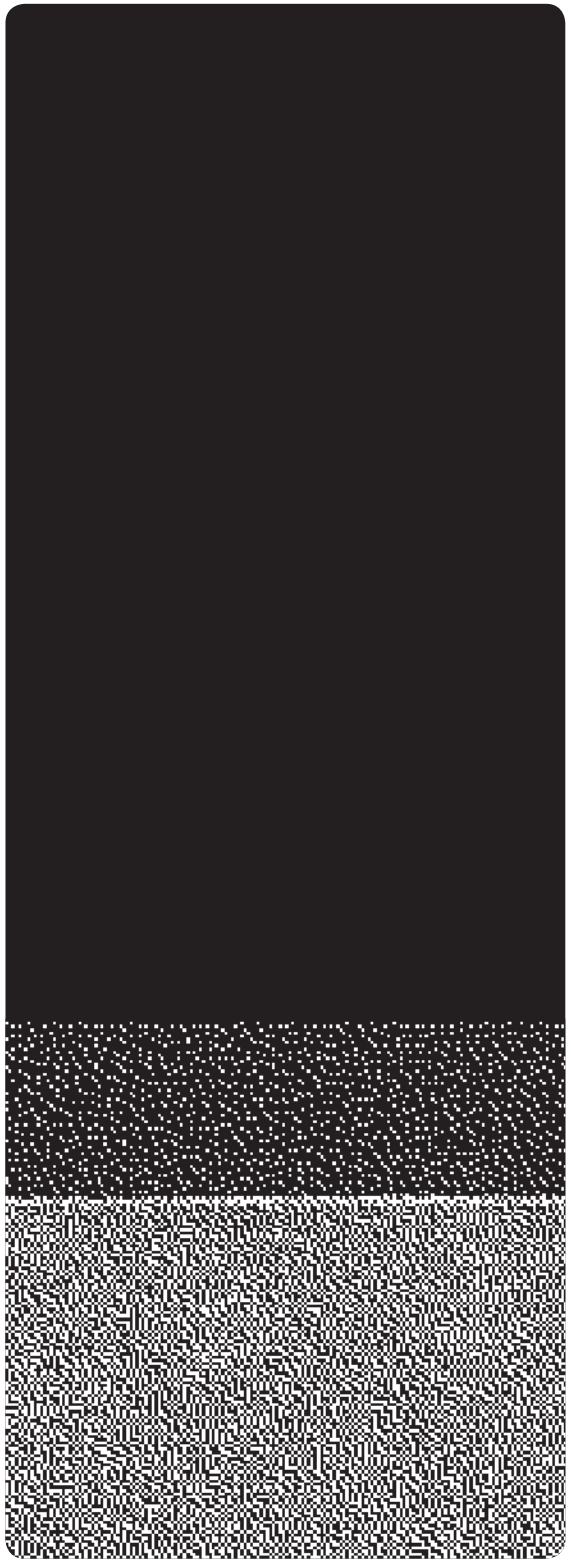

(...) *In sounds there is colour; in colours there is a music...*
(Charles Baudelaire, 1860, Les Paradis Artificiels)

APPENDICES

SUMMARY (ENG)

Psychiatric disorders in adulthood, including severe mental illnesses, commonly have antecedents in childhood or adolescence. A better understanding of the developmental pathways of psychiatric problems in early childhood might help to achieve a more comprehensive understanding of severe mental illness in adults. The general aim of this thesis was to gain insights into the neurodevelopmental pathways of children at increased risk for severe mental illness. Here we focussed on two prevalent yet impairing psychiatric phenotypes of childhood: psychotic phenomena and disruptive behaviour problems. Each of these constellations of psychiatric symptoms is in its own right predictive of substantially poorer psychosocial functioning in the long term. All studies described in this thesis were conducted in the context of the Generation R Study, a population-based cohort study in Rotterdam, the Netherlands. Below I present a brief overview of the individual studies and their results from this thesis.

In Chapter 2, we studied the occurrence of psychotic phenomena in ten-year-old children and the association of psychotic phenomena with (a) emotional and behavioural problems from as early as age three years, and (b) childhood adversities. In line with previous studies, psychotic experiences, such as hearing voices or sounds that are not there, were frequently reported by pre-adolescent children. Not surprisingly, these experiences were associated with more co-occurring emotional and behavioural problems, as well as with more lifetime exposure to childhood adversities, in particular physical or sexual maltreatment. What was novel was the finding that, after adjustment for the presence of other co-occurring psychiatric problems, we observed that pre-adolescent psychotic experiences were specifically predicted by earlier emotional difficulties. We concluded from this that these findings help us to better understand the extended psychosis continuum from a developmental perspective, and might aid in the early identification of children with increased risk of psychotic experiences and, possibly, subsequent psychiatric illness.

In Chapter 3, we examined whether cannabis use during pregnancy increases the odds for psychotic experiences in the offspring. Gestational exposure to cannabis has been associated with foetal growth retardation, lower birthweight and poorer cognitive performance. Furthermore, the link between the use of cannabis-containing products and the development of psychotic symptoms has been consistently reported, but previous studies on the association between parental cannabis use during pregnancy and psychotic experiences in their offspring were underpowered. In this study, we found that both maternal and paternal cannabis use were associated with more offspring psychotic experiences at age ten years. This may suggest that common aetiologies, rather than solely causal intra-uterine mechanisms, underlie the association between parental cannabis use and offspring psychotic experiences. These common backgrounds most likely reflect genetic vulnerabilities and shared familial mechanisms, shedding a potential new light on the debated causal path from cannabis use to psychotic phenomena.

Several previous studies have suggested that sleep problems, including insomnia and parasomnias, such as nightmares, trigger the onset of subsequent psychotic symptoms across various age groups. In Chapter 4, we investigated whether psychotic experiences in children were associated with actigraphic (i.e. objective) sleep measures, sleep problems, nightmares or other parasomnias. We observed that self-reported psychotic experiences of the child were not associated with objectively measured sleep duration, sleep efficiency, or arousal. However, psychotic experiences were associated with child self-reported sleep problems and with mother-reported parasomnias; the association was specifically strong for nightmares. This might suggest that parental-reported nightmares could serve as a risk indicator for psychotic experiences in children, and these observations will help us to better understand the relationship between the extended psychosis phenotype and sleep.

The aetiology of schizophrenia is multifactorial with early neurodevelopmental antecedents, likely to result from a complex interaction of genetic and environmental risk. In Chapter 5, we applied a gene by environment interaction approach to explore whether stress hormone level (i.e. hair cortisol) moderated the relationship between the polygenic liability for schizophrenia and child brain structure. Although no single measure exceeded the multiple testing threshold, nominally significant interactions were observed for total ventricle volume and global white matter microstructure – two of the most well replicated structural neuroimaging findings in schizophrenia. Given the widely replicated finding of ventricular enlargement and lower white matter integrity among schizophrenia patients, our findings generate novel hypotheses for future research on the gene–environment interplay affecting neurodevelopmental pathophysiology of schizophrenia.

In Chapter 6, we employed a similar approach to examine whether polygenic risk for schizophrenia is associated with a greater exposure to childhood adversities, and whether this explains the association between schizophrenia genetic risk and phenotypic manifestation of emotional and thought problems at age ten years. We found that the polygenic risk score for schizophrenia is associated with greater exposure to childhood adversities, and this association was mainly driven by adversities reported before age five years, although no distinct relationships were observed for person-related versus environment-related adversities. In addition, this association partly mediated the association of schizophrenia polygenic risk with child emotional, attention, and thought problems, but direct effects of genetic risk on behavior were also noted. Taken together, these findings contribute to a better understanding of gene-environment interplay of shaping behavioural development of children at higher genetic risk for schizophrenia. Further explorations of causal pathways are needed in order to identify modifiable risk factors which might be responsive to preventative interventions.

The second part of this thesis was centred around the theme of unruliness, or disruptive behaviour problems more specifically. In Chapter 7, we examined the presence of dimensions and distinct subgroups of childhood disruptive behaviour problems, and the cross-sectional and longitudinal associations between these dimensions. We employed a factor mixture model approach using data from three population-based cohorts, i.e. the Generation R Study, the Netherlands Twin Registry and the Swedish Twin Study of Child and Adolescent Development, and one clinical sample. Empirically obtained dimensions of disruptive behaviour problems were oppositional/disobedient behavior, rule-breaking/delinquent behavior, physical aggression, and irritability. Factor mixture models suggested that one-class solutions had the best model fit for all dimensions in all three population-based cohorts. We concluded that disruptive behaviour problems in childhood should be regarded as a multidimensional phenotype rather than comprising distinct subgroups. Incorporating multidimensionality will improve diagnostic accuracy and refine treatment.

In Chapter 8, we further examined the biological validity of the dimensions of disruptive behaviour problems observed in Chapter 7; with a focus on structural white matter neuroimaging. We studied associations of global and specific white matter connectivity with childhood disruptive behavior problems, while accounting for their complex multidimensionality. Global fractional anisotropy was negatively associated with delinquent behavior and global mean diffusivity was positively associated with delinquent behavior, suggesting reduced white matter microstructure in preadolescents with higher levels of delinquent behavior. Lower white matter microstructure in the inferior longitudinal fasciculus, superior longitudinal fasciculus, cingulum, and uncinate underlie these associations. Global white matter microstructure was not associated with physical aggression, irritability, or disobedient behavior. Against this background, our findings demonstrate that employing a multidimensional approach is advantageous in the search for neurobiological correlates of childhood disruptive behavior problems. We observed a negative association between global white matter microstructure and delinquent behavior, a relatively severe presentation of disruptive behavior at this developmental stage.

In Chapter 9, we used a multi-informant approach to assess the association of prenatal cannabis exposure with child behavioural and emotional functioning. To explore the possible causal nature of the association, we investigated whether maternal tobacco and paternal cannabis use during pregnancy were also associated with child behavioural and emotional problems. We observed that prenatal exposure to maternal cannabis use is specifically associated with offspring behavioural problems, but not emotional problems. This association is probably not due to an intrauterine cannabis exposure on foetal development, because both maternal and paternal cannabis exposure as well as maternal tobacco use during pregnancy were related to offspring externalizing problems. Our findings suggest that the association can be explained through residual confounding, most likely through shared genetic vulnerabilities for parental cannabis use and offspring behavioural problems.

In Chapter 10, we studied the structural neural correlates of callous traits during childhood (e.g. lack of remorse and shallow affect), which are a key risk marker for antisocial behaviour. Although callous traits have been found to associate with structural and functional brain alterations, evidence to date has been almost exclusively limited to small, high-risk samples of boys. Callous traits were associated with lower global brain (e.g. total brain) as well as decreased cortical surface area in frontal and temporal regions. Global mean diffusivity was negatively associated with callous traits, suggesting higher white matter microstructural integrity in children with elevated callous traits. Multiple individual tracts contributed to this global association, including the uncinate and cingulum. While no sex differences were observed for global volumetric indices, white matter associations were present only in girls. These findings extend previous work based on selected samples by demonstrating that childhood callous traits in the general population are characterized by widespread macro- and microstructural differences across the brain.

Attention-deficit/hyperactivity disorder is known to increase the risk for obesity development, but whether aggressive behavior is prospectively associated with a higher body mass index (BMI) or fat mass remains unknown. In Chapter 11, examined the prospective, and potentially bi-directional association of aggressive behavior with BMI and body composition across childhood in three population-based cohorts. We observed that aggressive behavior at six/seven years was associated with higher BMI at follow-up in all three cohorts. Furthermore, aggressive behavior was prospectively associated with higher fat mass, but not fat-free mass. There was no evidence that BMI or body composition preceded aggressive behavior. This association indicates that aggressive behavior problems contribute to the risk for obesity in childhood. Hence, it might be helpful to carefully screen for aggressive behavior problems in children with increased risk of obesity. In general, professionals as well as parents and other people involved in the care for children with weight difficulties should be aware of the possible behavioural mechanisms associated with higher BMI and fat mass.

Finally, in Chapter 12, we discuss the findings and interpretations of the individual studies in the broader context of the existing literature. More specifically, we discuss several methodological considerations pertaining to the neurodevelopmental model of psychosis, the neurodevelopmental model of disruptive behaviour, analytical methods of behavioural heterogeneity, assessment of psychotic experiences in children and the link between cannabis use and psychopathology. We conclude with several recommendations for improved clinical practice and future research.

SAMENVATTING (NL)

Psychiatrische stoornissen van de volwassenleeftijd, zoals ernstige psychiatrische aandoeningen, hebben vaak antecedenten in de kinderleeftijd of adolescentie. Een beter begrip van hoe kinderen met deze psychiatrische problemen zich ontwikkelen zou ons kunnen helpen ernstige psychiatrische aandoeningen van volwassenen beter te begrijpen. Het voornaamste doel van dit proefschrift was om inzichten te krijgen in de neurodevelopmental ontwikkeling van kinderen met een verhoogd risico op ernstige psychiatrische aandoeningen. Hier richtten we ons op twee veelvoorkomende doch zeer beperkende psychiatrische fenotypes van de kinderleeftijd: psychotische fenomenen en disruptieve gedragsproblemen. Elk van deze twee constellaties van psychiatrische symptomen is op zichzelf voorspellend voor substantieel slechter psychosociaal functioneren op de lange termijn. Alle studies beschreven in dit proefschrift waren uitgevoerd binnen de Generation R Studie, een algemeen populatie cohort uit Rotterdam, Nederland. Hieronder presenteer ik een kort overzicht van de individuele studies en hun resultaten van dit proefschrift.

In Hoofdstuk 2 bestudeerden we het voorkomen van psychotische fenomenen bij tien jaar oude kinderen, en de associatie van psychotische fenomenen met (a) emotionele en gedragsproblemen op een leeftijd vanaf 3 jaar oud, en (b) traumatische ervaringen. In overeenstemming met eerdere studies, werden psychotische ervaringen, zoals het horen van stemmen of geluiden die er niet zijn, frequent gerapporteerd door pre-adolescente kinderen. Niet verrassend was dat deze ervaringen geassocieerd waren met meer comorbide emotionele en gedragsproblemen, evenals met meer blootstelling aan traumatische ervaringen van de kinderleeftijd, met name lichamelijke en seksuele mishandeling. Een nieuwe bevinding was dat, na correctie voor de aanwezigheid van comorbide emotionele en gedragsproblemen, we observeerden dat psychotische ervaringen voornamelijk voorspeld werden door eerdere emotionele problemen. We concludeerden hieruit dat deze bevindingen ons het brede psychose spectrum beter laten begrijpen vanuit een ontwikkelingsperspectief, en zouden kunnen bijdragen aan het vroeg herkennen van kinderen met een verhoogd risico op psychotische ervaringen en, mogelijkerwijs, daaropvolgende psychiatrische aandoeningen.

In Hoofdstuk 3 onderzochten we of cannabisgebruik tijdens de zwangerschap geassocieerd is met meer psychotische ervaringen bij het nageslacht. Blootstelling aan cannabis tijdens de zwangerschap is in verband gebracht met foetale groeivertraging, lager geboortegewicht, en slechtere cognitieve vaardigheden. Bovendien wordt consistent beschreven dat er een link bestaat tussen het gebruik van cannabis bevattende producten en het ontwikkelen van psychotische symptomen, maar eerdere studies over de relatie tussen cannabisgebruik van ouders tijdens de zwangerschap en psychotische ervaringen in het nageslacht waren ontbraken aan statistische power. In deze studie vonden we dat cannabisgebruik van zowel de moeder als de vader gerelateerd is aan meer psychotische ervaringen bij het tien jaar oude kind. Dit suggereert dat gemeenschappelijke oorzaken, in plaats van alleen oorzakelijke intra-uteriene mechanismen, de associatie tussen cannabisgebruik van de ouders en psychotische ervaringen van het kind kunnen verklaren. Deze gemeenschappelijke oorzaken reflecteren hoogstwaarschijnlijk genetische kwetsbaarheden en gedeelde familiaire mechanismen, welke mogelijk een nieuw licht werpen op het veel bediscussieerde oorzakelijke verband tussen cannabisgebruik en psychotische symptomen.

Enkele eerdere studies hebben aangetoond dat slaapproblemen, inclusief insomnia en parasomnieën zoals nachtmerries, op verschillende leeftijden het ontstaan van psychotische symptomen in gang kunnen zetten. In Hoofdstuk 4 hebben we onderzocht of psychotische ervaringen bij kinderen gerelateerd zijn aan actigrafische (i.e. objectieve) slaapmaten, slaapproblemen, nachtmerries en andere parasomnieën. We observeerden dat zelf-gerapporteerde psychotische ervaringen van het kind niet geassocieerd waren met objectief gemeten slaapduur, slaapefficiëntie, of arousal. Daarentegen waren psychotische ervaringen wel gerelateerd aan door het kind gerapporteerde slaapproblemen en aan door de moeder gerapporteerde parasomnieën; de relatie was vooral sterk voor nachtmerries. Dit suggereert dat door de ouders geobserveerde nachtmerries kunnen dienen als een risicomarker voor psychotische ervaringen in kinderen, en deze observaties kunnen ons helpen de relatie tussen het brede psychose spectrum en slaap beter te begrijpen.

De etiologie van schizofrenie is multifactorieel met vroege neurodevelopmental antecedenten, hoogstwaarschijnlijk veroorzaakt door een complex samenspel van genetische en omgevingsrisico's. In Hoofdstuk 5 hebben een gen-omgevingsinteractie benadering toegepast om te exploreren of stresshormoonniveaus (i.e. cortisol gemeten in haar) de relatie tussen de genetische kwetsbaarheid voor schizofrenie en de kinderhersenstructuur modereert. Alhoewel geen enkele maat de drempel voor meervoudig testen overschreed, suggestief significante interacties werden geobserveerd voor totaal ventrikelvolume en globale witte stof microstructuur – twee van de best gerepliceerde structurele hersenbeeldvorming bevindingen in schizofrenie. Onze bevindingen zijn, gezien de op grote schaal gevonden observaties van ventrikelvergroting en lagere witte stof integriteit onder schizofrenie patiënten, interessant omdat ze inspireren tot nieuwe hypotheses voor toekomstig onderzoek over gen-omgevingsinteracties die van invloed zijn op de neurodevelopmental pathofysiologie van schizofrenie.

In Hoofdstuk 6 hebben we een vergelijkbare benadering toegepast om te onderzoeken of de polygenetische risicoscore voor schizofrenie gerelateerd is aan een meer blootstelling aan traumatische ervaringen, en of dit de relatie tussen schizofrenie genetisch risico en fenotypische manifestaties van emotionele en denkproblemen op tien jaar oud verklaart. We vonden dat de polygenetische risicoscore voor schizofrenie geassocieerd is met meer blootstelling aan traumatische ervaringen op de kinderleeftijd, en deze relatie werd met name gedreven door traumatische ervaringen gerapporteerd voor de leeftijd van vijf jaar. Echter vonden we geen verschillende relaties voor persoon-gerelateerde of omgeving-gerelateerde traumatische ervaringen. Bovendien medieerde deze relatie deels de associatie van schizofrenie polygenetisch risico met emotionele, aandachts-, en denkproblemen van het kind; maar directe effecten van genetisch risico op gedrag werden ook geobserveerd. Samengenomen dragen deze bevindingen bij aan een beter begrip van het samenspel tussen genen en omgeving, en hoe deze de gedragsontwikkeling van kinderen met een verhoogd genetisch risico op schizofrenie voorgeven. Verdere exploraties van oorzakelijke verbanden zijn nodig om modificeerbare risk factoren te kunnen identificeren; dit is belangrijk voor het ontwikkelen van preventieve interventies.

Het tweede deel van dit proefschrift was gericht op het thema van weerbarstigheid, of meer specifiek disruptieve gedragsproblemen. In Hoofdstuk 7 hebben we de aanwezigheid van dimensies en afzonderlijke subgroepen van disruptieve gedragsproblemen van de kinderleeftijd onderzocht, evenals de cross-sectionele en de longitudinale associaties tussen deze dimensies. We aanwendden een factor mixture model benadering, gebruikmakend van data van drie algemene populatie cohorten, i.e. de Generation R Studie, het Nederlands Tweelingregister en de Zweedse Twin Study of Child and Adolescent Development, en een klinisch cohort. Empirisch verkregen dimensies van disruptieve gedragsproblemen waren oppositioneel/ongehoorzaam gedrag, regel-overtredend/delinquent gedrag, fysieke agressie en prikkelbaarheid. Factor mixture modellen gaven aan dat één-klasse oplossingen de beste model fit hadden voor alle dimensies in alle algemene populatie cohorten. We concludeerden hieruit dat disruptieve gedragsproblemen van de kinderleeftijd benaderd zouden moeten worden als een multi-dimensioneel fenotype in plaats van bestaand uit afzonderlijke subgroepen. Het erkennen van deze multi-dimensionaliteit zal diagnostische precisie verbeteren en behandeling verfijnen.

In Hoofdstuk 8 hebben we de biologische validiteit van de dimensies van disruptieve gedragsproblemen (zoals beschreven in Hoofdstuk 7) verder onderzocht; dit was met een focus op structurele witte stof hersenbeeldvorming. We onderzochten de relaties van globale en specifieke witte stof connectiviteit met disruptieve gedragsproblemen, onderwijl rekening houdend met hun complexe multi-dimensionaliteit. Globale 'fractional anistropy' was negatief gerelateerd aan delinquent gedrag en globale 'mean diffusivity' was positief gerelateerd aan delinquent gedrag, suggererend dat verminderde witte stof integriteit samenhangt met hogere scores van delinquent gedrag. Lagere witte stof microstructuur in de fasciculus longitudinalis inferior, de fasciculus longitudinalis superior, het cingulum en de fasciculus uncinatus droegen bij aan deze globale associatie. Globale witte stof microstructuur was niet geassocieerd met fysieke agressie, prikkelbaarheid of ongehoorzaam gedrag. Deze bevindingen laten zien dat het toepassen van een multi-dimensionele benadering voordelig is in het onderzoeken van neurobiologische correlaten van disruptieve gedragsproblemen van de kinderleeftijd. Wij vonden een negatieve relatie tussen globale witte stof microstructuur en delinquent gedrag, een relatief ernstige presentatie van disruptief gedrag in deze ontwikkelingsfase.

In Hoofdstuk 9 gebruikten we een multi-informant benadering om de relatie van prenatale blootstelling aan cannabis met emotioneel en gedragsmatig functioneren van het kind te onderzoeken. Om de mogelijke causale aspecten van deze associatie te onderzoeken, bekeken we ook of maternaal tabaksgebruik en paternaal cannabisgebruik tijdens de zwangerschap gerelateerd waren aan emotionele of gedragsproblemen van het kind. We vonden dat prenatale blootstelling aan maternaal cannabisgebruik specifiek geassocieerd was met gedragsproblemen van het kind, maar niet met emotionele problemen. Dit verband komt waarschijnlijk niet door een intra-uteriene blootstelling of de foetale ontwikkeling, omdat zowel maternale als paternale blootstelling aan cannabis alsmede maternaal tabaksgebruik tijdens de zwangerschap gerelateerd waren aan externaliserende problemen van het kind. Onze bevindingen impliceren dat deze associatie verklaard kan worden door residual confounding (resterende verstorende factoren), meest waarschijnlijk door gemeenschappelijke genetische kwetsbaarheden voor cannabisgebruik van ouders en gedragsproblemen van het kind.

In Hoofdstuk 10 onderzochten we de structurele neurale correlaten van ongevoelige trekken tijdens de kinderleeftijd (zoals gebrek aan berouw en vlak affect), welke een belangrijke risicomarker zijn voor antisociaal gedrag. Alhoewel ongevoelige trekken in verband zijn gebracht met structurele en functionele breinveranderingen, de bevindingen hebben zich tot nu exclusief beperkt tot kleine, hoog-risico steekproeven van jongens. Hier vonden we dat ongevoelige trekken gerelateerd waren aan lagere globale hersenvolumes (zoals totaal breinvolume) evenals verminderd cortex oppervlakte in frontale en temporale regio's. Globale gemiddelde diffusiviteit was negatief gerelateerd aan ongevoelige trekken, suggererend dat kinderen met meer ongevoelige trekken een hogere witte stof microstructurele integriteit hebben. Meerdere individuele witte stofbundels droegen bij aan deze globale associatie, inclusief de fasciculus uncinatus en het cingulum. Alhoewel er geen sekse verschillen werden gevonden voor globale volumetrische indices, de witte stof verbanden waren alleen aanwezig in meisjes. Deze bevindingen breiden vorige bevindingen van geselecteerde steekproeven uit door te laten zien dat ongevoelige trekken van de kinderleeftijd in de algemene populatie gekarakteriseerd worden door wijd verspreide macro- en microstructurele verschillen in het brein.

Het is bekend dat aandachtsdeficiëntie-/hyperactiviteitsstoornis (ADHD) het risico op het ontwikkelen van obesitas verhoogd, maar of agressief gedrag prospectief geassocieerd is met een hogere body mass index (BMI) of vetmassa is onbekend. In Hoofdstuk 11 onderzochten we de prospectieve en mogelijk bi-directionele associatie van agressief gedrag met BMI and lichaamssamenstelling in drie algemene populatiecohorten. We observeerden dat agressief gedrag op de leeftijd van zes/zeven jaar oud gerelateerd was aan hoger BMI bij de follow-up; dit was gevonden in alle drie de cohorten. Bovendien was agressief gedrag prospectief geassocieerd met een hogere vetmassa, maar niet vetvrije massa. Er werd geen bewijs gevonden voor dat BMI of vetmassa voorafging aan agressief gedrag. Deze associatie impliceert dat agressieve gedragsproblemen bijdragen aan het risico voor obesitas op de kinderleeftijd. Daarom zou het nuttig zijn om te screenen op agressieve gedragsproblemen bij kinderen met een verhoogd risico op overgewicht. In het algemeen zouden professionals alsmede ouders en andere betrokken bij de zorg van kinderen met gewichtsproblemen zich bewust moeten zijn van de mogelijke gedragsmatige mechanismen die geassocieerd zijn met hogere BMI en vetmassa.

Tenslotte bediscussiëren we in Hoofdstuk 12 de bevindingen en interpretaties van deze individuele hoofdstukken en de bredere context van de bestaande literatuur. Specifieker, we bespraken enkele methodologische overwegingen betrekking hebbend op het neurodevelopmental model van psychose, het neurodevelopmental model van disruptief gedrag, analytische methodes van heterogeniteit van gedrag, het meten van psychotische ervaringen bij kinderen en het verband tussen cannabisgebruik en psychopathologie. We besluiten deze discussie met enkele aanbevelingen voor de klinische praktijk en toekomstig onderzoek.

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Principles of Research in Medicine and Epidemiology	2015	0.7
Principles of Genetic Epidemiology	2015	0.7
Genomics in Molecular Medicine	2015	1.4
Masterclass Advances in Genomics Research	2015	0.4
Practice of Epidemiologic Analysis	2015	0.7
Social Epidemiology	2015	0.7
Topics in Meta-analysis	2016	0.7
Clinical Trials	2016	0.7
Joint Models for Longitudinal and Survival Data	2016	0.7
Causal Inference	2016	0.7
Genome-Wide Association Studies	2016	0.7
Epigenetics	2016	0.7
Core Curriculum		
Study Design	2015	4.3
Advances in Genome-Wide Association Studies	2016	1.4
Family-Based Genetic Analysis	2016	1.4
Analysis of Next-Generation Sequencing Data	2016	1.4
Biostatistical Methods I: Basic Principles	2016	5.7
Linux for Scientists	2016	0.6
Genetic-Epidemiologic Research Methods	2016	5.1
SNPs and Human Disease	2016	2.0
Biostatistical Methods II: Classical Regression Methods	2016	4.3
Advanced Courses		
Psychiatric Epidemiology	2015	1.1
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Specific courses		
MRI Safety course, Erasmus MC, Rotterdam, the Netherlands	2015	0.3
Psychiatric Genetics Consortium (PGC) Amsterdam Workshop, Amsterdam the Netherland	2015	2.0
FMRIB Software Library (FSL) course, Giardini Naxos, Italy	2016	2.0
International conferences		
American Academy of Child and Adolescent Psychiatry annual meeting, Washington DC (poster presentation)	2017	1.2
Schizophrenia International Research Society meeting, Florence, Italy, 2018 (poster presentations)	2018	1.2
Workshops, Meetings and Symposia		
Phrenos Psychosecongres, Zwolle, the Netherlands (oral presentation)	2017	0.3
Biological Psychiatry meeting, Erasmus MC, Rotterdam, the Netherlands (oral presentation)	2017	0.3
Generation R Research meetings, Erasmus MC, Rotterdam, the Netherlands (oral presentation)	2015-2018	1.0
Child and Adolescent Psychiatry/Psychology Colloquia, Erasmus MC, Rotterdam, the Netherlands	2018-2018	1.0
ACTION Consortium meetings, Vrije Universiteit, Amsterdam, the Netherlands, (oral presentations)	2015-2018	1.0
De Amsterdamse School symposium, Amsterdam, the Netherlands (oral presentation)	2018	0.3
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Dragan Cibrev *(Health Sciences, Erasmus University Rotterdam)* Child- and parent-reported prevalence of thought problems	2016	3.0
Zeynep Yalcin *(Clinical Psychology, Erasmus University Rotterdam)* Childhood weight and obesity in relation to aggressive behaviour symptoms	2016-2017	3.0
Marieke Troost *(Clinical Psychology, Erasmus University Rotterdam)* Parental psychopathology in association with childhood aggressive behaviour	2016-2017	3.0
Selda Yalniz *(Clinical Psychology, Erasmus University Rotterdam)* Maternal cannabis use and risk of offspring psychotic-like experiences	2016-2017	3.0
Louise Otterman *(Child Psychology, Erasmus University Rotterdam)* The Prospective Association Between Executive Functioning and Traits of Neurodevelopmental Disorders in Early Childhood	2017-2018	3.0
Sandjana Parag *(Child Psychology, Erasmus University Rotterdam)* The association between maternal history of maltreatment of offspring psychotic experiences	2018	3.0
Other Teaching Activities		
Supervision of and giving lectures to 2nd and 3rd year medical students, Erasmus MC, Rotterdam, the Netherlands	2016-2017	2.0
Lectures for 1st-3rd year bachelor/master students, Erasmus University Rotterdam, the Netherlands	2016-2018	2.0
Lectures for Landelijke Onderwijsdag Kinder- en Jeugdpsychiatrie	2016-2018	1.5
3. Other Activities		
Coordination of Focus @13 measurements and questionnaires development	2015	6.0
Incidental findings MRI	2015-2016	2.0
Peer review (e.g. JAMA Psychiatry, JAACAP, Schizophrenia Bulletin, JCPP, British Journal of Psychiatry, Biological Psychiatry)	2015 - present	3.0

1 ECTS (European Credit Transfer System) is equal to a workload of 28 hours

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ABOUT THE AUTHOR

Koen Bolhuis was born on the 18th July 1989 in Amersfoort, the Netherlands. Koen studied Medicine at the Vrije Universiteit medical centre (VUmc) in Amsterdam. During his third year in medical school, Koen became involved in "Kleine Hans", the university's study group on child psychiatry, which was initiated by prof. em. Theo Doreleijers, head of the department of Child and Adolescent Psychiatry at the VUmc. Koen helped organise the first Kleine Hans study trip to London in 2010, for which he coordinated the educational program. Following this study trip, Koen started a research placement at the department of Child and Adolescent Psychiatry of the Institute of Psychiatry (London, UK) under the supervision of Dr. Argyris Stringaris and Dr. Isobel Heyman. His research project focused on mood problems, such as irritability and depression, in children suffering from obsessive-compulsive disorder. The work conducted in London resulted in two peer-reviewed publications and these jointly resulted in his master thesis, for which Koen was awarded the VUmc best thesis prize in 2012. Koen graduated from medical school in December 2014. In February 2015, he started the work described in this thesis at the Department of Child and Adolescent Psychiatry and the Generation R Study Group at the Erasmus Medical Centre-Sophia Children's Hospital in Rotterdam. Since June 2014, Koen has been involved with Doctors of the World, and with this organisation he has worked in the Mersinidi and Souda refugee camps on the island of Chios (Greece) in 2015, and continues to work in mobile clinics for undocumented migrants in Amsterdam. In addition, he is joint coordinator of the Doctors of the World's mental health support team for undocumented migrants. In 2018, Koen obtained a Master of Science degree in Genetic Epidemiology from the Netherlands Institute for Health Sciences. He was granted a Ter Meulen fellowship from the Royal Netherlands Academy of Arts and Sciences and a research fellowship from the Sophia Foundation to give him the opportunity to work in Dr. Ian Kelleher's psychiatric epidemiology group at the Royal Colleges of Surgeon in Ireland (Dublin, Ireland) from October 2018 to February 2019. In april 2019, Koen will start as a resident in psychiatry at the Amsterdam UMC – location AMC.

– *All people are not created equal. Some have real gifts and talents, and some have real problems right out of the starting block.* (...)

(...) *Once we accept that,* (...)

(...) *we can't dodge the responsibility for social action.*
(Terrie Moffitt, 2018)

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STUDIES ON THE CHILD-HOOD RISK FOR SEVERE MENTAL ILLNESS

WHICH LIVES IN PARALLEL TO SANITY, AND GIVEN THE RIGHT CIRCUMSTANCES OR EVEN JUST HALF A CHANCE, CREEPS LIKE A LICK OF FLAME OR A GROWING TUMOUR UP AND AROUND ORDINARY PERCEPTION, CONSUMING IT FOR A WHILE, AND CAUSING ONE, EVEN WHEN NOT AT THE MOVIES, TO QUAKE IN FEAR OF THE WORLD AND PEOPLE AND WHAT THEY – I MEAN, OF, WE – ARE CAPABLE OF. — JENNY DISKI , 2002, STRANGER ON A TRAIN