INFANT NEUROMOTOR DEVELOPMENT AND NEUROPSYCHIATRIC PROBLEMS

Modern Epidemiological approaches

FADILA SERDAREVIC

PROPOSITIONS

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Modern Epidemiological approaches

Dr. Molewaterplein 50, Rotterdam
Wednesday, June 19th 2019

Fadila Serdarevic

1. **Non-optimal infant neuromotor development is associated with poor executive functioning, mental rotation and immediate memory.**

2. **Minor neurological delays in infancy predict poor shifting and emotional problems during childhood.**

3. **A low muscle tone in infancy is associated with autistic symptoms.**

4. **Non-optimal senses and other observations, such as poor following eye movement, sweating, and startle reactions, mediate the association between the genetic susceptibility for attention deficit hyperactivity disorder and autistic symptoms in boys.**

5. **Schizophrenia but not bipolar genetic susceptibility is as sociated with non-optimal neuromotor development.**

6. **The most difficult subjects can be explained to the most slow-witted man if he has not formed any idea of them already; but the simplest thing cannot be made clear to the most intelligent man if he is firmly persuaded that he knows already, without a shadow of doubt, what is laid before him. (Lav Tolstoy)**

7. **The move from "alternative facts" to "truth isn't truth" paves the way from a threshold to a continuous approach.**

8. **If you try and take a cat apart to see how it works, the first thing you have on your hands is a non-working cat.**
(Douglas Adams)

9. **Randomness might essentially be a model of human ignorance or incomplete information.**

10. **Thinking on local level may decrease health disparities, a goal that many national level attempts have failed to achieve.**

11. **The octopus has an enormous range of possible movements and the capacity to process a huge amount of sensory information, consequently, the octopus like humans is good at tasks involving memory and learning.**

Infant Neuromotor Development and Neuropsychiatric Problems Modern Epidemiological approaches

Fadila Serdarević

"For my tree parents, my mom Mevlida (Maza), who thought me to think critically and to never give up, my aunt Azijada (Đena), a neuropsychiatrist who inspired me for this thesis and my dad Ismet (Imo), who gave me all the love of this world and who died from Alzheimer's disease during this PhD."

Drawing by Bakir Rokvic (age 12). Bakir is diagnosed with autism spectrum disorder at age 4 years. This book features Bakir's artwork from age 5 till age 12

ACKNOWLEDGEMENT

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Een moderne epidemiologische insteek

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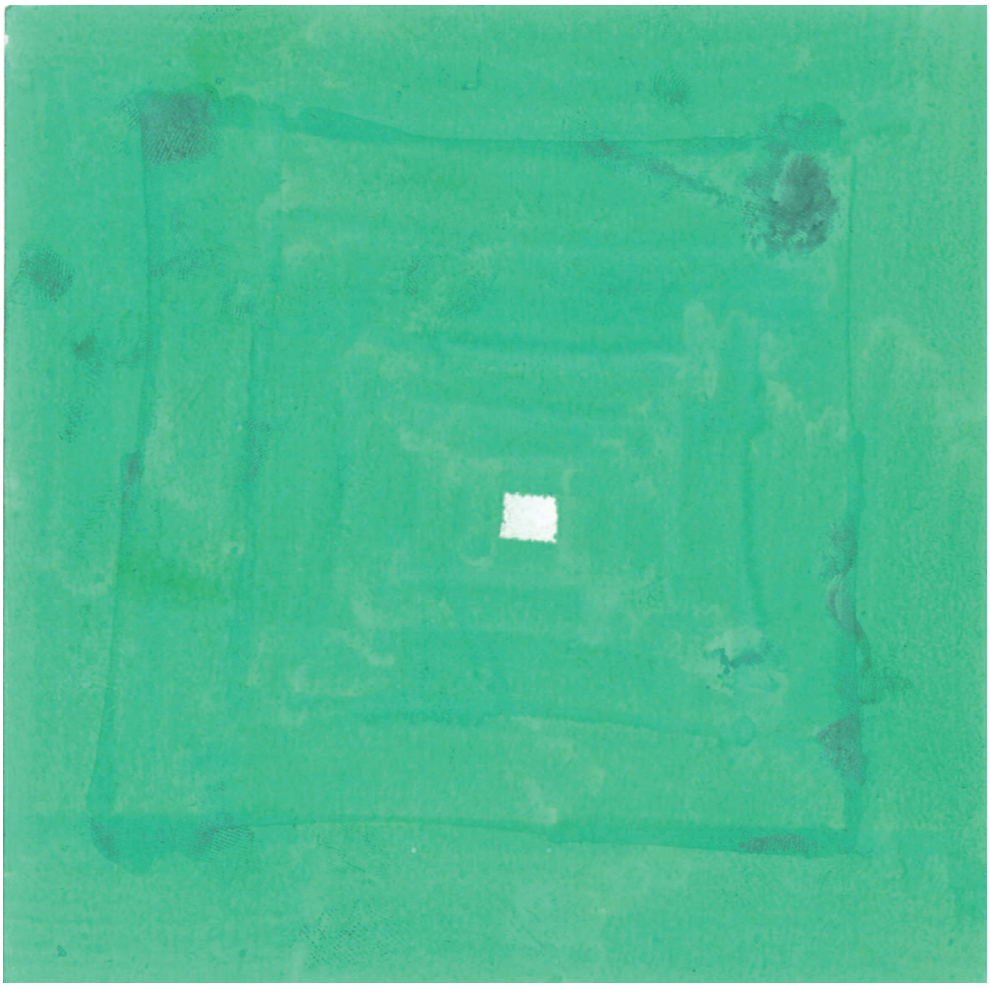

Painting by Bakir Rokvic (age 5 years)

1 INTRODUCTION

Neuromotor development is an accepted mean of measuring the maturity and the integrity of infant central nervous system (CNS).[1] Neurodevelopment is a dynamic process with new forms of motion emerging through intrinsic processes and interaction with the environment.[2,3] At the same time, neuromotor development is highly variable from child to child because each individual has distinctive neural and physical properties and grows up in a unique environment.[4] Motor skills are at the core of everyday actions and interactions during infancy and childhood, affecting physical, perceptual, cognitive, and social development in young children.[5,6] Therefore, these skills may initiate a cascade of events influencing subsequent development.[2]

Aberrant development cannot be understood without studying normal development, which in turn can benefit from insights obtained in the study of clinical cases. Aberrant neuromotor development is common in many developmental disorders such as developmental coordination disorder (DCD), autism spectrum disorder (ASD), and attention-deficit/hyperactivity disorder (ADHD) and, at the same time, poorly understood.[7-9] Whereas the importance of early detection and intervention in the clinical practice has been widely recognised[10], prospective studies on quality of neuromotor development measured during infancy and child behavior in the general population remain scarce. Large and longitudinal population-based studies from infancy onwards utilizing hands –on assessments help scientists pinpoint the onset of the cascade of neurodevelopmental abnormalities and address the question of why some children develop abnormalities, while others do not. In this thesis I address the relationship of minor neurological dysfunction during infancy with child cognition and behavior in the general population.

Theoretical background

Studying infant and child neurodevelopment is important for several reasons. First, researchers and clinicians can evaluate the variation in specific functions, such as grasping, posture, and locomotion. This evaluation can help understand infant CNS maturity and integrity. For example, the use of scissors or a pincer type of grasping may indicate whether certain cortical structures have become operative. Second, scientists and clinicians understand adaptive functioning such as exploring and playing in children by observing spontaneous (general) movement pathways. [11,12] Quality of movements can indicate adaptive abilities of the child in terms of cognitive, social, and emotional functioning. Direct observations of neuromotor development during infancy and childhood are possible via video recording; however, these observations are time consuming and expensive.[11] Third, scientists and clinicians can assess motor milestones, a valuable approach to evaluate general motor development in young children. Such studies, nonetheless, are often based on the retrospective report of the caregiver. Fourth, scientists study the quality of neuromotor performance, in particular minor neurological dysfunctions (MND). MND stands for the presence of neurological symptomatology,

which is not present in the majority of normal functioning children. At the same time, MND does not necessary interfere with daily life behavior, although if demands are higher, motor performance often suffers. Studies of MND have demonstrated that the "how" is as important as the "when" in neurodevelopment; meaning motor functioning is as important as reaching milestones.[1] Describing the quality of performance of an impaired brain function during development can give an indication of how compensation or substitution is achieved, or how stereotyped performance may take the place of normal variability.[1] Importantly, MND is not a classical neurological diagnosis, but a description of a child's neurological profile, which describes difficulties like muscle tone regulation, posture, balance, mildly abnormal reflexes, and coordination.

Neuromotor assessment

There are many different instruments to assess neuromotor development during infancy and childhood, and each instrument has its specific characteristics. Traditional schools mainly assess tone and primitive reflexes, while more recent schools assess behavior and coping with environment (capacity to take, utilize and respond to the stimuli). Prechtl and Dubowitz[13] method of neurological examination for new born infants, and Touwen's[1] neurological examination of young infants combine these components, measuring tone, reflexes abnormal movements and behavior. In studies presented in this thesis, we chose the Touwen's instrument to assess neuromotor development at a corrected postnatal age between 2 and 5 months as during this period major transition in development takes place.[14] Most of other instruments are suitable for assessments of neonates only. However, there is a high rate of 'false positives' in the neonatal period,[15] as the nervous system at birth has high plasticity, and the majority of infants with neonatal neurological signs recover. Moreover, the nervous system is a very sensitive organ system, which may react to temporary stresses in a reversible way: for instance, hyperbilirubinemia of the newborn may result in a temporary depression of brain function, leading to reversible hypotonia and hypokinesia. Because it is difficult to identify abnormal development during infancy, a full and age-adequate neurological examination should always be carried. Therefore, we chose the adapted Touwen methods adding assessments introduced by de Groot et al, which are described in detail elsewhere.[16] We selected the age-appropriate items from Touwen's Neurodevelopmental Examination for infants aged 9-20 weeks, and categorized items in three groups: tone (24 items), responses (6 items), senses and other observations (6 items). [16] Muscle tone is the degree of passive resistance to movement. Tone is assessed in several positions –supine, horizontal, vertical, prone and sitting– and all items, such as adductor angle, are scored as normal, low or high tone. Responses are assessed in supine (e.g. asymmetrical tonic neck reflex), vertical (e.g., Moro response) or prone position (e.g. Bauer response) and were scored as present, absent or excessive. Senses and other observations (e.g. following movements) were scored as present, absent or excessive. An age-appropriate response was labeled 'optimal'. If the response indicated a delayed

development, the response was labeled 'non-optimal'. Scale values were calculated by summing the non-optimal items. This resulted in a total score and three subscale scores: tone, responses, and other observations. Higher score indicate less optimal neuromotor development. Assessment of overall neuromotor development and the subscales tone, responses, senses and other observations will be used in this thesis.

With this approach, we maintained the multiple domains of Touwen, and at the same time, we emphasized the notion that discrepancy between passive and active tone serves as an early sign of poor posture and non-optimal neuromotor development. This specific way of measuring neuromotor development is unique and crucial for addressing our aim of understanding the role of neuromotor functioning in behavioral development of children from the general population.

Aim of this thesis

The overall aim of this thesis is to understand the role of neuromotor development measured in infancy in relation to behavior. Specific aims are

1. To study how neuromotor development measured in infancy predicts later behavior and cognitive functioning
2. To examine how genetic susceptibility for psychiatric disorders influences neuromotor development
3. To understand the role of infant neuromotor development in the association of genetic susceptibility for psychiatric disorders and behavioral outcomes during childhood.

Setting

The studies presented in this thesis are embedded in the Generation R Study, a prospective population-based cohort from early fetal life onwards, in Rotterdam, the Netherlands.[17] This cohort was designed to study early environmental and genetic determinants of growth, development and health during fetal and postnatal life. From all eligible participants, 8879 pregnant women with an expected delivery date between April 2002 and January 2006 were enrolled in the cohort during pregnancy. Detailed measurements were planned in early pregnancy and included fetal ultrasound measurements, physical examinations, collection of biological samples, and self-administered questionnaires. Information on perinatal and maternal pregnancy outcomes, including intra-uterine growth, placental parameters, birth weight, gestational age at birth, gestational hypertension and pre-eclampsia were all available. At the age of 9-20 weeks, neuromotor development was assessed at home settings. Because assessments were conducted during home visits, it was not logistically possible to visit all children at exactly the same age. Therefore, neuromotor assessment was performed in 4721 children at corrected age between 9 and 20 weeks (response rate 67%). At the age of six and 10 years, all children were invited to visit the Generation R Research Center together with their mothers to

study their growth, development and cardiovascular health using innovative and detailed tools. The Generation R Study has been approved by the Medical Ethical Committee of the Erasmus MC, University Medical Center Rotterdam, and the medical ethical review boards of all participating hospitals. All participants provided written informed consent. The Generation R Study follows the STROBE guidelines.

Outline of the thesis

Chapter 1 is a general introduction describing the background and hypotheses for the studies presented in this thesis.

Chapter 2 describes the associations of infant neuromotor development with behavioral and cognitive development. In **Chapter 2.1**. we describe the associations of infant neuromotor development with cognition, language and executive functioning during childhood. In **Chapter 2.2.** we show the association of neuromotor development and autistic symptoms. In **Chapter 2.3.** we evaluate how motor development measured during infancy predicts behavior during childhood..

In **Chapter 3,** we present studies focused on potential genetic determinants of infant neuromotor development. In **Chapter 3.1**. we explore the associations of a genetic risk score for schizophrenia and bipolar disorder with infant neuromotor development. In **Chapter 3.2.** we study the role of infant neuromotor development in the relationship of genetic susceptibility for ASD and ADHD with autistic symptoms during childhood. **Chapter 4** presents a study on another possible cause of autistic behavior. In **Chapter 5** I discuss the findings. Finally, in **Chapter 6** I present a summary of the results.

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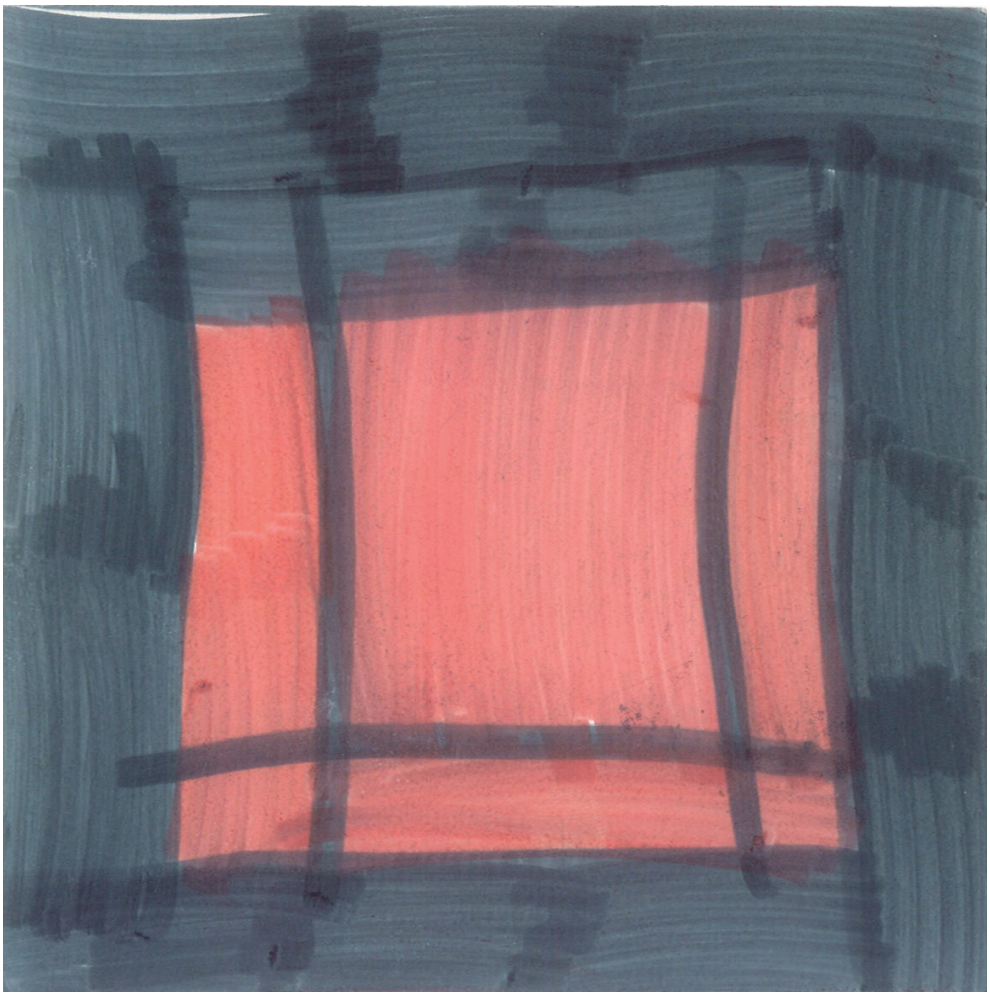

Painting by Bakir Rokvic (age 6 years)

2 NON-OPTIMAL NEUROMOTOR FUNCTIONING IN INFANCY AND CHILD NEURODEVELOPMENT

2.1

Relation of Infant Motor Development with Nonverbal Intelligence, Language Comprehension and Neuropsychological Functioning in Childhood. A Population-based Study

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Developmental Science. Dev Sci. 2016 Sep; 19(5):790-802.

ABSTRACT

We determined if infant neuromotor development is associated with cognition in early childhood. Within the Generation R, neuromotor development was assessed with an adapted version of Touwen's Neurodevelopmental Examination between 9-20 weeks. Parents rated executive functioning at 4 years. At age 6 years, children performed intelligence and language comprehension using Dutch test batteries. At age 7 years, neurocognitive development was measured using the validated NEPSY- II – NL neuropsychological battery. Less optimal infant neurodevelopment predicted poor mental rotation, immediate memory, shifting, and planning but not nonverbal IQ or language comprehension.

Infant neuromotor development is an important early indicator of central nervous system development. Research in children with severe neuromotor impairment demonstrated an increased risk of poor cognitive performance, learning disabilities and behavioral problems in these children[1,2]. Complex neuropsychological skills show a rapid change between five and eight years of age. Little is known about relation of infant neuromotor development with neuropsychological functioning in the preschool and early school age. [3,4].

Numerous clinical studies in children with developmental coordination disorder have demonstrated a close relation of infant neuromotor development with cognitive functions [3,5,6]. These children are characterized by poor performance in working memory, attention, inhibition, planning, monitoring and demanding tasks under speed. In a one-year follow-up study, Michel, Roethlisberger, Neuenschwander, Roebers [3] found that five-to-seven year old children with poor motor coordination scored equally accurate in executive functioning tasks compared to age-matched healthy controls, but were slower in inhibition and attention shifting tasks. Other studies focused on high-risk children. Korkman et al. examined a cohort of low birth weight children using a comprehensive neuropsychological battery. They found that only the very preterm children with motor coordination problems had slower neurocognitive development, but average intelligence [7]. Research in clinical and high-risk population suggests a relation between motor development and specific neurocognitive functioning in childhood.

Many population-based prospective studies related motor milestone achievement to cognition in children [8,9]. Achieving certain milestones at earlier age was associated with better intellectual performance and higher education level. Motor milestone assessment is a valuable approach to evaluate general gross motor development in young children. However, milestones can be assessed reliably only from the age of six months, and such studies of a child's motor development are often based on the retrospective report of the caregivers. Other researchers applied hands-on assessments of motor development in young children. Using population-based sample, they demonstrated an association between neuromotor assessment and certain aspects of cognitive functioning in the general population [10-12]. In a previous study embedded in the Generation R cohort, van Batenburg-Eddes, Henrichs, Schenk, Sincer, de Groot, Hofman, Jaddoe, Verhulst, Tiemeier [10] reported modest associations between less optimal infant neuromotor development and a delay in language development at the age of two and half years. In a cohort study of five to six-year old children, Wassenberg, Feron, Kessels, Hendriksen, Kalff, Kroes, Hurks, Beeren, Jolles, Vles [11] found no association between motor functioning and cognitive performance but only with specific aspects of executive functioning, such as working memory and visual motor integration. Similarly, (n=252), Jenni et al. [13] in a relatively small longitudinal study showed modest associations between motor functions and some intellectual domains in 7-18 years old children (r=0.15-0.37). Overall, in population-based studies, as in clinical studies, no consistent associations are found between motor development and general cognitive functions, whereas poor motor development possibly predicts problems in specific cognitive domains.

The inconsistent associations between the neuromotor development and cognitive functions may result from the typically small sample sizes, the cross-sectional design, the low prevalence of adverse neurodevelopment at this young age, or a true lack of association with general, whereas some specific cognition may easily be affected by poor development. In addition, within participant variability in neuropsychological performance is high because of the fast development at young age. Therefore, longitudinal population-based studies with large sample size and comprehensive assessment of cognitive abilities throughout childhood might yield different results.

In the present study, we utilized laboratory measures to study neuromotor development, intelligence and language comprehension. We also utilized two different procedures to study executive functioning in childhood: parental reports and laboratory assessment. The use of parent-reports provides an informative mean of examining executive functioning as parents are familiar with every day behavior of their children. Parental reports provide high ecological validity. On the other hand, laboratory measures can provide more objective quantitative measures of executive functioning but not the contextual information available from parental reports.

We hypothesized that less optimal neuromotor development in infants is related to cognition, in particular to executive functions. Specifically, we expected that less optimal neuromotor development predicts poor working-memory, planning and shifting.

METHOD

Participants

This study was conducted within the Generation R Study, a population-based prospective cohort from fetal life onwards, described in details elsewhere [14,15] Briefly, mothers were eligible if they were living in the Rotterdam area, the Netherlands, and when they had a delivery date between April 2002 and January 2006. When infants were 9-20 weeks old, their neuromotor development was assessed during a home visit by trained research assistants. In total, neuromotor development at age 9-20 was completed in 4055 children. These 4055 children were the eligible participants for this follow-up study. When the children were four years old, individual postal questionnaires were administrated to all care-givers in order to assess behavioral executive functioning. Out of 4055 participants, information on behavioral executive functioning at the age four years was available in 2592 (64 %) children. At age six years, all children were invited to visit the research center where, among other measures, nonverbal intelligence and language comprehension of the child were assessed. Nonverbal intelligence (IQ) and language comprehension assessments at the age six were completed in 2546 (63 %) and 2755 (68 %) participants, respectively. In total, 3356 (83% of 4055) children with neuromotor data participated in one or more of the cognitive follow-up assessments at ages 4-6 years.

At the age 5 to 10 years, a subgroup of children were invited to the research center for an extensive neuropsychological assessment using the NEPSY- II – NL- battery, which measures different domains of neuropsychological functioning. During this assessment wave, neuropsychological functioning was assessed in 495 children, as a part of imaging study. More detailed information on participant selection is provided elsewhere [16].

The Medical Ethics Committee of the Erasmus Medical Center approved the study and written informed consent was obtained from all adult participants.

Determinant

Neuromotor assesment. Infants underwent a neuromotor assessment at a corrected postnatal age between 9 and 20 weeks. There were two versions of neuromotor assessment instrument. One was appropriate for infants aged 9-15 weeks and the other for infants aged 15-20 weeks. We selected age-appropriate items from Touwen's Neurodevelopmental Examination, and categorized items in three groups: tone, responses, and other observations [17]. Tone was assessed in several positions – supine, horizontal, vertical, prone and sitting – and all tone items, such as adductor angle, were scored as normal, low or high tone. Responses were assessed in supine (e.g. asymmetrical tonic neck reflex), vertical (e.g. Moro response) or prone position (e.g. Bauer response) and were scored as present, absent or excessive. Other observations, such as following movements, were scored as present, absent or excessive. For each item, an age-appropriate response was labeled 'optimal' (with a value of 0). If the response indicated a delayed development, it was labeled 'non-optimal' (with a value of 1). By summing the values of all items, we obtained a total score, high values indicate a less optimal neuromotor development. We categorized the total sum score into tertiles in line with a previous study. Trained research assistants conducted the assessments during a home visit.

Nonverbal intelligence and language comprehension. Nonverbal intelligence was assessed at the age of six years (mean age=6.0±0.3 years). Children completed two subtests of the Snijders-Oomen nonverbal intelligence test – Revisie (SON-R 2½-7): Mosaics for visuospatial abilities and Categories for abstract reasoning [18]. Mosaics and Categories have good correlation with intellectual performance [19]. The raw test scores were converted into nonverbal IQ using norms tailored to exact age, making the obtained IQ score independent of age at assessment. The broad IQ ranged from mild mental retardation to superior intelligence.

During the same visit, children's language development was assessed using a comprehension subtest of a Dutch battery: Taal test voor Kinderen (TvK), that provides information about expressive and receptive language skills in children aged 4 to 6 years [20]. Each item consisted of two pictures and the child had to choose the alternative that matched the given words. For each child, the total number of correct answers were summed and divided by the total number of given answers, yielding a percent correct score. All testing was conducted in Dutch, at the Generation R research center.

Behavioural Executive functioning. The Behavior Rating Inventory of Executive Function-Preschool Version (BRIEF-P) was completed by parents and measures children's behavioural executive functioning at 4 years (mean age 4.1 years± 0.3) [21,22]. The BRIEF-P-contains 63 items within five related but non-overlapping clinical scales that measure children's ability in different aspects of executive functioning: inhibition (to stop his/her behaviour, 16 items), shifting (to change focus from one mind-set to another, 10 items), emotional control (to modulate emotional response, 10 items), working memory (to hold information with purpose of completing task, 17 items), planning/organization (to manage current and future task demands within the situational context, 10 items). A total score (the Global Executive Composite) is calculated by summing the scores across the five domains. The clinical raw scores and the composite scores yield T-scores based on gender and age. Higher scores indicate more problems with executive functioning.

In order to explore patterns of executive functioning further, we conducted principal component analysis (PCA). PCA with orthogonal rotation illustrated in Supplement Table 1 disclosed a four component solution, accounting for 94.8% of the variance. Component 1 consists of the planning and working memory which explained 58.1 % of the total variance. Component 2 which is comprised by the shifting alone explained 18.5 % of the total variance, whereas Component 3 is comprised by emotional control only and explained 11.0 % of the total variance. Component 4 consists of working memory and inhibition and explains 7.3% of the total variance.

Neuropsychological functioning. When the children were between 5 and 10 years (mean age 7.5 years ± 0.9) neuropsychological development was assessed in the research center using NEPSY- II – NL battery [23]. The NEPSY- II – NL measures five domains of neuropsychological functioning: attention and executive functioning, language, sensorimotor functions, memory and learning, and visuospatial functions. The attention and executive functioning domain consists of two subtests: auditory attention, which measure selective and sustained attention and the response set, which measure the ability to change and maintain a new complex set of rules and to inhibit previously learned responses. In the language domain, the child generates as many words as possible within specific category in 60 seconds. For sensorimotor domain, the participant is asked to draw lines with the dominant hand as quickly and neat as possible within a set of tracks. The memory and learning domain again consists of two subtests, memory for faces and narrative memory. In the first test the participant has to recall faces from memory, both immediately and with a delay. In the second test the child has to recall as many details as possible about a story. The visuospatial processing domain consists of three subtests. During the arrow test the child's ability to judge the direction of an arrow is assessed. The second test, geometric puzzles, measures the child's ability to recognize, match and mentally rotate difficult shapes. Finally, visuospatial functions are measured with a route finding task measuring orientation and direction. The battery of tasks that was selected

from the NEPSY- II– NL takes no more than 60 minutes. It was administered in one of four randomly selected counterbalanced orders.

Covariates

Socio-demographic characteristics, such as maternal age, family income, child or maternal ethnicity, maternal educational level, family size and family functioning, as well as maternal lifestyle, such as maternal smoking during pregnancy, were assessed by postal questionnaires. Ethnicity of the child was based on the parents' and child's country of birth. Children whose parents were born in the Netherlands were considered "Dutch". Children with parent born in European countries other than the Netherlands, or in US, Canada, Australia, or Japan were considered "Other Western". Children who had parent born in Cape Verde, Surinam, Morocco, Turkey, the Dutch Antilies, or in other economically disadvantaged countries were categorized "Other non-Western". Dutch ethnicity was used as a reference group. The highest completed education determined educational level of the mother, classified as "low" (no, only primary school education or less than 3 years of secondary school), "mid" (more than 3 years of secondary school, intermediate or first year higher vocational training), " and "high" (higher vocational education or university). Household income, defined as the total net month income of the household, was categorized into <1200 Euros (bellow social security level), 1200-2200 Euros (modal income), and >2200 Euros (more than modal income).

Birth characteristics including information about birth weight and gestational age as well as information on complications during pregnancy or delivery are obtained from the medical records and midwives' practices. Gestational age was determined by fetal ultrasound examination. Postnatal age was calculated as the difference between date of assessment and date of birth.

Statistical Analyses

We included children with an assessment of neuromotor development between 9 to 20 weeks in the analyses. Neuromotor development had skewed distribution and therefore it has been previously analyzed using tertiles in line with a prior study[10]. Low and mid tertiles were considered optimal neuromotor development and used as a reference category.

We used one-way Analysis of Variance (ANOVA) for a comparison of prenatal and demographic characteristics between groups of infants with optimal and less optimal neuromotor development and Analysis of Covariance ANCOVA to control for the effect of covariance. Selection of covariates was based on prior literature. Final models were adjusted for the child's age, gender, gestational age at birth, child ethnicity, family income, age-appropriate version of motor instrument, maternal age, maternal education, maternal IQ and maternal psychopathologic symptoms during pregnancy.

The associations between infant neuromotor development and nonverbal intelligence (IQ), language comprehension, behavioral (BRIEF-P) and neuropsychological functioning (NEPSY- II – NL) were assessed with linear regression. All the outcomes, except nonverbal intelligence and memory as assessed by the NEPSY II NL, had a skewed distribution, and were therefore transformed using logarithm function or square root. The first analysis was performed using total scores of neuromotor and cognitive measures, further analysis were performed using individual subdomains.

We present the correlation between different cognitive outcomes in Supplement Table 2.

Missing values were imputed using multiple imputations. Five copies of the original data set were generated. Standardized effect sizes were calculated as the average effect size of five imputed data sets. For testing the associations between neuromotor development and nonverbal intelligence, language comprehension and behavioral executive functioning, we imputed missing values on covariates and outcomes, if at least one cognitive measure was present. For testing the associations between neuromotor development and experimental neuropsychological functioning, we imputed only covariates, as there were no missing values on the outcome.

We conducted a sensitivity analysis. We rerun analysis excluding all children with autistic symptoms above a pre-defined cut-off in the analysis of the subcohort with neuropsychological assessment.

Non-response analysis. We compared child and maternal characteristics of the children included in the analysis (n=3356) with those excluded because of missing data on infant neuromotor development (n=699) Children of responding mothers were more likely to be Dutch (54.7% vs 30.5%, $p<0.001$) than children of nonresponding mothers. Responding mothers were more likely to be highly educated (61.9% vs 43.6%, $p<0.0010$) and to have a high family income (79.3% vs 58.8%, $p<0.001$) than no responding mothers.

RESULTS

In Table 1 subject characteristics are presented. In the cohort with data on neuromotor development and BRIEF-P (all, n=2573), 48.9% children were males and 60.3% had Dutch ethnic background; 53.3% mothers completed higher education and 84.1% families had high income (>2000 Euros). Neuromotor development was assessed at an average postnatal age of 12.6 weeks (SD±2). In the cohort with data on neuromotor development and nonverbal intelligence/language comprehension (n=2755), 47.9% children were males and 52.4% had Dutch ethnic background; 53.8 % mothers completed higher education and 78.0 % families had high income (>2000 Euros). Neuromotor development was assessed at an average postnatal age of 12.6 weeks (SD±2). In the subcohort with

data on neuromotor development and experimental neuropsychological functioning (n=486), the distribution of baseline covariates was similar. Of 486 children with NEPSY-II-NL measurements, 35.8% were 6 years old, 36.8 % 7 years old, 24.5 % 8 years old and 2.9 % of children were 9 years old.

Associations of infant neuromotor development with nonverbal intelligence, language comprehension and behavioral executive functioning in children are presented in Table 2. Neuromotor development and nonverbal intelligence were significantly associated in the unadjusted model (beta= –1.12, 95% CI: -1.83,-0.41 , p =0.002). However, adjustment for ethnicity and education strongly attenuated the association (adjusted beta= –0.30, 95% CI:-0.99, 0.39, p = 0.39). Likewise, we found no association between neuromotor development and language comprehension (adjusted beta= 0.00, 95% CI:-0.05, 0.05, p = 0.99).

Table 2 also shows that neuromotor development was significantly associated with shifting (adjusted beta=0.07, 95% CI:0.02,0.12, p = 0.004) and with planning/organizing (adjusted beta= 0.05, 95% CI:0.00,0.10, p = 0.040) in the adjusted linear regression analyses.

In order to further explore patterns of executive functioning domains and help interpret their association with neuromotor development, we conducted principal component analysis (PCA) of the domains assesses with the BRIEF-P. PCA with orthogonal rotation illustrated in Supplemental Table 2 disclosed a four-component solution, accounting for 94.8% of the variance. Component 1 consists of the planning and working memory, which explained 58.1 % of the total variance. Component 2, which is comprised by the shifting alone, explained 18.5 % of the total variance, whereas Component 3 is comprised by emotional control only and explained 11.0 % of the total variance. Component 4 consists of working memory and inhibition and explains 7.3% of the total variance.

As shown in the figure 1, non-optimal neuromotor development was associated with certain aspects of poor neuropsychological functioning in children. Less optimal neurodevelopment in infants was associated in particular with more number of errors in the visuomotor precision task (adjusted beta for inversely coded number of errors=-0.12, 95% CI:-0.23,-0.02, p = 0.041), poor immediate memory for faces (adjusted beta= -0.12, 95% CI:-0.23, -0.002, p = 0.047) and poor geometric puzzling (adjusted beta= -0.20, 95% CI:-0.32,-0.08, p = 0.001). These results hardly changed if children with autistic symptoms were excluded (see Supplemental Figure 3), although the association between neuromotor development and immediate memory was not significant anymore.

DISCUSSION

This population-based study showed that infant neuromotor development did not predict nonverbal intelligence, language comprehension, and overall executive functioning in preschool and early school age children if carefully adjusted for covariates

such as ethnicity, income and education. However, infant neuromotor development was significantly associated with shifting, and planning, as reported by parents. In addition, neuromotor development assessed during infancy predicted children's performance in visuospatial processing, sensorimotor functioning, immediate memory, and inhibition as assessed in the laboratory.

Our study did not provide support for an association between infant neuromotor development and intelligence at school age. Most previous studies showed that motor and intellectual domains are largely independent in childhood and adolescence. [13]. Also, we did not find association of infant neuromotor development with language comprehension in contrast to an earlier follow up study using parent report only [10]. Although, language development at school age is strongly influenced by socioeconomical and environmental factors (for a review, see Rescorla et al., 2011)[24] (24), evidence shows that language delay in preschool children, like motor development, is mainly explained by genetic factors and biological background characteristics, such as birth weight and family language delay [25]. Moreover, early word production and language comprehension have poor predictive value for later vocabulary scores [26]. Therefore, we speculate that there are different developmental patterns for preschool and school children.

Consistent with most existing studies our study also showed no association between infant neuromotor development and overall executive functioning in this large cohort of children [5,12]. We observed moderate associations between infant neuromotor development and specific executive functioning measures only, particularly shifting immediate memory and planning. In particular planning reflects higher executive demands of the more complex tasks with self-regulatory demands. In addition, we observed that that non-optimal infant neuromotor development predicts commission error on auditory attention. This is in line with Rigoli et al. [27] and Michel, Roethlisberger, Neuenschwander, Roebers [3], who argued that motor development predicts shifting and inhibition.

These findings can be explained using the concept introduced by Zelazo et al. who made a distinction between hot and cold executive functions in children. Hot executive functions (emotional control and inhibition) involve tasks with affective components, in which rewards and punishments are often present. Cold executive functions involve tasks that are mostly cognitive in nature. We did not find a significant association between infant neuromotor development and hot executive functions such as emotional control or the "hot" part of inhibition as measured by the BRIEF-P. In contrast, our results suggest an association between infant non-optimal neuromotor development and cold executive functions: planning problems, low scores on the test of "cool" inhibition (commission error on auditory attention), and immediate memory problems. The expected association with working memory as measured by the BRIEF-P though was not observed. However, non-optimal infant neuromotor development predicted low scores in geometric puzzling and immediate memory. Geometric puzzling is a complex task designed to assess attention to detail, mental rotation and visuospatial analysis, that requires immediate memory and

well performed executive functioning. Possibly, working memory measured by BRIEF-P represents different construct than (visuo-spatial) working memory measured by NEPSY- II – NL. In particular, the items of the BRIEF-P do not tap into visual domains in contrast to the geometrical puzzle tap of the NEPSY- II – NL. Therefore, similarly to Michel, Roethlisberger, Neuenschwander, Roebers [3], we can not conclude about visual-spatial working memory skills. In addition, we found an association between neuromotor development and shifting. Our principal component analysis suggests that shifting presents a domain separate from other executive function domains, in line with prior studies [28-30]. Children with shifting problems are often described as rigid, inflexible, or upset with a change in routines. Shifting difficulties characterize children with brain damages, a pervasive developmental disorders, a coordination disorder. Possibly, shifting particularly addresses the ability to automate behavior, which has repeatedly been related to neuromtor development. [31]

Our research extends findings of two prior, small studies which explored the association between motor development and mental rotation. One was a cross sectional study of nine-months-old infants (N=48) demonstrating an association between crawling and mental rotation, the other was a follow-up study (N=40) of six-months-old infants which demonstrating an association between a child's milestone achievements such as walking with assistance and mental rotation 4 months later [32,33]. These studies raise the question why locomotor experiences are so closely related to mental object transformation. Frick et al. [33] reason that "the onset of independent locomotion has a strong influence on a variety of cognitive (spatial) (…) abilities." The authors discussed that as the child starts to move, he or she becomes independent from his location and refers to the environment differently. On the other hand, walking skills may be an unspecific indicator of healthy motor development. In the current study, we measured neuromotor development at much younger age than previous studies: at 9 to 20 weeks. At this age children are able to look at the objects only from a stationary position. This suggests that very early neuromotor development already predicts mental rotation later in life, independently of crawling and independently of the occurrence of walking.

Our results can be discussed in the context of the theory of developmental stages, originally formulated by Piaget at al. [34]. He was the first to point out that early motor experience is important activity for both visuospatial abilities and memory. Children develop immediate memory by searching for hidden objects. Very young children are able to mentally rotate the object if they had opportunity to manually explore the object before [35]. Present evidence for an association between neuromotor development and cognition comes from neuroimaging studies. In normative samples of children, neuroimaging techniques have shown that motor functioning and sensory regions of the brain are the first to mature [36]. Kagan and Diamond et al. [37] showed that maturation of dorsolateral cortex may underlie both motor experience and active exploration of the world shaping these cognitive functions.

Some methodological issues must be discussed. The assessment of impaired neuromotor development preceded the cognitive measurements. Yet, the possibility of reverse causality must be discussed. Cognitive function may affect neuromotor development by influencing social activities and exploration in childhood. Sergeant [38] suggested a tiered cognitive energetic model. This model links prior executive functioning abilities to late motor behavior. Yet, this explanation is less likely in our study as we measured neuromotor development at infancy, whereas executive functioning typically develop later on during childhood.

Confounding by parental characteristics, including genetics, parents intelligence (IQ), health and behaviour, cannot be ruled out because these factors affect both offspring neuromotor development and cognition [39]. For example, parents IQ and soocioeconomical status (SES) influence offspring IQ and child's neuropsychological functioning. It is possible that some children benefit from greater economic resources and social or cultural capital. [39,40]. While we did not have access to paternal intelligence data, we were able to control for paternal lifestyle characteristics and education.

Some selection effects were observed in our non-response analysis regarding ethnic minorities, lower education and younger age. Non-response may have reduced generalizability but it is less likely to bias the associations between variables [41]. Also, we do not know if any of children in our study received intervention for neuromotor delay.

The present study has several strengths including longitudinal design with a large sample size from the general population. We were able to adjust for variety of prenatal and postnatal covariates. Also, we assessed executive functions with a comprehensive test battery in the laboratory, as well as using inventory based information. The BRIEF-P-P items measured by parents reports have been validated in a large sample of children and it has been widely used to identify problem behaviors.

CONCLUSION

Less optimal infant neuromotor development predicts poor visuospatial abilities, as well as problems with shifting, planning, auditory attention, and immediate memory. In contrast, infant neuromotor development is not associated with nonverbal intelligence, language comprehension or specific executive functions such as emotional control, working memory, in early childhood. To the best of our knowledge, this is one of the first longitudinal population-based studies that shows the specificity of the long-term impact of very early neuromotor development on higher cognitive functions in childhood.

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Table 1. Participant characteristics

	Infant neuromotor development		
	BRIEF-P n=2573	Nonverbal intelligence/ language comprehension n=2755	NEPSY-II-NL n=486
Maternal characteristics			
Age at enrollment, yr	31.5 (4.6)	30.8 (5.0)	30.7 (5.0)
Education %			
Primary	14.2	19.5	11.0
Secondary	32.6	26.6	28.8
High	53.3	53.8	60.2
Psychopathology score in pregnancy	0.13 (0.06, 0.29)	0.15 (0.06, 0.33)	0.21 (0.10, 0.52)
Intelligence	98.9 (9.8)	97.3 (10.6)	98.2 (9.8)
Household income per month %			
<€1200	4.3	6.7	9.9
>€1200 & <€2000	11.6	15.2	17.6
>€2000	84.1	78.0	74.6
Child characteristics			
Age at neuromotor assessment visit, weeks	12.6 (2.0)	12.6 (2.0)	12.6 (2.2)
Age at BRIEF-P assessment, years	4.1 (1.3)[#]	-	-
Age at Nonverbal intelligence/ Language, years comprehension assessment, years		6.0 (0.4)[#]	-
Age at NEPSY- II – NL, years		-	7.5 (0.8)[#]
Sex, boy%	48.9	47.9	51.2
Ethnic background %			
Dutch	60.3	52.4	60.6
Other Western	12.2	11.2	9.0
Non-Western	27.5	35.4	30.4
Gestational age at birth, weeks	40.1 (39.1, 41.0)	40.1 (39.1, 41.0)	40.3 (39.3, 41.0)
Birth weight	3445 (3100, 3810)	3440 (3080, 3770)	3500 (3125, 3840)
Low birth weight %	4.5	4.5	3.9
Overall neuromotor development, raw score	3.7 (3.3)	3.8 (3.4)	3.6 (3.4)
Tone, raw score	2.9 (2.8)	3.0 (2.9)	2.69 (2.6)
Nonverbal Intelligence	103.1 (14.7)	101.2 (15.1)	100.1 (14.3)
Language	22.0 (2.9)	21.7 (3.1)	21.6 (3.2)
Inhibition	47.6 (8.8)	47.4 (8.6)	50.7 (10.3)
Shifting	48.2 (8.5)	48.2 (8.3)	49.9 (9.9)
Emotional control	48.0 (10.2)	47.9 (10.2)	51.8 (12.8)
Working memory	47.1 (9.6)	47.0 (9.3)	50.1 (11.7)
Planning/Organizing	45.6 (9.3)	45.5 (9.1)	48.7 (11.1)

Numbers are mean (SD) for variables with normal distribution, median (quartile range) for non-normally distributed variables, and percentages for categorical variables.

Table 2. Infant neuromotor delay and nonverbal intelligence, language comprehension and behavioral executive functioning in children at age 6 years (n=3356)

	Neuromotor delay per tertile n =3356			
	Model I		Model II	
Outcome measure	beta (95%CI)	p	beta (95%CI)	p
Nonverbal intelligence, score	-1.12 (-1.83, -0.41)	0.002	-0.30 (-0.99, 0.39)	0.39
Language comprehension, ln (score) per SD	-0.04 (-0.08, 0.01)	0.14	0.00 (-0.05, 0.05)	0.99
Executive functioning, ln (score) per SD				
Inhibition	0.02 (-0.03, 0.08)	0.42	0.00 (-0.06, 0.05)	0.86
Shifting	0.07 (0.03, 0.12)	0.002	0.07 (0.02; 0.12)	0.004
Emotional control	0.01 (-0.04,0.06)	0.83	0.00 (-0.05, 0.05)	0.90
Working memory	0.03 (-0.01, 0.08)	0.13	0.37 (-0.03, 0.07)	0.21
Planning/Organization	0.07 (0.02, 0.12)	0.004	0.05 (0.00, 0.10)	0.040

Model I: adjusted for gender and gestational age
Model II: adjusted for age child, gender, gestational age at birth, household income, ethnicity child, age mother, education mother, IQ mother, instrument, maternal psychopathology in pregnancy, epilepsy, seizures, birth weight.
Predictor: Neuromotor development was measured at 9-20 weeks, the outcomes nonverbal IQ and language comprehension at 6 years of age, executive functioning problems at 4 years of age

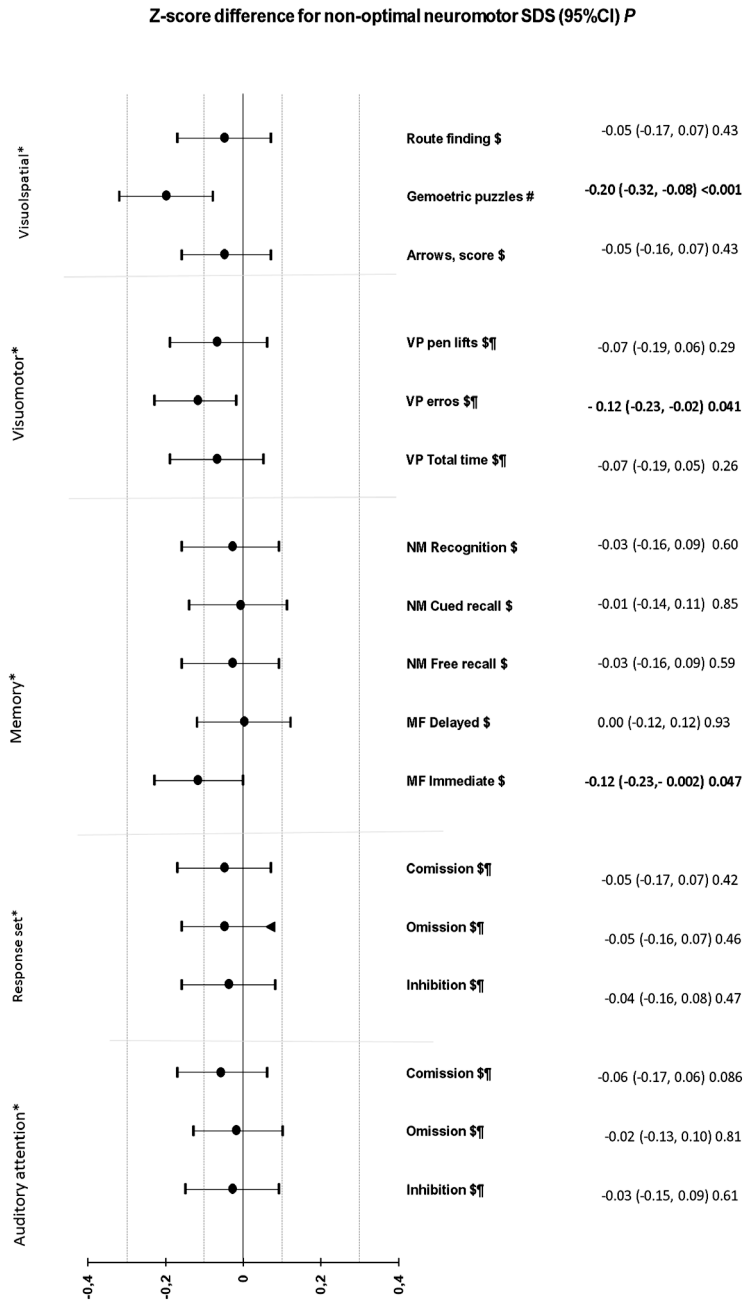

Figure 1. Infant neuromotor delay and neuropsychological functioning in children (n=486)

Models are adjusted for age child, gender, gestational age at birth, household income, ethnicity child, age mother, education mother, IQ mother, instrument, maternal psychopathology in pregnancy, maternal smoking, birth weight.
Predictor: Neuromotor development. #Score per SD, $ ln (score) per SD, ¶ error inverted. * Domains : every domain consists of subtests tapped by NEPSY-II-NL
VP: Visuospatial Processing, MF: Memory for Faces, NM: Narrative Memory

Supplemental table 1. Factor Pattern Coefficients (n=3356)

	Component 1	Component 2	Component 3	Component 4
Plan/Organize	0.98			
Working Memory	0.62			0.42
Inhibition				0.94
Shifting		**1.00**		
Emotional Control			-0.92	

Supplemental table 2. Correlations between Nonverbal Intelligence, Verbal Intelligence and Behavioral Executive Functioning (n=3056)

	Nonverbal IQ	Verbal Intelligence	Inhibition	Shifting	Emotion Control	Working Memory	Planning
Nonverbal IQ	-						
Verball Intelligence	0.38**	-					
Inhibition	-0.13**	-0.10**	-				
Shifting	**-0.02**	-0.04*	0.27**	-			
Emotion Control	-0.04*	-0.03	0.51**	0.44**	-		
Working Memory	-0.16**	-0.14**	0.64**	0.30**	0.36**	-	
Planning/Organizing	-0.10**	-0.10**	0.53**	0.27**	0.36**	0.66**	-

*p values<0,05 ** p values <0,0001

2.2

Infant Muscle Tone and Childhood Autistic Traits. A Longitudinal Study in the General Population

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ABSTRACT

Objective: In a longitudinal population-based study of 2905 children, we investigated if infants' neuromotor development was associated with autistic traits in childhood.
Methods: Overall motor development and muscle tone were examined by trained research assistants with an adapted version of Touwen's Neurodevelopmental Examination between ages 9-20 weeks. Tone was assessed in several positions and items were scored as normal, low or high tone. Parents rated their children's autistic traits with the Social Responsiveness Scale (SRS) and the Pervasive Developmental Problems (PDP) subscale of the Child Behavior Checklist at 6 years. We defined clinical PDP if scores were >98th percentile of the norm population. Diagnosis of autism spectrum disorder (ASD) was clinically confirmed in 30 children.
Results: We observed a modest association between overall neuromotor development in infants and autistic traits. Low muscle tone in infancy predicted autistic traits measured by SRS (adjusted beta=0.05, 95% CI for B: 0.00-0.02, p=0.01), and PDP (adjusted beta=0.08, 95% CI for B: 0.04-0.10, p<0.001). Similar results emerged for the association of low muscle tone and clinical PDP (adjusted OR=1.36, 95% CI: 1.08-1.72, p=0.01) at age 6 years. Results remained unchanged if adjusted for child intelligence. There was no association between high muscle tone and SRS or PDP. Exclusion of children with ASD diagnosis did not change the association.
Conclusion: This large study showed a prospective association of infant muscle tone with autistic traits in childhood. Our findings suggest that early detection of low muscle tone might be a gateway to improve early diagnosis of ASD.

Key words: infant muscle tone, autistic traits, autism spectrum disorder, prospective

ABBREVIATIONS

ASD: Autism Spectrum Disorder
SRS: Social Responsiveness Scale
CBCL/1½-5: Child Behavior Checklist for toddlers
PDP: Pervasive Developmental Problems
SCQ: Social Communication Questionnaire

Autism spectrum disorder (ASD) is a developmental disorder characterized by persistent impairments in social communication and interaction, and repetitive stereotyped behaviors that manifest in early childhood [1]. Subclinical deficits in social communication or some degree of repetitive behaviors that do not meet the diagnostic criteria for ASD are defined as autistic traits and exist in the general population [2].

Neuromotor function during infancy is an important early indicator of central nervous system development. Numerous studies in clinical or high-risk populations have demonstrated a close relationship between neuromotor development and ASD [3-5]. For example, Landa, Gross, Stuart, Faherty [6] reported a worsening of fine motor performance over the first 3 years of life in infants later diagnosed with ASD. Bhat, Landa, Galloway [7] proposed that early motor delays within the first two years of life may contribute to the social impairments of children with ASD.

As individuals with ASD are at the high end of the distribution of autistic traits [8], infant neuromotor development may also be associated with autistic traits in children from the general population. In a population-based cohort, Bolton, Golding, Emond, Steer [9] showed that maternal report of fine motor delays at 6 months predicted later diagnoses of ASD, as well as autistic traits. Although fine and gross motor milestone assessment is a valuable approach to evaluate motor development in children, it is reliable only from 6 months of age, and often based on retrospective report of caregivers.

Several studies reported problems with muscle tone in children with ASD. Adrien, Lenoir, Martineau, Perrot, Hameury, Larmande, Sauvage [10] rated family home movies of 12 infants who were later diagnosed as autistic and 12 typically developing infants. They observed a high prevalence of low muscle tone in children with autistic traits. Ming, Brimacombe, Wagner [11] investigated a cohort of children with ASD using retrospective clinical record review and found a higher prevalence of gross and fine motor impairment among 2-6 year old children with ASD. The children typically had mild to moderate hypotonia early in life.

It remains unknown if differences in infant neuromotor development, and in particular muscle tone, as early as 9-20 weeks may serve as a prodromal sign of autistic traits. Such information can facilitate early detection of children at risk for ASD and potentially allow for early intervention. The purpose of the current study was to explore whether variations in infant neuromotor development are associated with childhood autistic traits in the general population. We hypothesized that non-optimal neuromotor development, and in particular muscle tone, in infants are related to autistic traits in childhood.

We applied two parental rating scales to study autistic traits in childhood: the Social Responsiveness Scale (SRS) and the Pervasive Developmental Problems (PDP) scale of the Child Behavior Checklist for toddlers (CBCL/1½-5). The use of parental reports on children's social behavior provides an informative mean of examining autistic traits, as parents are familiar with everyday behavior of their children. Parental reports provide the contextual information with high ecological validity. We also obtained information on ASD diagnosis, if available.

METHODS

This study was conducted within the Generation R Study, a population-based cohort that follows children from fetal life onwards [12,13]. Briefly, mothers were eligible if they were living in the Rotterdam area, the Netherlands, and had a delivery date between April 2002 and January 2006. When infants were 9-20 weeks old, their neuromotor development was assessed during a home visit by trained research assistants. Neuromotor assessment was completed in 4055 infants. These 4055 children were the eligible participants for this follow-up study. When the children were 6 years old, questionnaires were mailed to caregivers in order to assess autistic traits in the children. Information on autistic traits was available in 2905 children (72 % of 4055). The Medical Ethics Committee of the Erasmus Medical Centre approved the study and written informed consent was obtained from all adult participants.

Neuromotor and Muscle Tone Assessment

Infants underwent a neuromotor assessment at a corrected postnatal age between 9 and 20 weeks. Two versions of neuromotor assessment instrument were used: One for infants aged 9-15 weeks and the other for infants aged 15-20 weeks. We selected age-appropriate items from Touwen's Neurodevelopmental Examination, and categorized items in three groups: tone, responses, and other observations [14] (see Supplemental Table 1). Tone was assessed in several positions –supine, horizontal, vertical, prone and sitting– and all items, such as adductor angle, were scored as normal, low or high tone. Responses were assessed in supine (e.g. asymmetrical tonic neck reflex), vertical (e.g. Moro response) or prone position (e.g. Bauer response) and were scored as present, absent or excessive. Other observations, such as following movements, were scored as present, absent or excessive. Assessment of overall neuromotor development and tone was used in this analysis. For each item, an age-appropriate response was labelled 'optimal'. If the response indicated a delayed development, it was labelled 'non-optimal'. By summing the raw values of all items, we obtained a total score, with high values indicating less optimal neuromotor development.

Research assistants were trained by a movement scientist who also was a child physiotherapist specialized in motor development. Training consisted of a lecture about the theory of neuromotor development. Furthermore, the neuromotor assessment and the practical procedure and protocol were explained in detail. To investigate interobserver reliability, two research assistants independently conducted a neuromotor assessment in a sample of 76 children. The intra-class correlation coefficient was 0.64.

Child Autistic Traits

Social Responsiveness Scale (SRS). Due to length of the original questionnaire, and the need to minimize subject burden, we used a short-form SRS with 18 items for assessment of autistic traits based on parent's observation of the child's social behavior in a naturalistic setting. Each item is rated from '0' (never true) to 3 (almost always true), covering social, language, and repetitive behaviors; higher scores indicate more problems [2,15]. In a sample of 3857 children aged 4-18 years (part of the Social Spectrum Study, a multi-center study on social development of children referred to a mental health care institution in the South-West of the Netherlands from 2010-2012) the correlation between total scores derived from the SRS short-form (18 items) and the SRS scores derived from the complete instrument was r=0.95 ($p<0.001$). The correlation between SRS short-form and SRS total scores in Missouri Twin Study was 0.93 in monozygotic male twins (n=98) and 0.94 in dizygotic male twins (n=134).

Pervasive Developmental Problems (PDP) subscale of the CBCL/1½-5. The CBCL/1½-5 is a validated instrument to measure behavioral and emotional problems of children at young age. The Dutch version is reliable and well-validated [16]. The PDP is one of the five scales that can be derived from the CBCL/1½-5, consistent with the Diagnostic and Statistical Manual of Mental Disorders 4th edition diagnostic categories. The PDP has been shown to be a useful screening instrument to identify children with ASD when compared with Autism Diagnostic Observation Schedule-Generic [17]. It has a good predictive validity to identify preschoolers at risk of ASD (sensitivity=0.85. specificity=0.90) [18]. At 6 years, the correlation coefficient between the PDP and SRS scores was r=0.6 ($p<0.001$, n=2275)

ASD diagnoses. Only diagnoses made by a specialist as part of the regular clinical care were used. These diagnoses were retrieved from general practitioners; in the Dutch health care system, all specialists are obliged to inform the general practitioner as the primary health care provider, who holds the central medical records. To the aim of checking general practitioners' records, we selected those children for which one of three sources of information signaled possible ASD. First, children who screened positive on the Social Communication Questionnaire (SCQ), a 40-item parent-reported screening instrument for ASD. All children who scored in the top 15th percentile on the CBCL/1½-5 total score or those in the top 2nd percentile on the PDP subscale were screened with this instrument [19]. Second, we retrieved medical records from the general practitioners of all children who had weighted scores over 1.078 for boys and 1.000 for girls on the SRS-short form [20]. Third we retrieved medical records in all children of whom the mother at any contact moment up to age 8 years had reported that the child had undergone a diagnostic procedure for possible ASD. Only children for whom a diagnosis of ASD could be confirmed by specialist medical records were considered ASD cases in the analyses. The specialist diagnoses of ASD were generally based on clinical consensus by a multidisciplinary team. The standard

diagnostic work-up involves an extensive developmental case history obtained from parents, as well as school information, and repeated observations of the child.

Covariates

Characteristics such as maternal age, household income, child ethnicity, maternal educational level, as well as maternal lifestyle, were assessed by questionnaires. The child's national origin was defined based on the national origin of parents and grandparents. Dutch ethnicity was used as the reference group. The education level of the mother was assessed by the highest completed education and reclassified into 3 categories: "low" (less than 3 years of secondary school), "mid" (at most intermediate vocational training), and "high" (at least college education). Household income, defined as the total net monthly income of the household, was categorized into <1200 Euros (below social security level), 1200-2200 Euros (low income), and >2200 Euros (modal income and above). We used the Brief Symptom Inventory, a validated self-report questionnaire, to measure maternal psychopathology during pregnancy [21]. Child nonverbal IQ was assessed during the child's visit to the research center at the age of 6, using two subtests of the validated Dutch test battery Snijders-Oomen Niet-verbale intelligentietest-Revisie [22]. These subtests were Mosaics (assesses spatial visualization abilities), and categories (accesses abstract reasoning abilities). Raw non-verbal IQ scores were standardized using age-defined norms. In our previous study, we showed that infant neuromotor development is not associated with verbal expressive language ability and therefore we did not include this variable as a covariate in the analysis [23].

Information about birth weight and gestational age at birth as well as complications during pregnancy or delivery were obtained from the medical records and midwives' practices. Gestational age was determined by fetal ultrasound examination. Postnatal age was calculated as the difference between date of assessment and date of birth.

Statistical Analyses

We included children with an assessment of neuromotor development between 9 to 20 weeks and at least one autistic trait measure in the analyses (n=2905). Missing values on covariates and autistic traits were imputed using multiple imputations. Five copies of the original data set were generated. Standardized effect sizes were calculated as the average effect size of five imputed data sets.

Infant's overall neuromotor development and muscle tone were determinants in all analyses. The neuromotor assessment scores were highly skewed in this non-clinical population. Therefore, we categorized the sum scores into tertiles in line with a prior study [24]. The lowest tertile represents the most optimal neuromotor development and the highest the least optimal neuromotor development. These tertiles were analyzed continuously, as well as categorically. The associations of neuromotor development and muscle tone with autistic traits were assessed with linear regression. Both outcome

measures, i.e. SRS and PDP, had a skewed distribution, and were therefore transformed using a logarithm function. We also categorized PDP scores in order to facilitate the clinical interpretation of the findings. For this purpose, the 98th (clinical) percentile of a Dutch norm group was used as a cut-off score to classify children with behavioral problems within the clinical range of the PDP [16]. We explored the associations of neuromotor development and muscle tone with clinical PDP using logistic regression.

To assess whether our results were driven by children in the clinical end of the spectrum, we conducted a sensitivity analysis excluding children with a confirmed diagnosis of ASD. We also explored the associations with confirmed diagnosis of ASD using logistic regression.

Selection of covariates was based on prior literature. Final models were adjusted for the child's age, gender, gestational age at birth, Apgar score at 5 minutes, child ethnicity, child IQ, household income, age-appropriate version of motor instrument, maternal age, maternal education, and maternal psychopathology during pregnancy.

Attrition Analysis. We compared child and maternal characteristics of the children included in the analysis (n=2905) with those excluded because of missing data on outcome (n=1190). Children of responding mothers were more likely to have higher IQ (mean 103.1) compared to children of nonresponding mothers (mean 96.4). They were also more likely to be Dutch (61.2% vs 34.0%, $p<0.001$) compared to children of nonresponding mothers. Responding mothers also had less severe psychopathology symptoms (mean 0.24) compared with nonresponding mothers (mean 0.37). However, children included scored similarly on muscle tone compared to children not included (score: 1.92 vs 1.96, $p=0.54$).

RESULTS

Participants' characteristics are presented in Table 1. In this cohort (n=2905), 48.8% children were male and 54.5% had Dutch ethnic background; 55.0% mothers completed higher education and 79.3% families had a monthly income >2000 Euros. Neuromotor development was assessed at an average age of 12.6 weeks (SD=2) postnatally.

Associations of neuromotor development and muscle tone with autistic traits measured continuously by the SRS are presented in Table 2. Overall neuromotor development and autistic traits were significantly associated in the unadjusted model (beta=0.06, 95% CI: 0.01, 0.02, $p=0.001$). Adjustment for maternal education, age and psychopathology attenuated the association (adjusted beta=0.04, 95% CI: 0.00, 0.02, $p=0.05$). Muscle tone in infants predicted autistic traits in children measured by the SRS (adjusted beta=0.05, 95%CI: 0.00, 0.02, $p=0.016$). This association remained significant for low muscle tone and SRS after adjustment for all confounders (adjusted beta=0.05, 95%CI: 0.00, 0.02, $p=0.006$), while there was no association between high muscle tone and

SRS in unadjusted and adjusted analyses (adjusted beta=0.03, 95%CI:-0.004, 0.02, p=0.23). The association between neuromotor development and autistic traits measured by PDP scores is presented in Table 3. We found an association between overall muscle tone and PDP scores in children. Less optimal muscle tone was significantly associated with PDP scores (adjusted beta=0.05, 95%CI: 0.02, 0.09, p=0.010). Low muscle tone predicted PDP scores (adjusted beta=0.08, 95%CI: 0.04, 0.10, p<0.001), with the third tertile being strongly associated with outcome (adjusted beta=0.09, 95%CI: 0.08, 0.21, p<0.001), whereas the second tertile was not (adjusted beta=0.03, 95% CI:-0.04, 0.13, p=0.29). Results adjusted for child IQ remained essentially unchanged (Table 3).

In an additional analysis, we present the association between neuromotor development and the dichotomized PDP scale using the clinical cut off. We found that the odds of having PDP scores in the clinical range was not associated with overall neuromotor development (adjusted OR=1.29, 95% CI: 0.99, 1.68, p=0.066) or high muscle tone (adjusted OR=0.87, 95%CI: 0.64, 1.17, p=0.36). Consistent with the above results, the odds of having PDP scores in the clinical range was associated with low muscle tone in infancy (adjusted OR=1.36, 95%CI: 1.08, 1.72, p=0.01) (Supplemental Table 2). Children with low muscle tone in the highest tertile had higher odds of PDP in the clinical range compared to other children.

To assess whether results were driven by children with ASD, we repeated the analyses excluding children with a confirmed diagnosis of ASD (n=30, 0.9%). This exclusion did not materially change the results (adjusted beta of the second tertile of low muscle tone=0.02, 95%CI: -0.01, 0.03, p=0.25, adjusted beta of the third tertile of low muscle tone=0.05, 95%CI: 0.00, 0.04, p =0.01).

Next, we tested the association between low muscle tone and a confirmed diagnosis of ASD. We observed that a non-optimal low muscle tone increased the odds of ASD (adjusted OR for the second tertile=1.99, 95%CI: 0.69, 5.70, p=0.20, adjusted OR for the third tertile=1.44, 95%CI: 0.54, 3.83, p=0.46), although results did not reach significance due to small number of confirmed ASD cases.

DISCUSSION

In this population-based study, we investigated whether infant neuromotor development is associated with autistic traits. Our study showed a modest association between overall neuromotor delay early in life and autistic traits in school age. In particular, low muscle tone in infants aged 9-20 weeks was prospectively associated with autistic traits in 6 year-old children. High muscle tone during infancy did not predict autistic traits.

The majority of studies to date exploring early motor development of ASD children used case-control designs of clinically diagnosed patients with ASD [25,26]. While these studies are very beneficial to help understanding of ASD, they cannot answer the question

whether subtle variations in neurodevelopment precede autistic traits in the general population. In the past, videos and child care registries have been used to overcome such limitations in developmental studies of autism [27]. Also, registry studies using routine assessment of motor milestones have yielded important findings in psychopathology research, but they have several limitations, e.g. a restricted number of confounders that can be addressed and the lack of precision of results in small samples [28,29]. The studies assessing motor functioning in large numbers of participants relied on age of motor milestone achievement as reported by parents, whereas full neurological examinations to assess neuromotor development as a precursor of psychopathology at a later age were often conducted in small or clinical samples only [11].

Several large studies measured fine motor skills from the age of 6 months onwards in the general population or in children at high-risk for ASD [6,9,30]. Some studies on children at high risk for autism reported differences in gross motor skills [5], other studies observed that high risk children had delays in fine moto and grasping skills [31] On the other hand, Ozonoff, Young, Goldring, Greiss-Hess, Herrera, Steele, Macari, Hepburn, Rogers [32] found motor abnormalities in children with developmental delays, but not ASD-specific movement abnormalities in children who later developed ASD. Importantly, the authors recommended motor screening for the detection of developmental delays in general pediatric settings. One potential explanation for these inconsistent findings is that high-risk children from multiplex families may have different risk profiles than children from the general population [33-35]. Our results are in line with studies suggesting that motor coordination deficits are pervasive across ASD subtypes and a potential core feature of ASD [36].

Motor problems are commonly reported in children with ASD, but little is known about the prospective association between early muscle tone development and autistic traits at later age [11,37]. Our research extends the findings of two small prior studies focusing on muscle tone in children with ASD. Ming, Brimacombe, Wagner [11] found a higher prevalence of motor impairment in 2-6 year old children with ASD based on retrospective record reviews. The degree of hypotonia was mild to moderate and observed throughout the body. In a sample of 398 twin pairs (aged 8–17 years) from an Italian twin registry, Moruzzi, Ogliari, Ronald, Happe, Battaglia [38] showed a genetic overlap between low muscle tone and autistic traits. They hypothesized that the genes with effects in brain regions underlying autistic behaviors also affect motor development, arguing that low muscle tone might be an early, preclinical marker of autism. Even if hypotonicity is not necessarily autism-specific, hypotonia may be important to recognize and evaluate in children who are at risk for autism [7]. The authors recommended measures of clumsiness as an endophenotype of disease. Some of the most consistently reported structural brain abnormalities in ASD occur within regions of the brain involved in movement, including the frontal lobe and cerebellum [39]. However, there are other possible explanations. For example, teratogens, like diseases or nutrition and maternal stress, could cause brain

abnormalities associated with hypotonia or autistic traits during prenatal development. Alternatively, postnatal influences could underlie both poor motor development and autistic traits.

In this study, we controlled for numerous external factors in our study, e.g. gestational age or Apgar score. Importantly, we included child nonverbal IQ in our analysis and our results remained largely unaltered, which is consistent with our previous work showing no association between motor development and IQ [23]. Similarly, Bolte, Poustka, Constantino [40] showed low correlation between IQ and social responsiveness scale in children with ASD. It is possible that there is substantial degree of genetic independence between IQ and autistic symptoms [41] in the general population, although autistic symptoms closely co-occur with low IQ in clinical population.

Our study has several strengths, including the longitudinal design and large sample size recruited from the general population. The prospective nature of the study ensured that infant neuromotor development measurements were assessed blind to the eventual later autistic traits. Research nurses assessed neuromotor development using a hands-on test battery, independently of mothers, eliminating common method bias, which can affect motor mile stone research based on parent report [42]. Also, we were able to adjust for large number of prenatal and postnatal covariates.

The present study also has limitations. Selection effects were observed in our non-response analysis. The non-response may reduce the generalizability but is less likely to bias the associations between variables [43]. Also, we do not know if any of children in our study received intervention for neuromotor delays, but this is unlikely, as most children with subclinical traits receive no treatment.

CONCLUSION

Less optimal infant low muscle tone predicted autistic traits at age 6 years. High muscle tone was not associated with autistic traits. To the best of our knowledge, this is the first longitudinal population-based study that shows the long-term association of very early low infant muscle tone development with autistic traits in childhood. The earliest observable traits of ASD involve motor behaviour. There is a growing awareness of the developmental importance of impaired motor function in ASD and its association with social skill. This study suggests that muscle tone as part of the motor system development might be a gateway to improving early detection of ASD. Motor problems, in particular low muscle tone in early infancy as an early symptom or precursor of autistic traits requires increased attention.

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Table 1. Participant characteristics (n=2905)

Maternal characteristics	
Age at enrolment, year	31.3 (4.7)
Education %	
Primary	16.5
Secondary	28.5
High	55.0
Psychopathology score in pregnancy	0.13 (0.00, 0.87)
Household income %	
Poor	6.2
Low	14.5
Modal and above	79.3
Child characteristics	
Age at neuromotor assessment, month visit, weeks	12.6 (2.0)
Sex, boy%	48.8
Nonverbal Intelligence	102.4 (14.7)
Ethnic background %	
Dutch	57.8
Other Western	11.5
Non-Western	30.7
Gestational age at birth, week	40 (1.7)
Age at SRS assessment, year	6.0 (1.3)
Age at PDP, year	5.9 (0.4)

Numbers are mean (SD) for variables with normal distribution, median (quartile range) for not-normally distributed variables, and percentages for categorical variables. SRS: Social Responsiveness Scale; PDP: Pervasive Developmental Problems scale of the Child Behavior Checklist for toddlers.

Table 2. Infant neuromotor development and Social Responsiveness Scale (SRS) scores in children at age 6 years (n=2905)

	Social Responsiveness Scale (SRS) scores N =2905					
Predictor measure	Basic beta (95% CI)	p	Adjusted for covariates beta (95% CI)	p	Adjusted for child IQ beta (95% CI)	p
Overall neuromotor development, per tertile	0.063 (0.005, 0.022)	**0.001**	0.042 (0.000, 0.018)	**0.050**	0.039 (-0.001, 0.017)	**0.073**
Muscle tone, per tertile	0.057 (0.004, 0.020)	**0.003**	0.045 (0.002, 0.017)	**0.016**	0.043 (0.001, 0.017)	**0.023**
Hypertone						
High muscle tone, per tertile	0.035 (-0.001, 0.015)	0.075	0.028 (-0.004, 0.015)	0.230	0.027 (-0.004, 0.015)	0.236
First tertile	Reference		Reference		Reference	
Second tertile	0.006 (-0.013,0.017)	0.782	-0.001 (-0.015, 0.014)	0.930	-0.002 (-0.015, 0.014)	0.915
Third tertile	0.039 (-0.001, 0.030)	0.069	0.041 (-0.005, 0.034)	0.132	0.041 (-0.005, 0.034)	0.134
Hypotone						
Low muscle tone, per tertile	0.057 (0.004, 0.020)	**0.005**	0.054 (0.003, 0.019)	**0.006**	0.053 (0.003, 0.019)	**0.007**
First tertile	Reference		Reference		Reference	
Second tertile	0.030 (-0.004,0.026)	0.146	0.027 (-0.009, 0.029)	0.306	0.025 (-0.010, 0.028)	0.339
Third tertile	0.061 (0.007, 0.041)	**0.006**	0.058 (0.006, 0.039)	**0.007**	0.058 (0.006, 0.039)	**0.008**

Model I: adjusted for gender and gestational age
Model II: additionally adjusted for a child's age, ethnicity, Apgar score at 5 min, and household income, and maternal age, education, and psychopathology in pregnancy, and version of instrument for assessment of tone.
Model III: additionally adjusted for child IQ
For muscle tone first tertile was as the reference category. The SRS scores were transformed using natural logarithm. Confidence intervals are reported for B.

Table 3. Infant neuromotor development and autistic traits measured by Pervasive Developmental Problems (PDP) scores in children at age 6 years (n=2792)

	Pervasive Developmental Problems (PDP) scores N =2905					
Predictor measure	Basic beta (95%CI)	p	Adjusted for covariates beta (95% CI)	p	Adjusted for child IQ beta (95%CI)	p
Overall neuromotor development, per tertile	0.066 (0.015, 0.082)	**0.004**	0.060 (0.024, 0.095)	**0.001**	0.058 (0.023, 0.093)	**0.001**
Muscle tone, per tertile	0.042 (0.011, 0.081)	**0.021**	0.052 (0.020, 0.090)	**0.010**	0.051 (0.020, 0.090)	**0.010**
Hypertone						
High muscle tone, per tertile	-0.003 (-0.033, 0.028)	0.878	0.024 (-0.019,0.059)	0.312	0.024 (-0.019, 0.059)	0.316
First tertile	Reference		Reference		Reference	
Second tertile	0.006 (-0.054,0.073)	0.778	0.005 (-0.055,0.071)	0.805	0.005 (-0.055, 0.070)	0.811
Third tertile	-0.003 (-0.067, 0.056)	0.858	0.032 (-0.036,0.132)	0.260	0.032 (-0.036, 0.131)	0.263
Hypotone						
Low muscle tone, per tertile	0.080 (0.036, 0.102)	**<0.001**	0.082 (0.039,0.104)	**<0.001**	0.081 (0.039, 0.104)	**<0.001**
First tertile	Reference		Reference		Reference	
Second tertile	0.011 (-0.044,0.078)	0.588	0.030 (-0.038,0.128)	0.286	0.029 (0.039,0.126)	0.300
Third tertile	0.092 (0.083, 0.216)	**<0.001**	0.089 (0.080, 0.211)	**<0.001**	0.089 (0.079, 0.211)	**<0.001**

Model I: adjusted for gender and gestational age
Model II: additionally adjusted for a child's age, ethnicity, Apgar score at 5 min, and household income, and maternal age, education, and psychopathology in pregnancy, and version of instrument for assessment of tone.
Model III: additionally adjusted for child IQ
For muscle tone first tertile was used as the reference category. The PDP scores were transformed using natural logarithm.
Confidence intervals are reported for B.

2.3

Infant Neuromotor Development and Problem Behavior across Childhood

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ABSTRACT

Background Research in adult and school-aged children suggests a neurodevelopmental basis for major psychiatric disorders. Less clear is whether emotional and behavioral problems in young children, classified as internalizing and externalizing problems, also have a neurodevelopmental basis.

Methods In Generation R, a population-based cohort in the Netherlands (2002-2006), we examined whether infant neuromotor development predicted internalizing and externalizing problems through childhood. In 4,006 infants aged 2-5 months, trained research assistants evaluated neuromotor development with an adapted version of Touwen's Neurodevelopmental Examination (tone, responses, senses and other observations). We defined non-optimal neuromotor development as scores in the highest tertile. Mothers and fathers rated their children's behavior at age 1.5, 3, 6 and 10 years with the Child Behavior Checklist (n=3,474, response: 86.7%). The associations were tested with generalized linear mixed models.

Results Overall neuromotor development predicted internalizing scores; but no association was observed with externalizing scores. Non-optimal muscle tone was associated with higher internalizing scores (mothers' report beta=0.07, 95%CI: 0.01-0.13; fathers' report beta=0.09, 95%CI: 0.00-0.16). In particular, non-optimal low muscle tone was associated with higher internalizing scores (mothers' report beta=0.12, 95%CI: 0.06-0.18; fathers' report beta=0.13, 95%CI: 0.04-0.22). We also observed an association between senses and other observations with internalizing scores. There was no relationship between high muscle tone or reflexes with internalizing scores.

Conclusions These findings suggest that common emotional problems in childhood have a neurodevelopmental basis in infancy. Neuromotor assessment in infancy may help identifying vulnerability to early internalizing symptoms and offer the opportunity for targeted interventions.

Keywords: neuromotor development, problem behavior, cohort study, longitudinal

INTRODUCTION

Childhood psychiatric problems are common and are associated with adverse mental health outcomes, poor school achievements, and delinquent behavior in later ages.[1-3] Problem behavior in children often coincides with early developmental problems. In a community-based sample of twins, a modest association was reported between problem behavior and cognitive problems.[4] Similarly, in a population-based follow-up study, delays in motor function and deviant language development predicted later psychiatric disorders.[5] These observations are in accordance with the notion that an association exists between early developmental problems and later problem behavior. A possible explanation for this link comes from research on developmental disorders.[6] For example, children with autism are shown to have problems with muscle tone in infancy.[7] Other abnormalities such as difficulties in motor coordination were also observed in children at risk of autism.[8] Neuromotor abnormalities including abnormal movement and motor coordination problems can also be a precursor in schizophrenia.[9] Overall this line of research suggests that early motor impairment, reflecting diffuse neural dysfunction, represents a vulnerability marker for psychopathology.[10,11] Nonetheless, the association with later problem behavior in young children and in particular emotional problems is poorly understood. The association between neuromotor development and later psychopathology could be a potential treatment target for early detection and prevention. If early neuromotor impairment can predict specific patterns of behavioral traits in the general population, we may be able to better influence disease development with early interventions.

Registry studies using routine assessment of motor milestone have yielded important findings in psychopathological research, but they have several limitations, e.g., appropriate adjustment for confounders.[11,12] Also, studies that assess motor functioning and psychiatric disorders in large samples have relied on parents' report on the age of achieving motor milestone.[10] In contrast, the studies based on full neurological examinations carried out by professionals to assess neuromotor development as a precursor of psychopathology are typically conducted in small or clinical samples.[10]

Thus, our goal was to study the prospective association between objective measures of neuromotor development conducted in infancy with repeated measures of children's internalizing and externalizing problems through age ten years. Based on our previous work demonstrating the importance of later executive functioning in infants with suboptimal motor development, children's shifting and planning were tested as the underlying mechanisms of any potential association.[13] While child behavior was measured using parental rating, the infant neuromotor development was assessed by research nurses, eliminating common method bias.[14] We hypothesized that infants with non-optimal neuromotor development had an increased risk of internalizing and externalizing problems.

METHODS

Participants

Participants were from the Generation R Study, a population-based cohort in the Netherlands, which follows children and their parents from fetal life onwards.[15,16] Briefly, pregnant women with expected delivery date between 2002 and 2006 in Rotterdam, were invited to participate.

A flow chart of the study population is shown in the Supplemental Figure 1. A total of 4,006 infants underwent a neuromotor assessment at corrected ages between 2 to 5 months during a home visit. Information on one or more assessments of child behavior up to age 10 years was available in 3,474 children (86.7% of 4,006). Since random exclusion of siblings did not change our results, they were included in the analyses. The study was approved by the Medical Ethics Committee of the Erasmus University Medical Centre. Written informed consent was obtained from adult participants.

Neuromotor development

We performed a full neurological age-adequate examination, encompassing assessment of tone, elicited responses, senses and other observations, as spontaneous movements at age 2-5 months. There were two versions of the neuromotor assessment instrument (for 9-15 weeks and for 15-20 weeks) adapted from Touwen's Neurodevelopmental Examination.[17] All measured items were categorized in three groups: tone, responses, senses and other observations. Tone items were scored as normal, low or high tone; most responses, senses and other observations were scored as present, absent, or excessive. An age-appropriate response was labeled 'normal'; a response that indicated delayed development was labeled 'not-normal'. Senses and other observations comprise strabism, fixation eyes, following movement eyes, hearing, sweating and startle reactions. Within the subscale measuring tone, a further distinction was made between low tone and high tone, resulting in two additional scales for tone: 'low tone symptoms' and 'high tone symptoms'. As we studied a non-clinical population, the outcome measures were skewed; thus we categorized the sumscores of the total and the subscales into tertiles, subsequently classifying the lowest and the middle tertiles as optimal neuromotor development, and the highest as non-optimal.[18]

We examined the short-interval test-retest intraobserver reliability and the interobserver reliability of the assessment (n=76). The intra-class correlation coefficient for the interobserver reliability was 0.64.[13]

Child behavior

Preschool and school versions of the Child Behavior Checklists (CBCL/$1_{1/2}$-5 and CBCL/6-18) were used to obtain standardized parent reports of children's problem behavior. By summing scores of specific questions from the CBCL/$1_{1/2}$-5, the following syndrome scales

were obtained: emotionally reactive, anxious/depressed, somatic complaints, withdrawn, sleep problems, attention scores, and aggressive behavior. The CBCL/6-18 includes similar scales as the CBCL/$1_{1/2}$-5, with the exception of the emotionally reactive and sleep problems scores. There are two broadband scales: internalizing comprises the anxious/depressed, withdrawn-depressed, and somatic complaints scales; whereas externalizing comprises attention problems and aggressive behavior. According to the taxonomy of the CBCL, internalizing behaviors comprise problems that manifest mainly within the self, such as sad mood or anxiety, somatic complaints without known medical cause, and withdrawal from social contacts. These problems that pertain to the emotion are often more difficult to detect than overt behavioral problems. Externalizing problems that are outer-directed generate discomfort and conflict.[19,20] Good reliability and validity have been reported for both versions of the CBCL.[21] Scale values were used continuously following a square root transformation to approximate normality. Since the CBCL/6-18 scores had different scales compared to the version for younger ages, we standardized all CBCL scale scores by dividing them by the corresponding standard deviation. This method enables us to compare two scores that are from different distributions.

Covariates

Questionnaires were used to obtain information on parents' highest level of education completed, age, antenatal psychiatric symptoms, child ethnicity, history of smoking in pregnancy, and household income. Maternal smoking was assessed using questionnaires at enrolment, in mid-pregnancy, and in late pregnancy. Family income was defined by the total net monthly income of the household. The Brief Symptom Inventory was used to access parents' antenatal psychiatric symptoms.[22] Child ethnicity was based on the parents' countries of birth. Fetal ultrasound examinations were used to establish gestational age at birth. Midwife and hospital registries provided information on infant's date of birth, birth weight, and sex. Parents reported on their children's autistic-like behaviors at age six years using a short form of the Social Responsiveness Scale.[21] Parents also rated their children's executive functioning at ages 4 years in domains of shifting and planning using the Behavior Rating Inventory of Executive Function-Preschool version.[23]

Statistical analyses

Differences in characteristics between children with and without data on problem behavior were compared by chi-square tests and independent samples *t-tests*. We observed that mothers of infants with missing data were lower educated and suffered from more antenatal psychological symptoms compared to those included. There were no differences in other characteristics between the two groups. Table 1 provides percentage of missing for covariates.

We used generalized linear mixed models (GLMM) to estimate the standardized coefficients (beta) and 95% confidence intervals (CI) of the association between neuro-

motor development and internalizing and externalizing scores up to age ten years. Subsequently we examined the associations with the different CBCL syndrome scales. All models included a child-level random intercept and slope to account for repeated measures of child behavior and to model the child-specific variable effect. These models utilized children's repeated CBCL scores age 1.5 years through age ten years. GLMM are robust to loss to follow-up under the missing at random assumption.[24,25] Testing for missing assumptions[26] confirmed that the missingness in externalizing scores but not internalizing was completely at random. All models were rerun with neuromotor development as a continuous variable.

Models were adjusted for confounders, selected based on the change-in-estimate method or the theoretical framework of the study question. We also adjusted the models for the version of the instrument used. In an additional step, we adjusted the models for a child's autistic-like behaviors to assess whether our results were independent of autistic traits.[27] Interaction terms of neuromotor development with the age of each CBCL assessment, sex, ethnicity, and educational level were tested. Since the likelihood ratio tests did not show significant differences between nested models, except for the model for low muscle tone and age in relation to externalizing problems, the interaction terms were not included in other final models.

We ran mediation models with 99 % bias-corrected bootstrap confidence intervals applying 5,000 bootstrap samples using a SPSS macro.[28] We explored the indirect effects of infant neuromotor development on child's internalizing behavior through shifting and planning (scores square-root transformed).

To handle the missing values on covariates, we used multiple imputations. Imputations were based on information on the infant neuromotor development and all covariates measured. Five independent datasets were generated and pooled estimates for those datasets were calculated. Multiple imputations were performed using SPSS (version 22.0; IBM Statistics). All other analyses were performed using SAS version 9.3 (SAS Institute Inc., Cary, NC).

RESULTS

Participants' characteristics are presented in Table 1. Neuromotor development was assessed at an average age of 12.6 weeks (SD=2 weeks). Table 2 shows the relationship between infant neuromotor development and internalizing scores through age ten years. Non-optimal overall neuromotor development predicted internalizing scores across childhood. Low muscle tone in infancy was associated with higher internalizing scores (mothers' report: adjusted beta=0.12, 95% CI: 0.06, 0.18; fathers' report: adjusted beta=0.13 95% CI: 0.04, 0.22). Supplemental Figure 2 shows associations between low muscle tone and internalizing scores at each assessment (adjusted for covariates and also

additionally for prior observations). There was also an association between senses and other observations in infancy and internalizing scores (Table 2), but not between high muscle tone or reflexes and internalizing scores.

There was no association between non-optimal overall neuromotor development in infancy and externalizing scores. Neither low nor high muscle tone was associated with externalizing scores (Table 3). We depict how infants' low muscle tone relates to internalizing and externalizing scores over time in Figures 1 and 2. When we repeated the analysis with continuous neuromotor variables, results remained essentially unchanged (Supplemental Table 1).

When the interaction of motor development with age at CBCL assessment was tested, we observed that for externalizing scores, the likelihood ratio test showed model fit improvement compared to the model with no interaction term. There was no constant overall association between infant low muscle tone and externalizing scores through age ten; at age 3 years, children with non-optimal neuromotor development had higher externalizing scores than children with an optimal development (Figure 2).

We examined the association between infant neuromotor development and internalizing subscales (mother and father report) and found that children with non-optimal neuromotor development were more likely to have higher withdrawn, emotionally reactive, and anxious/depressed scores (latter with father's report only) (Supplemental Tables 2 and 3). In particular, a low muscle tone was consistently associated with higher withdrawn scores (mothers' report: adjusted beta=0.09, 95%CI: 0.05, 0.14; fathers' report: adjusted beta=0.09, 95%CI: 0.03, 0.16). No association was observed with high muscle tone.

Using a bootstrapping technique, we observed a significant indirect effect of low muscle tone on internalizing and, in particular, withdrawn problems explained by shifting problems (adjusted beta: 0.05, 95%CI for internalizing: 0.00, 0.10; adjusted beta: 0.02, 95%CI: 0.01, 0.15 for withdrawn). We did not observe any mediation by planning.

In all analyses, adjustment for autistic traits did not change the results. With exclusion of 41 children with physical impairments, the results remained unchanged. There was no sex difference in the relation between infant neuromotor development and child behavior (data not shown).

DISCUSSION

We found that non-optimal infant neuromotor development and, in particular, low muscle tone and non-optimal senses were consistently associated with internalizing scores, as repeatedly reported by mothers and fathers through age ten years. This association was mainly accounted for by withdrawn problems and was partly mediated by problems in the shifting domain. We observed no association between non-optimal neuromotor development and externalizing scores.

Common genetic and environmental factors might underlie both non-optimal neurodevelopment and child problem behavior. Subtle genetic variations, represented by single nucleotide polymorphisms or copy number variation, have been associated with subtle abnormalities of brain development.[29] Recent genome wide meta-analysis showed that internalizing problems are heritable and moderately genetically stable from childhood to adulthood.[30] At the same time, teratogens may cause brain abnormalities during prenatal development,[31] which in turn lead to neurodevelopmental problems during the course of life. Examples of these adverse influences are nicotine and alcohol exposure during gestation[32,33] viral infections,[34,35] as well as maternal stress, nutrition, and age.[36,37] In our analysis, we controlled for several environmental factors. We observed that gestational age and birth weight as indicators of insults during pregnancy did not substantially affect the associations under study. Likewise parental psychiatric symptoms, possible indicators of stress, did not explain our observations.[38,39] With adjustment, the relationship between early neuromotor development and problem behavior was attenuated for parental psychopathology, but remained. Postnatal factors, e.g., a child's physical health, may also underlie the association between infant neuromotor development and internalizing problems in children.[40,41] Motor skills are at the core of infants' and children's everyday actions and interactions consequently affect perceptual, cognitive, and social development.[42] Therefore, motor skills may initiate a cascade of events influencing subsequent development. Since Piaget's original observations that infants own sensorimotor experiences are critical for their learning about the environment, several studies have reported evidence for relations between motor skills and development in seemingly unrelated domains –such as object perception, face processing, and language skills.[43] For example, early experiences of successful reaching at 3 months have been found to be associated with infants' attention to faces over objects.[44] Similarly, in another study, the onset of sitting independently at 3–5 months predicted language development at 10 and 14 months.[45] Low muscle tone affects how infants move and develop and may mean that the infants achieve the major developmental milestones late. These infants get upset when confronting new situations on motor tasks and therefore spend less time exploring objects and different ways to do things. This cautious/fearful infant behavior style may have long-term consequences for communications, and emotional and cognitive development. As a consequence, infants can become more reactive.

We showed an association between low muscle tone and internalizing and, in particular, withdrawn problems, which was independent of autistic traits. This finding is compatible with Touwen's theory that describes infants with suboptimal neuromotor development, i.e. mild neurological signs, as typically displaying "clumsy behavior" that worsens between 4 and 9 years.[46] Infants with low muscle tone might have difficulties in initiating movement, interactions with the environment, and therefore show symptoms of withdrawal behavior during toddlerhood and school age. Our results suggest that low muscle tone in infancy might be an independent precursor of withdrawal behavior in these

children. It is unclear if children withdraw because they lack the drive for social interaction, or because of fear and anxiety. Whatever the origins of minor and mild neurological signs, their presence play a role in the development of behavioral and learning problems, most likely in combination with other factors.

To our knowledge, this study is the first to investigate whether executive functioning underlies the path from low muscle tone to internalizing problems. We found that a higher-order cognitive process such as shifting is an important factor in children's vulnerability to psychopathology. Infants with low muscle tone face more problems when adapting to new circumstances during childhood. These more "rigid" children are at a higher risk of developing internalizing and in particular withdrawn behavior.[47]

Other than at age 3 years, we did not find an association between infant neuromotor development and externalizing scores. Externalizing problems, like aggression, are highly prevalent in young children[48] and probably often reflect a normal developmental stage of children as evident by the decline in externalizing symptoms seen in both the children with optimal and non-optimal motor development. Developing a sense of autonomy and determination to become independent of caregivers typically involve conflicts with parents and other caregivers. This could explain why in young children, scores in the borderline range of externalizing behavior are less likely to have origins in deviant neuromotor development. Twin studies have shown that while externalizing behaviors have unique genetic and a shared environmental basis, internalizing behaviors are associated with unique genetic influences only.[49] Our findings are compatible with the notion that externalizing behaviors are sensitive to environmental influence as indicated by an early non-normative development in children with more hypotonus at baseline, although in theory, such an age-limited difference could also be of genetic origin.

This study has several strengths including a large sample, considering many potential confounders, and objective assessments of neuromotor development, independently of the mother. This is important, as relying on parents' report for both neurodevelopment and the child's behavior may introduce shared method bias.[14] Also, we used mother's and father's reports of child behavior, to test consistency and obtain more accurate description of children's problems.[50] But we faced limitations. First, we experienced loss to follow up. However, we used GLMM that are robust to loss to follow-up if the assumption holds that missing outcome happened at random. Second, we had only parental rating data on child behavior. Parents who are aware of their infant's delay in neuromotor development could have been more attentive to their child's problem behavior. In addition, parents were blinded to delayed neuromotor development and therefore, their report was independent of neuromotor development in infancy. We considered many confounders; however, we cannot rule out residual confounding. We performed several numbers of tests. Therefore, chance finding should be considered when interpreting data. Also, the observed betas indicate small effects as expected in the general population.

CONCLUSIONS

In a prospective population-based study, we found that infants with non-optimal neuromotor development, and in particular low muscle tone, have higher internalizing scores during childhood. Our findings suggest that internalizing problems in childhood might have origins in early neurodevelopment. Future studies are needed to examine whether evaluation of early neuromotor development might help identifying vulnerability to internalizing symptoms and can be used for targeted interventions in young children. For example, scaffolded reaching experiences have been shown to improve developmental parameters in 3 months old infants at risk for motor delay.[51]

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Table 1. Participants' characteristics (n=3,474)

Maternal characteristics	
Age at enrolment, year	30.9 (5.0)
Missing data	0
Education, n (%)	
Primary	630 (18.1)
Secondary	945 (27.2)
High	1674 (48.2)
Missing data	*225 (6.5)*
Antenatal psychiatric symptoms	0.15 (0.06, 0.31)
Missing data	
Smoking during pregnancy, n (%)	672 (20.8)
Missing data	250 (7.2)
Household income, n (%)	
Poor	168 (4.8)
Low	389 (11.2)
Modal and above	2098 (60.4)
Missing data	*819 (23.6)*
Partner characteristics	
Age at enrolment, year	33.5 (5.4)
Missing data	977
Education %	
Primary	395 (11.4)
Secondary	594 (17.1)
High	1239 (35.7)
Missing data	*1246 (35.9)*
Child characteristics	
Age at neuromotor assessment, weeks	12.6 (2.0)
Missing data	0
Sex, boy%	1702 (49.0)
Missing data	0
Ethnic background, n (%)	
Dutch	1858 (53.5)
Other Western	388 (11.2)
Non-Western	1167 (33.6)
Missing data	61 (1.8)
Gestational age at birth, week	39.9 (1.7)
Missing data	5
Instrument (9-15 w), %	2802 (80.7)
Instrument (15-20 w), %	672 (19.3)
Missing data	0

Numbers are mean (SD) for variables with normal distribution, median (quartile range) for not-normally distributed variables, and percentages for categorical variables.

Table 2. Infant neuromotor development and internalizing scores across childhood within the Generation R Study

	Internalizing Scores					
	Mother report a (1.5, 3, 6 and 10 years)			Father report b (3 and 10 years)		
	N	β (95% CI)	*p*	N	β (95% CI)	*p*
Non-optimal neuromotor development						
Overall Neuromotor Development						
Unadjusted	3402	0.13 (0.07, 0.20)	<0.001	3023	0.11 (0.03, 0.19)	0.009
Adjusted	3402	0.08 (0.03, 0.14)	0.005	3023	0.10 (0.02, 0.18)	0.022
Muscle Tone						
Unadjusted	3321	0.10 (0.04, 0.17)	0.001	2954	0.09 (0.01, 0.17)	0.031
Adjusted	3321	0.07 (0.01, 0.13)	0.018	2954	0.09 (0.00, 0.16)	0.041
Low Tone Symptoms						
Unadjusted	3327	0.14 (0.07, 0.21)	<0.001	2959	0.14 (0.05, 0.22)	0.003
Adjusted	3327	0.12 (0.06, 0.18)	<0.001	2959	0.13 (0.04, 0.22)	0.004
High Tone Symptoms						
Unadjusted	3441	0.12 (0.04, 0.20)	0.005	3056	0.09 (-0.01, 0.20)	0.091
Adjusted	3441	0.06 (-0.02, 0.13)	0.147	3056	0.07 (-0.04, 0.18)	0.211
Responses						
Unadjusted	3267	0.04 (-0.02, 0.10)	0.237	2901	0.05 (-0.03, 0.14)	0.195
Adjusted	3267	0.00 (-0.05, 0.06)	0.905	2901	0.05 (-0.03, 0.13)	0.258
Senses						
Unadjusted senses	3452	0.12 (0.06, 0.18)	<0.001	3068	0.13 (0.04, 0.22)	0.003
Adjusted senses	3452	0.07 (0.01, 0.13)	0.029	3068	0.10 (0.02, 0.19)	0.019

Note. The association between non-optimal neuromotor development and internalizing through age ten years were estimated with generalized linear mixed models to account for repeatedly assessed outcome. All models adjusted for a child's age, sex and ethnicity, gestational age at birth, antenatal maternal psychiatric symptoms, household income, maternal history of smoking in pregnancy, and the version of neuromotor instrument.
a in addition, all models are adjusted for age mother and education level mother
b in addition, all models are adjusted for age father and educational level father

Table 3. Infant neuromotor development and externalizing scores across childhood within Generation R Study

	Externalizing Scores					
	Mother report a (1.5, 3, 6 and 10 years)			Father report b (3 and 10 years)		
	N	β (95% CI)	*p*	N	β (95% CI)	*p*
Non-optimal neuromotor development						
Overall Neuromotor Development						
Unadjusted	3402	0.03 (-0.03, 0.09)	0.320	3023	0.04 (-0.04, 0.11)	0.353
Adjusted	3402	-0.01 (-0.07, 0.04)	0.717	3023	0.03 (-0.05, 0.10)	0.520
Muscle Tone						
Unadjusted	3321	0.01 (-0.05, 0.07)	0.752	2954	0.01 (-0.08, 0.10)	0.704
Adjusted	3321	-0.01 (-0.07, 0.04)	0.679	2954	0.02 (-0.06, 0.09)	0.737
Low Tone Symptoms*						
Unadjusted	3327	-[c]	-	2959	0.04 (-0.04, 0.12)	0.386
Adjusted	3327	-[c]	-	2959	0.03 (-0.05, 0.11)	0.446
High Tone Symptoms						
Unadjusted	3441	0.08 (0.01, 0.16)	0.030	3056	0.02 (-0.10, 0.15)	0.764
Adjusted	3441	0.02 (-0.05, 0.09)	0.551	3056	-0.02 (-0.12, 0.09)	0.765
Responses						
Unadjusted	3267	0.01 (-0.05, 0.07)	0.668	2901	0.04 (-0.03, 0.12)	0.276
Adjusted	3267	-0.01 (-0.06, 0.05)	0.793	2901	0.04 (-0.03, 0.11)	0.299
Senses						
Unadjusted senses	3452	0.03 (-0.02, 0.09)	0.251	3068	0.03 (-0.05, 0.11)	0.445
Adjusted senses	3452	0.00 (-0.06, 0.05)	0.864	3068	0.01 (-0.07, 0.08)	0.807

Note. The association between non-optimal neuromotor development and externalizing through age ten years were estimated with generalized linear mixed models to account for repeatedly assessed outcome. All models adjusted for a child's age, sex and ethnicity, gestational age at birth, antenatal maternal psychiatric symptoms, household income, maternal history of smoking in pregnancy, and the version of neuromotor instrument

[a] in addition, all models are adjusted for age mother and education level mother

[b] in addition, all models are adjusted for age father and educational level father

[c] because of interaction effect of low muscle tone with age, results cannot be presented here. See Figure 2 for the beta's per time point and confidence intervals

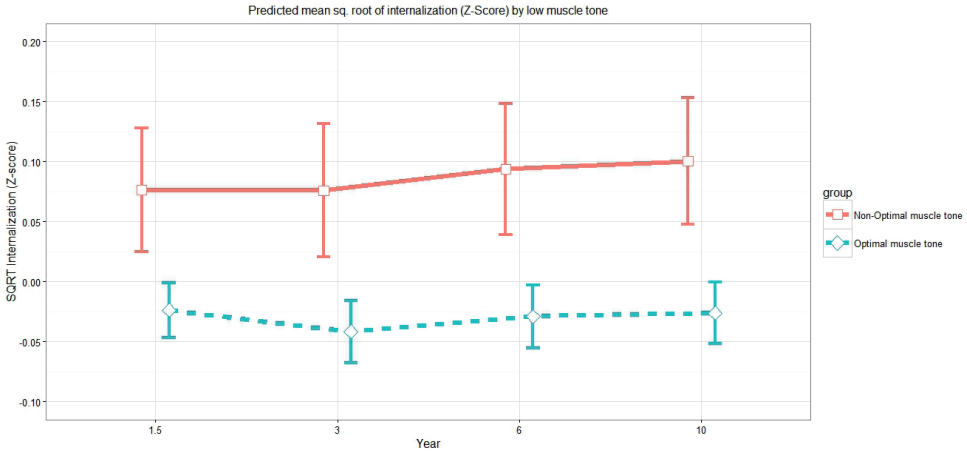


Figure 1. Internalizing Z-scores in children with optimal and non-optimal neuromotor development in infancy (fitted values from the linear mixed model) with bootstrap confidence intervals from 1000 runs

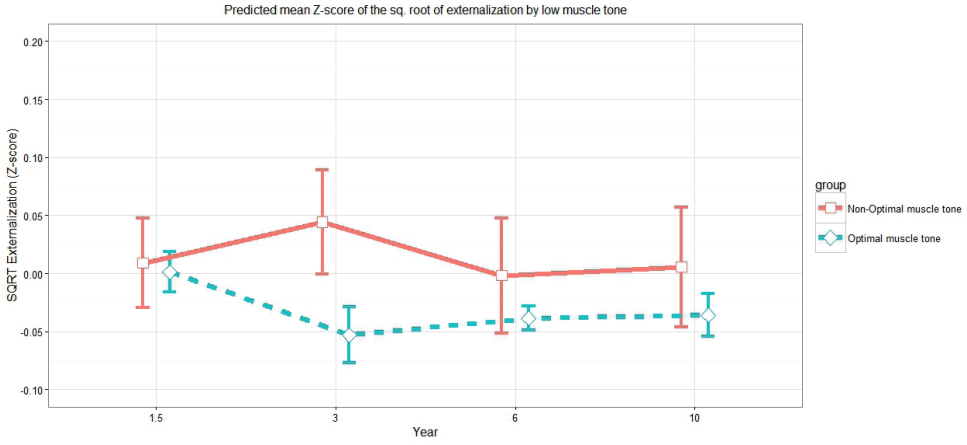
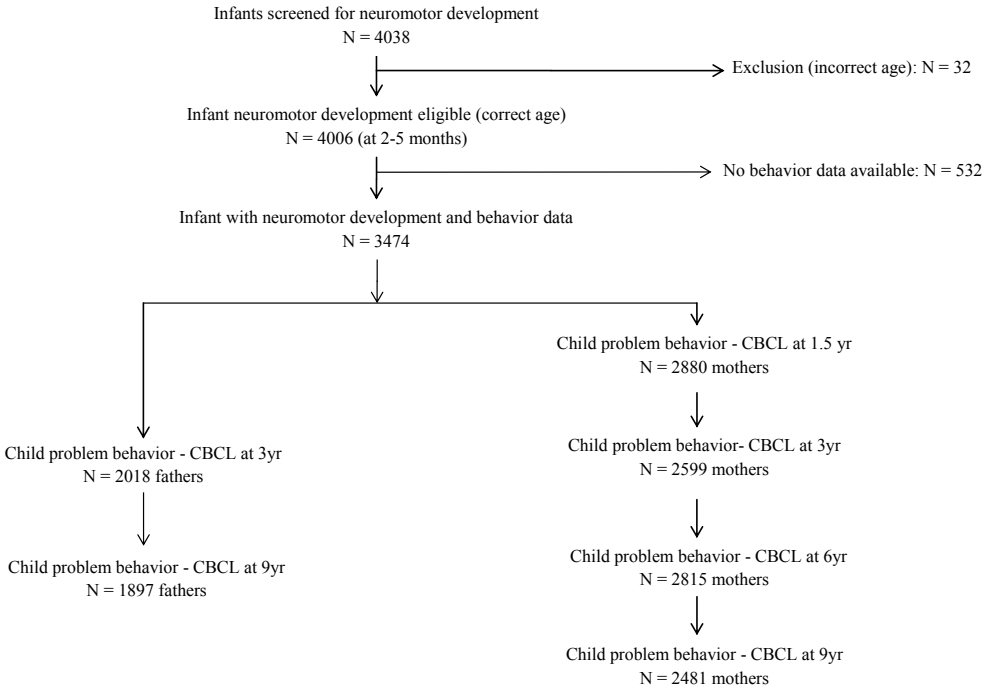


Figure 2. Externalizing Z-scores in children with optimal and non-optimal neuromotor development in infancy (fitted values from the linear mixed model) with bootstrap confidence intervals from 1000 runs



Supplement Figure 1

Supplemental Table 1. Infant neuromotor development and internalizing scores across childhood within Generation R Study

	Internalizing Scores					
	Mother report [a] (1.5, 3, 6 and 10 years)			Father report [b] (3 and 10 years)		
	N	β (95% CI)	*p*	N	β (95% CI)	*p*
Non-optimal neuromotor development						
Overall Neuromotor Development						
Unadjusted	3402	0.02 (0.01, 0.03)	<0.001	3035	0.02 (0.01, 0.03)	<0.001
Adjusted	3402	0.01 (0.00, 0.02)	0.013	3035	0.02 (0.01, 0.03)	0.001
Muscle Tone						
Unadjusted	3321	0.02 (0.01, 0.03)	0.001	2970	0.02 (0.01, 0.03)	0.001
Adjusted	3321	0.01 (0.00, 0.02)	0.012	2970	0.02 (0.01, 0.03)	0.002
Low Tone Symptoms						
Unadjusted	3327	0.02 (0.01, 0.03)	0.007	2959	0.03 (0.01, 0.04)	0.002
Adjusted	3327	0.02 (0.00, 0.03)	0.014	2959	0.03 (0.01, 0.04)	0.003
High Tone Symptoms						
Unadjusted	3441	0.03 (0.01, 0.05)	0.007	3056	0.03 (0.00, 0.06)	0.021
Adjusted	3441	0.02 (-0.03, 0.08)	0.060	3056	0.03 (0.00, 0.05)	0.033
Responses						
Unadjusted	3267	0.02 (-0.02, 0.06)	0.298	2901	0.04 (-0.01, 0.10)	0.130
Adjusted	3267	0.00 (-0.04, 0.04)	0.991	2901	0.04 (-0.01, 0.10)	0.142
Senses						
Unadjusted senses	3452	0.09 (0.05, 0.13)	<0.001	3068	0.06 (0.01, 0.11)	0.030
Adjusted senses	3452	0.04 (0.00, 0.08)	0.042	3068	0.04 (-0.02, 0.09)	0.163

Note. The association between infant neuromotor development and internalizing through age ten years were estimated with generalized linear mixed models to account for repeatedly assessed outcome. All models adjusted for a child's age, sex and ethnicity, gestational age at birth, antenatal maternal psychiatric symptoms, household income, maternal history of smoking in pregnancy, and the version of neuromotor instrument.

a in addition, all models are adjusted for age mother and education level mother

b in addition, all models are adjusted for age father and educational level father

Supplemental Table 2. Associations between infant neuromotor development and mother report of child problem behavior

	Internalizing scales (1.5, 3, 6 and 10 years)								
		Emotionally Reactive*		Anxious /Depressed		Somatic Complaints		Withdrawn	
	N	β (95% CI)	*p*	β (95% CI)	*p*	B (95% CI)	*p*	β (95% CI)	*p*
Non-optimal neuromotor development									
Overall neuromotor development	3402	**0.07 (0.02, 0.12)**	**.009**	0.03 (-0.02, 0.07)	.189	0.02 (-0.03, 0.06)	.510	**0.06 (0.02, 0.10)**	**.003**
Tone									
	3321	**0.06 (0.01, 0.11)**	**.024**	0.02 (-0.03, 0.06)	.434	0.01 (-0.03, 0.05)	.724	**0.05 (0.01, 0.09)**	**.024**
Low tone symptoms	3327	**0.09 (0.03, 0.14)**	**.001**	0.04 (-0.01, 0.09)	.117	0.04 (-0.01, 0.08)	.094	**0.09 (0.05, 0.14)**	**<0.001**
High tone symptoms	3441	0.04 (-0.03, 0.11)	.215	0.04 (-0.03-0.10)	.257	0.00 (-0.01, 0.00)	.870	0.03 (-0.02, 0.09)	.275
Responses	3267	-0.01 (-0.07, 0.04)	.568	0.01 (-0.03, 0.05)	.644	-0.01 (-0.05,0.03)	.543	0.00 (-0.04, 0.04)	.810

Note. The association between non-optimal neuromotor development and mother report of internalizing through age ten years were estimated with generalized linear mixed models to account for repeatedly assessed outcome.. All models adjusted for a child's age, sex and ethnicity, gestational age at birth, antenatal maternal psychiatric symptoms, household income, maternal history of smoking in pregnancy, and the version of neuromotor instrument

*Emotional Reactivity measured at child age 1.5, 3 and 6 years.

Supplemental Table 3. Associations between infant neuromotor development and father report of child problem behavior

	Internalizing scales (1.5, 3, 6 and 10 years)								
		Emotionally Reactive*		Anxious /Depressed		Somatic Complaints		Withdrawn	
	N	β (95% CI)	*p*	β (95% CI)	*p*	B (95% CI)	*p*	β (95% CI)	*p*
Non-optimal neuromotor development									
Overall neuromotor development	3023	0.01 (-0.08, 0.09)	0.875	**0.07 (0.01, 0.14)**	**0.030**	0.05 (-0.02, 0.11)	0.138	**0.05 (-0.01, 0.11)**	**0.11**
Tone	2954	-0.01 (-0.10, 0.07)	0.746	**0.07 (0.00, 0.13)**	**0.049**	0.03 (-0.04, 0.09)	0.392	**0.06 (0.00, 0.11)**	**0.069**
Low tone symptoms	2959	0.01 (-0.08, 0.09)	0.886	**0.13 (0.06, 0.20)**	**0.000**	**0.07 (0.00, 0.14)**	**0.041**	**0.09 (0.03, 0.16)**	**0.003**
High tone symptoms	3056	0.01 (-0.11, 0.12)	0.880	0.05 (-0.04, 0.14)	0.253	0.04 (-0.04, 0.13)	0.335	0.04 (-0.04, 0.12)	0.295
Responses	2901	0.07 (-0.01, 0.15)	0.096	0.06 (0.00, 0.13)	0.059	-0.02 (-0.09,0.04)	0.463	0.00 (-0.06, 0.05)	0.847

Note. The association between non-optimal neuromotor development and father report of internalizing through age ten years were estimated with generalized linear mixed models to account for repeatedly assessed outcome. All models adjusted for a child's age, sex and ethnicity, gestational age at birth, antenatal maternal psychiatric symptoms, household income, maternal history of smoking in pregnancy, and the version of neuromotor instrument.

* CBCL scales measured at child age 3 years and 5 years. Emotional Reactivity measured at age 3 years.

Painting by Bakir Rokvic (age 6 years)

3

GENETIC SUSCEPTIBILITY FOR PSYCHIATRIC DISORDERS AND NON-OPTIMAL INFANT NEUROMOTOR DEVELOPMENT

3.1

The Association of Genetic Risk for Schizophrenia and Bipolar Disorder with Infant Neuromotor Development

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INTRODUCTION

Schizophrenia and bipolar disorder (BD) are highly heritable disorders with similarities in clinical symptoms and onset typically after puberty.[1] While research showed that impaired motor coordination can be an early precursor of schizophrenia,[2] only few studies have examined childhood development preceding BD or mania. Murray et al. proposed a developmental model for similarities and dissimilarities between schizophrenia and BD.[1] It remains unknown if dissimilarities exist in early infancy and if they covary with genetic liability for these disorders. Utilizing polygenic risk scores (PGRS), we explored if genetic risk for schizophrenia and BD are associated with neuromotor development in infancy.

METHODS

From a population-based cohort, Generation R, we included a large pediatric sample of European ancestry (defined by genetic principal components) with genotype data (n=2,830).[3] Of these, 1,174 infants (41%) underwent neuromotor examination at 2.9 months (range: 2 to 5 months). PGRS were calculated for schizophrenia and BD using genome-wide association study (GWAS) summary statistics, and were standardized to a mean of 0 and SD 1 for interpretability. Additive PGRS were calculated for each individual by multiplying the allele count by the allele log of the odds ratio (OR). SNPs were clumped prior to calculation of the score.[4] Full details have been described elsewhere.[4] Trained research nurses assessed neuromotor development during a home visit using an adapted version of Touwen's Neurodevelopmental Examination (Table 1).[5] Non-optimal neuromotor development was defined as an age-corrected score in the highest tertile. We performed logistic regression adjusted for gender and population structure by including the first four genetic principal components. Informed consent was obtained from participants. Erasmus Medical Center Medical Ethics Committee approved the study.

RESULTS

A higher PGRS for schizophrenia was associated with non-optimal overall infant neuromotor development at age 2-5 months (p-value thresholds (P_T) <0.05 OR=1.15, 95%CI: 1.01-1.30, p=0.031). The results remained essentially unchanged across the range of P_T (0.05 to 0.0005). A PGRS for BD was not consistently associated with non-optimal overall infant neuromotor development (P_T<0.05 OR=0.95, 95%CI: 0.84-1.08, p=0.443) (Table 2).

DISCUSSION

This report showed that the PGRS for schizophrenia are associated with non-optimal overall infant neuromotor development, whereas no consistent associations were observed for BD PGRS. Similarly, Burton et al. found an association between motor development at age seven with familiar risk for schizophrenia, but not with familiar risk for BD.[6] To date, the earliest manifestation of genetic predisposition for schizophrenia was reported by Jansen et al in three-year-old children.[4] Research suggests that impaired neuromotor development precedes schizophrenia onset, although most children with impaired neuromotor development do not develop schizophrenia.[2] In contrast, children who later met criteria for mania exhibited better motor performance during childhood than controls.[1] Our results highlight that the genetic predisposition for schizophrenia covaries with motor deficits observable during infancy in the general population. Given that the prevalence of schizophrenia is low, these early features represent indices of liability rather than a precursor of the disorder. Genetic pleiotropy or early environmental factors, could also explain the observed association.[1] Selective non-response to neuromotor assessment could bias the analysis. However, we did not detect any difference between infants with and without neuromotor assessment (data not shown). The power of the BD GWAS might have been insufficient to detect associations between BD PGRS and neuromotor development. Despite limitations, this study has several strengths including an objective and prospectively assessed measure of neuromotor development in a large homogenous sample of infants. This is the first evidence showing that genetic liability for schizophrenia may covary with altered neuromotor development in infancy. Future research will show whether early neuromotor development can support early screening of susceptible groups possibly defined by genetic risk.

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Table 1. Items neuromotor developmental assessment

Subscale	Position	Item description	Answering categories		
			Optimal	**Non-optimal**	**Non-optimal**
Tone	Supine	Resting posture	Semi-flexed legs; slight abduction at the hips	Legs flat on the surface	Legs stretched
		Adductor angle	> 80° - < 140°	> 140°	< 80°
		Popliteal angle	90°-130°	130°-180°	< 90°
		Ankle angle	> 20° - < 90°	< 20°	> 90°
		Head preference	No	Yes	
		Opening & closing hands	Yes	Sometimes closed	Always closed
		Alternating leg movements	Yes	Decreased	Absent
		Grasps with one hand	Yes	Decreased	Absent
		Hyperextension	No	Sometimes	Yes
		Dyskinesia	No	Sometimes	Yes
	Supine-to- sit	Traction response	Arms moderately flexed	Arms fully extended, no resistance	Strong resistance, flexion elbows, legs extended
		Traction response-head control	Active lift of head	Head lag	Exaggerated
	Horizontal	Ventral Tone	Normal tone	Low tone	Back and limbs stretched
	Vertical	Head	Normal tone	Low tone	High tone
		Shoulders	Normal tone	Low tone	High tone
		Trunk	Normal tone	Low tone	High tone
		Legs	Normal tone	Low tone	High tone
	Prone	Pulls arms up	Yes	No	
		Turns head	Yes	No	
		Lifts head	Yes	No	Overstretched
	Sitting	Needs support	Yes	No	
		Head control	Yes	No	
		Shoulder retraction	No	Yes	
		Shape of the back	Round	Straight	Scoliosis
Responses	Supine	Asymmetrical Tonic Neck Reflex	Weak	Yes	Exaggerated
		Babinski	Yes	Exaggerated	Spontaneous
	Prone	Bauer	Yes / weak	Exaggerated	
	Vertical	Stepping movements	No	Yes	Exaggerated
		Moro intensity	Yes / weak	Exaggerated	
		Moro opening hands	Yes	No	
Other	Supine	Strabismus	No	Sometimes	Yes
		Fixation eyes	Yes	Decreased	No
		Following movements eyes	Smooth	Decreased	No
		Hearing	Yes	Moderate	No
		Sweating	No	Yes	
		Startles	No	Sometimes	Yes

Table 2. Associations between schizophrenia and BD PGRSs with infant non-optimal neuro-motor development, corrected for age (N=1,174)

		Non-optimal overall neuro-motor development	
Schizophrenia	OR (95% CI)	*p*	*Number of SNPs*
$P_T < 0.0005$	1.14 (1.00; 1.29)	**0.046**	**2,965**
$P_T < 0.001$	1.14 (1.00; 1.29)	**0.043**	**4,148**
$P_T < 0.005$	1.14 (1.01; 1.30)	**0.039**	**9,547**
$P_T < 0.01$	1.14 (1.01; 1.30)	**0.034**	**13,916**
$P_T < 0.05$	1.15 (1.02; 1.30)	**0.029**	**34,947**
$P_T < 0.1$	1.12 (0.99; 1.27)	0.081	52,256
$P_T < 0.5$	1.12 (0.99; 1.26)	0.084	126,674
Bipolar Disorder			
$P_T < 0.0005$	0.87 (0.77; 0.98)	**0.023**	**525**
$P_T < 0.001$	0.92 (0.82; 1.04)	0.203	915
$P_T < 0.005$	0.99 (0.88; 1.11)	0.853	2,946
$P_T < 0.01$	0.95 (0.84; 1.07)	0.403	4,992
$P_T < 0.05$	0.95 (0.84; 1.08)	0.443	16,461
$P_T < 0.1$	0.91 (0.81; 1.03)	0.138	27,366
$P_T < 0.5$	0.92 (0.81; 1.03)	0.151	79,569

$P_{T:\ p\text{-value threshold}}$
Note: The models are adjusted for gender and four genetic principal components

3.2

Polygenic risk scores for developmental disorders, neuromotor functioning during infancy, and autistic traits in childhood

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ABSTRACT

Background: Impaired neuromotor development is often one of the earliest observations in children with autism spectrum disorder (ASD) and non-optimal infant neuromotor development predicts autistic traits in the general population. Common genetic variations may underlie this association. We investigated if a genetic predisposition to developmental disorders was associated with non-optimal neuromotor development during infancy and if this association contributed to autistic traits in children with high genetic risk.

Methods: In a population-based cohort in the Netherlands (2002–2006), we calculated polygenic risk scores for ASD (PRS_{ASD}) and for attention-deficit/hyperactivity disorder (PRS_{ADHD}) using genome-wide association study summary statistics. In 1921 children with genetic data, parents rated autistic traits at age 6 years. Among them, 1174 (61.1%) children underwent neuromotor examinations (tone, responses, senses and other observations) during infancy (age 9-20 weeks). Higher scores for both measures indicated more problems.

Results: Higher PRS_{ASD} were associated with higher scores in overall infant neuromotor development, in particular low muscle tone. Higher PRS_{ADHD} were associated with higher scores on senses. PRS_{ASD} and PRS_{ADHD} were both associated with autistic traits at age 6 years. Neuromotor development mediated the association between PRS_{ASD} and autistic traits. We observed an indirect effect of PRS_{ADHD} on autistic traits through senses in boys only.

Conclusions: This is the first evidence showing that the genetic liabilities for ASD and ADHD covary with neuromotor development during infancy. Furthermore, a genetic predisposition to ASD or ADHD might partly explain the association between non-optimal neuromotor development during infancy and autistic traits in childhood.

INTRODUCTION

Autism spectrum disorder (ASD) are lifelong developmental disorders characterized by social interaction impairment, communication deficits, and repetitive behavior. Evidence confirms that autistic traits, or behaviors that mimics ASD symptomatology are continuously distributed in the general population [1]. ASD has a strong genetic basis and shares common genetic risks with other neurodevelopmental disorders of childhood, e.g., attention-deficit/hyperactivity disorder (ADHD) [2, 3]. Similar to ASD, autistic traits show moderate to high heritability in the general population as well as in the clinical samples [2, 4].

Behavioral signs of ASD typically emerge mostly from second year of life and few symptoms that occur during the first year of life mainly belong to the sensorimotor domain [5]. Non-optimal neuromotor development is often one of the earliest identifiable clinical observations in children with ASD [6]. The motor manifestations encompass a wide range of impairments including floppiness, delays in gross motor milestones such as sitting, and poor motor coordination and control, for example difficulties in grasping objects. Many studies have reported co-occurrence of motor impairments with autistic traits and autism [7, 8]; follow-up studies have shown early sensorimotor manifestations in the prodromal stages of the neurodevelopmental trajectory potentially leading to autism diagnosis in high risk infants [9, 10]. We have previously shown that neuromotor development measured during infancy is often an early predictor of autistic traits in the general population [11]. While impaired neuromotor development can be a symptom of an underlying brain abnormalities related to autistic symptoms, inflexible sensorimotor development during infancy is also posited to precede and contribute to an abnormal developmental trajectory of the brain and may subsequently lead to autism [12, 13] Studies have suggested that infant sensorimotor variations are associated with increased risk of autistic symptoms; with recent evidence from follow up and twin studies suggesting direction from the former to the later [12, 14]. However, the possible genetic contribution to this association is unknown. Common genetic risk or antecedent environmental factors might explain the observed association between non-optimal neuromotor development and autistic traits.

Accordingly, the aim of this study was to investigate the associations between genetic predisposition to neurodevelopmental disorders and neuromotor functioning during infancy and explore if the genetic susceptibility has a role in observed association between neuromotor functioning and autistic traits. We examined the association of polygenic risk scores for ASD (PRS_{ASD}) and for ADHD (PRS_{ADHD}) with infant neuromotor development, because it is less clear whether minor neuromotor deficits are specifically related to genetic predisposition to ASD or constitute a broader reflection of genetic susceptibility to neurodevelopmental disorders, in general [15]. We further investigated whether the association between PRS and non-optimal neuromotor development

during infancy contributed to the development of autistic traits in children with high genetic risks.

METHODS AND MATERIALS

This study was embedded in the Generation R Study, a population-based birth cohort in Rotterdam, the Netherlands, which recruited more than 9000 pregnant women with a delivery date from April 2002 until January 2006 to study early determinants of development and health in childhood and adolescence [16]. From this birth cohort, we included a pediatric sample of European ancestry with available genotype data (n=2830) [17]. Between 9 to 20 weeks of age, 1174 infants (41% of 2830 with genotyping data) underwent a neuromotor examination during home visits. When the children were 6 years old, questionnaires were mailed to caregivers for assessments of autistic traits (n=1921 children, 68% of 2830).

We compared child and maternal characteristics of the children included in this analysis (n=1921) with those excluded because of missing data on autistic traits (n=909). Children included in the analyses were more likely to have higher nonverbal IQ scores than children excluded (mean IQ score 103.1 vs. 96.4). Responding mothers also had lower scores of psychopathology symptoms during pregnancy compared to nonresponding mothers (mean scores based on the Brief Symptoms Inventory: 0.24 vs. 0.37). However, two groups of children did not differ on neuromotor assessments during infancy.

The study protocol was approved by the Medical Ethics Committee of the Erasmus Medical Centre. Written informed consent was obtained from the legal representatives of all participants.

Genotyping and imputation

DNA samples were collected from the cord blood or venipuncture at age 6 years on Illumina 610K and 660K single nucleotide polymorphism (SNP) arrays depending on collection time (Illumina, San Diego, CA, USA). Others have previously reported further details on genotype calling procedures in Generation R [18]. Quality control of the genotype and individual level data was conducted using PLINK (v1.9) [19]. Genotype data quality control included filtering variants for minor allele frequency (MAF< 0.01), Hardy-Weinberg disequilibrium (HWE, $P<1x10^{-5}$), and missing rate (> 0.05). Individuals were excluded according to relatedness, genetic and phenotypic sex mismatch, and genotype quality (<5% missing).

Imputation was carried out to the 1000 Genomes (phase I version 3) using prephasing in SHAPEIT [20] and imputation in IMPUTE v2 [21]. Post-imputation filtering on imputation quality (INFO score <0.9) resulted in a total number of 6,561,671 variants. To correct for population stratification, we calculated four genetic principal components using principal component analysis in EIGENSOFT and included them in regression models [22, 23].

Polygenic scoring

We used imputed genotype data that passed quality control to compute PRS based on genome wide association studies (GWAS) of ASD and ADHD performed by the Psychiatric Genomics Consortium. PRS_{ASD} and PRS_{ADHD} were created using PRSice [24]. This software calculates individual PRS by summing up all the single nucleotide polymorphism (SNP) alleles associated with the trait carried by the participants weighted by the SNP allele effect size estimated in a previous GWAS. Polygenic scoring was performed in clumped variants according to linkage disequilibrium using r^2<0.1 cutoff within a 250-kb window. We calculated PRS for each trait based on six different *P*-value thresholds (P_T) with P_T <0.001, P_T <0.01, P_T <0.05, P_T <0.1, P_T <0.5, and P_T <1. Supplementary Table 1 presents the number of variants included in the PRS for each P_T. All PRS were standardized to a mean of 0 and a standard deviation of 1.

Infant neuromotor development

During home visits, trained research assistants carried out full neuromotor assessments for infants aged 9 to 20 weeks using an age-appropriate and modified version of the Touwen's Neurodevelopmental Examination (Supplementary Table 2) [25]. We used two age-adapted versions of the neuromotor instrument, for 9-15 weeks and 15-20 weeks; both versions encompassed assessments of tone, responses, and senses, and other observations (such as spontaneous movements) [26]. Tone was assessed in several positions – supine, horizontal, vertical, prone and sitting – and all tone items, such as adductor angle, were scored as normal, low or high tone. We added items to measure both active and passive muscle tone, as a discrepancy between active and passive tone serves as an early sign of deviant motor development. Within the subscale measuring tone, a further distinction was made between low tone and high tone, resulting in two additional subscales for tone: "low muscle tone" and "high muscle tone". Responses were assessed in vertical, supine or prone position and were scored as present, absent or excessive. Senses and other observations comprise strabismus, fixation eyes, following movement eyes, hearing, sweating, and startled reactions [27]. The later items were scored as present, absent or excessive. For each item, we labeled an age-appropriate response as 'optimal'. If the response indicated a delayed development, the item was labeled 'non-optimal'. By summing the raw values of all items, we obtained a total score; for which high values indicated less optimal neuromotor development. All scores were used as continuous variables after square root transformation for normality. The intraclass correlation coefficients for the short-interval test-retest reliability and the interobserver reliability were 0.52 and 0.64 respectively, similar to earlier reports [28].

Autistic traits

When the children were 6 years old, we administered the Social Responsiveness Scale (SRS) to parents to obtain a quantitative measure of child's autistic traits during the past

six months in a naturalistic setting [1]. Due to the length of the original questionnaire, we used a short version of SRS with 18 items. The 18-items questionnaire contained items concerning social cognition, social communication and autistic mannerism. In Generation R, the Cronbach's alpha indicated high inter-item reliability for the SRS (α=0.79). In a sample of 3857 children aged 4-18 years in the South-West of the Netherlands (2010-2012) the correlation between total scores derived from the selected 18 items and the scores derived from the complete test was r=0.95. Each item is rated from "0" (never true) to "3" (almost always true). After summing the items, higher scores indicate more problems. SRS scales were used continuously after square root transformation and standardization.

Covariates

We used medical records completed by community midwives and obstetricians to obtain information on the child's sex. Information on maternal age and educational levels was obtained from self-reported questionnaires. Maternal education was defined by the highest attained educational level and classified as primary (no or only primary education), secondary (lower or intermediate vocational education), and higher education (higher vocational education or university).

Statistical analysis

We performed linear regression analysis to examine whether PRS_{ASD} and PRS_{ADHD} were associated with infant neuromotor development scores (overall, tone, and senses and other observations) and autistic traits at age 6 years. We also reran the models with the domains of senses and other observations using negative binominal regression. This dimension comprises scores on items (e.g., vision) that can also be conceptualized as count data. Models were adjusted for a child's sex and age at neuromotor assessment, the version of the neuromotor instrument, and four principal components of the population structure. In an additional step, we adjusted the models for maternal age and education. Given that previous analyses of PRS_{ASD} and PRS_{ADHD} showed sex differences in the associations with autistic traits, we tested interactions by sex [29]. Effect sizes were reported as standardized beta (β) coefficients.

Next, we ran a mediation model using R (package mediation) with 99 % bias-corrected bootstraps confidence intervals (CIs). We applied 1000 bootstrap samples to identify the indirect effect of PRS on autistic traits through neuromotor development during infancy. We performed a mediation analysis only if linear regression showed associations between the PRS_{ASD} or PRS_{ADHD} and domains of neuromotor development. We estimated the direct effect, indirect effect, and total effect. The DE represents the effect of PRS on autistic traits scores after controlling for infant neuromotor development, and the indirect effect is the estimated effect of PRS operating through infant neuromotor development [30]. The proportion of mediation by infant neuromotor development was calculated as the ratio

of indirect to total effect. Based on *a priori* hypothesis, we also studied if the magnitude of the indirect effect depended on sex by formally testing effect modification [31].

In a sensitivity analysis, we used inverse probability weighting to correct for selective loss to follow-up at age 6 years in the sample with genotyping data. We used information on mother and child characteristics to predict the probability of having SRS data and used the inverse of these probabilities to rerun the models with weights [32].

All analysis were performed using the R statistical software package, version 3.3.1.[33]

RESULTS

Children had an average neuromotor score of 1.67 [standard deviation (SD) = 0.96]; mean age at neuromotor assessment was 12.6 weeks (SD= 20); and 48.7 % of children were girls. Mothers were on average 31.3 years old (SD=4.7) and 55% of them completed higher education.

A higher PRS_{ASD} was positively associated with higher scores in overall neuromotor development during infancy (e.g., with GWAS P_T<0.1, β=0.067, 95%CI: 0.015, 0.120, p=0.01) (Supplementary Table 3, Supplementary Figure 1). There was a relationship between PRS_{ASD} and overall muscle tone and, in particular, low muscle tone (e.g., with GWAS P_T <0.5, β=0.068, 95%CI: 0.015, 0.120, p=0.01). Stratified analysis showed that this association was present in boys only (Table 1, Figure 1). Also, boys with a higher PRS_{ASD} had higher scores in senses and other observations in infancy (e.g., with GWAS P_T <0.1, β=0.043, 95%CI: 0.003, 0.084, p=0.04). A higher PRS_{ADHD} was associated with higher scores in senses and other observations (P_T <0.01, β=0.035, CI: 0.006, 0.065, p=0.02), in particular in boys (e.g., with GWAS P_T <0.01, β: 0.057, 95%CI: 0.015, 0.099, p=0.01) (Table 2, Figure 1). There was no association between PRS_{ASD} or PRS_{ADHD} and high muscle tone or responses (Supplementary Figure 1, Supplementary Table 3).

Children with higher PRS_{ASD} had higher autistic traits scores at age 6 years (P_T <0.5, β=0.08, 95%CI: 0.03, 0.13, p=0.01); with no interaction with sex (p for interaction: 0.91) (Supplementary Figure 1). In contrast, we found that the association between PRS_{ADHD} and autistic traits at age 6 depended on sex (p for interaction <0.03). Boys but not girls with a higher genetic liability for ADHD had higher autistic traits scores (P_T <0.1, β=0.176, 95%CI: 0.090, 0.266, p ≤ 0.001) (Table 2, Figure 1).

Mediation analysis revealed that 11% of the association between PRS_{ASD} and autistic trait scores at age 6 years was mediated by neuromotor development (95% CI for indirect effect: 0.01, 0.50, p=0.03). The indirect path was in particular present for low muscle tone (13%, p for mediation=0.05). We observed an effect modification by sex, showing that 24% of the association between PRS_{ASD} and autistic traits scores were mediated by neuromotor development in boys (p=0.02). Further analysis in boys showed that higher scores in senses and other observations mediated the associations of both PRS_{ASD} and PRS_{ADHD} with

autistic traits. We did not perform the latter mediation analysis in girls, because there was no association between PRS and neuromotor development or autistic traits in girls (Table 2).

The results on the subscale of senses and other observations were unchanged when we performed regressions with negative binominal regression distribution. If anything, the observed association became stronger (e.g., for PRS_{ADHD}, for P_T <0.01, β=0.24, CI: 0.07, 0.40, p=0.005). Additional adjustment for maternal education and age did not essentially change the findings. Results remained unchanged when weights were used (data not shown). In addition, we used the Imai method to assess how sensitive our results were to unmeasured confounding of the mediator to outcome relationship. We found limited evidence that an unknown confounder affected our results.

DISCUSSION

In this population-based study of children from European ancestry, a higher genetic liability for ASD was associated with less optimal overall infant neuromotor development and low muscle tone at age 9-20 weeks. Less optimal neuromotor development, in particular low muscle tone, mediated the relationship between the ASD genetic susceptibility and autistic traits at age 6 years. Less optimal senses and other observations mediated the association of both ASD and ADHD genetic risks with autistic traits in boys only.

Previous studies have shown associations of PRS_{ASD} and PRS_{ADHD} with neuropsychiatric symptoms in the general population [34, 35]. Using large ASD consortia and population-based resources (n>38,000), Robinson et al. have found that multiple types of genetic risk for ASD influence a continuum of socio-behavioral and developmental traits, the extreme of which comprise of children with ASD or neuropsychiatric disorders [4, 35]. In the Avon Longitudinal Study of Parents and Children, Martin et al. have reported associations between PRS_{ADHD} and ADHD symptoms, and pragmatic language abilities but not with social cognition [34]. Riglin et al. have examined the association between PRS_{ADHD} and trajectories of symptoms for ADHD childhood and have shown associations with autistic traits.[36]. In line with these earlier studies, we found that PRS_{ASD} and PRS_{ADHD} were associated with autistic traits in children from the general population. Moreover, our study extends these findings to infants from the general population, showing that PRS_{ASD} and PRS_{ADHD} predicted neuromotor development at a very early age before the emergence of problem behavior. Early delays in motor development are a common feature in children with autistic symptoms [11], ASD [37], and ADHD [38]. In a cohort of 114 children with ASD and their siblings, co-occurrence of motor impairment and attention problems predicted high autistic symptoms and diagnosis of ASD in the siblings of ASD children [14]. Typically motor impairment and attention problems jointly appear before ASD is diagnosed, accounting for more than 50% of the autistic symptom variation in siblings

of children with ASD. In this sample, motor proficiency score was the most important predictor of autistic symptoms and ASD diagnostic status in siblings of ASD children [14]. Yet, it has remained unclear why motor impairment tracked so closely with attention and social communication problems and what defines the cascade of impairments from genetic predisposition to early symptoms during infancy and later in childhood. While motor impairments can be a comorbidity of ASD, alternatively, autistic symptoms and ASD might partly arise as consequences of early sensorimotor impairments [39]. In our prospective study, the timing of neuromotor assessment makes it very likely that non-optimal performance of infants precedes and contributes to the development of autistic symptoms rather than opposite. Neuromotor development was measured as early as 9 to 20 weeks. Therefore, it most probably preceded autistic traits, because the early sensorimotor symptoms of autism, such as eye contact decline are first exhibited from age 1-6 months [40, 41], while behavioral signs typically emerge during second part of the first year of life [5, 42].

We also found that the PRS_{ASD} and PRS_{ADHD} were associated with less optimal senses and other observations (i.e., strabism, fixation eyes, following movement eyes, hearing, sweating, and startled reactions) in infancy. In our population-based sample, less optimal senses and other observations mediated the associations between PRS_{ADHD} and autistic traits in boys. The connection between sensory and perception processes of an individual with the environment is key to the execution of a motor task. Poor sensorimotor integration plays an important role in the disturbances of motor control, typically seen in autistic children [43, 44]. Over 90% of children with ASD present with abnormalities in sensory function, manifesting as hypo- or hyper-sensitivity [45]. This observation differentiates ASD from ADHD as children with ADHD more typically present hypersensitivity [46]. It remains unclear whether hypo or hyper-sensitivity observed early in childhood with ASD reflects stimulus-specific mechanisms or moment to moment fluctuations in attention. Atypical maturation of early sensorimotor functioning may affect the integrity of developmental trajectories, although the mechanism is not fully understood [13, 47]. Our results support a genetic origin of sensory abnormalities which partly mediates the association of genetic susceptibility for ASD and ADHD with autistic traits in boys.

We observed effect modification by sex in the relationship between ADHD genetic susceptibility and autistic traits, in the way that this association was only present in boys. PGS_{ADHD} are on autosomal genes and therefore the distribution of genetic loci related to ADHD is similar in boys and girls. However, certain genetic loci could convey more vulnerability to environmental and social influences in boys than girls. Sex differences also exist in developmental trajectories, with girls more likely to experience an escalation of autistic traits later than boys during early and mid adolescence [48, 49]. Slower maturation of the fetal and postnatal brains extends the window of vulnerability and puts boys at greater risk to environmental toxins and prolonged stress than girls [50]. It is also possible that autistic traits are more typically displayed by boys. Autistic traits in girls are often

misclassified as being withdrawn and anxious, and such symptoms considered part of their typical development.

Strength and limitations

This study has several strengths including genotyping in a homogeneous group of children from the general population, comprehensive neurological examinations during infancy, and a prospective design and evaluations of autistic traits in childhood. Nonetheless, we faced limitations that require caution in the interpretation of our findings. First, selective non-response to neuromotor assessment could potentially be the source of bias in the analysis. However, we did not detect any differences in neuromotor measurements between infants with and without genetic assessment. Second, we employed a PRS approach to estimate individual-level genetic propensities and predicted developmental outcomes in an independent target sample. But, genetic markers reflect only a fraction of total genetic and disease risk [51]. Also, predictive power of PRS does not simply reflect the genetic correlation between discovery and target trait, but depends on the genetic architecture of both traits and sample size [52].

CONCLUSIONS

We found that the associations between genetic predisposition to ASD and ADHD and non-optimal neuromotor development during infancy contribute to the development of autistic traits in children with high genetic risks. This finding suggests that infants with genetic liability for developmental disorders and early motor abnormalities should be followed for signs of atypical development.

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Table 1. Associations between polygenic risk score for autism spectrum disorder (PRS_{ASD}) with neuromotor development during infancy and autistic traits at age 6 years (boys and girls).

	Polygenic risk score for autism spectrum disorder (PRSASD)											
	$P_T < 0.001$			$P_T < 0.01$			$P_T < 0.1$			$P_T < 1$		
	β	95%CI	p	β	95%CI	p	β	95%CI	P	β	95%CI	p
Girls												
Overall neuromotor	-0.040	-0.115, 0.035	0.30	0.005	-0.070, 0.079	0.91	0.028	-0.048, 0.103	0.47	0.053	-0.021, 0.134	0.16
Overall tone	-0.030	-0.105, 0.045	0.43	-0.001	-0.082, 0.067	0.84	0.023	-0.052, 0.099	0.54	0.046	-0.027, 0.120	0.22
Low muscle tone	-0.034	-0.105, 0.038	0.35	0.013	-0.058, 0.084	0.72	0.029	-0.043, 0.100	0.43	0.048	-0.023, 0.118	0.18
High muscle tone	0.020	-0.034, 0.073	0.47	-0.021	-0.074, 0.033	0.45	0.004	-0.049, 0.058	0.88	0.019	-0.033, 0.072	0.47
Responses	0.006	-0.039, 0.051	0.79	0.020	-0.024, 0.065	0.37	0.033	-0.012, 0.078	0.15	0.046	0.002, 0.090	0.04
Senses and others	-0.030	-0.068, 0.010	0.14	0.005	-0.034, 0.044	0.79	-0.001	-0.041, 0.037	0.93	0.004	-0.035, 0.043	0.84
Autistic traits	-0.059	-0.157, 0.038	0.23	0.080	-0.016, 0.177	0.10	0.057	-0.004, 0.156	0.26	0.062	-0.036, 0.160	0.21
Boys												
Overall neuromotor	0.025	-0.049, 0.099	0.51	0.049	-0.026, 0.124	0.20	**0.106**	**0.032, 0.181**	**0.01**	**0.109**	**0.033, 0.185**	**0.01**
Overall tone	0.011	-0.062, 0.085	0.76	0.035	-0.039, 0.110	0.35	**0.082**	**0.008, 0.156**	**0.03**	**0.091**	**0.016, 0.167**	**0.02**
Low muscle tone	0.010	-0.061, 0.081	0.79	0.028	-0.044, 0.099	0.45	0.069	-0.002, 0.040	0.06	**0.086**	**0.014, 0.158**	**0.02**
High muscle tone	0.015	-0.037, 0.067	0.57	0.025	-0.027, 0.077	0.34	0.038	-0.015, 0.089	0.16	0.030	-0.023, 0.083	0.26
Responses	0.001	-0.04, 0.042	0.96	0.009	-0.033, 0.050	0.68	0.018	-0.024, 0.059	0.40	0.008	-0.034, 0.051	0.07
Senses and others	0.036	-0.005, 0.076	0.08	0.040	0.000, 0.080	0.06	**0.043**	**0.003, 0.084**	**0.04**	**0.048**	**0.007, 0.090**	**0.02**
Autistic traits	0.032	-0.059, 0.122	0.49	0.054	-0.037, 0.144	0.25	0.052	-0.038, 0.142	0.26	**0.094**	**0.002, 0.186**	**0.05**

Models were adjusted for a child's sex and age at assessment and four genetic principal components (and for the version of the neuromotor instrument in models with neuromotor development). *P*-value thresholds (P_T): significance threshold for inclusion of variants in the polygenic score.

Table 2. Associations between polygenic risk score for attention-deficit/hyperactivity disorder (PRS_{ADHD}) with neuromotor development during infancy and autistic traits at age 6 years (boys and girls).

	Polygenic risk score for attention-deficit/hyperactivity disorder											
	$P_T < 0.001$			$P_T < 0.01$			$P_T < 0.1$			$P_T < 1$		
	β	95%CI	p	β	95%CI	p	β	95%CI	p	β	95%CI	p
Girls												
Overall neuromotor	0.021	-0.056, 0.098	0.59	-0.028	-0.107, 0.052	0.49	-0.031	-0.108, 0.046	0.43	-0.020	-0.094, 0.055	0.60
Overall tone	0.023	-0.054, 0.099	0.56	-0.033	-0.112, 0.045	0.41	-0.036	-0.112, 0.041	0.36	-0.029	-0.010, 0.045	0.44
Low muscle tone	0.020	-0.053, 0.093	0.59	-0.029	-0.104, 0.046	0.45	-0.027	-0.101, 0.046	0.46	-0.021	-0.093, 0.050	0.56
High muscle tone	0.009	-0.046, 0.064	0.75	-0.033	-0.090, 0.023	0.24	-0.020	-0.075, 0.035	0.47	-0.008	-0.061, 0.045	0.77
Responses	-0.014	-0.060, 0.032	0.54	-0.015	-0.062, 0.033	0.55	-0.015	-0.061, 0.030	0.51	0.001	-0.043, 0.046	0.96
Senses and others	0.023	-0.017, 0.063	0.27	0.011	-0.031, 0.052	0.62	0.015	-0.025, 0.055	0.47	0.007	-0.032, 0.046	0.74
Autistic traits	-0.075	-0.174, 0.024	0.14	-0.023	-0.124, 0.079	0.66	-0.112	-0.101, 0.097	0.97	0.008	-0.087, 0.103	0.87
Boys												
Overall neuromotor	0.034	-0.044, 0.112	0.39	0.026	-0.052, 0.104	0.52	0.023	-0.053, 0.100	0.55	0.023	-0.054, 0.099	0.56
Overall tone	0.016	-0.060, 0.092	0.68	0.007	-0.070, 0.083	0.86	0.008	-0.068, 0.084	0.83	0.015	-0.061, 0.091	0.70
Low muscle tone	0.037	-0.036, 0.111	0.31	0.022	-0.052, 0.096	0.55	0.027	-0.046, 0.099	0.48	0.021	-0.053, 0.094	0.58
High muscle tone	-0.011	-0.064, 0.043	0.70	-0.002	-0.056, 0.052	0.94	-0.007	-0.061, 0.046	0.79	0.001	-0.052, 0.054	0.96
Responses	0.013	-0.030, 0.056	0.56	0.013	-0.030, 0.056	0.54	-0.002	-0.044, 0.041	0.95	-0.004	-0.047, 0.038	0.84
Senses and others	0.040	-0.003, 0.082	0.07	**0.057**	**0.015, 0.099**	**0.01**	0.039	-0.003, 0.081	0.07	0.035	-0.006, 0.077	0.09
Autistic traits	0.090	-0.02, 0.182	0.06	**0.131**	**0.040, 0.221**	**0.01**	**0.176**	**0.090, 0.266**	**<0.001**	**0.187**	**0.097, 0.276**	**<0.001**

Models were adjusted for a child's sex and age at assessment and four genetic principal components (and for the version of the neuromotor instrument in models with neuromotor development). *P*-value thresholds (P_T): significance threshold for inclusion of variants in the polygenic score.

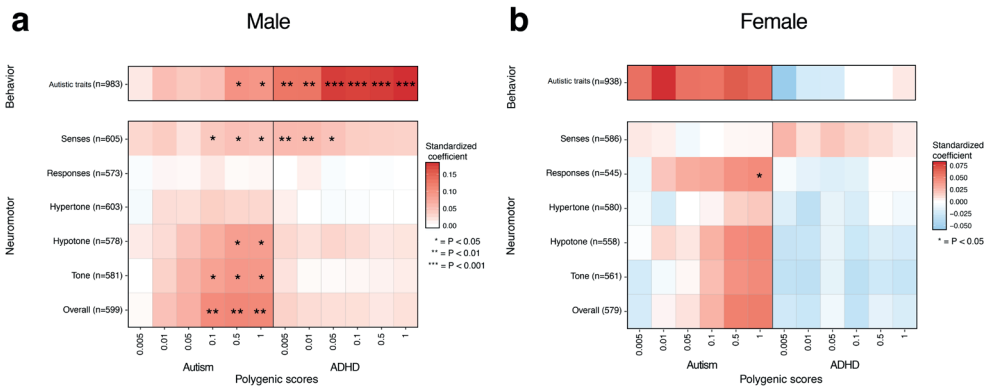


Figure 1. Associations of polygenic risk score for autism spectrum disorder (PRS_{ASD}) and for attention-deficit/hyperactivity disorder (PRS_{ADHD}) with neuromotor development scores during infancy and autistic traits at age 6 years (a) in boys and (b) in girls.

Effect sizes were estimated using linear regression models with neuromotor development and autistic traits as outcomes. PRS_{ASD} and PRS_{ADHD} were selected based on different *P*-value thresholds (P_T): P_T <0.005, P_T <0.01, P_T <0.05, P_T <0.1, P_T < 0.5, and P_T <1.

Models were adjusted for a child's age at assessment and four genetic principal components (and for the version of the neuromotor instrument in models with neuromotor development).

Supplementary Table S1. Number of variants included in the polygenic scores for each p-value threshold (P_T)

P-value threshold (P_T)	Autism spectrum disorder	Attention-deficit/ hyperactivity disorder
0.001	947	1745
0.005	3834	5080
0.01	6765	8294
0.05	24417	25305
0.1	41214	40393
0.5	121530	108728
1	163219	143070

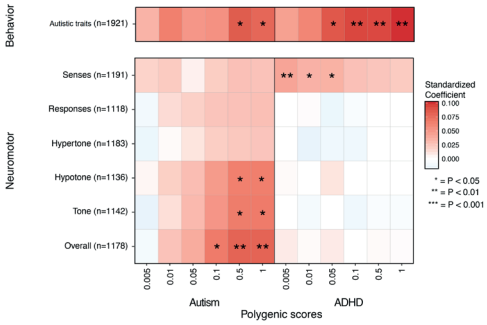
Supplementary Table 2. Items for assessing neuromotor development (adapted from van Batenburg-Eddes et al. 2009)[53].

Subscale	Position	Item description	Answering categories		
			Optimal	Non-optimal	Non-optimal
Tone	Supine	Resting posture	Semi-flexed legs; slight abduction at the hips	Legs flat on the surface	Legs stretched
		Adductor angle	> 80° - < 140°	> 140°	< 80°
		Popliteal angle	90°-130°	130°-180°	< 90°
		Ankle angle	> 20° - < 90°	< 20°	> 90°
		Head preference	No	Yes	
		Opening & closing hands	Yes	Sometimes closed	Always closed
		Alternating leg movements	Yes	Decreased	Absent
		Grasps with one hand	Yes	Decreased	Absent
		Hyperextension	No	Sometimes	Yes
		Dyskinesia	No	Sometimes	Yes
	Supine-to- sit	Traction response	Arms moderately flexed	Arms fully extended, no resistance	Strong resistance, flexion elbows, legs extended
		Traction response-head control	Active lift of head	Head lag	Exaggerated
	Horizontal	Ventral Tone	Normal tone	Low tone	Back and limbs stretched
	Vertical	Head	Normal tone	Low tone	High tone
		Shoulders	Normal tone	Low tone	High tone
		Trunk	Normal tone	Low tone	High tone
		Legs	Normal tone	Low tone	High tone
	Prone	Pulls arms up	Yes	No	
		Turns head	Yes	No	
		Lifts head	Yes	No	Overstretched
	Sitting	Needs support	Yes	No	
		Head control	Yes	No	
		Shoulder retraction	No	Yes	
		Shape of the back	Round	Straight	Scoliosis
Responses	Supine	Asymmetrical Tonic Neck Reflex	Weak	Yes	Exaggerated
		Babinski	Yes	Exaggerated	Spontaneous
	Prone	Bauer	Yes / weak	Exaggerated	
	Vertical	Stepping movements	No	Yes	Exaggerated
		Moro intensity	Yes / weak	Exaggerated	
		Moro opening hands	Yes	No	
Other	Supine	Strabismus	No	Sometimes	Yes
		Fixation eyes	Yes	Decreased	No
		Following movements eyes	Smooth	Decreased	No
		Hearing	Yes	Moderate	No
		Sweating	No	Yes	
		Startles	No	Sometimes	Yes

Supplementary Table 3. Associations between polygenic risk scores for autism spectrum disorder (PRS_{ASD}) and for attention-deficit/hyperactivity disorder (PRS_{ADHD}) with infant neuromotor development and autistic traits.

	Polygenic risk score for autism spectrum disorder											
	$P_T < 0.001$			**$P_T < 0.01$**			**$P_T < 0.1$**			**$P_T < 1$**		
	β	**95%CI**	**p**	β	**95%CI**	**p**	β	**95%CI**	**p**	β	**95%CI**	**p**
Overall neuromotor	-0.006	-0.059, 0.046	0.81	0.027	-0.026, 0.080	0.32	**0.067**	**0.015, 0.120**	0.01	**0.081**	0.028, 0.134	**0.003**
Overall tone	-0.009	-0.062, 0.043	0.73	0.013	-0.039, 0.066	0.62	0.052	-0.001, 0.104	0.05	**0.068**	0.015, 0.120	**0.01**
Low muscle tone	-0.011	-0.061, 0.039	0.67	0.020	-0.030, 0.071	0.43	0.048	-0.002, 0.099	0.06	**0.066**	0.016, 0.116	**0.01**
High muscle tone	0.017	-0.020, 0.054	0.37	0.002	-0.035, 0.039	0.90	0.021	-0.016, 0.058	0.27	0.025	-0.012, 0.062	0.19
Responses	0.004	-0.027, 0.034	0.81	0.015	-0.015, 0.045	0.33	0.026	-0.004, 0.057	0.09	0.028	-0.002, 0.059	0.07
Senses and others	0.004	-0.024, 0.033	0.76	0.022	-0.006, 0.051	0.12	0.021	-0.007, 0.050	0.14	0.027	-0.002, 0.055	0.06
Autistic traits	-0.011	-0.077, 0.055	0.75	0.065	-0.001, 0.130	0.05	0.055	-0.011, 0.121	0.10	**0.079**	**0.013, 0.0146**	**0.02**
	Polygenic risk score for attention-deficit/hyperactivity disorder											
Overall neuromotor	0.027	-0.028, 0.082	0.33	0.004	-0.052, 0.059	0.90	-0.002	-0.057, 0.052	0.93	0.002	-0.051, 0.056	0.94
Overall tone	0.019	-0.034, 0.073	0.48	-0.008	-0.063, 0.046	0.76	-0.013	-0.066, 0.041	0.64	-0.007	-0.060, 0.046	0.80
Low muscle tone	0.030	-0.022, 0.081	0.26	0.001	-0.052, 0.053	0.98	0.001	-0.051, 0.052	0.98	-0.001	-0.052, 0.050	0.98
High muscle tone	-0.002	-0.040, 0.036	0.92	-0.015	-0.054, 0.024	0.44	-0.013	-0.051, 0.025	0.51	-0.002	-0.040, 0.035	0.91
Responses	-0.001	-0.032, 0.031	0.97	0.002	-0.030, 0.034	0.90	-0.007	-0.038, 0.024	0.67	0.000	-0.031, 0.030	0.99
Senses and others	**0.043**	**0.001, 0.060**	**0.04**	**0.035**	**0.006, 0.065**	**0.02**	0.028	-0.001, 0.057	0.06	0.022	-0.006, 0.051	0.12
Autistic traits	**0.094**	**0.029, 0.160**	**0.01**	**0.098**	**0.031, 0.164**	**0.004**	**0.098**	**0.032, 0.164**	**0.004**	**0.111**	**0.047, 0.176**	**0.001**

Models were adjusted for a child's sex and age at assessment and four genetic principal components (and for the version of the neuromotor instrument in models with neuromotor development). *P*-value thresholds (P_T): significance threshold for inclusion of variants in the polygenic score.



Supplementary Figure 1. Associations of polygenic score for autism spectrum disorder (PRS_{ASD}) and for attention-deficit/hyperactivity disorder (PRS_{ADHD}) with neuromotor development scores during infancy and autistic traits at age 6 years

Effect sizes were estimated using linear regression models with neuromotor development and autistic traits as outcomes. PRS_{ASD} and PRS_{ADHD} were selected based on different *P*-value thresholds (P_T): P_T <0.005, P_T <0.01, P_T <0.05, P_T <0.1, P_T < 0.5, and P_T <1.

Models were adjusted for a child's sex and age at assessment and four genetic principal components (and for the version of the neuromotor instrument in models with neuromotor development).

Painting by Bakir Rokvic (age 11 years)

4 EARLY FAMILY ENVIRONMENT AND CHILD BEHAVIOR

4.1

The Complex Role of Parental Separation in the Association Between Family Conflict and Child Problem Behavior[7]

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ABSTRACT

Background: Parental separation is a major adverse childhood experience. Parental separation is generally preceded by conflict, which is itself a risk factor for child problem behavior. Whether parental separation independent of conflict has negative effects on child problem behavior is unclear. **Method:** This study was embedded in Generation R, a population-based cohort followed from fetal life until age 9 years. Information on family conflict was obtained from 5808 mothers and fathers. The four-way decomposition method was used to apportion the effects of prenatal family conflict and parental separation on child problem behavior into four non-overlapping components. Structural equation modeling was used to test bidirectional effects of child problem behavior and family conflict over time. **Results:** Family conflict from pregnancy onwards and parental separation each strongly predicted child problem behavior up to pre-adolescence according to maternal and paternal ratings. Using the four-way decomposition method, we found evidence for a strong direct effect of prenatal family conflict on child problem behavior, for reference interaction, and for mediated interaction. The evidence for interaction implies that prenatal family conflict increased the children's vulnerability to the harmful effect of parental separation. There was no evidence of a pure indirect effect of parental separation on child problem behavior. **Conclusions:** Overall, results indicated that if parental separation occurs in families with low levels of conflict, parental separation does not predict more child problem behavior. Moreover, the bi-directional pattern suggested that child problem behavior influences the persistence of family conflict.

Key words: *family conflict, parental separation, child problem behavior, mediation, interaction*

INTRODUCTION

Parental separation affects approximately a third of all marriages in many societies. Parental separation has been related to diverse negative outcomes of the child, including mental and physical health problems [1]. Many children from separated families show difficulties in functioning, including frequent emotional and behavioral problems [2-4]. However, family conflict often long precedes the actual physical separation, thus making it difficult to determine whether the negative effects on children are caused by the parental separation or by the family conflict [5], which increases the risk of separation as well as causing child maladjustment [6,7]. Furthermore, child maladjustment can often trigger or exacerbate family conflict [8,9]. In some families, family conflict may start before the child is born and escalate over time. However, in other families, family conflict begins sometime after the child is born and increases over time, particularly if the child has physical, developmental, regulatory, emotional, or behavioral problems [10-12]. Given this complex set of factors, it is important to consider the effects of prenatal family conflict on later family conflict, on separation, and on child maladjustment. Additionally, it is important to test mediation and interaction effects linking prenatal conflict and separation with child maladjustment. Finally, bi-directional effects between child maladjustment and family conflict are important to test. Before detailing our specific hypotheses, we summarize previous research relevant to associations between family conflict, separation, and child maladjustment.

Family Conflict

Many studies show that family conflict plays a central role in child maladjustment [13-15]. Parents in high-conflict marriages are less warm towards their children, more rejecting, harsher in their discipline, and more withdrawn and depressed than parents in low-conflict marriages [16-18]. When family conflict increases parental harshness, rejection, and inconsistency, it may lead to child maladjustment, such as internalizing and externalizing problems [19,20]. Additionally, the effects of family conflict may vary depending on the age of the child, with toddlers showing developmental, self-regulatory, and attachment issues but preschoolers showing self-blame, fear, confusion, guilt and sadness [21,22]. As children age, they develop a more sophisticated understanding of interactions between people, but they are still troubled by loyalty conflicts when their divorced parents remain locked in conflict [22].

Few studies have examined the stability of family conflict over time and even fewer have tested this stability starting prenatally. However, Kluwer, Johnson [23] reported that a high level of conflict during pregnancy predicted worse marital relationships after the child was born. This may be because the stresses of parenting are added onto an already conflictual relationship [24].

Separation/Divorce

Separation and divorce represent a cascade of potentially stressful changes in the social and physical environment of families. Separation is often associated with increased parental distress, reduced attention paid to the child by one or both parents, disruption of the home environment, conflict over money and custody/visitation, and reduced economic circumstances, all of which are stressors for children [3,25,26]. Parental preoccupation with issues pertaining to separation/divorce and adjustment to the new domestic arrangements can also interfere with effective parenting, which can lead to problems in their children [19,20].

Most prospective studies have found that both family conflict and parental separation stress children and can lead to maladjustment [27]. Furthermore, the level of conflict preceding the separation influences child emotional and behavioral problems [17,28]. Some research indicates that family conflict is a more important predictor child maladjustment than parental separation [29]. Interaction effects between conflict and separation are likely, though they have not been widely studied. For example, separation may have fewer negative effects on children when conflict is low and parents can collaborate for their children's welfare before, during, and after the separation process [30]. On the other hand, when conflict is high before, during, and after the separation, then the compound effects of conflict and separation may result in many negative consequences for the children. However, a few longitudinal studies have found that children in high-conflict families showed improved wellbeing after parental separation. [16,17]. This outcome may be contingent on the discrepancy between pre- and post-separation level of contact and conflict.

Gaps in Previous Research

Few studies thus far have explored the extent to which the association between parental separation and child maladjustment depends on family conflict and even fewer have tested this in young children. Most previous research has considered the effects of family conflict and divorce individually, but the two are likely to interact. The few studies [18,31] that have considered both family conflict and parental separation did so by adjusting the regression analyses of separation predicting child behavior for family conflict. However, these studies have generally not tested the interaction effect between family conflict and parental separation. Moreover, family conflict has typically been assessed after the child was born. Because child behavior can influence family conflict and separation, reverse causality can create a bidirectional feedback loop, but this has been largely unexplored in previous studies [32]. Measuring family conflict prenatally controls for such bidirectional effects. Furthermore, measuring both family conflict and child maladjustment at successive time points in a longitudinal design permits analysis of the bidirectional associations between parental and child behavior over time [33,34]. Additionally, many studies of divorce/separation do not obtain ratings of child emotional and behavioral problems from both

parents, although discrepancies between maternal and paternal ratings are a well-documented finding [35,36].

Goals of our Research

To address these limitations in the literature, we examined effects of family conflict and parental separation on child maladjustment using a large, multi-ethnic population-based prospective cohort from the Generation R study [37]. Both parents provided reports of family conflict prenatally and at age 9, and mothers reported on family conflict at age 5. Information about marital status (i.e., married/living together vs. separated/divorced) was obtained prenatally and at ages 3, 5, and 9. The parents each reported child behavioral and emotional problems at age 3 and 9 and mothers also provided reports at age 5. We used these data to test the following hypotheses: (a) prenatal family conflict is associated with later family conflict, separation, and child maladjustment; (b) parental separation is associated with child maladjustment; (c) parental separation might not affect child maladjustment independent of prenatal family conflict; and (d) bidirectional associations would be found between child maladjustment and family conflict.

METHOD

Participants

Our research was embedded in the Generation R Study, a multi-ethnic population-based cohort from fetal life onwards. The Generation R Study has been described in detail previously [37]. Briefly, all pregnant women living in Rotterdam, the Netherlands, with an expected delivery date between April 2002 and January 2006 were invited to participate. The study was approved by the Medical Ethics Committee of the Erasmus Medical Center, Rotterdam. Written informed consent was obtained from all adult participants. Of the 8879 pregnant women enrolled during pregnancy, we excluded 1266 mothers with no partner and 490 with missing family conflict data, leaving 7123 mothers and 4561 fathers. Of the 7123 mothers who completed questionnaires on family conflict before the child was born, 1315 (18%) mothers were lost to follow-up, leaving 5,808 remaining mothers with child report data. Not all of these 5,808 mothers were seen at every time point (i.e., ages 3, 5, and 9). We tabulated the number of mothers who reported being separated from their partners at each time point and calculated this as a percent of the mothers seen at that time point, as follows: (a) by age 3 (342/4174 = 8.2%); (b) from ages 3 to 5 (430/5163 = 8.9%); and (c) from ages 5 to 9 (298/4543 = 7.9%). Overall by the time the child was 9-years-old, 1,070 (23.6%) mothers were separated/divorced from their partner. At age 9 years, 4062 mothers reported data on child problem behavior (4223 and 5063 had reported child problem behavior at age 3 and 5 years, respectively (see supplementary

Figure 1). At age 9 years, 3080 fathers reported data on child problem behavior (3556 had reported child problem behavior at age 3years, respectively).

Measures

Family Assessment Device

Family functioning was assessed with the General Functioning (GF) subscale of the Family Assessment Device - FAD [38,39], at 20 weeks pregnancy, as well as when the child was 5 and 9 years old. Both mothers and fathers completed this measure prenatally and at age 9, but only maternal report was available at age 5. The General Functioning scale is a validated self-report measure of family health and pathology consisting of 12 items. Half of the items describe healthy functioning, e.g., 'In times of crisis, we can turn to each other for support'. The other half describe unhealthy functioning, e.g., 'There are a lot of unpleasant and painful feelings in our family'. Parents were asked to rate how well each item described their family by selecting from four different responses ranging from 1 to 4: strongly agree, agree, disagree or strongly disagree. So that a higher total FAD score could indicate less well-functioning families, the six positively worded healthy items were reverse-coded. Then, all 12 items were summed and divided by 12, yielding a total score from 1 to 4. FAD score will therefore be referred to henceforth as *family conflict*. In the current study, internal consistencies (Cronbach's alpha) ranged from 0.82 to 0.87.

Child Behavior Checklist

The Child Behavior Checklist for Ages 1½-5 (CBCL/1½-5; [40]), and the Child Behavior Checklist for Ages 6-18 (CBCL/6-18; [41]), were used to obtain standardized parent reports of children's emotional and behavioral problems. The CBCL/1½-5 contains 99 problems items, which are scored on seven empirically based syndromes and three broadband scales (Internalizing, Externalizing, and Total Problems). Each item used a three point rating scale 0 ='not true', 1 = 'somewhat or sometimes true', and 2= 'very true or often true', based on the preceding two months. The CBCL/6-18 has 118 problem items, also yielding syndrome scales and the same three broadband scales, with ratings based on the preceding 6 months. Good reliability and validity have been reported [40], and the scales were found to be generalizable across 23 societies, including The Netherlands [42]. We used the continuous Total Problems score (the sum of ratings on all problem items) as our outcome measure because it reflects all the behavioral and emotional problems tapped by the CBCL and is thus the best overall index of maladjustment. Cronbach's alpha at the different time points ranged from 0.77 to 0.80.

Parental Separation/Divorce

Marital status questions from the Generation R Study parental questionnaires were used to measure the occurrence of parental separation at four different data collection rounds: during pregnancy and when the child was 3, 5, and 9 years old. At each time point,

marital status was scored dichotomously: "married/living together" and "separated/divorced". If parents reported "not living together anymore" or "divorced" the child was coded as having experienced separation. In the Netherlands, many unmarried couples have a registered partnership. Marriage and registered partnership are similar in many ways. They are both relationships formalized by law. When registered partners who live together with their children decide to separate, the procedure must be conducted as if it were divorce. For our study, once a family was classified as separated/divorced, that classification remained for all subsequent time points. With our data, we were not able to differentiate children who were exposed to multiple separation/divorces from those exposed to a single such event.

Covariates

Descriptive statistics for the parent and child characteristics used as possible confounders are presented in Table 1. Parental age, ethnicity, education, and parental psychopathology are well-established predictors of children's problems in existing separation/divorce studies [3], as well as in many studies from the Generation R group. Maternal religion (e.g., Muslim vs. non-Muslim) has been an important variable in previous Generation R studies [43,44]. Gestational age at birth was included as a confounder because perinatal problems are known risk factors for psychopathology. The divorce literature generally considers child gender as an important variable, given that separation/divorce often has differential effects on boys versus girls. For example, boys often become more oppositional and aggressive, whereas girls often show more dependency, anxiety, and depression [45].

Maternal and paternal age were assessed at intake. Parental ethnicity was categorized into Dutch, non-Western and other Western national origin [46]. Parental education was classified in three levels: 'low' (maximum of three years general secondary school), 'medium' (>3 years general secondary school; intermediate vocational training), and 'high'(higher vocational training, Bachelor's degree, higher academic education). Information on maternal religion was obtained with questionnaires filled in by the mothers during pregnancy. Based on their responses to two questions about religion, mothers were classified into four categories: not religious, Christian, Islamic and other religion. Date of birth and gender of the infant were obtained from community midwife and hospital registries at birth. Information on gestational age was established by fetal ultrasound examinations within the Generation R Study. Parental psychopathological symptoms were assessed at 20 weeks of pregnancy and when the child was 3 years old using the Brief Symptom Inventory (BSI), a validated self-report questionnaire with 53 items to be answered on a five-point scale, ranging from '0 = not at all' to '4 = extremely'[47,48]. High validity and reliability have been reported for the Dutch translation [49]. Cronbach's alpha was $\alpha = 0.86$. In summary, it is important to control for factors such young maternal age, low education, minority status, child gender, religion, gestational age and parental psychopathology, as they are often associated with family conflict, parental separation,

and/or child maladjustment [3,12].

Statistical Analyses

Prior to our data analyses, missing values of the covariates were imputed using multiple imputations. With the Markov Chain Monte Carlo multiple imputation technique, 10 complete data sets were created [50]. Multivariate analyses were performed on each imputed data set, and effect estimates were pooled. The data were analyzed using SAS 9.4 software.

To address our first hypothesis, we computed concurrent and predictive correlations among family conflict scores over time and CBCL Total Problems scores over time. Then, we used logistic regressions to analyze prenatal family conflict as a predictor of separation at ages 3, 5, and 9. We then analyzed with separate linear regressions the prospective associations of prenatal family conflict and parental separation with CBCL Total Problems scores over time. In a sensitivity analysis, we used generalized estimating equations (GEE; (Litman et al., 2007), to test the interaction with age in the associations between family conflict and maladjustment. This analysis tested if the association of family conflict (as reported by both mothers and fathers) with child problem behavior depends on the age of the child by comparing the single estimate of the repeatedly assessed family conflict.

Our main analysis involved the use of the four-way decomposition method [51], to test if the association of prenatal family conflict with child problem behavior is due to mediation by, or interaction with, parental separation. To this aim, the association of prenatal family conflict with child problem behavior mediated by parental separation (referred as the total effect - TE) was decomposed into four non-overlapping components: (i) the controlled direct effect (CDE) of prenatal family conflict on child problem behavior with parental separation absent; (ii) the reference interaction (INTref), which is the additive interaction of prenatal family conflict and parental separation on child's problem behavior; this only operates if the effects of prenatal family conflict and parental separation on child problem behavior differ from the sum of the effect of being exposed to only family conflict and the effect of only separation; (iii) the mediated interaction (INTmed), which operates when parental separation is causally dependent on prenatal family conflict, and the interaction of the two has an effect on child problem behavior (i.e., parental separation occurs due to family conflict, and separation has an effect on child problem behavior only at certain levels of family conflict); and (iv) the pure indirect effect (PIE), which operates when parental separation is associated with child problem behavior independent of prenatal family conflict (i.e. pure mediated effect). This regression-based approach was used to estimate these direct and indirect effects and involved combining parameter estimates according to the analytic expressions in the literature [51]. Confidence intervals were obtained from standard errors for these effects using the delta method.

We first ran the four-way decomposition model adjusting for all previously mentioned confounders. We then adjusted the model for child problem behavior at 1.5 years as

an additional confounder. These primary analyses assumed no additional unmeasured confounding. However, because it is possible that potential unmeasured confounders could have affected our results [52], we posited and evaluated an unmeasured confounder in a sensitivity analysis. That is, an unobserbved covariate that correlates with parental separation and child problem behavior to such an extent that it would substantially reduce or eliminate the natural direct and indirect effects (details can be found in Supplementary, Table 1).

The four-way decomposition model extends the formula from Baron, Kenny [53] to take account of exposure-mediator interactions in mediation analysis. Several previous studies in the social science field have reported mediated effects in the presence of interaction, but in the past it was difficult to decompose the total effect into direct and indirect effects in these studies [54]. Such a decomposition is important because, in many studies, the exposure and mediator do interact to affect the outcome [55].

Finally, we examined the bidirectional relations between child problem behavior and postnatal family conflict. Structural equation modeling methods were used with the covariance matrices as input. The goodness-of-fit of the estimated SEM models with the data was considered acceptable if the following criteria were met: the root mean square error of approximation (RMSEA) had a value of 0.05 or less, and the comparative fit index (CFI) and Tucker-Lewis index (TLI) had a value of 0.90 or higher [56]. A baseline model was identified in which all paths were free to vary across time and across maternal and paternal reports. Then, for each type of effect (child-effect on mother, child-effect on father, mother effect on child, and father-effect on child), a model was run in which these effects were constrained to be equal across time.

RESULTS

Predictions from Prenatal Family Conflict

The correlations in Table 2 show that mothers' and fathers' reports of family conflict were moderately associated both in the prenatal period and at age 9 (*r*s = .44). Within-informant longitudinal stability in family conflict ratings (*r*s = .38 -.53 for mothers and .40 for fathers) was higher than cross-informant longitudinal stability (*r*s = .25). Prenatal ratings of family conflict had modest correlations with CBCL Total Problems score at age 3 (*r*s = .13 -.25), age 5 (*r*s = .13-.21), and age 9 (*r*s = .11-.19), consistent with our first hypothesis.

Also consistent with our first hypothesis, the odds ratios (ORs) results derived from logistic regressions (see Table 3) indicate that prenatal family conflict was associated with parental separation across childhood, after adjusting for parent age, ethnicity, education, religion, and psychopathology as well as child sex and gestational age at birth. The largest ORs were for separation by age 3 (ORs = 2.8 for mothers' ratings and 3.14 for fathers ratings). However, ORs predicting separation between ages 3 and 5 and by age 9 were

all > 2.0. Thus, regardless of the informant, each unit increase in prenatal family conflict doubled the relative risk of later parental separation.

Family Conflict and Child Problem Behavior

Table 4 presents results from the regression analyses predicting CBCL Total Problems across childhood from family conflict as reported by both mothers and fathers at various time points. For mothers' ratings of prenatal family conflict, prediction of CBCL Total Problems scores was as strong for age 9 as for age 3, with a slight dip at age 5. For fathers' reports of prenatal family conflict, prediction to age 9 was slightly weaker than prediction to age 3. For later reports of family conflict, concurrent associations between family conflict and CBCL Total Problems scores were stronger than associations for both informants. Overall, a child exposed to family conflict was more likely to have higher levels of behavioral and emotional problems at both concurrent and later ages, consistent with our first hypothesis.

Our GEE sensitivity analysis tested the interaction between levels of family conflict as assessed by each informant and age in predicting child problem behavior at age 9. The GEE estimates were very similar to the results in Table 5, only the CIs varied slightly because this method takes into account within-individual correlation across the time points. Tests for homogeneity of the varying family conflict effects at different ages showed a significant interaction between levels of family conflict across time in predicting child problem behavior at age 9 (GEE: F = 10.97, p_{int} = .001 for mothers' report and GEE: F = 16.37, p_{int} = <.001 for fathers' report). Specifically, the strongest association with child problem behavior at age 9 was found when family conflict at age 9 was the predictor.

Parental Separation and Child Problem Behavior

To address our second hypothesis, we conducted regression analyses predicting CBCL Total Problems scores at different ages from parental separation at different ages. As shown in Table 5, parental separation was consistently related to higher CBCL Total Problems scores as reported by both mothers and fathers. However, consistent with our third hypothesis, no associations of parental separation were observed after prenatal parental family conflict was added to the model for all the regressions presented in Table 5 except for the "separation by age 9" results for mother-reported Total Problems score, which had a B = 1.67, 95% CI: 0.12, 3.22, p = .034.

Four-Way Decomposition Analysis

Our four-way decomposition analysis provided an integrated test of our first three hypotheses, namely that prenatal conflict and parental separation would both associated with child emotional and behavioral problems but that separation might not be a significant predictor independent of prenatal family conflict. In this analysis, we tested direct, mediation, and interaction effects of prenatal family conflict and parental

separation on CBCL Total Problems scores at age 9. Because the four components sum to the total effect, each component's proportional share of the total effect can be obtained by dividing the coefficient for each effect (which approximates a beta value) by the total effect.

As shown in Table 6, a strong 'direct effect' (CDE) of prenatal family conflict on child problem behavior was present, with a large effect size. That is, in families with high levels of prenatal conflict, children had higher CBCL Total Problems scores at age 9. Second, there was evidence for a 'reference interaction effect' (INTref) of prenatal family conflict and parental separation on child problem behavior, with a small effect size. The direction of this effect suggests that when prenatal family conflict was high, the children were more vulnerable to the harmful effects of parental separation. Third, if parental separation was preceded by prenatal family conflict, the interaction of the two 'mediated' the effect on child problem behavior with a small effect size (INTmed). The direction of this effect suggests that parental separation had a negative effect on child problem behavior at high levels of family conflict, allowing for prenatal family conflict and separation to interact. As noted above, traditional methods of mediation do not allow for interaction between the effects of exposure (family conflict) and the effects of the mediator (parental separation). The 'pure indirect effect' (PIE) of parental separation on child problem behavior in the absence of prenatal family conflict was not significant and the confidence interval spanned zero, as shown in Table 6. Although the direction of this effect could suggest that parental separation might have some inverse (i.e., beneficial) effect on child behavior, this cannot be inferred from our data given the broad confidence interval and non-significant *p* value. In summary, we found that parental separation partially mediated the association between prenatal family conflict and CBCL Total Problems scores.

It should be noted that the results in Table 6 and reported here represent adjustment for our potential confounders, namely maternal age, ethnicity, education, religion, maternal psychopathology, gestational age at birth, child sex. We additionally adjusted for child emotional and behavioral problems at age 1.5 years, yielding results that were essentially unchanged. Our sensitivity analysis [52] indicated that is unlikely to be eliminated by the influence of an unobserved confounder (details in Supplementary, Table 1). This suggests that even under the scenario of substantial unmeasured confounding, the effect of prenatal family conflict on child problem behavior is not purely mediated by parental separation.

Bi-Directional Analysis

To address our last hypothesis, we examined bi-directional effects between child maladjustment and family conflict. Structural equation modeling showed good fit to the data (RMSEA = 0.08, CFI = 0.99, TLI = 0.89), (Figure 1.). For cross-lagged standardized paths, coefficients are shown. The long-term bidirectional effects between child problem behavior and family conflict were positive for both directions based on maternal and

paternal report. Thus, the structural equation model showed that both parent-to-child effects and child-to-parent effects operated, such that child maladjustment led to increased family conflict and vice versa.

DISCUSSION

We tested the longitudinal effects of family conflict and parental separation on child maladjustment using a large, multi-ethnic population-based prospective cohort from the Generation R Study. Innovative aspects of our study include that we measured family conflict prenatally as well as periodically up to age 9 and that we obtained ratings of family conflict and child problems from mothers and fathers both prenatally and at age 9. Also, we used an association pathway mediation analysis to better understand the interaction of prenatal family conflict with postnatal parental separation as they relate to child problem behavior. Findings generally supported our four major hypotheses, as summarized below.

As hypothesized, prenatal family conflict predicted later family conflict, with longitudinal stability in family conflict ratings that were moderate to high for both maternal and paternal reports of conflict up to age 9. Also, as we hypothesized, prenatal family conflict, whether reported by mother or father, strongly predicted later parental separation across childhood, with the strongest association for separation by age 3. These findings replicated previous studies [16,18,27], showing that family conflict is associated with separation.

Also consistent with our first hypothesis, prenatal ratings of family conflict modestly predicted child maladjustment up to age 9. This replicates findings from previous studies showing that family conflict is consistently related to maladjustment in childhood. This study extends previous findings by using paternal reports.Thus our findings from family conflict and parental separation analyzed and measured separately confirm previous research showing that both family conflict and parental separation predict child behavioral and emotional problems [3,57], consistent with our first two hypotheses. However, we advanced that research by showing that parental separation was no longer predictive of maladjustment once prenatal parental family conflict was added to the regression model, except for the "separation by age 9" results for mother-reported Total Problems score, consistent with our third hypothesis.

To further test our hypothesis that parental separation might not affect child maladjustment independent of prenatal family conflict, we used the 4-way decomposition model. Results indicated that prenatal family conflict was strongly related to maladjustment. Furthermore, the interaction of prenatal family conflict with separation predicted child maladjustment. High levels of prenatal family conflict increased the vulnerability to the effects of separation on child problem behavior. The observed mediated interaction

effect suggests that family conflict to some extent leads to separation and also interacts with the effects of separation on child problem behavior. This result support the notion that prenatal family conflict to some extent affects child problem behavior through a pathway of parental separation.

An important benefit of the 4-way decomposition model used in this study is the ability to estimate interaction and mediation effects of prenatal family conflict and parental separation on child problem behavior. Although these effects were small in size, both were observed and significant. Whether parental separation has a direct and independent effect on child problems as opposed to family conflict leading to parental separation, which then increases child problems, has to our knowledge not been previously studied. When prenatal family conflict was not included in the model (by setting it to 0), we found no substantive "pure indirect effect' (PIE). In other words, parental separation was not related to child problem behavior in the absence of family conflict.

Thus, our two interaction results support the hypothesis that parental separation did not increase child problem behavior if the level of prenatal family conflict was low. Our sensitivity analyses modelling unobserved confounders underscores these conclusions; the direct effect of prenatal family conflict on child problem behavior increased, whereas the indirect effect decreased. Traditional methods of mediation could not have shown that family conflict both causes separation and also interacts with the effect of separation. Furthermore, many studies have noted considerable difficulties of drawing conclusions about separation [6,7], leading to uncertainty regarding whether family conflict plays a more important role for child problem behavior than parental separation. Our results indicate that parental separation did not have a negative effect on child problem behavior at low levels of prenatal family conflict. Indeed, low family conflict has previously been identified as one of the major protective factors for children's of separated parents [25].

Generally, parental psychopathology and family conflict are closely interwoven and predispose each other [58]. Yet, in the current study, when we adjusted for parental psychopathology we found no change in results. Thus, our findings for the associations between family conflict, separation and child held regardless of other maternal, paternal, child and family factors. Our findings also did not depend on the gender of the parent reporting on the family conflict, which we could test because we obtained both mother and father reports prenatally and at age 9.

The associations of family conflict on the child have often been explained by the effects of parenting stress [13,59], and consequent negative parenting [60,61]. Parental separation also may cause many stressful life changes for children, such as transition to a new home and/or school, changed relations with peers, financial insecurities, and visitation issues [62]. To enable comparison with these studies we also analyzed family conflict and separation independently. While we replicated many findings reported in the literature, parental separation was not associated with child problem behavior after adjustment for family conflict. This is in contrast with some studies, which found that parental separation

independently predicts child problems [25,31,63]. These longitudinal studies found that children of high conflict families that separated experienced improvement in well-being [17,30]. We did not find this in our study perhaps because we used different analytical approaches and ours was not a high risk sample exposed to extremely high levels of conflict.

Finally, our hypothesis about bidirectional associations between child maladjustment and family conflict was also confirmed. Parent-reported family conflict was associated with increases in child maladjustment across childhood and child maladjustment, which in turn, was associated with later family conflict levels. These findings underscore the importance of measuring problem behavior early in childhood, which can help further clarify the directionality of the associations between family conflict and child problem behavior.

Some possible limitations of this study should be discussed. First, we measured parental separation repeatedly only by mother reports. That is, we obtained reports of both mothers and fathers for family conflict as well as child problem behavior, but parental separation was reported only by mothers. However, this can be considered factual information. An important limitation is that information about post-separation family conflict was not available. It is likely that the degree of post-separation family conflict could moderate the effects of separation on children's mental health. Additionally, we should be careful generalizing our findings to clinical populations, as this study was performed in a general population sample. Family conflict and parental separation cannot be easily studied as a cause of child problem behavior. In particular, separation is a predictor or indicator of a process, "a series of dominos cascading in several directions" [64]. At the individual level, once a given family separates one cannot know what the outcome of the children in that family might have been if the separation had not occurred. However, future research might statistically stratify families for the level of family conflict and then compare post separation family conflict and child outcomes in families in which separation then occurred or did not. Lastly, another limitation of this study is the absence of information for children who were exposed to more than one separation and/or divorce as a distinct group.

On the other hand, the study has several strengths. It is a population-based study with a large sample size, which made it possible to take into consideration numerous confounders. We used validated questionnaires with good reliability and validity. We also had repeated measurements of family conflict, parental separation, and child emotional and behavioral problems. Mothers and fathers participated in this study, and information about family conflict and child problem behavior as reported by both parents was available. Thus, our study used multiple informants, which increases the reliability of our findings and reduces the risk of reporter bias. Although we replicated that child problem behavior can increase the risk of family conflict [65,66], our primary conflict measure was prenatal, thus obviating this reverse causality issue in part. Also, we ensured temporal ordering by adjusting for pre-existing child emotional and behavioral problems.

Clinical Implications

Our study has several important clinical implications for prevention and treatment of emotional and behavioral disorders in children. Our findings that both family conflict and parental separation predict child maladjustment and that prenatal family conflict predicted child emotional and behavioral problems up to age 9 underscore that conflict and separation are significant risk factors for children. Practitioners should be aware that if parental separation occurs in families with high levels of conflict, some proactive intervention may be needed to help the children adjust. These children remain at risk for behavioral and emotional problems even after separation. Family counselors and practitioners should address conflict arising around new domestic arrangements, financial problems, parental care or guardianship even after separation. Furthermore, school-based or health-care based screening for emotional and behavioral problems in children experiencing family conflict and /or separation would be helpful as a prevention measure [67].

In cases of severe family conflict, separation is seen by many parents and family counselors as a potential solution. Also, we did not find a positive effect of separation on child behavioral and emotional problems; the association was tentative at best, given the lack of statistical significance and broad confidence intervals. However, because clinicians sometimes do find beneficial effects of separation on children, examination of possible beneficial effects of separation merits further research. The interaction of family conflict and parental separation could be explored in adolescence and incorporated into studies addressing the impact of family conflict on emotional and behavioral problems.

CONCLUSIONS

Using the large and diverse Generation R sample, we found that family conflict from pregnancy onwards and parental separation each strongly predicted child problem behavior up to pre-adolescence according to maternal and paternal ratings. Our use of the four-way decomposition method yielded evidence prenatal family conflict increased the children's vulnerability to the harmful effect of parental separation but no evidence of a beneficial effect of parental separation on child problem behavior. Overall, our findings indicated that if parental separation occurs in families with low levels of conflict, parental separation does not predict more child problem behavior. Moreover, our bi-directional findings suggested that child problem behavior influences the persistence of family conflict.

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POTENTIAL CONFLICTS OF INTEREST

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AVAILABILITY OF DATA AND MATERIALS

Data can be obtained upon request. Requests should be directed to the towards the management team of the Generation R study (secretariaat.genr@erasmusmc.nl), which has a protocol of approving data requests. Because of restrictions based on privacy regulations and informed consent of participants, data cannot be made freely available in a public repository.

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Table 1. Baseline Characteristics for Participants with Information on Family Conflict (FAD)

	Mother *(n=5,808)*	Father *(n=4,561)*
Age, M (SD)	30.9 (4.8)	33.3 (5.3)
Ethnicity		
Dutch, (%)	62.6	67.9
Other Western, (%)	9.3	6.9
Non Western, (%)	28.1	25.2
Education level		
High, (%)	52.4	54.8
Middle, (%)	28.9	25.7
Low, (%)	18.7	19.5
Religion		
Yes, (%)	57.7	
No, (%)	42.3	
Parental psychopathology score, M (SD)	0.26 (0.34)	0.13(0.21)
Gestational age at birth, weeks, M(SD)	39.81 (1.83)	
Gender, (% boy)	49.5	
Family functioning (FAD-score) prenatal, M (SD)	1.54 (0.46)	1.51 (0.39)
Family functioning (FAD-score) at age 5, M (SD)	1.50 (0.41)	
Family functioning (FAD-score) at age 9, M (SD)	1.52 (0.44)	1.49(0.41)
Parental separation by age 3 years		
Yes, (%)	8.2	
Parental separation between age 3-5 years		
Yes, (%)	8.9	
Parental separation between age 5-9 years		
Yes, (%)	7.9	
Parental separation by age 9 years		
Yes, (%)	23.6	
Child problem behavior (CBCL-score) at age 1.5, M (SD)	22.47 (14.7)	
Child problem behavior (CBCL-score) at age 3, M (SD)	20.33 (14.6)	22.34 (15.6)
Child problem behavior (CBCL- score) at age 5, M (SD)	19.16 (16.1)	
Child problem behavior (CBCL- score) at age 9, M (SD)	17.18 (15.0)	17.30 (14.9)

Note: Numbers denotes children included in one or more analyses. Values are frequencies for categorical and means and standard deviations (M ±SD) for continuous measures.

Table 2. Correlation Coefficients Between Family Conflict and Child Problem Behavior

	1	2	3	4	5	6	7	8	9	10
1 Family conflict (FAD) prenatal-mother report	-									
2 Family conflict (FAD) prenatal-father report	.44**	-								
3 Family conflict (FAD) at age 5-mother report	.40**	.28**	-							
4 Family conflict (FAD) at age 9-mother report	.38**	.25**	.53**	-						
5 Family conflict (FAD) at age 9-father report	.25**	.40**	.34**	.44**	-					
6 CBCL Total Problems scores at age 3-mother report	.25**	.13**	.23**	.24**	.15**	-				
7 CBCL Total Problems scores at age 3-father report	.14**	.14**	.13**	.14**	.19**	.55**	-			
8 CBCL Total Problems scores at age 5-mother report	.21**	.13**	.27**	.24**	.15**	.60**	.42**	-		
9 CBCL Total Problems scores at age 9-mother report	.19**	.12**	.20**	.29**	.20**	.43**	.31**	.59**	-	
10 CBCL Total Problems scores at age 9-father report	.11**	.12**	.13**	.17**	.31**	.29**	.41**	.41**	.61**	-

**Correlation is significant at the 0.01 level (2-tailed).

Table 3. Associations between Mother and Father Reported Prenatal Family Conflict and Later Parental Separation

	Parental Separation							
	by age 3		between age 3-5		between age 5-9		by age 9	
	OR (95% CI) (n = 4,174)	*p*	OR (95% CI) (n = 4,821)	*p*	OR (95% CI) (n = 3,771)	*p*	OR (95% CI) (n = 4,543)	*p*
Mother Reported								
Prenatal family conflict (FAD), per score	2.80 (2.20, 3.56)	<.001	2.18 (1.74, 2.72)	<.001	1.32 (1.00,1.74)	.048	2.16 (1.84, 2.53)	<.001
Father Reported								
Prenatal family conflict (FAD), per score	3.14 (2.11, 4.66)	<.001	2.14 (1.51,3.02)	<.001	1.15 (0.77, 1.71)	.476	2.07 (1.62,2.64)	<.001

Note: Binary logistic regression analysis of FAD and separation outcome. OR are averaged from 10 imputed datasets. The models are adjusted for age, ethnicity, education and religion, parental psychopathology, child sex and gestational age at birth reported by mother and father.
Separated mothers by age 3, (8.2%); between age 3-5, (8.9%); between age 5-9, (7.9%); by age 9, (23.6%).

Table 4. The Association of Family Conflict and Child Problem Behavior

	Child problem behavior (CBCL –total score, per point)					
	age 3		age 5		age 9	
	B (95% CI) (n=4,223)	*p*	B (95% CI) (n=5,063)	*p*	B (95% CI) (n=4,062)	*p*
Mother Reported Family Conflict						
Prenatal Family conflict (FAD), per score	5.01 (4.01, 6.02)	<.001	4.20 (3.17, 5.22)	<.001	5.08 (4.01, 6.16)	<.001
Age 5 Family conflict (FAD), per score	-		8.53 (7.49, 9.57)	<.001	6.32 (5.17, 7.48)	<.001
Age 9 Family conflict (FAD), per score	- (n=3,556)		-		9.26 (8.24, 10.2) (n=3,091)	<.001
Father Reported Family Conflict						
Prenatal Family conflict (FAD), per score	3.87 (2.27, 5.47)	<.001	-		3.45 (1.73, 5.16)	<.001
Age 9 Family conflict (FAD), per score	-		-		10.84 (9.61, 12.0)	<.001

Note: Linear regression analysis of FAD and CBCL outcome. Betas are averaged from 10 imputed datasets. The models are adjusted for age, ethnicity, education and religion, parental psychopathology, gestational age at birth and child sex reported by mother and father.

Table 5. The Association of Parental Separation and Child Problem Behavior

	Child problem behavior (CBCL - total score, per point)					
	age 3		age 5		age 9	
	B (95% CI)	*p*	B (95% CI)	*p*	B (95% CI)	*p*
			Mother reported			
	(n=4,223)		(n=5,063)		(n=4,062)	
Separation by age 3, (yes)						
Model 1	1.90 (0.28, 3.52)	.021	1.98 (0.68,3.89)	.042	3.01 (1.03,4.99)	.003
Model 2	1.08 (-1.14, 3.30)	.341	1.65 (-0.97, 4.28)	.218	0.94 (-1.79, 3.68)	.499
Separation between age 3 - 5, (yes)						
Model 1	-		2.58 (0.98, 4.18)	.002	2.24 (0.38, 4.10)	.018
Model 2			1.50 (-0.65, 3.66)	.172	0.84 (-1.55, 3.23)	.490
Separation between age 5 - 9, (yes)						
Model 1	-		-		3.93 (2.07, 5.80)	<.001
Model 2					1.21 (-1.06, 3.48)	.296
Separation by age 9, (yes)						
Model 1	-		-		3.28 (2.08, 4.48)	<.001
Model 2					1.67 (0.12, 3.22)	.034
			Father reported			
	(n=3,556)				(n=3,091)	
Separation by age 3, (yes)						
Model 1	3.29 (0.46, 6.13)	.023	-		4.88 (1.64, 8.12)	.003
Model 2	1.09 (-2.31, 4.49)	.530			2.78 (-1.08, 6.64)	.159
Separation between age 5-9, (yes)						
Model 1	-		-		3.40 (0.93, 5.87)	.007
Model 2					1.27 (-1.63, 4.18)	.391
Separation by age 9, (yes)						
Model 1	-		-		3.05 (1.34, 4.76)	<.001
Model 2					1.13 (-0.92, 3.18)	.280

Note: Linear regression analysis of parental separation and CBCL outcome. Betas are averaged from 10 imputed datasets.
Model 1 is adjusted for age, ethnicity, education and religion, parental psychopathology, gestational age at birth and child sex reported by mother and father. Model 2: model 1 + prenatal family conflict reported by mother and father.

Table 6. Estimates of Direct and Indirect Effects Mediated Through Parental Separation of the Association Between Prenatal Family Conflict and Child Problem Behavior

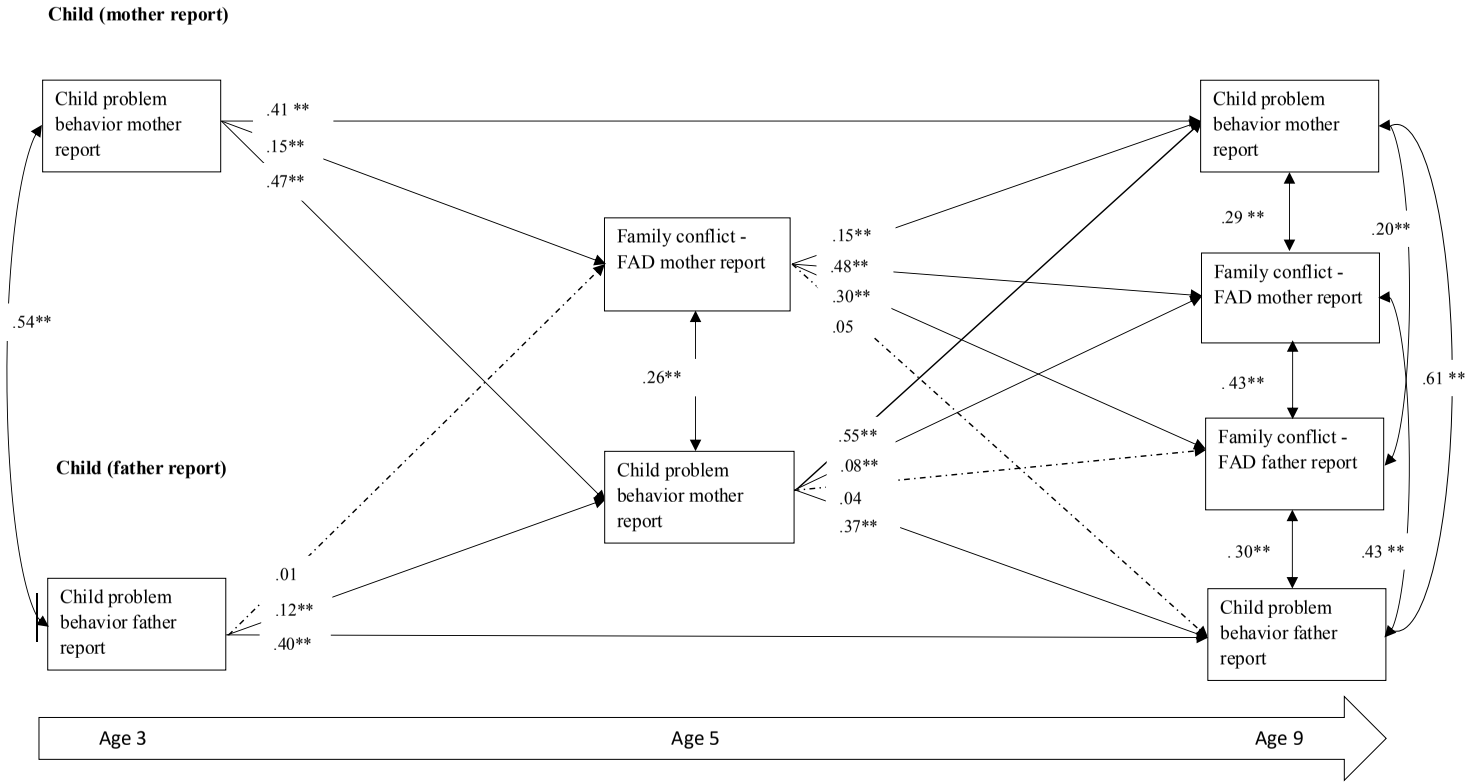
	Child problem behavior (CBCL-total score, per point), (n=3,787)														
	Mother reported														
Mediator: Parental separation															
	Controlled direct effect			Reference interaction			Mediated interaction			Pure indirect effect			Total effect		
		(95% CI)	*p*		(95% CI)	*p*		(95% CI)	*p*		(95% CI)	*p*		(95% CI)	*p*
Family conflict (FAD) prenatal, per score	2.90	(1.69, 4.10)	<.001	0.19	(0.03, 0.33)	.013	0.18	(0.04, 0.31)	.008	-0.14	(-0.37, 0.08)	.206	3.12	(1.94, 4.29)	<.001

Note: The models are adjusted for maternal age, ethnicity, education, religion, maternal psychopathology, gestational age at birth, child sex and prior child problem behavior when child was 1.5 years reported by mother.

CI obtained from delta method standard errors.

Parental separation mediated through prenatal family conflict were estimated as follows: TE= (CDE + INTref + INTmed + PIE), where INTref and INTmed refer to the corresponding betas for controlled direct effect and pure indirect effect mediated through parental separation respectively.

Overall proportions are not presented because the natural direct effect and indirect effect are in the opposite directions.



Note: Structural equation modeling of child problem behavior and family conflict. Numeric values are standardized path regression coefficients averaged from 10 imputed datasets. The models are adjusted for parental age, ethnicity, education and religion, gestational age at birth, child sex and age, prenatal parental psychopathology and prenatal family conflict reported by mother and father, (RMSEA =0.08; CFI=0.99; TLI=0.89).
*p<0.01. **p<0.001.

Figure 1. Bidirectional Associations of Child Problem Behavior and Family Conflict.

SUPPLEMENTARY MATERIAL

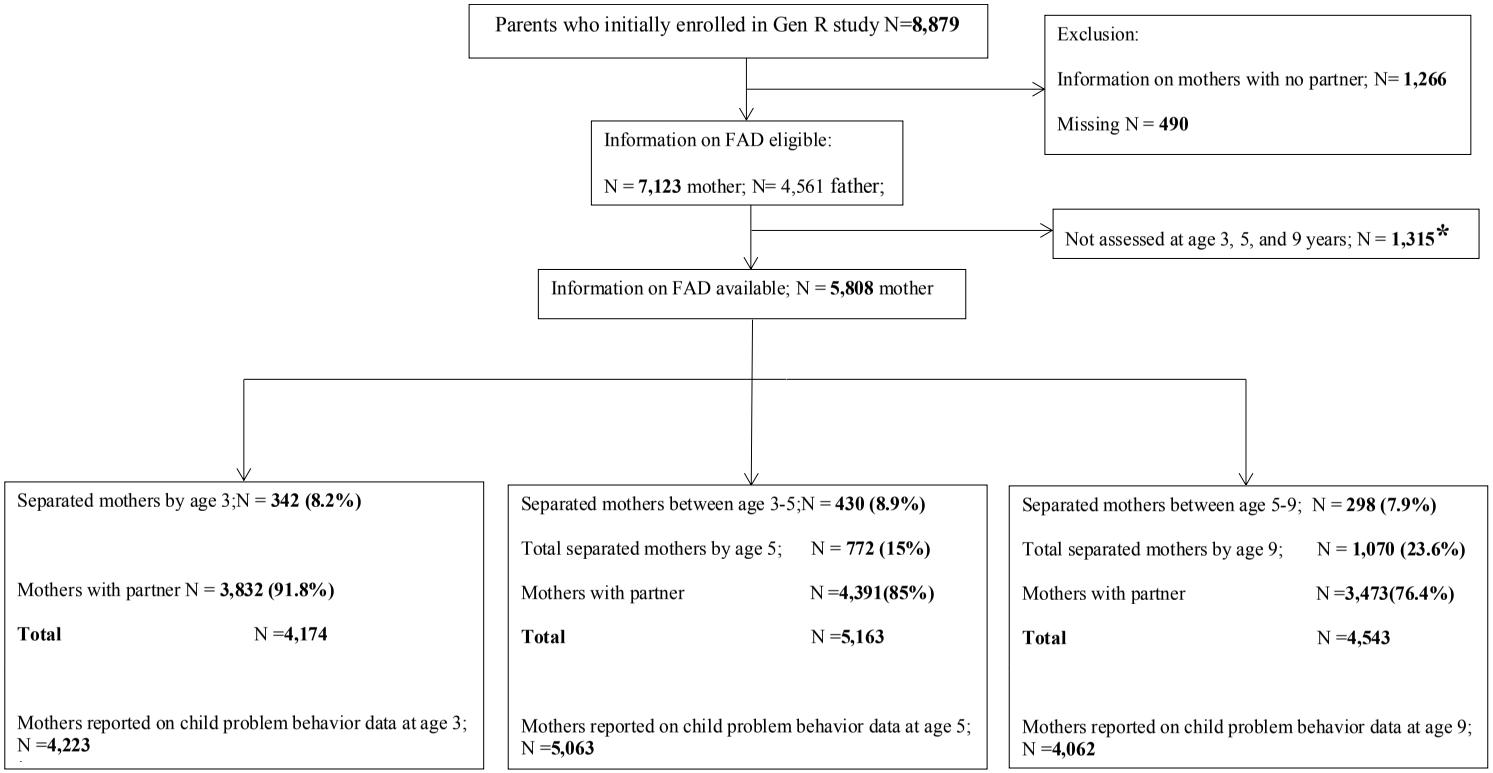


Figure 1. Inclusion of Study Sample

Sensitivity analysis approach Given a hypothetical unmeasured confounder under simplifying assumptions, we assessed how robust our mediation analysis is to violations of unmeasured confounding. The sensitivity parameters of the correlation ρ between parental separation and child problem behavior regressions were tested (Imai et al., 2010). If unobserved variables exist that confound the associations between parental separation and child problem behavior, even after conditioning on the observed prenatal family conflict, we expect that the unmeasured confounding assumption is violated and ρ is no longer zero. The sensitivity analysis was conducted by varying the value of ρ and examining how the estimated Total Natural Indirect Effect (TNIE) changes. The results of the average mediation effect is -0.30, 95%CI: -0.005, 0.40 for a correlation that would reduce the effect of parental separation to zero. That is, the unobserved confounder would have to explain 30% of the variance in the child problem behavior for the estimate of TNIE (natural direct effect - NDE + natural indirect effect - NIE) to be zero (Supplementary, Table 1).

Table 1. Sensitivity Analysis for Unmeasured Confounding (N=3,787)

Average of NDE and NIE	Estimate	(95% CI)	*p*
Natural indirect effect - (NIE)	-0.30	(-0.007, 0.43)	.014
Natural direct effect - (NDE)	3.42	(2.08, 4.23)	.001
Total natural indirect efeffct	3.12	(1.70, 4.18)	<.001

Note: Average mediation effect corresponding to unobserved confounder. Estimates represents the variance explained by the unobserved confounder for the mediator (parental separation) and the outcome (child problem behavior) respectively. The models are adjusted for maternal age, ethnicity, education, religion, maternal psychopathology, gestational age at birth, child sex and prior child problem behavior when child was 1.5 years reported by mother. Natural direct effect estimated as follows: NDE = (CDE+INTref). Natural indirect effect estimated as follows: NIE = (PIE+INTmed).

Painting by Bakir Rokvic (age 11 years)

5 DISCUSSION

Psychiatric disorders and the high prevalence of psychiatric symptoms in childhood place a large short- and long-term burden on these children, their families and the society.[1] [2] To prevent the disease and to decrease the burden, it is of high priority to know the etiology of psychiatric symptoms in early childhood. Considering the developmental origins of these disorders, early life might be a critical window to identify first symptoms and prevent disorders.[3] Early motor and vocalization skills can be observed in the very first months of life. All mothers carefully observe their children during early life, when they start to crawl or walk and they easily worry if a child slows down in this process. Indeed, neuromotor development is a reflection of brain function that predicts child development.[4, 5] Neuromotor delay during infancy can be the first indicator of growth difficulties and behavioral problems in children. Any deviation from typical milestones of motor development in toddlers has always been a concern for parents and pediatricians. Pre-, peri-, and postnatal parental and child risk factors (e.g., pre-gestational body mass index and psychopathology before and during pregnancy) are related to both motor development and child behavior.[6-8] The early neuromotor development has diagnostic and prognostic values for child neurodevelopment. This study increased the insights into disease mechanism underlying impaired development. The knowledge gained can, in return, benefit children at risk of behavioral problems.

MAIN FINDINGS

Non-optimal neuromotor development in infancy and child neurodevelopment

In **Chapter 2**, I combined a series of studies examining neurodevelopment in children with non-optimal neuromotor functioning in infancy. The non-optimal neuromotor functioning of children with neurodevelopmental disorders such as autism spectrum disorder (ASD) has been widely studied. Children with neurodevelopmental disorders (e.g., ASD) are at the tail of the distribution of neurodevelopmental traits (e.g., autistic symptoms). The question is whether infant neuromotor development may also be associated with neurodevelopmental symptoms (cognition and behavior) across the whole distribution in children from the general population. In my studies, I aimed to examine if infant neuromotor development is related to childhood emotional and behavioral problems, autistic symptoms, as well as executive and cognitive functioning in the general population. Furthermore, I explored potential intermediates in this relation. In the Chapter 2.1. I observed an association between overall neuromotor development and cold executive functions (planning, immediate visual memory problems and mental rotation), but not hot executive functions (e.g., emotional control) measured at age 4 years. In addition, non-optimal overall neuromotor development was related to shifting. Using Principal Component Analysis, I showed that shifting is an independent domain

of executive functioning. Furthermore, non-optimal motor development in infancy was related to immediate memory and mental rotation at age 4 years. Piaget was the first to show that early motor experience is important for visuospatial abilities and memory.[9] He found that children develop immediate memory by searching for hidden objects. Infants are able to mentally rotate objects if they had the opportunity to manually explore the object.[10] Optimal neuromotor development is prerequisite for reaching and grasping skills, allowing object exploration as early as age 3 months.

In Chapter 2. 2 I showed that non-optimal neuromotor development, and in particular low muscle tone measured during infancy is associated with autistic symptoms in childhood. Similarly, in a twin study, delays in early learning developmental trajectories in high risk infants (co-twins of ASD children) were predicted by worse stochastic patterns in their spontaneous head movements as early as 1–2 months after birth, relative to low risk infants, who showed a more rapid developmental progress.[11] This observation suggests that an inflexible sensorimotor systems and/or an atypical transition between behavioral states may interfere with the development of the capacity to extract structure and important cues from sensory input at birth. In Chapter 2.3. I studied if neuromotor development measured during infancy predicts child problem behavior repeatedly measured during childhood. In order to answer this question, I applied generalized linear mixed model analysis. Neuromotor development measured at age 9-20 weeks predicted emotional, but not behavioral development to the age 9 years. In particular, low muscle tone and non-optimal senses and other observations predicted withdrawn behavior, anxiety, somatic complains and emotional reactions. The most prominent relationship was with withdrawn problems independent of social communication problems. This means that children can score high on the withdrawn scale because they lack the drive for social interaction, or because of fear and anxiety.

My findings are compatible with Touwen's theory that describes infants with suboptimal neuromotor development, i.e., minor and mild neurological signs, as typically displaying "clumsy behavior" that worsens between 4 and 9 years.[5] Infants with low muscle tone might have difficulties in initiating movement, interactions with the environment, and therefore show symptoms of withdrawn behavior during toddlerhood and school age. Our results suggest that low muscle tone in infancy might be an independent precursor of withdrawn behavior in children.

Further, I explored if specific domains of executive functions measured at age 4 years mediated association between infant neuromotor development and internalizing behavior. Shifting, but not planning, mediated the association between low muscle tone and internalizing problems. Adjustment for pre-existing internalizing problems did not alter my results. Indeed, higher-order cognitive processes such as shifting are important factors in children's vulnerability to psychopathology.[12]

In summary, I found associations between different indicators of non-optimal neuromotor in infancy (e.g., low muscle tone, senses, overall non-optimal neuromotor

development) and children's executive functioning, autistic symptoms, and internalizing problems (withdrawn, anxiety, emotional reactive, somatic complains). The consistent findings on the association of low muscle tone with autistic symptoms and withdrawn problems (reported by both parents) indicate the importance of "clumsiness" during infancy. I showed that low muscle tone is related to internalizing behavior via shifting at age 4 years. Indeed, these more "rigid" children are at a higher risk of developing internalizing problems and in particular withdrawn behaviour.[13, 14] Withdrawn is the most prominent autistic symptom. These children face more problems when adapting to new circumstances during childhood. Several studies have reported evidence for relations between motor skills and developmental problems in seemingly unrelated domains – such as object perception, face processing, and language skills.[15] For example, early experiences of successful reaching at 3 months are associated with infants' attention to faces over objects.[16] The onset of sitting independently at 3–5 months predicts language development at 10 and 14 months.[17] Low muscle tone affects how infants move and develop and may mean that the infants achieve the major developmental milestones late. These infants get upset when confronting new motor tasks and therefore spend less time exploring objects. This cautious/fearful infant behavior style may have long-term consequences for communication and emotional and cognitive development.

Genetic susceptibility for psychiatric disorders and non-optimal infant neuromotor development

In **Chapter 3**, I have further studied whether observed associations between non-optimal neuromotor development during infancy and psychiatric problems in the general population are mostly driven by environmental factors or whether neuromotor abnormalities index genetic predisposition. I found that genetic risk for schizophrenia were associated with non-optimal overall neuromotor development during infancy, whereas no consistent association were observed for bipolar disorder (Chapter 3.1.). I also found that the genetic risk for ASD predicts non-optimal neuromotor development and in particular low muscle tone, while the genetic risk for ADHD predicts non-optimal senses and other observations (Chapter 3.2.). Finally, I explored the mediating role of infant neuromotor development in the association between genetic predisposition to neurodevelopmental disorders (ASD and ADHD) and autistic symptoms. In boys, low muscle tone and senses mediated the association of genetic risk for autism with autistic symptoms, while senses and other observations mediated the association of genetic risk for ADHD and autistic symptoms. The interplay between genetic and environmental factors in neuromotor development is poorly understood. Within the field of genetics, the role of pleiotropy in neurodevelopmental traits and disorders should be explored. It is also unclear if infant neuromotor development mediates the association between genetic susceptibility and neurodevelopmental outcomes. Moreover, any investigation on the relationship between genetic variants and development can be the subject of

distortion by bias, if environmental influences (e.g., maternal psychopathology) are not considered. [18] Large population-based studies, such as Generation R, provide the opportunity to correct for possible confounders, including pre- and perinatal factors as well as parental and child characteristics. Importantly, in the last years, there have been a few observational and experimental studies demonstrating the effectiveness of early interventions targeting neuromotor development to improve behavioral outcomes.[19-21] For example, Libertus et al. showed that systematically varying reaching experiences in 3 months-old infants, who were not reaching on their own, predicted object engagement in a longitudinal follow-up assessment 12 months later.[21] Further, the grasping activity after - but not before - reaching training predicted infants' object exploration 12 months later.[21] These findings provide evidence for the long-term effects of reaching experiences and illustrate the cascading effects initiated by early motor skills.

In summary, the genetic risk for psychiatric disorders predicts infant neuromotor development. While the genetic risk for schizophrenia predicted overall non-optimal neuromotor development, the genetic risk for autism predicts low muscle tone, and the genetic risk for ADHD senses and other observations. Among boys, neuromotor development during infancy mediates the association of genetic risk for ASD and ADHD with autistic symptoms, providing further evidence for a potential causal role in infancy. Early identification of non-optimal neuromotor development in infants with a high genetic risk, followed by early intervention, could potentially reduce autistic symptoms in children.

Early family environment and child behavior

I studied not only infant neuromotor development but also other etiological factors that influence child problem behavior. In **Chapter 4**, we showed that both family conflict from pregnancy onwards and parental separation strongly predicted child problem behavior up to pre-adolescence. Using the 4-way decomposition method we found 1) evidence for a strong direct effect of prenatal family conflict on child problem behavior, 2) evidence for an interaction and 3) evidence for a mediated interaction. The observed interactions imply that at high levels of family conflict and parental separation can increase the risk of child problem behavior. There was less clear evidence of pure mediation suggesting, if any, modest beneficial effects of parental separation on child problem behavior. Sensitivity analysis suggested that conclusions are reasonably robust to unmeasured confounding.

Future studies should clarify if non-optimal neuromotor development mediates these associations between family problems and poor child development. A previous publication using data from Generation R showed an association between maternal stress and infant neuromotor development.[7] Maternal stress is associated with family conflict. [22] Therefore, it is possible that prenatal family conflict influences infant neuromotor development and, in reverse, infants with non-optimal neuromotor development might increase family chaos making families susceptible to separation and children to problem

behavior. In the future, big data will allow a comprehensive approach that can identify not only vulnerable children, but also the window of vulnerability during development, when the intervention would show the best results.

METHODOLOGICAL CONSIDERATIONS

Descriptive, diagnostic, predictive, discovery or/and interventional research?

In general, research is divided into five global categories: descriptive (who, when, where), diagnostic (what is happening), predictive (what is likely to happen in the future), discovery or etiological (why did it happen), and interventional (what should we do about it). Studies of risk factors can belong to more than one of these domains. For example, research on non-optimal neuromotor development can fall into any of all five categories. In this thesis, I have mostly focused on descriptive, predictive, and etiological role of infant neuromotor development. In Chapters 2.1 and 2.2. I **described the associations** between infant neuromotor development and neurodevelopmental outcomes. In Chapter 2.3, I showed how infant neuromotor development **predicts** behavioral problems in childhood, and in Chapter 3.2 and in Chapter 4, I aimed **to explain** if and how neuromotor development measured during infancy mediates the association between a genetic predisposition for neurodevelopmental disorders and autistic symptoms.

Answering each question requires different assumptions and statistical techniques. To answer a research question in diagnostic studies, authors mostly apply diagnostic analytical techniques (e.g., Receiver Operating Curves or likelihood ratio). To answer causal questions in etiological research, I controlled for known and unknown confounding (sensitivity analysis, Chapter 3.2 and Chapter 4). Interventional research questions require a similar approach. Although predictive or diagnostic questions might not require controlling for confounding (confounding can increase the prediction power), in Chapter 2.3. I controlled for known confounders in my prediction models, but I was not very elaborate about unknown confounders. Furthermore, measurable data are often not very accurate representations of their underlying constructs. [23] Therefore, research objectives should be clearly stated in terms of research question and the use of appropriate techniques in order to give an answer. Authors should be required to specify whether they are interested in a diagnostic factor, a prognostic factor, an etiological factor, or interventional effect. [23]

Prediction

In Chapter 2.3. I used generalized linear mixed models (GLMM) to estimate the standardized coefficients (beta) of the association between neuromotor development and internalizing and externalizing scores up to age 10 years. In contrast to causal analysis where

experimental data (e.g., Randomized Clinical Trials) are greatly preferred, in prediction models, observational data are preferable to "overly clean" experimental data, if they better demonstrate the realistic prediction framework in regard to the noise, uncontrolled confounders, and the measured response. Simple models often better predict new data, while complex models are needed to fit the old data.[24] Also, underspecified model might produce more accurate predictions when the sample is small, parameter estimates are small, predictors are highly correlated, or data are very noisy. In contrast, more complex predictive models capture small nuances that enhance predictive accuracy. [25] Hastie T. et al stated: "Typically the more complex I make the model, the lower the bias but the higher the variance."[26]

Variance coming from confounding is informative for prediction, but not for explaning causality. For example, shrinkage methods such as principal components regression and ridge regression, "shrink" predictor coefficients in order to reduce estimation variance. Another class of methods, such as bagging [28], random forests [29], boosting [30], and Bayesian alternatives. [31] are considered the most influential development in Data Mining and Machine Learning. [27] It is also possible to combine several models to create more precise predictions by averaging predictions from different models.[23]

For causal models, it is necessary to enter main effects in a model that contains an interaction term between those effects. This it is not always required in the predictive context, due to the acceptability of uninterpretable models and the potential decrease in sampling variance when dropping predictors. Also, because of the focus in explanatory modelling on causality and on bias, there is a large evidence on detecting reverse causality and solutions like creating instrumental variables. [23]

In the future behavioral research, psychological outcomes should be predicted as dynamics of system change (e.g., sudden vs gradual problem onset) rather than as a category. The novel prediction studies (e.g. joint modeling and time series analysis) should more accurately model the dynamic nature of psychopathology and identify factors, such as interpersonal dynamics and the individual variability in system architecture. This modern statistical approach would enable identifying critical periods of risk and therefore have important treatment implications.[32] The next generation research would identify dynamic nature of neurotomor development as well as critical periods, important for further behavior, enabling proper interventions.

Causality

It is important to realize that some of the strongest correlations may lack any causal relationships. The challenge of causality cannot be addressed through mathematical tools alone (i.e., in absence of direct causal investigations). When a causal research question is addressed, the hypotheses section should include a causal diagram illustrating theoretical background and the hypothesized causal relationship between the constructs (an example is illustrated in Figure 1).

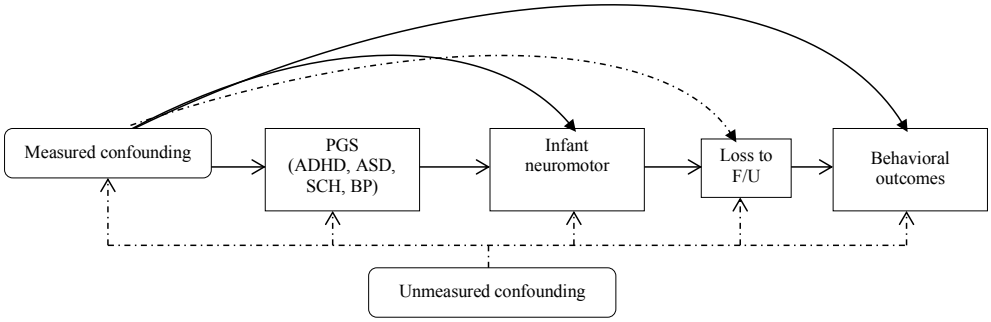


Figure 1. A simplified directed acyclic graph (DAG) depicting the hypothesized causal association between genetic predisposition to psychiatric disorders and behavioral symptoms during childhood with infant neuromotor development as the mediator. The role of both observed and unobserved confounders in these causal pathways are shown, along with loss to follow-up.

The observed association between infant neuromotor development and neurodevelopmental problems may be clarified by three not mutually exclusive mechanisms: (1) an aberrant neuromotor development leads directly to neurodevelopmental problems, (2) neurodevelopmental problems or their precursors (genetic liability) increase the risk of non-optimal neuromotor development, or (3) the association results from confounders—risk factors that predispose to both aberrant neuromotor development and neurodevelopmental problems. The resolution of these mechanisms is crucial because prior evidence demonstrate that early motor development might influence a cascade of events leading to child problem behavior. [9, 21, 33, 34] The possible achievement of intervention programs to minimize the consequences of aberrant neurodevelopment depends on the relative importance of these three mechanisms. Scientists address causal questions using different approaches. The most popular option for testing causality in observational studies is specialized causal inference methods for observational data (e.g., mediation analysis[18] and causal diagrams).[35-38]

In Chapter 2.3, Chapter 3.2 and in Chapter 4, I performed mediation analysis with 99 % bias-corrected bootstraps confidence intervals (CIs) to answer causal questions. I applied 1000 bootstrap samples to identify the indirect effect of exposure on outcomes through proposed mediators. First, I applied 4-way mediation analysis using mediation R package. As there was no evidence for interaction between exposure and mediator in Chapter 2.3 and 3.2, I applied 3-way mediation analysis. I estimated the direct effect (DE), indirect effect (IE) and total effect (TE). In Chapter 3.2., the DE represents the effect of genetic susceptibility on autistic symptoms after controlling for infant neuromotor development, and the IE is the estimated effect of genetic susceptibility operating through infant neuromotor development. The proportion of mediation by infant neuromotor development was calculated as the ratio of IE to TE. Based on *a priori* hypothesis that the prevalence and presentation of autistic symptoms differ between boys and girls,[39] I also studied if the magnitude of the IE depended on sex by formally testing effect modification.

My observations in Chapter 2.3 show that infant neuromotor development and, in particular, infant low muscle tone are associated with emotional problems during childhood through shifting (which we measured at age 4 years). I adjusted this analysis for a spectrum of confounders, including pre-existing emotional problems and therefore ensured the likelihood of temporality affecting the relationship between exposure and outcome. My results pose the question if neuromotor development measured in infancy presents only a marker of behavioral development or whether there is a causal relationship between infant neuromotor functioning and behavioral outcomes. A possible explanation for the causal relationship is that low muscle tone affects how infants move and develop. This may mean that the infants achieve the major developmental milestones with delay. Although, infant neuromotor development is related to later physical activity,[40] we did not include these variables in our final models as it could create collider bias (false positive associations). Both, infant neuromotor development and later physical activity influence child behavior. In addition, behavior, as well as gestational age could be affected by parental pre-pregnancy psychopathology, which in turn affect pre-moto behavior, infant neuromotor development and child behavioral outcomes (see figure 1). This could be a violation of one of the important assumptions in mediation analysis, mediator –outcome confounding affected by exposure. Indeed, parental psychopathology is related to gestational age and weight, and early childhood behavioral measurements, which in turn affect infant neuromotor development and later behavior. [41, 42]

In addition to the method I used in my studies to define causality, other methods for causal inference in observational studies include co-twin studies and applying propensity scores [46, 47].

Finally, although often difficult to implement, the gold standard for addressing causality questions are considered RCT as they allow counterfactual approach and therefore the isolation of mechanisms by which an intervention causes changes. Randomization assures absence of unknown and known confounding in the association between exposure and outcome. In observational studies, this is not guaranteed if subjects may self-select into the treatment group. In these circumstances, a common approach is to gather as many pretreatment confounders as possible so that the ignorability of group assignment is more plausible once the observed differences in these confounders among the treatment and control groups are adequately adjusted. Given the observed confounding, the genetic assignment should be statistically independent of potential mediators and ourcomes. In genetics, this is usually met assumption. [48] Therefore, we did not need to collect many pretreatment confounders for our studies in Chapter 3 as it is usual scenario in observational studies.

Another assumption is questionable even when interpreting results of randomized clinical trials. Although randomization assures absence of unknown and known confounding between exposure and outcome, it cannot guarantee absence of confounding between mediator and outcomes, influenced by exposure.[36] In Chapter 3, among those children

who share the same genetic susceptibility and the same pretreatment characteristics, infant neuromotor assessment as a mediator can be regarded as if were randomized. [48]

Unfortunately, in practice association-based statistical models applied to observational data are frequently used and interpreted as causal, although we often lack an adequate debate on confounding bias. [49] Even when confounding bias is discussed, authors are in general convinced that it is not relevant to their findings and they do not often call for cautious interpretation. When causal question is clearly stated and assumptions are satisfied, causal language is necessary in order to improve the quality of observational research. [50]

I showed that aberrant motor development has at least a partially causal effect on some areas of children's mental health in middle childhood, in particular social withdrawn and internalizing problems. Associations between aberrant neuromotor development and emotional and behavioral problems in children could also reveal a non-causal process underlying familial environmental and/or genetic factors that dispose to both. For example, autistic symptoms and low muscle tone are related through a shared underlying genetic predisposition that makes children with low muscle tone particularly vulnerable to future behavioral problems. The identification of preexisting risk factors that emerge from a shared liability with aberrant motor development is crucial for understanding how to support children with multiple areas of vulnerability.

Our approach is innovative and the results are convincing. There are causal and non-causal processes underlying behavioral problems in children who have aberrant neuromotor development that have direct clinical implications for lowering the effect of non-optimal motor development on the child behavior. These include two approaches: First, reducing motor problems. This can be achieved by directly intervening on child motor functioning and by addressing preexisting risk factors resulting from genetic liability. The identification of such preexisting genetic and/or shared environmental risk factors should be a priority of future research. Second, as the time course of the causal and non-causal influences on aberrant motor development likely differs, the short-term causal effect of aberrant motor development should be contrasted to longer-term effects.

CLINICAL IMPLICATIONS

I have shown that neuromotor development in the general population is genetically determined and can be predicted by risk scores of psychiatric disorders, such as genetic risk for schizophrenia. Therefore, in combination with genetic risk scores, early screening for non-optimal neuromotor development might be a gateway to improving detection of developmental disorders, such as autism spectrum disorder (ASD), developmental coordination disorder (DCD), internalizing problems, and attention-deficit/hyperactivity disorder (ADHD). Recently, several studies, including one randomized trial, demonstrated

the effectiveness and importance of early intervention during neuromotor development, but early screening for developmental disorders in the general population remains a major challenge. [16, 17, 21, 34] It is of high priority to identify novel genetic loci underlying non-optimal neuromotor development in infancy, understand the genetic relationship of neuromotor development with developmental disorders, and importantly, to develop a screening model integrating genetic, clinical, and environmental information.

Future Directions

I would not like to give general advice only, but to present specific research aims for Generation R Next or any new samples. Here is an agenda for future motor research in settings like the Generation R cohort. I recommend:

1. to perform a hypothesis-free genetic association analysis utilizing standard GWAS methodology.
2. to investigate correlations between genetic risk for developmental disorders and neuromotor development using LD score regression.
3. to investigate the pleiotropy between genetic variants and neuromotor traits using individual data of multivariate GWAS from the cohorts.
4. to test the mediating role of infant neuromotor development with four-way decomposition methods.
5. to develop a screening model that enable sensitivity and specificity calculation of a screening program. Within Generation R, cut-offs should be determined for the infant neuromotor development screening tool in order to maximize the trade-off between optimal sensitivity and specificity. This model should be implemented in the Generation R Next and compared to the screening performance of the existing non-validated Van Wiechen Schema used in Youth Health Care NL.

The knowledge gained would advance early diagnosis of developmental problems and support interventions for patients with neuromotor abnormalities. Using a population-based prospective design with objective hands-on assessments of neuromotor development in infancy, combined with genetic information and intrauterine growth data is unique. Because of the potential relevance for very early screening, and treatment of these disorders in infancy, this proposal is of high public health relevance. Finally, this approach would develop screening tool based on not only neuromotor data but also genetic risk and environmental factors. Our findings would have important implications for clinical services and follow-up programs that at the moment rely on the Van Wiechen Schema (VWS) for the assessment of non-optimal development.

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Painting by Bakir Rokvic (age 12 years)

6 SUMMARY/ SAMENVATTING

SUMMARY

Neuromotor development is an accepted mean of measuring the maturity and the integrity of the infant central nervous system (CNS). Motor skills are at the core of everyday actions and interactions during infancy and childhood, affecting physical, perceptual, cognitive, and social development in young children. Therefore, these skills may initiate a cascade of events influencing subsequent development. In this thesis, I focused on the role of infant neuromotor development in relation to neuropsychiatric problems. I have used genetics to further our understanding of these issues. All the studies described in this thesis were performed in the generation R Study, a large prospective population-based cohort in Rotterdam, the Netherlands. In a large subsample of children at age 9-20 weeks, 15 research assistants assessed neuromotor development (tone, responses, senses and other observations) using Touwen instrument. The aims of this thesis were 1) to study how neuromotor development measured in infancy predicts later behavior and cognitive functioning, 2) to examine how genetic susceptibility for psychiatric disorders influences neuromotor development 3) to understand the role of infant neuromotor development in the association of genetic susceptibility for psychiatric disorders and behavioral outcomes during childhood. The main findings, described in chapter 2, 3 and 4 are summarized here.

In Chapter 2, I focused on associations between neuromotor development and later behavior and cognitive functioning. I showed that infant neuromotor development is associated with shifting and cold, but not hot executive functioning (Chapter 2.1). I also observed associations between infant neuromotor development, and in particular low muscle tone with autistic symptoms (Chapter 2.2). I further found that infant neuromotor development (low muscle tone and senses) predicts internalizing, but not externalizing symptoms during childhood.(Chapter 2.3) Shifting partly mediated association between low muscle tone and later internalizing symptoms. (Chapter 2.3). The consistent findings on the association of low muscle tone with autistic symptoms and withdrawn problems (reported by both parents) indicate the importance of "clumsiness" during infancy. These "clumsy" children with minor neurological deficit that usually do not interfere with daily life, are at a higher risk of developing internalizing problems and in particular withdrawn behavior. Low muscle tone affects how infants move and develop and may mean that the infants achieve the major developmental milestones late. These infants get upset when confronting new motor tasks and therefore spend less time exploring objects. This cautious/fearful infant behavior style may have long-term consequences for communication and emotional and cognitive development.

In Chapter 3, I explored the rule of infant neuromotor development in relation to genetic susceptibility for neuropsychiatric disorders and autistic symptoms. A higher genetic risk for schizophrenia and a lower genetic risk for bipolar disorder was associated with non-optimal overall neuromotor development during infancy (Chapter 3.1.). I also found that the genetic risk for ASD predicts non-optimal neuromotor development and in

particular low muscle tone, while the genetic risk for ADHD predicts non-optimal senses and other observations in boys (Chapter 3.2.). Infant neuromotor development mediates the association of genetic risk for ASD and ADHD with autistic symptoms. In particular, low muscle tone mediates the association of genetic risk for ASD with autistic symptoms, while senses and other observations mediate association of genetic risk for ASD and ADHD with autistic symptoms. Early identification of non-optimal neuromotor development in infants with a high genetic risk, followed by early intervention, could potentially reduce autistic symptoms in children.

In Chapter 4 I studied not only infant neuromotor development but also other etiological factors that influence child problem behavior. In Chapter 4, we showed that both family conflict from pregnancy onwards and parental separation strongly predicted child problem behaviour up to pre-adolescence. Using the 4-way decomposition method we found 1) evidence for a strong direct effect of prenatal family conflict on child problem behavior, 2) evidence for reference interaction and 3) evidence for mediated interaction. These interactions imply that at high levels of family conflict and parental separation can increase the risk of child problem behaviour. There was no evidence of pure mediation suggesting no beneficial effects of parental separation on child problem behavior. Future studies should clarify if non-optimal neuromotor development mediates these associations.

In Chapter 5, I discussed findings of the studies provided in this thesis. Furthermore, I discussed major methodological considerations, as well as major implications and offered directions for future research.

SAMENVATTING

Neuromotorische ontwikkeling is een veelgebruikte manier om de rijping van het centrale zenuwstelsel (CZS) van het kind te meten. Motorische vaardigheden zijn de kern van alledaagse handelingen en interacties, met name gedurende de kindertijd. De motorische vaardigheden zijn van invloed op de fysieke, perceptuele, cognitieve en sociale ontwikkeling van jonge kinderen. Dit is de reden dat deze vaardigheden een aaneenschakeling van gebeurtenissen in gang zetten die de verdere ontwikkeling van het kind beïnvloeden. In dit proefschrift bestudeerde ik de rol van neuromotorische ontwikkeling van kinderen in relatie tot neuropsychiatrische problemen. Ik heb gekeken naar genetica om het begrip van deze problemen te verbeteren. De studies beschreven in dit proefschrift zijn uitgevoerd binnen de Generation R Studie, een groot prospectief cohort binnen de algemene populatie in Rotterdam. In een grote groep kinderen van 9-20 weken oud werd de neuromotorische ontwikkeling (tonus, reacties, zintuigen en andere observaties) beoordeeld door 15 onderzoeksmedewerkers met behulp van de Touwen neurologische ontwikkelingsschaal voor zuigelingen. De doelen van dit proefschrift

waren: 1) bestuderen hoe vroeg in de kindertijd gemeten neuromotorische ontwikkeling later gedrag en cognitief functioneren voorspelt; 2) onderzoeken hoe genetische kwetsbaarheid voor psychiatrische stoornissen de neuromotorische ontwikkeling beïnvloedt; 3) de rol van neuromotorische ontwikkeling van zuigelingen in de associatie van genetische kwetsbaarheid voor psychiatrische stoornissen en gedragsuitkomsten gedurende de kindertijd. De belangrijkste bevindingen, beschreven in hoofdstuk 2, 3 en 4, zijn hier samengevat.

In hoofdstuk 2 heb ik me gericht op de associaties tussen neuromotorische ontwikkeling en later gedrag en cognitief functioneren. Ik toonde aan dat neuromotorische ontwikkeling van zuigelingen is geassocieerd met slechter executief functioneren zoals moeilijkheden met shifting en auditieve aandacht, maar niet met andere executieve functies (hoofdstuk 2.1). De consistente bevindingen over de associatie van lage spierspanning met autistische symptomen (gemeld door beide ouders) wijzen op het belang van "onhandigheid" tijdens de kindertijd. Deze "onhandige" kinderen kunnen een minimaal neurologisch tekort hebben, wat gewoonlijk niet interfereert met het dagelijks leven. Een lage spierspanning beïnvloedt hoe baby's bewegen en zich ontwikkelen, dit kan betekenen dat de baby's de belangrijkste ontwikkelingsmijlpalen later bereiken. Deze baby's raken van slag als ze nieuwe motorische taken moeten uitvoeren en zullen daarom minder tijd besteden aan het exploreren van objecten. Deze voorzichtige/angstige gedragsstijl van het kind kan op lange termijn gevolgen hebben voor de communicatie en de emotionele en cognitieve ontwikkeling (hoofdstuk 2.2). Ik heb verder vastgesteld dat de neuromotorische ontwikkeling van de zuigelingen (lage spierspanning en -zintuigen) internaliserende, maar niet externaliserende symptomen tijdens de kindertijd voorspellen. Deze associatie wordt gedeeltelijk gemedieerd door een lage spierspanning (hoofdstuk 2.3).

In hoofdstuk 3 heb ik de rol van neuromotorische ontwikkeling van kinderen onderzocht in relatie tot genetische kwetsbaarheid voor neuropsychiatrische stoornissen en autistische symptomen. Een hoger genetisch risico voor schizofrenie en een lager genetisch risico voor een bipolaire stoornis was geassocieerd met een niet optimale algehele neuromotorische ontwikkeling tijdens de jonge kindertijd (hoofdstuk 3.1.). Ik vond ook dat het genetische risico voor autismespectrumstoornis (ASS) een sub-optimale neuromotorische ontwikkeling, in het bijzonder een lage spierspanning, voorspelt. Het genetische risico voor aandachtstekort-hyperactivitieitsstoornis (ADHD) voorspelt sub-optimale sensomotoriek bij jongens (hoofdstuk 3.2). De neuromotorische ontwikkeling van zuigelingen medieert de associatie van genetische risico's voor ASS en ADHD met autistische symptomen. Vroege identificatie van sub-optimale neuromotorische ontwikkeling bij zuigelingen met een hoog genetisch risico, gevolgd door vroege interventie, zou mogelijk autistische symptomen bij kinderen kunnen verminderen.

In hoofdstuk 4 heb ik niet alleen de neuromotorische ontwikkeling van de baby bestudeerd, maar ook andere etiologische factoren die het gedrag van kinderen beïn-

vloeden. Zowel familieconflicten vanaf de zwangerschap als de scheiding van ouders voorspelden het probleemgedrag van kinderen tot de pre-adolescentie. Met behulp van de 4-weg-decompositiemethode vonden we 1) bewijs voor een sterk direct effect van prenatale familieconflicten op probleemgedrag bij kinderen, 2) bewijs voor referentie-interactie en 3) bewijs voor gemedieerde interactie. Deze interacties suggereren dat veel conflict in het gezin en scheiding van de ouders het risico op gedragsproblemen bij kinderen kan vergroten. Er was geen bewijs voor pure mediatie, wat suggereert dat er geen gunstige effecten zijn van de scheiding van de ouders op het probleemgedrag van kinderen. Toekomstige studies moeten duidelijk maken of een sub-optimale neuromotorische ontwikkeling deze associaties medieert.

In hoofdstuk 5 besprak ik de bevindingen van de studies die in dit proefschrift zijn beschreven. Verder besprak ik belangrijke methodologische overwegingen, evenals belangrijke implicaties en bood ik aanbevelingen voor toekomstig onderzoek.

Painting by Bakir Rokvic (age 12 years)

7 APPENDICES

Authors and affiliations

List of publications

PhD portfolio

Final words

About the author

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List of publications

SCIENTIFIC PAPERS

1. Soares HP, Kumar A, Daniels S, Swann S, Cantor A, Hozo I, Clark M, **Serdarevic F**, Gwede C, Trotti A, Djulbegovic B. Evaluation of new treatments in radiation oncology: are they better than standard treatments? JAMA. 2005;293:970-8.

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20. Dizdarevic J, Ridanovic Z, **Serdarevic F**, et al. Evidence-based guideline on abortion procedures, UNFPA, BH office, 2007.

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24. Jatić Z, **Serdarević F**, Designing Effective Center for Continuing Medical Education at the Faculty of Medicine, University of Sarajevo, Bosnia and Herzegovina, Folia Med. Fac. Med. Univ. Saraeviensis 2015; 50(2): 127-12

PhD Portfolio

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Erasmus MC Department: Child and Adolescent Psychiatry/Psychology
Research School: Netherland Institute for Health Sciences (NIHES)
PhD period: July 2014-March 2019
Promotors: Prof. dr. Henning Tiemeier, Prof. dr. Frank C. Verhulst
Copromotor: Dr. Akhgar Ghassabian

1. PhD training	Year	ECTS*
General academic skills		
Research Integrity	2017	2.0
Advanced Medical Writing and Editing	2016	0.7
Writing Successful Grant Proposals	2017	0.7
In-depth courses		
Netherland Institute for Health Sciences		
Psychiatric Epidemiology	2015	0.7
Principals of Genetic Epidemiology	2017	0.7
Genomics in Molecular Medicine	2017	0.7
Joint Models for Longitudinal and Survival Data	2016	0.7
Genome-Wide Association Studies	2017	1.4
Topics in Meta-analysis	2016	0.7
Clinical Trials	2016	0.7
Methods of Health Services Research	2016	0.7
Human Epigenomics	2016	0.7
Repeated measurements in Clinical Studies	2014	1.4
Screening	2017	0.7
Preventing Failed Interventions in Behavioral Research	2014	1.4
Methods of Public health Research	2014	0.7
Quality of Life Measurements	2014	0.9
Conceptual Foundation of Epidemiological Study Design	2014	0.7
Principals of Epidemiologic Data-Analysis	2014	1.4
Bayesian Statistics	2014	0.7
Causal Mediation Analysis	2014	0.7
Other courses		
Harvard T.H. Chan		
Short Course on Causal Inference	2018	
Presentations		
18thEPA Meeting in Epidemiology and Social Psychiatry, Gothenburg, Sweden (Oral presentation)	2016	1
Society for Epidemiological Research annual Meeting, Baltimore, USA	2018	1
Joseph Dancis Pediatrics Research Day, New York, USA	2018	1
Wetenschapslunch Psychiatry, Rotterdam, NL (Oral presentation)	2018	0.5
Cochrane Collaboration, Sarajevo, B&H (Oral presentation on EBM guideline adaptation)	2015	1

Workshop organized by Agency for Healthcare Quality and Accreditation , Tuzla, B&H (Oral presentation on evidence based medicine)	2018	0.5
Regional conference led by Hubert H. Humphrey Alumni "Brain Gain in the Balkans: Economic and Development Strategies for a Better Future", Zagreb, Croatia	2019	
Internships, Seminars and Workshops and other activities		
Fellowship in Department of Environmental Pediatrics, New York University, USA	2018	
Research Meetings, the Generation R Study Group	2016-2018	1
Research grants		
Eraweb PhD scholarshio		
Eraweb postdoctoral scholarship		
KNAW Ter Meulen Beurs, personal grant		
Reviewing papers		
Bosnian Journal of Basic Science	2015	0.2
Bosnian Journal of Basic Science	2016	0.2
European Journal of Epidemiology	2018	0.5
Pediatrics	2019	0.5
Other activities		
Established the Association: South-East European Network for Medical Research-SOVE (board member)	2018	

2. Teaching activity		
Supervision of students		
Supervising Emina Zoletic, Master student, Erasmus MC	2017	4
Project title: High Infant Muscle Tone and Attention Deficit Hyperactivity Disorder		
Supervising Lejla Halilovic Master student International University of Sarajevo	2016	2
Project title: Analysis of *Irs1* Genetic Variations In Type 2 Diabetic Patients from Bosnia and Herzegovina		
Supervising Nur Hamad Master student, International University of Sarajevo	2016	2
Project title: Genetic variation of ADRA2A in type 2 Diabetes		
Supervising Sanne Buitendijk and Daniël Dumas, Medical students, Erasmus MC	2014	2
Project title: Early Neuromotor Development and Autism Spectrum Disorder		
Other activities		
Senior teaching assistant at the Institute of Epidemiology and Biostatistics, Sarajevo, B&H. Teaching epidemiology and introduction to biostatistics	Until 2016	
Senior research associate at the University of Sarajevo, B&H. Contributed to the development of Continuing Medical Educational (CME) Program	2015-2016	
Introduced the following CME courses:		
Henning Tiemeier, Fadila Serdarević: Study Design I		
Henning Tiemeier: Basics of Psychiatric Research		
Fadila Serdarević, Zoran Riđanović, Ahmed Novo: How to adapt EBM guidelines		

Costanza Pizzi, Daniela Zugna, Fadila Serdarević : Causal inference methods in epidemiology *Reference: Jatić Z, Serdarević F, Designing Effective Center for Continuing Medical Education at the Faculty of Medicine, University of Sarajevo, Bosnia and Herzegovina, Folia Med. Fac. Med. Univ. Saraeviensis 2015; 50(2): 127-12*

*1 ECTS (European Credit Transfer System equal to workload of 28 hrs)

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My committee members: Prof.dr. M. A. Ikram, Prof. dr. S. Kushner and, Prof.dr.M.A.J. de Koning-Tijssen, I would like to thank you for revising and evaluating my thesis. My special thanks to Prof. dr. M. A. Ikram who accepts to be the Secretary of the reading committee.

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Haley Desai, we all admire your beautiful cover painting "Öctopicasso" (one should notice small octopus on the shoulder). You are only 10 year old girl now, but it is already obvious that you will grow into a great artist.

My sincere and deep appreciation goes to my friend Vanja Resić in Sarajevo. With Vanja's permission, this book features her son Bakir's artwork. Bakir has autism spectrum disorder and was diagnosed with ASD at the age of 4 years. We could track Bakir's art through this book from age 5 till age 12. One can observe significant progress and happiness on his later paintings. Bakir is now 13 years old and just started regular primary school. Today, he is a very social child. Vanja has been successfully treating her son Bakir by following the Son-Rise program that incorporates motor interventions (fine and gross) in addition to abundance of trust and love.

Without my family: my mom, my aunt Đena, my sister Selma, my brother Mustafa, their kids Hamza, Arif, Kerim and Ajna and my cousins Dunja, Tarik, Slađa, this journey would not had been possible!

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About the author

Fadila Serdarevic is a medical doctor with extensive training specializing in the areas of evidence-based guideline development, surveillance, neuropsychiatric and disaster epidemiology. Her academic credentials include a M.D. from the University of Sarajevo, Bosnia and Herzegovina, a MPH from Tulane University, New Orleans, Lousiana, a M.Sc and D.Sc in epidemiology from the Erasmus MC University, Netherlands (NIHES). Her doctoral study at Erasmus MC under the supervision of prof. Henning Tiemeier, examines causal relationship between infant neuromotor development and the psychiatric effects. Her postdoctoral training at New York University under supervision of Dr. Akhgar Ghassabian focuses on environmental epidemiology.

Fadila is an alumna of Hubert Humphrey Fellowship in Public Health, a prestigious and competitive professional Fulbright program and also a graduate of the Center for Disease Control (CDC)'s special professional Epidemic Intelligence Service program in disease surveillance and outbreak investigation. She has been a long-term consultant with the International Center for Migration, Health and Development (ICMHD) on disaster response and non-communicable disease epidemiology. In addition to being a doctor, researcher, consultant, scholar and a student, Fadila is also a seasoned epidemiology lecturer. Fadila is especially interested in further exploring the causal inference methods and her long-term plan is to lead a first chronic PTSD cohort in the Balkans operating out of Sarajevo.