Long-term associations between early-life family functioning and preadolescent white matter microstructure.

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ABSTRACT

Objective: Causes of childhood behavior problems remain poorly understood. Enriched family environments and corresponding brain development may protect against their onset, but research investigating white matter neurodevelopmental pathways explaining associations between the family environment and behavior remains limited. We tested whether healthier early-life family functioning would be associated with lower global mean diffusivity (MD) and higher global fractional anisotropy (FA) in preadolescence, which have been associated previously with reduced problem behaviors.

Methods: Data are from 2,653 children in the Generation R Study in Rotterdam, the Netherlands. Mothers reported family functioning using the McMaster Family Assessment Device (range 1 – 4, higher scores indicate healthier functioning) both prenatally and in mid-childhood (mean age 6.0 years). In preadolescence (mean age 10.1), the study collected diffusion-weighted scans. We computed standardized global (i.e., multi-tract mean) MD and FA values by averaging metrics from 27 white matter tracts and used adjusted OLS models to examine global and tract-specific outcomes.

Results: Estimates from fully adjusted, weighted models for a one-unit increase in prenatal family functioning score were $\beta_{\text{global MD}}$ = -0.13 (95% CI: -0.25, -0.02) and $\beta_{\text{global FA}}$ = 0.10 (95% CI: -0.01, 0.21). These magnitudes were 76% and 57% of estimates associated with a one-year increase in age at scan for global MD and FA, respectively. Tract-specific analyses supported these global findings. We found no evidence of an association between mid-childhood functioning and global outcomes.

Conclusion: Healthy early-life family functioning may induce white matter microstructural differences in preadolescence linked previously to reduced problem behaviors.

INTRODUCTION

The origins of child behavior disorders remain poorly understood. Increasingly, investigators have called for a population neuroscience approach both to identify factors shaping brain structure and function, and to understand how variations in the brain cause child behavior problems.[1–3] Empirical studies suggest elements of the social environment impact brain development in both positive and negative ways, with effects on aspects of brain function that have been associated with behavior problems.[4–6] Neuroscience research often characterizes the childhood social environment as a monolithic experience measured by parental socioeconomic status. In contrast, social scientific models of the social environment include experiences related to one's family, friends, schools, organized activities, neighborhood, and place of worship.[7] The relative importance of these domains may change throughout childhood, with the family environment most influential early in life. As such, a healthy early-life family environment may drive healthy brain development and protect against behavior disorders.

However, the neurodevelopmental effects of family-based exposures have not been thoroughly explored. Among studies in this area, most focus on family dysfunction and its link to poor outcomes.[8] For example, a broad spectrum of research links child maltreatment, which occurs most often within the family environment, to structural alterations in corticolimbic regions of the brain involved in cognitive and affective processes underlying behavior problems.[9–11] Similarly, functional imaging studies report that family conflict is associated with increased adolescent risk-taking behavior.[12,13]

In contrast to research on family dysfunction, some neurodevelopmental studies assess positive family-based experiences, which may confer benefits beyond those associated with a mere absence of negative exposures. For example, greater maternal support and positive parenting behavior have been associated with brain structural changes thought to be advantageous, including accelerated hippocampal growth in childhood and adolescence, and attenuated amygdala growth in adolescence.[14,15] Some functional imaging studies also report associations between healthy parent-child relationships, decreased risk taking behavior, and increased cognitive control in adolescence and early adulthood.[16–18]

These studies are limited insofar as they focus on parenting practices—typically maternal practices—rather than on broader measures of overall family functioning that may capture important characteristics within a complex family ecology. Many of these studies also assess aspects of the family environment during a narrow time period in a child's life. As a result, they cannot quantify how the family environment's influence may change throughout childhood. And despite the importance of white matter to

healthy brain development, prior imaging studies of family-based exposures assess only brain function or gray matter structural outcomes.

Studies suggest both negative and positive experiences occurring prenatally, postnatally, and in childhood alter white matter structural development.[19,20] These studies generally report associations between negative exposures (e.g., maternal prenatal anxiety) and properties of white matter microstructure that may decrease neural efficiency, and between positive ones (e.g., breastfeeding) and the opposite.[21–25] Studies associating childhood experiences with white matter microstructure differences are complemented by a separate body of mostly cross-sectional research associating white matter microstructure with behavioral outcomes. In these studies, microstructural properties related to more efficient neural processing are generally associated with fewer behavior problems, while microstructural properties related to less efficient neural processing are associated with antisocial behavior, attention deficit hyperactivity disorder, bipolar disorder, and disruptive behavior problems.[20,26,27]

To investigate whether a positive family environment may impact white matter microstructure, this study used prospective data from the Generation R Study, a population-based birth cohort tracking child development from pregnancy through adolescence. Study staff collected data on family functioning from mothers prenatally and in mid-childhood, and their children completed an MRI brain scan in preadolescence. We hypothesized that more positive family functioning at each time point would be associated with more organized white matter microstructure across all areas of the brain (i.e., global effects), even after extensive adjustment for plausible confounders selected based on prior literature and theory.[20]

METHODS

Participants

This study uses data from the Generation R Study, a prospective, population-based birth cohort in Rotterdam, the Netherlands, seeking to identify social, environmental, and genetic factors affecting child health and development.[28] The Generation R Study enrolled 8,879 pregnant women living in Rotterdam between 2002 and 2006 and another 898 women at the birth of their child during the same time period. Study researchers have collected data through clinic visits and postal questionnaires from children and their caregivers at multiple time points through the present after securing written informed consent and assent from all participants. All study protocols are approved by the Medical Ethics Committee of the Erasmus University Medical Center.

Women completed a postal questionnaire about their family functioning prior to the birth of their enrolled child (gestational age range 18 – 25 weeks) and again when their child was in mid-childhood (mean age 6.0 years; range 4.0 – 9.1 years). Mothers enrolled at the birth of their child (i.e., not while pregnant) completed only the mid-childhood questionnaire. In sum, 8,271 women completed at least one of these questionnaires. Later, study researchers obtained diffusion-weighted magnetic resonance imaging (DWI) scans from 3,992 children in preadolescence (mean age 10.1 years; range 8.6 – 12.0 years).[29] The current study included participants if they had a usable DWI scan with no missing tract-specific scalar data (described below) and either prenatal or mid-childhood family functioning data. Among these participants, we excluded those whose mothers reported using cocaine or heroin while pregnant. And because Generation R includes a number of twins and triplets, we randomly selected only one sibling for inclusion in these cases. Our final analysis sample included 2,653 children. Supplement Section 1 details selection into our analysis sample.

Measures

Family Functioning

To measure family functioning, parents completed the McMaster Family Assessment Device, General Functioning Subscale.[30] This is a self-report survey of established reliability and validity in Dutch and several other populations, in which parents respond on a 4-point Likert scale to 6 positively framed and 6 negatively framed items.[31–33] Representative questions include, "If there are problems, we can count on each other for support," and, "There are a lot of unpleasant and painful feelings in our family." Because these questions do not reference specific family members or roles, parents can respond regardless of their family's structure. We derived a family functioning score at each time point by reverse-scoring negatively framed items, then averaging response scores across all 12 items to yield a family functioning score range of 1 to 4 for each participant and time period, where higher scores indicated more positive functioning. Cronbach's alpha in the analytic sample was strong (0.89) at both prenatal and mid-childhood time periods.

Brain Imaging

Generation R researchers have described diffusion-weighted imaging collection protocols and preprocessing pipelines elsewhere.[29,34] All DWI images were acquired using a 3T GE MR-750W scanner (General Electric, Milwaukee, Wisconsin) and an eight-channel head coil. Sequence parameters yielded 2 mm isotropic resolution and 35 diffusion-weighted volumes. Study staff preprocessed the resulting images using the FMRIB Software Library (FSL), version 5.0.9, and the FSL AutoPtx plugin to compute tract-specific scalar metrics of white matter microstructure, including mean diffusivity

(MD), fractional anisotropy (FA), axial diffusivity (AD), and radial diffusivity (RD) for 27 white matter tracts. These included three brainstem tracts (middle cerebellar peduncle; left and right medial lemniscus), ten projection fibers (left and right corticospinal tracts and acoustic radiations, and bilateral anterior, posterior, and superior thalamic radiations), eight association fibers (bilateral superior and inferior longitudinal fasciculi, and bilateral inferior fronto-occipital and uncinate fasciculi), four limbic system fibers (left and right cingulate gyrus part of the cingulum and parahippocampal part of the cingulum), and two callosal fibers (forceps major and forceps minor).[35] Supplement Section 2 further details scan acquisition and processing. Researchers inspected all raw images, selected tractography data, and slice signal intensities to assess scan quality. Scans deemed poor quality were excluded from analysis.

Following prior research on white matter microstructure, we focused our primary analyses on two measures, MD and FA.[36,37] MD is a measure of the extent to which water molecules in white matter tissue move freely in all directions. FA assesses the extent to which white matter microstructure constrains water molecule diffusion in a single direction. In post hoc analyses, we also assessed AD and RD, which quantify how much water molecules are able to move in certain directions.[38] All four measures provide complementary information from which inferences about white matter microstructural anatomy can be made. As children age, MD values decrease, and FA values increase.[20] Lower MD and higher FA values suggest more organized white matter, which in turn may enable more efficient neural functioning.[20]

Because complex human behavior manifests from coordinated neural activity across many distinct brain regions connected by many different white matter tracts, we constructed multi-tract mean measures of white matter microstructure incorporating information from all 27 tracts delineated by AutoPtx by averaging and standardizing all tract-specific MD, FA, AD, and RD values (hereafter referred to as "global" or "multi-tract mean" values). While tracts vary substantially in size, calculating arithmetic means ensured each tract contributed equal information to our "global" outcomes regardless of its size, which enabled us to test our primary hypothesis that family functioning affects all (or nearly all) white matter tracts in the brain.

Separately, for the 24 tracts with analogues in both hemispheres (e.g., left and right uncinate fasciculus), we averaged and standardized measures from both hemispheres. For example, we averaged left and right MD values for each participant's uncinate fasciculi, resulting in a single mean MD value for the uncinate fasciculus. Because three tracts (middle cerebellar peduncle, forceps major, and forceps minor) do not have independent analogues in both hemispheres, this process resulted in 15 sets of tract-specific values used in our analyses.

Covariates

Researchers retrieved child birthdate and sex data from birth records. Parents self-reported the following: their country of origin and ethnicity, which we used to categorize child ethnicity as European (including Dutch but excluding Turkish), Turkish, Moroccan, and Other Ethnicity; household income during pregnancy (more or less than €2200 / month); highest maternal and paternal completed education level at study enrollment (< high school equivalent; high school or intermediate vocational training; advanced vocational training, bachelor's degree, or higher); maternal and paternal history of psychotic episodes (yes, no); maternal age at childbirth; maternal smoking history during pregnancy (never, until pregnancy was known, or through pregnancy); and parental psychopathology symptoms at two time points: (1) prenatally, included in models of prenatal family functioning and measured using the full 53-item Brief Symptom Inventory (BSI); and (2) at child age 3 years, included in models of mid-childhood family functioning and measured using a subset of the 21 items from the BSI available at that time point.[39] We calculated continuous BSI sum scores for each parent at each time point. We considered paternal-report education, psychosis, and psychopathology symptoms in addition to the maternal factors as covariates because paternal factors may also confound the relationship between maternal-report family functioning and child white matter structure.

Statistical Analyses

We assessed and removed as appropriate outliers in MD, FA, AD, and RD using standard methods (n = 162 removed). Supplement Section 3 details our methods and rationale.

To investigate whether family functioning was associated with our primary measures of white matter microstructure (i.e., MD and FA only), we used ordinary least squares linear regression. We imposed a hierarchical structure to these analyses with initial models examining global multi-tract mean outcomes and subsequent models evaluating specific tracts. We adjusted p-values for multiple comparisons in tract-specific models via the Simes procedure.[40] For each outcome, we fit (1) minimally adjusted models accounting for each child's age at scan, sex, and ethnicity; and (2) fully adjusted models adding all other covariates listed above. We ran separate models to assess associations with prenatal and mid-childhood family functioning, after which we considered models including functioning scores from both time points simultaneously. We also fit fully adjusted models weighted to account for differential loss to follow-up by important sociodemographic characteristics (see below for details of inverse probability weights). In post hoc analyses, we followed the same analysis plan for global AD and global RD.

We conducted several sensitivity analyses with respect to our primary outcomes (i.e., global multi-tract mean MD and FA). First, we evaluated whether prenatal family

Figure 1. Associations between prenatal family functioning and tract-specific FA and MD.

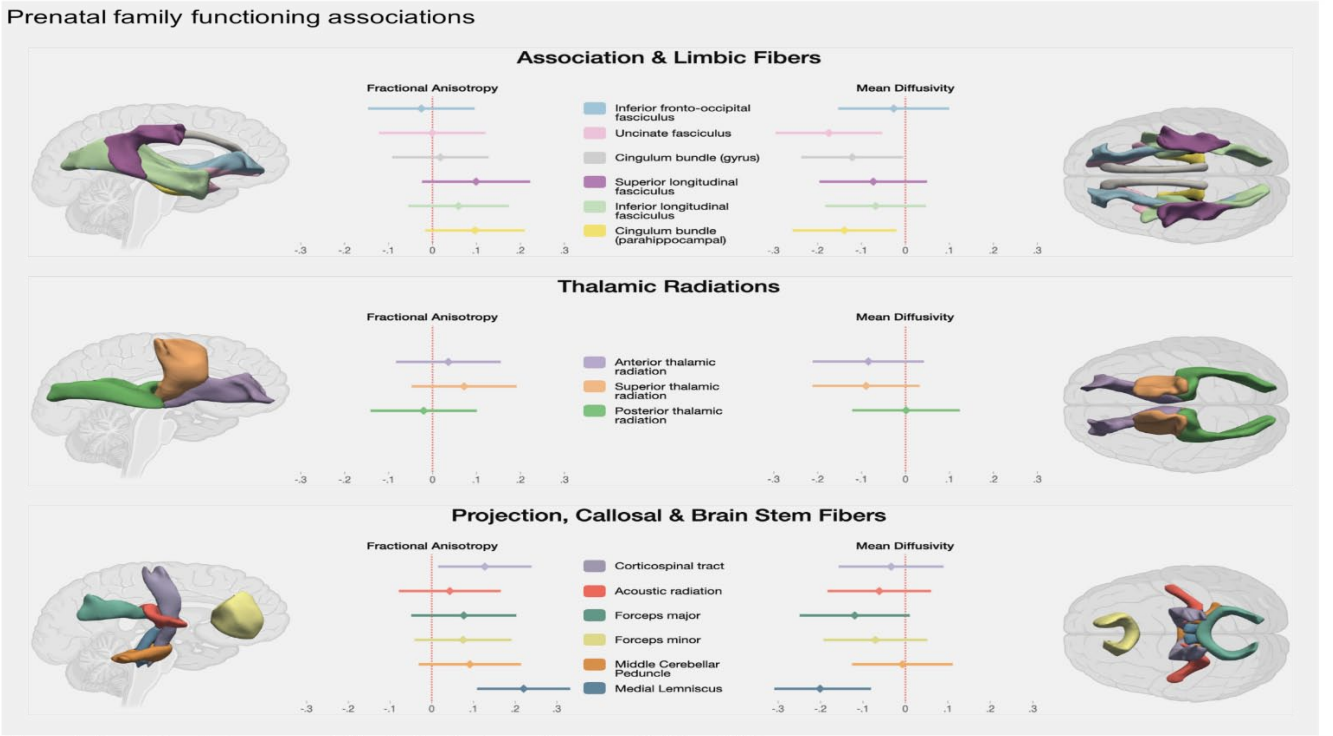

Figure 1. Associations between prenatal family functioning and tract-specific FA and MD.

Estimates are from fully adjusted and weighted models accounting for child age at MRI scan, sex, ethnicity, household income, highest level of parental education achieved, maternal and partner history of psychosis, prenatal maternal and partner psychopathology symptoms, maternal age at child's birth, and child in utero exposure to smoking.

Coefficient plot estimates are standard deviation differences associated with a one-point increase in prenatal family functioning score (score range 1 - 4).

functioning modified effects of mid-childhood family functioning by incorporating an interaction term between prenatal and mid-childhood functioning scores using continuous measures in fully adjusted models. Second, we evaluated associations between both global outcomes and mean family functioning by averaging functioning scores from both prenatal and mid-childhood time points. Third, because there was substantial left skew in the functioning score distributions (see below for more detail), we fit fully adjusted piecewise continuous linear spline models of prenatal functioning and both global outcomes. Based on *a priori* considerations of the family functioning scale and score distributions in our sample, we initially modeled a knot at a score of 3.0, after which we iteratively modeled alternative knots below 3.0 in score decrements of 0.1.

Missing data.

To account for differential loss to follow-up by sociodemographic characteristics, we calculated inverse probability of attrition weights (IPWs). We deemed lost to follow-up participants enrolled at baseline but excluded from our analytic sample for any reason. We multiply imputed missing exposure and covariate data using chained equations to construct 50 imputed datasets, then combined imputation-specific mean and variance measures using Rubin's Rules.[41] Supplement Sections 4 and 5 include additional details of our IPWs and imputation models.

RESULTS

Analytic sample characteristics

Included versus excluded participants were more likely to be of European ethnicity (71% versus 58%); to have parents with at least advanced vocational training or a bachelor's degree (63% versus 44%); to be from higher-income households (65% versus 49%); and to be born to older mothers (mean maternal age at birth 31.7 years versus 29.8 years).

Table 1 details sociodemographic characteristics in our analytic sample according to family functioning scores. Mothers of European children reported higher family functioning scores at both time points than mothers of children of other ethnicities, as did mothers of higher-income or education households. Prenatal and mid-childhood scores were moderately correlated, r = 0.38. Functioning scores at both time points were left skewed. Prenatal mean and median scores were 3.48 and 3.58, respectively, with 75% of mothers in the analysis sample reporting scores greater than or equal to 3.0 (scale range 1.0 – 4.0). Similarly, mid-childhood mean and median scores were 3.50 and 3.58, respectively, with fully 83% of mothers reporting mid-childhood scores 3.0 or higher. Supplement Section 6 details mean outcome values (global FA and MD) by sociodemographic characteristics.

Table 1. Distribution of exposure measures by participant characteristics in the final analysis sample. n = 2,653.

		Prenatal Family Functioning		Mid-Childhood Family Functioning	
	%	$\bar{x}$	*s*	$\bar{x}$	*s*
Total Sample	100	3.48	0.46	3.50	0.42
Child biological sex					
Female	51	3.49	0.46	3.51	0.41
Male	49	3.47	0.46	3.49	0.43
Child race / ethnicity / country of origin					
Dutch / Other European	71	3.56	0.42	3.55	0.40
Turkish	5	3.26	0.48	3.29	0.49
Moroccan	4	3.26	0.47	3.27	0.40
Surinamese	7	3.25	0.50	3.39	0.45
Other	13	3.30	0.53	3.40	0.43
Highest Household Education					
Less than high school equivalent	4	3.18	0.49	3.22	0.47
High school or intermediate vocational trainin	33	3.36	0.49	3.44	0.43
Adv. vocational training, bachelor's, or higher	63	3.60	0.41	3.56	0.40
Household Income					
€2200 / month or less	35	3.30	0.51	3.38	0.46
More than €2200 / month	65	3.59	0.40	3.57	0.38

a. This table is based on observed values for each characteristic and does not account for missing data.
b. Family functioning scores are based on the McMaster Family Assessment Device - General Functioning Subscale and range from 1 to 4.

Global multi-tract mean outcomes.

In both weighted and unweighted fully adjusted models, prenatal family functioning was negatively associated with preadolescent global multi-tract mean MD (Table 2), with more modest evidence of a positive association with global multi-tract mean FA. For comparison, the magnitudes of the prenatal functioning effect estimates were approximately 76% and 57% of those associated with a one-year increase in age at scan in our weighted, fully adjusted models for global MD and global FA, respectively. Effect estimates from weighted and unweighted models were nearly identical, though standard errors were greater in weighted versus unweighted models. In contrast, we found no evidence of an association between mid-childhood functioning and either global measure of white matter microstructure. Notably, in models of mid-childhood functioning adjusting for prenatal functioning, prenatal functioning remained statistically significantly associated with both outcomes. For example, in weighted, fully adjusted models of mid-childhood functioning, effect estimates for prenatal functioning were $\beta_{\text{global MD}} = -0.13$ (95% CI: -0.25, -0.00), and $\beta_{\text{global FA}} = 0.14$ (95% CI: 0.03, 0.26). Supplement Section 7 includes post hoc model results for global multi-tract mean RD and AD, which suggest global RD—but not AD—is associated with prenatal family functioning.

Table 2. Associations between family functioning and global multi-tract mean measures of white matter microstructure in preadolescence. n = 2,653.

		Global FA			Global MD		
		β	95% CI	*p*	β	95% CI	*p*
Prenatal Family Functioning							
Min. adjusted	Unweighted	0.13	(0.04, 0.22)	0.01	-0.10	(-0.19, -0.01)	0.03
Fully adjusted	Unweighted	0.10	(0.00, 0.19)	0.04	-0.13	(-0.23, -0.04)	0.01
Fully adjusted	Weighted	0.10	(-0.01, 0.21)	0.07	-0.13	(-0.25, -0.02)	0.03
Mid-Childhood Family Functioning - Baseline Unadjusted							
Min. adjusted	Unweighted	0.00	(-0.10, 0.09)	0.93	0.00	(-0.10, 0.09)	0.95
Fully adjusted	Unweighted	-0.02	(-0.12, 0.08)	0.70	-0.01	(-0.11, 0.09)	0.81
Fully adjusted	Weighted	-0.04	(-0.17, 0.08)	0.48	0.00	(-0.12, 0.12)	0.98
Mid-Childhood Family Functioning - Baseline Adjusted							
Min. adjusted	Unweighted	-0.06	(-0.16, 0.04)	0.24	0.04	(-0.06, 0.14)	0.46
Fully adjusted	Unweighted	-0.06	(-0.16, 0.04)	0.27	0.03	(-0.07, 0.13)	0.59
Fully adjusted	Weighted	-0.08	(-0.21, 0.04)	0.19	0.03	(-0.09, 0.16)	0.58

a. Minimally adjusted models include covariates for child age at scan, sex, and ethnicity.
b. Fully adjusted models account for child age at scan, sex, ethnicity, household income, highest level of parental education achieved, maternal and partner history of psychosis, prenatal maternal and partner psychopathology symptoms (for prenatal models), early-childhood maternal and partner psychopathology symptoms (for mid-childhood models), maternal age at child's birth, and child in utero exposure to smoking.
c. "Global" measures are standardized mean values of weighted-average FA and MD across all 27 tracts delineated by AutoPtx.

Tract-specific outcomes.

Exploratory tract-specific models revealed associations between prenatal functioning and MD in the uncinate fasciculus, medial lemniscus, and both parts (parahippocampal and gyral) of the cingulum bundle; however, the latter two associations did not survive adjustment for multiple testing (Figure 1, Supplement Section 8). The remaining tract-specific MD effect estimates had larger standard errors and thus did not evince associations based on statistical significance, but all MD effect estimates were uniform in direction (Figure 1). A similar pattern emerged from models assessing prenatal functioning and tract-specific FA. Effect estimates were mostly uniform in direction, though only the association with medial lemniscus FA remained statistically significant after adjustment for multiple testing (Figure 1, Supplement Section 8).

Sensitivity analyses.

In weighted, fully adjusted models of global multi-tract mean MD and FA, we found no evidence of statistical interaction between prenatal and mid-childhood functioning scores. Interaction terms were $\beta_{\text{global MD}}$ = -0.08 (95% CI: -0.33, 0.18) and $\beta_{\text{global FA}}$ = 0.15 (95% CI: -0.09, 0.40). Separately, weighted, fully adjusted models of mean family functioning scores yielded only marginal or no evidence that mean functioning was associated with either outcome: $\beta_{\text{global MD}}$ = -0.11 (95% CI: -0.23, 0.01); and $\beta_{\text{global FA}}$ = 0.05 (95% CI: -0.06, 0.17). Piecewise continuous linear spline models suggested

effects of greater magnitudes for lower prenatal functioning scores, i.e., scores between 1.0 and 3.0. However, given the relatively fewer number of participants with lower functioning scores, these effect estimates were uncertain. Among the relatively greater number of participants with higher functioning scores (i.e., above 3.0), effect estimates were smaller. See Supplement Section 9 for estimates from these models.

DISCUSSION

This study provides evidence to support our hypothesis that early-life family functioning may affect white matter neurodevelopment. Specifically, more positive prenatal family environments (i.e., supportive and accepting families with high problem-solving capacity) were associated with lower MD and higher FA, on average, across the brain's white matter tracts in preadolescence. While the effect estimate magnitudes were relatively small in absolute terms, they can be compared to other known contributors to white matter microstructure. For example, the difference in global MD associated with a one-unit increase in prenatal family functioning score was about three-quarters of the difference associated with a one-year increase in scan age. The three-unit range of the family functioning scale (i.e., from 1 to 4) renders these estimates more substantial when comparing children of families with exceedingly low scores to those with very high scores. In contrast to our prenatal findings, we found no evidence suggesting a relationship between mid-childhood family functioning and our global outcomes. One possible explanation for the diverging results between prenatal and mid-childhood functioning relates to the decreasing relative importance of the family environment to children's broader social environment (and thus to their neurodevelopment) over time. As children grow older, they attend school, spend more time with friends, and establish influential relationships outside the family.

Secondary analyses suggest effects of prenatal family functioning on white matter microstructure may be widespread throughout the brain. For example, with respect to MD, only associations with the uncinate fasciculus and medial lemniscus among the 15 tract-specific outcomes tested remained statistically significant after adjustment for multiple testing, but the uniform direction and similar magnitude of the remaining tracts' estimates suggest a model of global effects rather than one in which effects are targeted at specific tracts. Moreover, if effects were tract-specific (rather than global), one might postulate the uncinate fasciculus and medial lemniscus would share a common structural feature or functional role. However, the uncinate fasciculus connects the brain's temporal and frontal lobes and is involved in memory, language, and social-emotional processing, while the medial lemniscus is a brainstem tract involved in sensory information transport to the brain.[42,43] While both tracts emerge at around 15 gestational weeks,

many other tracts for which effect estimates were not strictly statistically significant also appear to emerge between 13 and 19 gestational weeks.[44] Thus, taken together, our tract-specific analyses suggest prenatal family functioning may have global rather than targeted effects.

Our findings are consistent with the limited available prior work in this area. The only other study to assess prenatal and early childhood life experiences and white matter microstructure in a population-based cohort also found lasting effects of prenatal exposures. Using diffusion-weighted images obtained when participants were in early adulthood, Jensen et al. (2018) reported maternal prenatal stressful experiences were associated with a decrease in the magnetization transfer ratio (MTR) in the splenium, a marker of lower white matter microstructure.[21] Thus, our findings correspond with those of Jensen et al. (2018) because they reported *stressful* prenatal experiences were associated with *less* microstructure, while our study reports *enriched* prenatal environments are associated with *more* microstructure.

Moreover, our results support findings from prior studies reporting that positive parenting practices or healthy parent-child relationships confer neurodevelopmental advantages associated with decreased risky behavior. Because many of these studies assess the family environment after the children are born, they are vulnerable to reverse causation, since child behavior likely influences family functioning. Our study, however, found similar effects using a measure of prenatal family functioning obtained before the child's birth, thereby reducing concerns about recall bias and reverse causation. Together, these findings suggest additional investigation is warranted to explore whether, how, and to what extent prenatal and early-life experiences induce lasting white matter microstructural changes.

The period from the last weeks of gestation through the first years of life is critical to a number of foundational white matter developmental processes, which may be affected by the family environment and may also explain lasting microstructural differences. Our prenatal measure of the family environment is unlikely to measure the prenatal environment exclusively. More likely, it captures the perinatal and early-childhood family environment, spanning some time period both before and after the child's birth. Interestingly, we found prenatal and mid-childhood functioning scores were only moderately correlated ($r = 0.38$), suggesting that the family environment may change modestly through the child's first six years of life. Follow-up research may investigate whether and to what extent family functioning fluctuates during this time period. Measures of prenatal and immediate postnatal functioning may be particularly interesting as families adjust to the presence of a new infant while the infant continues rapid white matter development.

Jensen et al. (2018) propose at least three complementary mechanisms that could explain how prenatal stress may affect white matter microstructure. The first is the balance between neurogenesis (neuron production) and apoptosis (neuron death). Both processes occur in the prenatal and, at least within the hippocampus, the very early postnatal period. The balance between these processes affects neuronal density by influencing the number of neurons (and thus axons) that comprise the brain's white matter. Studies in humans and other animals suggest both processes are in part experience dependent. For example, maternal psychological stress—and the resulting increase in stress hormones—may reduce neuronal density by decreasing neurogenesis and increasing apoptosis, while enriched environments may increase neuronal density by doing the opposite.[20,21] Increased neuronal density could result in higher FA and lower MD values.[21] Importantly, if the associations observed in this study are both (1) reflective of biological reality and (2) caused by a change in balance between neurogenesis and apoptosis, then neuroplastic processes in childhood and adolescence that enable the brain to reorganize itself might able to compensate partially for these prenatal effects later in a child's life, but they would be unable to undo them entirely because neurogenesis largely ends prior to or just after birth.

Another possible mechanism is altered developmental myelination, or the process by which axons develop an insulating myelin sheath to enhance their efficiency. Myelination begins in the late prenatal period and extends well into childhood. Enriched environments have been associated with increased FA and decreased MD, which suggest greater myelination. Likewise, stressful environments have been associated with decreased FA and increased MD, which suggest lesser myelination.[21] Positive family functioning may have effects similar to those of enriched environments.

A third potential mechanism relates to changes in axonal diameter and the thickness of the myelin sheath. Larger axons have thinner myelin sheaths compared to smaller axons, resulting in different microstructural profiles. Because enriched environments entail novel and healthy stimuli, they may increase neuronal activity and promote axonal growth.[21] Both FA and MD may be influenced by these changes, such that a greater density of large-diameter axons (perhaps resulting from enriched environments) would manifest as higher FA and lower MD.

Our study has limitations. First, the sample included few families reporting low functioning scores, perhaps due either to selection or social desirability bias. This inhibits our ability to examine effects of scores at the low end of the continuum. Second, with only one DWI scan per participant, we cannot fully assess changes in neurodevelopmental trajectories due to our exposures. Third, as with all observational studies, confounding and reverse causation may bias our results. For example, certain parental genetic profiles

may predispose parents to report higher or lower family functioning while also affecting their child's white matter structure. We partly addressed this concern by adjusting for maternal and paternal psychopathology symptoms and psychosis history. Fourth, our study is limited by challenges inherent in large, population-based pediatric neuroimaging studies. For example, we excluded several participants due to poor scan quality, which can be patterned by child behavior and sociodemographic profiles. Relatedly, the study's generalizability is limited by differential attrition in the cohort by sociodemographic characteristics, although our use of inverse probability weights to account for attrition helps to address this concern. Selection in utero may also induce survival bias, wherein frail fetuses of mothers reporting high prenatal family functioning may have survived and been included in our analyses when they would not have done so if they were from lower functioning families.[45] In turn, prenatal functioning effect estimate magnitudes are likely to be underestimated. Finally, our analyses do not account for possible partial volume effects related to head size that may influence DTI scalar metrics.[46]

Our study also has several strengths. First, we used a longitudinal design, leveraging prospectively collected exposure data predating the child's birth and linking it to outcomes measured fully ten years later, which enabled us to investigate relatively long-term effects of the perinatal family environment. This mitigates concerns about reverse causation and recall bias. We also avoid many challenges associated with studies using maternal reports of both exposures and outcomes (e.g., behavior measures) by using objective outcomes constructed from DWI scans. Finally, this study is nested within a large, population-based birth cohort, which reduces the risk of selection bias common to many neuroimaging studies relying on case-control designs.

CONCLUSION

In a sample of 2,653 children, more positive prenatal family functioning—a measure of the perinatal family environment—was associated with greater white matter microstructure in preadolescents, suggesting healthy perinatal family functioning may confer lasting neurodevelopmental advantages. Our results also suggest the emphasis on parenting practices in family-focused child neurodevelopmental research may be too narrow, and that more general measures of family functioning agnostic to family structure may capture important health-relevant dimensions of the family environment. Subsequent studies of family functioning and its neurodevelopmental effects should consider developing new tools to assess better variation at both the lower and the higher end of the scale, and they should emphasize participation of low-functioning families. Capturing positive aspects of early-life family functioning may provide important in-

sight into novel pathways by which facets of the social environment become biologically embedded and link to child health and well-being.

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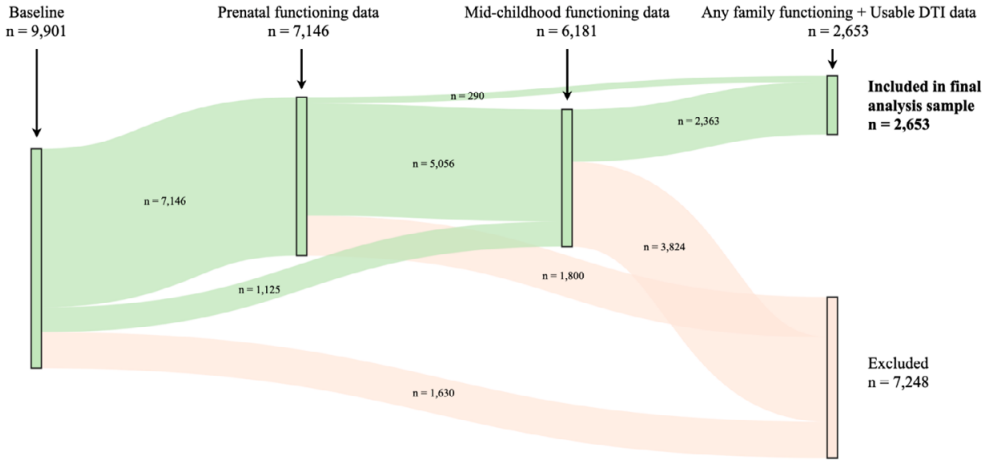
APPENDIX OF SUPPLEMENTAL INFORMATION

Section 1.

Supplement Figure 1.



Supplement Table 1. Bases for excluding participants from analysis sample.

1,630	Missing both prenatal and mid-childhood family functioning data
4,063	No MRI / DTI scan data
1,282	Unusable DTI scan (poor quality image)
12	Incidental finding on MRI scan
8	In utero exposure to heroin or cocaine
55	Missing DTI data for some tracts
162	Outlier DTI data
36	Randomly selected twin removed
7,248	**Total excluded participants**

Section 2. Brain imaging details.

All MRI and DTI brain scans were acquired by a GE MR-750W scanner (General Electric Healthcare, Chicago, IL) at 3T with an eight-channel head coil.[29] Sequence parameters included 2 mm isotropic resolution and 35 diffusion-weighted volumes. Study staff preprocessed the resulting images using the FMRIB Software Library (FSL), version 5.0.9, which stripped non-brain tissue, corrected for artifacts from eddy currents and head motion, and fit a diffusion tensor to each voxel using the RESTORE method from the Camino diffusion MRI toolkit. This pipeline calculated fractional anisotropy (FA), mean diffusivity (MD), axial diffusivity (AD), and radial diffusivity (RD) metrics for each voxel.

Next, study researchers conducted fully automated probabilistic fiber tractography on each participant's diffusion-weighted image in native space using the AutoPtx plugin for FSL.[35] This method generates subject-specific, probabilistic representations of 27 large white matter tracts that can be consistently and robustly identified across brain regions. The process identifies each tract's connectivity distribution, normalizes it given the number of successful seed-to-target attempts, and then removes voxels unlikely to be part of the tract's distribution. Thereafter, the process automatically computes tract-specific scalar metrics (MD, FA, AD, RD) of microstructural properties by weighting voxel-specific metrics by the probability that each voxel is part of the specific tract. To ensure the quality of all scans and reconstructions, researchers visually inspected all raw images and examined signal intensity in each slice to assess attenuation by various artifacts. They also visually inspected all probabilistic tractography data. Scans deemed poor at any point in the quality control process were excluded from analysis.

Section 3. Outlier analyses.

We assessed statistical outliers in four measures of white matter microstructure: tract-specific MD, FA, AD, and RD. Though our primary outcomes are composite MD and FA metrics, we included AD and RD outcomes in the outlier analyses because they are based more directly on tensor eigenvalues describing diffusion anisotropy than MD and FA and therefore may be less likely to obscure extreme values. For example, while MD is the mean of all three tensor eigenvalues (λ_1, λ_2, and λ_3), RD is merely the mean of two (λ_2 and λ_3), and AD is simply λ_1, where $\lambda_1 > \lambda_2 > \lambda_3$.

In our first outlier identification strategy, we excluded participants with any tract-specific MD, FA, AD, or RD value greater than 5 standard deviations from the mean value for each respective tract because such values are either (1) biologically implausible or (2) so far from the sample means that they likely represent significant pathology or brain structural abnormality. Next, we calculated jackknife residuals of minimally adjusted models for the associations between prenatal and mid-childhood family functioning and tract-specific MD, FA, AD, and RD outcomes. Using Tukey's formula, we then excluded participants with any jackknife residual beyond Tukey's outer fences, i.e., greater than a cutoff value at 3 interquartile ranges above the respective residual distribution's 75th percentile or below its 25th percentile. When this test identified statistical outliers, we re-ran the original models after excluding the outliers and repeated diagnostic testing until the process revealed no additional outlier values. Finally, we visually inspected quantile-quantile plots of all outcomes and excluded any remaining participants with outlier outcome metrics.

Section 4. Inverse probability of attrition weights.

We defined participants lost to follow up as those enrolled at baseline (either prior to or at birth) but excluded from our analysis sample for any reason. To calculate our IPWs, we identified a broad set of variables theorized to predict who among originally enrolled participants satisfied our inclusion criteria. We used multiple imputation by chained equations (regression models for continuous dependent variables; predictive mean matching for all other variables, knn = 10, burn-in = 25) to address missing data in these variables, resulting in 50 imputed datasets. Thereafter, we fit a logistic regression model using these variables to predict the likelihood of each enrolled participant's inclusion in our analysis sample. The predictive accuracy of this model yielded an area under the receiver operating characteristic curve (AUC of ROC curve) of 0.800 (SE = 0.005). Last, we calculated IPWs for use in later analyses. Unstabilized weights had a mean of 3.40 and ranged from 1.00 to 22.72.

Section 5. Multiple imputation models.

We imputed missing exposure and covariate data. The proportion of missing data for most covariates was low to moderate (e.g., 12% for paternal age at birth), with the exception of household income, for which we were missing 20% of data. We used the 'mi impute chained' package in Stata 16.0/MP. For continuous variables, we specified linear OLS regression models. For ordinal and categorical variables, we specified predictive mean matching models, knn = 10 (i.e., 10 donor values). We specified a 25-iteration burn-in period for each chain to ensure convergence to a stationary distribution. Models included all outcomes as right-hand side variables with no missing data. We imputed 50 imputed datasets and combined the resulting estimates using Rubin's Rules.[41]

Section 6. Mean FA and MD values by sociodemographic characteristics.

On average, girls had lower global MD and FA scores than boys (p-values < 0.001 for both outcomes). European children had higher global FA values than children of other ethnicities (p < 0.001). Children of more socially advantaged households had higher global FA values than their less advantaged counterparts (p = 0.002 and < 0.001 for parental education and household income, respectively). No differences in global MD by ethnicity, parental education, or household income were evident.

Supplement Section 6. Distribution of standardized outcome measures by participant characteristics in the final analytic sample. n = 2,653.

	%	Mean Global FA	*p*	Mean Global MD	*p*
Total Sample	100	0.00		0.00	
Child biological sex			< 0.001		< 0.001
Female	51	-0.11		-0.13	
Male	49	0.11		0.13	
Child race / ethnicity / country of origin			< 0.001		0.229
Dutch / Other European	71	0.06		-0.01	
Turkish	5	-0.21		0.04	
Moroccan	4	-0.13		0.21	
Surinamese	7	-0.24		0.00	
Other	13	-0.10		0.01	
Highest Household Education			0.002		0.484
Less than high school equivalent	4	-0.11		-0.08	
High school or intermediate vocational trainin	33	-0.07		-0.03	
Adv. vocational training, bachelor's, or higher	63	0.07		0.01	
Household Income			< 0.001		0.955
€2200 / month or less	35	-0.12		0.00	
More than €2200 / month	65	0.06		0.00	

1. This table is based on observed values for each characteristic and does not account for missing data.
2. p-values are from one-way ANOVA F-tests for differences in outcomes by each respective participant characteristic.

Section 7. Global axial diffusivity and global radial diffusivity.

Supplement Section 7. Associations between family functioning and global measures (AD and RD) of white matter microstructure in preadolescence.

		Axial Diffusivity			Radial Diffusivity		
		β	95% CI	*p*	β	95% CI	*p*
Prenatal Family Functioning							
Min. adjusted	Unweighted	-0.01	(-0.09, 0.08)	0.90	-0.13	(-0.22, -0.04)	< 0.01
Fully adjusted	Unweighted	-0.07	(-0.17, 0.02)	0.13	-0.14	(-0.23, -0.04)	< 0.01
Fully adjusted	Weighted	-0.07	(-0.20, 0.06)	0.29	-0.15	(-0.26, -0.03)	0.01
Mid-Childhood Family Functioning - Baseline Unadjusted							
Min. adjusted	Unweighted	0.00	(-0.10, 0.09)	0.95	0.00	(-0.10, 0.09)	0.96
Fully adjusted	Unweighted	-0.03	(-0.12, 0.07)	0.56	0.00	(-0.10, 0.10)	0.99
Fully adjusted	Weighted	-0.04	(-0.16, 0.09)	0.57	0.02	(-0.10, 0.14)	0.74
Mid-Childhood Family Functioning - Baseline Adjusted							
Min. adjusted	Unweighted	0.00	(-0.10, 0.10)	0.99	0.05	(-0.05, 0.15)	0.30
Fully adjusted	Unweighted	-0.01	(-0.11, 0.09)	0.80	0.05	(-0.06, 0.15)	0.38
Fully adjusted	Weighted	-0.03	(-0.16, 0.10)	0.68	0.06	(-0.06, 0.18)	0.29

1. Minimally adjusted models include covariates for child age at scan, sex, and ethnicity.
2. Fully adjusted models account for child age at scan, biological sex, ethnicity, household income, highest level of parental education achieved, maternal and partner history of psychosis, prenatal maternal and partner psychopathology symptoms (for prenatal models), early-childhood maternal and partner psychopathology symptoms (for mid-childhood models), maternal age at child's birth, and child in utero exposure to smoking.
3. Global measures are standardized mean values of weighted-average AD and RD across all 27 tracts delineated by AutoPtx.

Section 8. Prenatal functioning score and tract-specific MD and FA.

Supplement Section 8. Weighted associations between prenatal family functioning and tract-specific measures of white matter microstructure.

	Fractional Anisotropy			Mean Diffusivity		
	β	95% CI	*p*	β	95% CI	*p*
Association Fibers						
Superior Longitudinal Fasciculus	0.10	(-0.02, 0.22)	0.11	-0.07	(-0.20, 0.05)	0.24
Inferior Longitudinal Fasciculus	0.06	(-0.06, 0.17)	0.31	-0.07	(-0.18, 0.05)	0.25
Inferior Fronto-Occipital Fasciculus	-0.02	(-0.15, 0.10)	0.68	-0.03	(-0.15, 0.10)	0.68
Uncinate Fasciculus	0.00	(-0.12, 0.12)	0.99	-0.17	(-0.30, -0.05)	< 0.01 *
Limbic System Fibers						
Cingulum (Cingulate Gyrus Part)	0.02	(-0.09, 0.13)	0.75	-0.12	(-0.24, -0.00)	0.04
Cingulum (Parahippocampal Part)	0.10	(-0.02, 0.21)	0.09	-0.14	(-0.26, -0.02)	0.02
Projection Fibers						
Corticospinal Tract	0.13	(0.02, 0.24)	0.03	-0.03	(-0.16, 0.09)	0.59
Acoustic Radiation	0.04	(-0.08, 0.17)	0.49	-0.06	(-0.18, 0.06)	0.32
Anterior Thalamic Radiation	0.04	(-0.08, 0.16)	0.55	-0.08	(-0.21, 0.04)	0.19
Superior Thalamic Radiation	0.07	(-0.05, 0.19)	0.24	-0.09	(-0.21, 0.03)	0.15
Posterior Thalamic Radiation	-0.02	(-0.14, 0.10)	0.74	0.00	(-0.12, 0.12)	0.99
Callosal Fibers						
Forceps Major	0.08	(-0.05, 0.20)	0.23	-0.12	(-0.25, 0.01)	0.07
Forceps Minor	0.07	(-0.04, 0.19)	0.21	-0.07	(-0.19, 0.05)	0.26
Brainstem Tracts						
Middle Cerebellar Peduncle	0.09	(-0.03, 0.21)	0.14	-0.01	(-0.13, 0.11)	0.91
Medial Lemniscus	0.22	(0.11, 0.33)	< 0.001 *	-0.20	(-0.32, -0.08)	< 0.01 *

1. Fully adjusted models account for child age at scan, sex, ethnicity, household income, highest level of parental education achieved, maternal and partner history of psychosis, maternal and partner psychopathology symptoms, maternal age at child's birth, and child in utero exposure to smoking.

2. MD and FA values are averaged across hemispheres where appropriate and standardized.

** Starred results remain statistically signficant after adjustment for multiple comparisons.*

Section 9. Prenatal functioning and global FA and MD – spline model results.

Supplement Section 9. Piecewise continuous linear spline model results for the association between prenatal family functioning, global MD, and global FA.

Global mean diffusivity

	Pre-knot slope		Post-knot slope	
Knot	β	95% CI	β	95% CI
No knot	-0.13	(-0.25, -0.02)	-0.13	(-0.25, -0.02)
3.0	-0.21	(-0.54, 0.11)	-0.10	(-0.26, 0.05)
2.9	-0.24	(-0.62, 0.14)	-0.10	(-0.25, 0.04)
2.8	-0.25	(-0.70, 0.19)	-0.11	(-0.25, 0.03)
2.7	-0.23	(-0.76, 0.30)	-0.12	(-0.26, 0.02)
2.6	-0.26	(-0.89, 0.36)	-0.12	(-0.25, 0.01)
2.5	-0.39	(-1.12, 0.33)	-0.11	(-0.24, 0.01)

Global fractional anisotropy

Knot	β	95% CI	β	95% CI
No knot	0.10	(-0.01, 0.21)	0.10	(-0.01, 0.21)
3.0	0.20	(-0.09, 0.49)	0.07	(-0.09, 0.22)
2.9	0.20	(-0.13, 0.53)	0.08	(-0.07, 0.22)
2.8	0.23	(-0.15, 0.61)	0.08	(-0.06, 0.22)
2.7	0.31	(-0.12, 0.73)	0.07	(-0.06, 0.21)
2.6	0.42	(-0.05, 0.91)	0.07	(-0.06, 0.20)
2.5	0.59	(0.05, 1.13)	0.06	(-0.06, 0.19)

1. Fully adjusted models account for child age at scan, biological sex, ethnicity, household income, highest level of parental education achieved, maternal and partner history of psychosis, maternal and partner psychopathology symptoms, maternal age at child's birth, and child in utero exposure to smoking.
2. The post-knot β is the estimated absolute slope, i.e., not merely the change in slope relative to the pre-knot β.