Eat2beNICE

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Executive summary:

Within Deliverable 1.05 ("Offspring candidate gene pathways x maternal diet/lifestyle interactions during pregnancy on offspring"), one manuscript has been prepared. The manuscript details the results from the examination of the effects that maternal diet quality during pregnancy and genetics (both parental and offspring) has on the development of ADHD symptoms in children.

The effect of overall diet quality during pregnancy and genetics on the development of attention deficit/hyperactivity disorder (ADHD) symptoms at 3, 5 and 8 years of age was examined in the Norwegian Mother, Father and Child Cohort Study (MoBa), a prospective population-based pregnancy cohort study. ADHD is a common neurodevelopmental disorder characterized by hyperactivity and impulsivity, inattention, or a combination of both. Further, ADHD is of polygenic and environmental origins. Diet is one of the environmental factors that are potentially modifiable to target prevention of neurodevelopmental disorders. It has been reported that ADHD symptoms are associated with the quality of the diet.

Our study sample consisted of 2,463 mother-father-child trios, with available information on maternal fiber intake from the Moba food frequency questionnaire (FFQ) during gestational weeks 17-22, with information about maternal education, and with completed questionnaires on ADHD symptoms when the offspring was either 3, 5 or 8 years of age. Our exposures were ADHD polygenic risk scores from child and both parents that were calculated using MoBa genetic data and the largest genome-wide association study of clinically diagnosed ADHD to date (20,183 cases and 35,191 controls). Our outcome was a constructed „ADHD score" for offspring at either 3 , 5 or 8 years of age, utilizing information from several instruments collected through MoBa questionnaires. To examine the effect of maternal diet during pregnancy, we utilized the total fiber intake as a proxy for overall diet quality. Our results indicate that common genetic variants associated with ADHD, captured by PRS, are associated with ADHD-related behaviour at 3, 5 and 8 years of age. The contribution of all predictors explained 1.3-1.9% of variability in ADHD scores at the examined three timepoints. Further, change in ADHD scores between 5 and 8 years of age was associated with paternal ADHD PRS, showing change in ADHD scores from 5 to 8 years of age for those whose fathers have higher ADHD PRS. Related to maternal dietary intake of fiber during pregnancy,

we found that higher intake of fiber during pregnancy was associated with a lower levels of ADHD scores at 3 and 5 years of age, but not at 8 years of age, and the change model suggests that higher maternal intake of fiber during pregnancy was associated with a lower baseline level of ADHD at 3 years of age. Further analyses in larger samples are needed before definite conclusions can be drawn.

1. DELIVERABLE REPORT

Note: publishers' policies prevent sharing of detailed results prior to publication in a peer-reviewed journal. Detailed results, tables and uncompromised figures will be made available upon acceptance and/or as soon as the publisher's embargo has been lifted.

Effects of overall diet quality during pregnancy and genetics on the development of attention deficit/hyperactivity symptoms at 3, 5 and 8 years of age. A longitudinal study in The Norwegian Mother, Father and Child Cohort.

Attention-deficit/hyperactivity disorder (ADHD) is a common neurodevelopmental disorder characterized by hyperactivity and impulsivity, inattention or a combination of both (American Psychiatric Association, 2013). Further, ADHD is of polygenic and environmental origins, with an estimated heritability of 70-80% found in twin- and family studies (Chen et al., 2016; Faraone & Larsson, 2019; Pettersson et al., 2019). A recent genome wide association (GWA) study of clinically diagnosed ADHD identified 12 genome wide significant loci and estimated that common variants explain ~22% of the single nucleotide polymorphism (SNP) heritability (Demontis et al., 2019). A GWA study on ADHD symptoms estimated the SNP heritability to be up to 34% in population-based samples (Middeldorp et al., 2016). Genetic studies indicate that different biological mechanisms contribute to the risk of ADHD through rare and common genetic variants, gene-by-environment interactions, as well as parent-of-origin effects (Faraone & Larsson, 2019; Zayats, Johansson, & Haavik, 2015).

A study of ADHD polygenic risk score's (ADHD PRS) association with ADHD trajectories in 13,457 children estimated the ADHD PRS to explain ~0.50% of the variance in hyperactivity/impulsivity symptoms (Brikell et al., 2018). Another recent study by Agnew-Blais and colleagues found that ADHD PRS explained 0.2% of the variance in the mother-reported

total ADHD symptoms (inattention and hyperactivity/impulsivity combined) among 5- and 7-years old children, respectively (Agnew-Blais et al., 2021).

Both genetic and environmental factors contribute to the development of ADHD symptoms and their trajectories, with intrauterine exposures potentially playing a critical role in brain development. Understanding what environmental factors drive the development of ADHD is important. Diet is one of the environmental factors that are potentially modifiable to target prevention of neurodevelopmental disorders (Adan et al., 2019). It has been reported that ADHD trajectories are associated with the quality of diet (Li, Francis, Hinkle, Ajjarapu, & Zhang, 2019). The amount of fiber in a diet to which an offspring is exposed prenatally is suggested to play a role in fetal brain development through a gut-brain pathway (Dalile, Van Oudenhove, Vervliet, & Verbeke, 2019). In contrast, no consistent associations have been found between ADHD symptoms in offspring and maternal intake of such nutritional factors as folic acid, multivitamins, iodine, polyunsaturated fatty acids, seafood, and caffeine during pregnancy (Li et al., 2019). The observed associations between diet quality during pregnancy and offspring ADHD symptoms or ADHD trajectories found in epidemiological studies may be subject to unmeasured familial confounding (Larsson et al., 2013; Skoglund, Chen, D'Onofrio, Lichtenstein, & Larsson, 2014). Thus, there is a need for studies that can address the contributions from both environmental (dietary) and genetic factors, as well as their interactions, on the risk for ADHD.

Here, we aimed to examine the effects of prenatal maternal fiber intake (a proxy for overall diet quality) and common genetic variants on the development of ADHD symptoms in a sample of a unique prospective population-based pregnancy cohort. To obtain information about offspring ADHD symptoms and their trajectories, ADHD symptoms were collected at multiple time points, i.e. at 3, 5 and 8 years of age.

This study is based on data from The Norwegian Mother, Father and Child Cohort Study (MoBa) and the Medical Birth Registry of Norway (MBRN). MoBa is a prospective population-based pregnancy cohort study conducted by the Norwegian Institute of Public Health. Pregnant women were recruited from across Norway from 1999 to 2008 (Magnus et al., 2016). The women consented to initial participation in 40.6% of the pregnancies. The total cohort includes 114 500 children, 95 200 mothers and 75 200 fathers. Data from MoBa

participants are routinely linked to the MBRN, a national health registry based on mandatory reporting of information on pregnancies and birth outcomes for all births in Norway since 1967 (Irgens, 2000).

The current study was approved by The Regional Committee for Medical Research Ethics (2015/2055). The phenotypes used in this study are based on version 12 of the quality-assured phenotypic data files released for research in 2019, while the genotypic data were obtained through MoBaGENETICS version 1.0 (https://github.com/folkehelseinstituttet/mobagen/wiki/MoBaGenetics1.0).

The genetic data were obtained from blood samples collected from parents during pregnancy and from children (umbilical cord) at birth (Paltiel et al., 2014). The genotyping was carried out in several batches as a collaborative effort, performed mainly in three research projects (HARVEST, ROTTERDAM and NORMENT).

Our study sample consisted of mother-father-child trios from the HARVEST sample, with available information from the food frequency questionnaire (FFQ) in gestational weeks 17-22 and with completed questionnaires on behavioural phenotypes when the offspring was either 3, 5 or 8 years of age.

Quality control (QC) and imputation of genetic data are described in details elsewhere (Helgeland et al., 2019) https://github.com/folkehelseinstituttet/mobagen). After imputation, the data were converted to best guess genotypes and subjected to the following QC: (1) SNPs were removed if their imputation quality (INFO score) was below 0.80, call rate was below 98%, minor allele frequency (MAF) was below 0.5% and if the Hardy-Weinberg equilibrium was not met ($p<1.00x10^{-06}$) and (2) individuals were removed if their call rate was below 98%, had inbreeding coefficient outside of +/- 0.2 value or had sex mismatch. Homogenous population of European descent was visually selected based on principal components analyses with anchor populations from one thousand genomes, taking into account family relatedness. The relatedness was confirmed by kinship estimates in KING software. SNPs with Mendelian errors of more than 1% as well as families with Mendelian errors of more than 5% were removed.

The PRSes for the child, mother, and father were calculated in MoBa genetic data using PRSice2 software and the largest genome-wide association study of clinically diagnosed ADHD to date (20,183 cases and 35,191 controls) (Demontis et al., 2019).

To examine ADHD symptoms in our sample, we constructed an „ADHD score" as our outcome variable for offspring at 3 (ADHD3), 5 (ADHD5), and 8 years (ADHD8) of age, utilizing information from several instruments collected through MoBa questionnaires (Table 1). Each questionnaire was filled out by mothers and contained information on frequency of offspring behaviours on Likert scales. The scores were constructed by summing up the Likert ratings, so that the larger score reflected a more frequent ADHD behaviour.

We utilized the following instruments to construct the ADHD score, including both inattention and hyperactivity/impulsivity items: (1) Child Behavior Checklist (CBCL) at 3 and 5 years of age (Achenbach, 2019), (2) Child Behaviour and Manner questionnaire (CBM) at 3 years of age (American Psychiatric Association, 2000), (3) Conner's Parent Rating Scale-Revised, Short Form (CPRS-R (S)) at 5 years of age (Conners, Sitarenios, Parker, & Epstein, 1998) and (4) the Parent/Teacher Rating Scale for Disruptive Behaviour Disorders (RS-DBD) at 8 years of age (Silva et al., 2005)(Table 2). The CBCL and CBM items were rated on a three-point Likert response scale (1 = never/rarely, 2 = sometimes, 3 = often/very often), while the CPRS-R (S) and the RS-DBD on a four-point Likert scale (1 = never/rarely, 2 = sometimes, 3 = often, 4 = very often). For the CPRS-R (S) and RS-DBD, the response options 3 and 4 were collapsed to correspond to those of CBCL and CBM. ADHD scores were calculated only for individuals with no more than one item missing at either at 3, 5 or 8 years of age.

Maternal diet was assessed through an FFQ answered around week 22 that covered habitual diet during the first half of pregnancy (included from 2002). The MoBa FFQ is a semi-quantitative questionnaire designed to capture dietary habits and intake of dietary supplements during the first 4–5 months of pregnancy (Meltzer et al., 2008). Nutrient calculations were performed with the use of FoodCalc (Lauritsen J (2019) Foodcalc v.1.3. https://github.com/jesperldk/FoodCalc (accessed August 2019) and the Norwegian food composition table (Norwegian Food Composition Database 2020. Norwegian Food Safety Authority, 2020). The MoBa FFQ has been validated with regard to nutrients, foods, and

dietary supplement use (Brantsaeter, Haugen, Alexander, & Meltzer, 2008; Meltzer et al., 2008). To examine maternal diet, we utilized the total fiber intake as it was reported to be representative of the overall diet quality (Erlund-Ögge, 2014, Borge 2017 og Borge 2019, Torjusen, 2012). Maternal fiber intake was calculated based on the intake of food containing fiber (bread, cereals, fruits, vegetables, legumes), registered in the FFQ. Respondents were instructed to report their average intake since they became pregnant. The total intake of fiber was calculated in grams per day.

In this study, we excluded participants for whom calculated kilocalories (kcal) were lower than 900 kcal (0.25 percentile) and higher than 6000 kcal (99.75 percentile), and a total fiber intake during pregnancy lower than 8 grams (0.25 percentile) and higher than 85 grams (99.75 percentile) (Flowchart, Figure 1).

We aimed to estimate the effects genetics and maternal fiber intake during pregnancy may have on the development of ADHD symptoms in children recruited in this large prospective population-based pregnancy cohort. To achieve our aim, we utilized structural equation modelling (SEM) (Figure 2A/2B). We used previous knowledge to choose items for our ADHD outcome variables as described above and used confirmatory factor analyses (CFA) to examine the relationship between the ADHD outcome variable as indicators of the underlying construct, the ADHD score at 3, 5 and 8 years of age (Figure 3). A good CFA model should show high and relatively homogenous factor loadings. In addition, all covariations between the indicators should be explained by the model. Thus, no substantial relations between the indicators should be left after what is explained by the factor model.

The SEM framework gives possibility to analyse both measurement models and structural models. Two main models were examined: (1) the effects of genetics and maternal fiber intake on the overall level of ADHD scores at three different timepoints at 3, 5, or 8 years of age (ADHD3, ADHD5, and ADHD8) and (2) the effects of genetics and maternal fiber intake on ADHD scores at 3 years of age (ADHD3, considered to be the baseline level) and change over time in offspring's ADHD scores from 3 to 5 years (ADHD3 to ADHD5) and from 5 to 8 years of age (ADHD5 to ADHD8) (Figure 2A/2B). The model 1 was analysed with ordinary multiple regression and model 2 with Latent Growth Curve (LGC) modelling (Bollen & Curran, 2006). Model fit was evaluated using chi-square test, comparative fit index (CFI), Tucker–Lewis index

(TLI) and root mean square error of approximation (RMSEA) with a standard confidence interval of 90% (Bollen & Curran, 2006). We used PRSes from parents and children, maternal fiber intake, and maternal education as predictors and ADHD score as the outcome in all models. Sex and parental age were not included at this time.

We followed a standard procedure for LGC modelling (model 2). First, an unconditional linear model was fitted to the observed data. An LGC model describes level and change over time in the ADHD scores, both at group level and at individual levels, estimated as means and variance in the intercept and slopes of the model. This model also includes the relationship between the level of ADHD symptoms at baseline (ADHD3 score) and the rate of change in ADHD scores over time. Since the model with linear change over time was not supported, with a poor model fit (χ^2=410.9, df = 1, p<0.001, CFI = 0.62, TLI=-0.15, RMSEA = 0.41, RMSEAc.i. =0.38–0.44, RMSEA close fit = 0.000), separate estimates of change were estimated for the two time intervals in a new unconditional model from 3 to 5 years of age (slope 1) and from 5 to 8 years of age (slope 2) (Figure 2B). For this model based on three measurement occasions, it is not possible to estimate with all parameter values included. We therefore constrained variances of the slopes and the covariances to zero. This model is identical to a mixed effect model with random intercept and fixed slopes. These necessary restrictions have negative consequences for model fit. Estimation of two intervals (3 to 5 years and 5 to 8 years of age) allows for different effects of the predictors on the outcome over time. After establishing a model that accounts for observed levels and change in ADHD scores, the predictors were entered in conditional models. The estimator was maximum likelihood with robust standard errors (MLR), which is robust to non-normality in data (Kline, 2016). The full information maximization likelihood method uses all available data under the 'Missing at Random' assumption; thus, the effect of missing data is minimized (Enders, 2010). The level of statistical significance was set at *p=0.05* (two-tailed). Based on our hypotheses, the structure model was tentatively specified, with all predictors directly related to the outcomes (direct effects). Further, the model also included indirect relationships from maternal education to ADHD outcomes via maternal fiber intake (Figure 2A/2B). There is a reason to believe that fiber intake could be different in mothers with different education levels. In all analyses, maternal

education was modelled as a categorical variable with three categories: low (less than high school), middle (high school), or high (4 years or more of college/university).

In addition, we tested interaction effects between child PRS and fiber intake in order to explore if the relationships between fiber intake and ADHD scores were different in children with different levels of genetic propensity for ADHD. Since the interaction term was not significant (b_{ADHD3}=0.001, 95% CI -0.02-0.02, *p=0.90 at age 3;* b_{ADHD5}=-0.002 (95% CI -0.0221-0.02, *p=0.87 at age 5;* b_{ADHD8}= 0.01, 95% CI -0.01-0.03, *p=0.34 at age 8*), we did not include it in the structural equation model 1 (reported below).

Analyses were carried out in Mplus (Muthén & Muthén, 2020) and STATA version 16.1 (StataCorp. 2015. StataCorp LP).

After quality control, a final sample of 3,259 genotyped full parent child trios from the HARVEST sample were identified and 2,463 trios had information on ADHD symptoms at either 3, 5 or 8 years of age (2,061; 1,817; and 1, 610, respectively). Of these offspring, 1,606 (49.3%) were girls (Table 1). The association models were executed on 2,405 individuals, as 58 individuals were excluded due to missing information on maternal education.

Maternal education and fiber intake during pregnancy correlated slightly (Spearmans rho=0.04, p=0.048).

Figure 3 shows the factor loadings for the CFA models for ADHD scores at 3, 5 and 8 years of age (ADHD3/ ADHD5/ADHD8). To some degree, the loadings are different for different indicators within each time of measurement, and also over time (for the same indicators). The magnitude of factor loadings showed that all indicator variables (ADHD items from questionnaires) are related to the underlying factor (ADHD score). The variation in factor loadings over time in each indicator suggested some changes in their importance and, thus, some change in the meaning of the construct (ADHD score), but not more than is acceptable. In such a large sample size, the magnitude of the factor loadings is much more important than if they are statistically significant or not. This only tell that these loadings in this sample are generalizable to the population the observations are sampled from.

SEM model 1: Effects of genetics and maternal fiber intake on the overall level of ADHD symptoms in children at 3, 5 and 8 years of age

The child's own ADHD PRS was associated with ADHD scores at 5 and 8 years of age (unstandardized regression coefficient (b)$_{ADHD5}$=0.32, *p=0.016* and b $_{ADHD8}$=0.47, *p=0.003*), but not at age 3. Higher ADHD PRS from the father was associated with a lower ADHD score at 8 years of age (b_{ADHD8}=-0.30, *p=0.03*). Higher intake of fiber during pregnancy was associated with a lower level of ADHD symptoms at 3 and 5 years of age (b_{ADHD3}=-0.02, *p=0.002*, and b_5=-0.01, *p=0.015*), but not at 8 years of age (b_{ADHD8}=-0.001, *p=0.84*). Higher level of maternal education was associated with lower ADHD scores at all three ages (b_{ADHD3}= -0.34, *p<0.001*; b_{ADHD5}=-0.29, *p=0.005*; b_{ADHD8}=-0.53, *p<0.001*). The contribution of all predictors on ADHD scores explained 1.3-1.9% (R^2 = 1.3–1.9%) at the three examined ages. Explained variance of maternal fiber intake during pregnancy on ADHD scores at 3,5 and 8 years of age was estimated to be 0.1% (*p=0.21*). The estimates from this model are presented in Table 3.

SEM model 2: Effects of genetics and maternal fiber intake on the level of change over time in offspring's ADHD scores at 3,5 and 8 years of age

The unconditional two slopes model estimated the following mean values at baselevel (ADHD3): $alfa_0$=3.62, p<0.001, and $alfa_{1\ (ADHD3\text{-}ADHD5)}$= -1.10, p<0.001 for the interval from 3 to 5 years of age, and $alfa_{2(ADHD5\text{-}ADHD8)}$= 0.72, p<0.001 for the interval from 5 to 8 years of age. Finally, the conditional two slopes model gave the following mean values at baselevel (ADHD3) of $alfa_0$=5.35, p<0.001, and the change from 3 to 5 years of $alfa_{1\ (ADHD3\text{-}ADHD5)}$= -1.44, p<0.001, and from 5 to 8 years $alfa_{2(ADHD5\text{-}ADHD8)}$= 0.83, p=0.048. In this model, the intercept (baseline level) was associated with fiber (b_{ADHD3} = -0.02, *p =0 .003*), suggesting that higher maternal intake of fiber during pregnancy was associated with a lower baseline level of ADHD score at 3 years of age. Similarly, higher maternal education was associated with lower ADHD score at 3 years of age (b_{ADHD3} = -0.34, *p <0 .001*). Change in ADHD scores during the first interval (between 3 and 5 years of age) was not associated with any of the predictors (*p >0 .05*). The change in ADHD scores between 5 and 8 years showed positive correlation with child's PRS and negative correlation with both maternal and paternal PRSes, although only the latter was significant ($b_{ADHD5\text{-}ADHD8}$ = -0.28, *p = 0.045*) suggesting change in ADHD score at 8 years of age

for those whose fathers have higher ADHD PRS. No other statistically significant relationships were found. The estimates from this model are presented in Figure 5.

Discussion and future directions

We found evidence that common genetic variants associated with ADHD, captured by PRS, were associated with ADHD symptoms in general population at 5 and 8 years of age. The contribution of all predictors explained 1.3-1.9% of variability in ADHD scores at the examined three timepoints. The change in ADHD scores between 5 and 8 years showed positive correlation with child's PRS and negative correlation with paternal PRSes, suggesting change in ADHD score at 8 years of age for those whose fathers have higher ADHD PRS. Related to maternal dietary intake of fiber during pregnancy, we found that higher intake of fiber during pregnancy was associated with lower level of ADHD symptoms at 3 and 5 years of age, but not at 8 years of age. The model estimating the change in ADHD symptoms over time suggests that higher maternal intake of fiber during pregnancy was associated with a lower baseline level of ADHD at 3 years of age.

Our results indicate that maternal fiber intake during pregnancy correlate with the development of ADHD symptoms in offspring in childhood, suggesting that maternal prenatal diet rich in fiber is associated with lower ADHD scores in offspring. This observation is in line with previous findings linking fiber intake to ADHD (Li et al., 2019).

Similarly, in line with previous studies (Agnew-Blais et al., 2021; Brikell et al., 2018), we noted that genetic effects of common variants associated with clinically diagnosed ADHD (captured by PRS) showed correlation with ADHD scores in children from general population at 5 and 8 years of age. Genetic correlation between genetics underlying clinical ADHD and those underlying ADHD symptoms in general population has been estimated to be ~90% (Demontis et al., 2019). This is echoed in our findings of association between PRS of clinically diagnosed ADHD and ADHD score in general population.

Apart from estimating the effect child's ADHD PRS may have on childhood ADHD symptoms, we also examined the effect of parental ADHD PRSes on these symptoms as well as their change (from 3 to 5 years of age and from 5 to 8 years of age). For change in ADHD scores

from 5 to 8 years of age, we observed that child's PRS showed positive correlation with the change, while parental PRSes showed negative correlation with the same change. It is noteworthy though that only paternal PRS revealed significant correlation (b_{ADHD8}=-0.30, *p=0.03*). Such negative correlation may indicate that children of parents with lower genetic ADHD loading have larger changes in their symptoms compared to children whose parents have high(er) ADHD genetic loading. As these results are noted in a small sample size, it is challenging to offer solid explanation at this time. Potentially, this observation may indicate that parenting practices of parents with probable ADHD symptoms (high parental PRS) might affect the rate of change in ADHD symptoms of their children. It is noteworthy that "change" in our model does not reflect the direction of the change itself (increase or decrease of symptoms). We are in process of obtaining additional genetic data as well as data on important covariates (e.g. sex and age). Thus, in the near future, we will be able to (1) increase our sample size and (2) better adjust the models for possible confounders. To examine this further and gain a better understanding of our preliminary results, we plan to explore the categorization of children into the following ADHD trajectories: (1) increase in ADHD score from 3 to 8 or 5 to 8 years of age, (2) decrease in ADHD score from 3 to 8 or 5 to 8 years of age and (3) similar score at age 3 and age 8. These categories will then be tested for the association with child's and parental PRSes. Alternatively, we are considering performing latent class analyses to derive the ADHD trajectories and test those for the associations with child's and parental PRSes. Finally, we plan to adjust our models for more confounders, such as parental age, offspring sex, gestational age and, potentially, medications (subject to data availability).

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2. TABLES AND OTHER SUPPORTING DOCUMENTS WHERE APPLICABLE AND NECESSARY

Figure 1. Flowchart

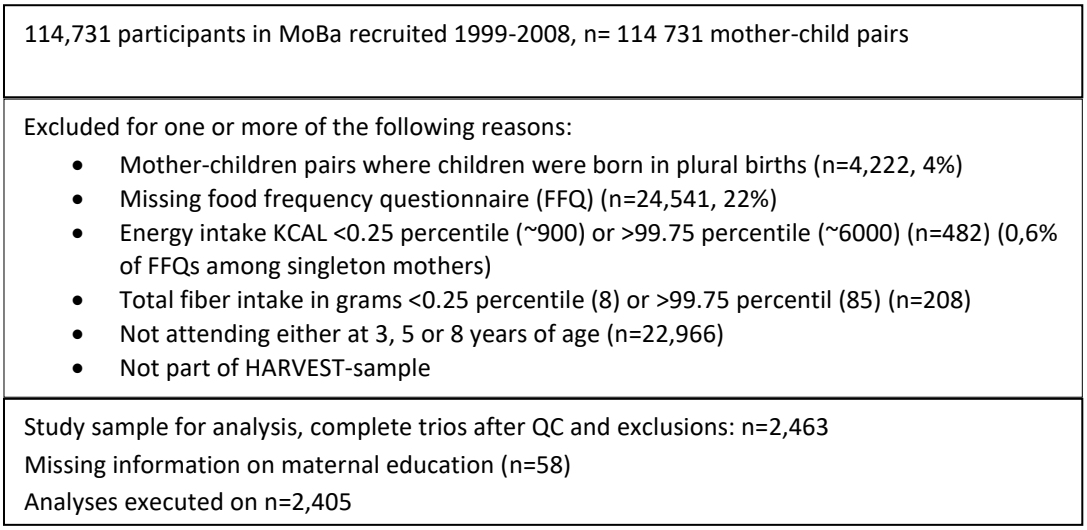
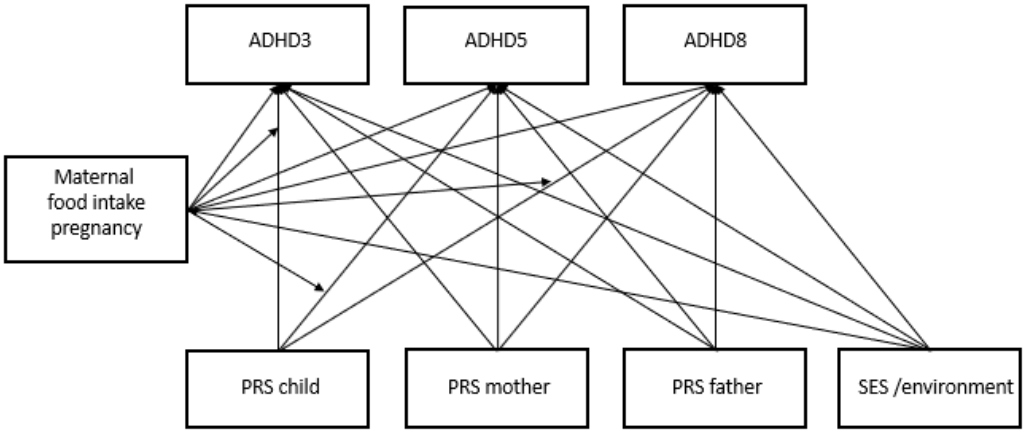


Table 1. ADHD-symptom items from CBCL/CBM/Conners at 3 and 5 years of age compared to Parent/Teacher Rating Scale for Disruptive Behaviour Disorders (RS-DBD) at 8 years of age

3 years CBCL/CBM	**5 years** CBCL/Conners	**8 years** RS-DBD
INATTENTION		
CBCL 2: Can't concentrate, can't pay attention for long	CBCL 2: Can't concentrate, can't pay attention for long	RS-DBD 2: Has difficulty sustaining attention in tasks or play activities
CBM 7: Doesn't seem to listen when he/she is being spoken to	Conners 8: Gets distracted when given instructions to do something	RS-DBD 3: Does not seem to listen when spoken to directly
CBM 1: Becomes distracted or diverted by outside stimuli (sounds or events)	Conners 1: Inattentive, easily distracted	RS-DBD 8: Is easily distracted
HYPERACTIVITY		
CBCL 3: Can't sit still, restless or overactive	CBCL 3: Can't sit still, restless or overactive	RS-DBD 10: Fidgets with hands or feet or squirms in seat (sits uneasily)
CBCL 20: Quickly shifts from one activity to another	CBCL 19: Quickly shifts from one activity to another	RS-DBD 14: Is "on the go" or acts as if "driven by a motor"
CBCL 4: Can't stand waiting, wants everything now	CBCL 4: Can't stand waiting, wants everything now	RS-DBD 17: Has difficulty awaiting turn

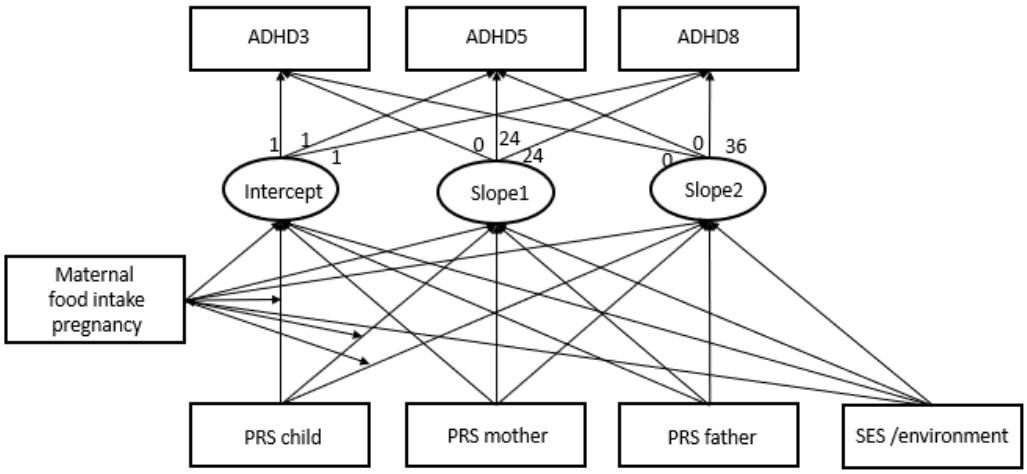
CBCL – Child Behavior Checklist; CBM – Child Behaviuor and Manner; Conners - Conners Parent Rating Scale-Revised (CPRS-R (S)); RS-DBD - Parent/Teacher Rating Scale for Disruptive Behaviour Disorders (DSM-IV)

Figure 2 A. SEM model 1. Effects of genetics and maternal fiber intake on the level of ADHD symptoms in children at 3,5 and 8 years of age



ADHD3 – ADHD-score at 3 years of age: ADHD5 – ADHD-score at 5 years; ADHD8 – ADHD-score at 8 years of age; PRS – polygenic risk score; SES – socioeconomic status

Figure 2 B. SEM model 2. Effects of genetics and maternal fiber intake on the level of change over time in offspring's ADHD scores at 3,5 and 8 years of age

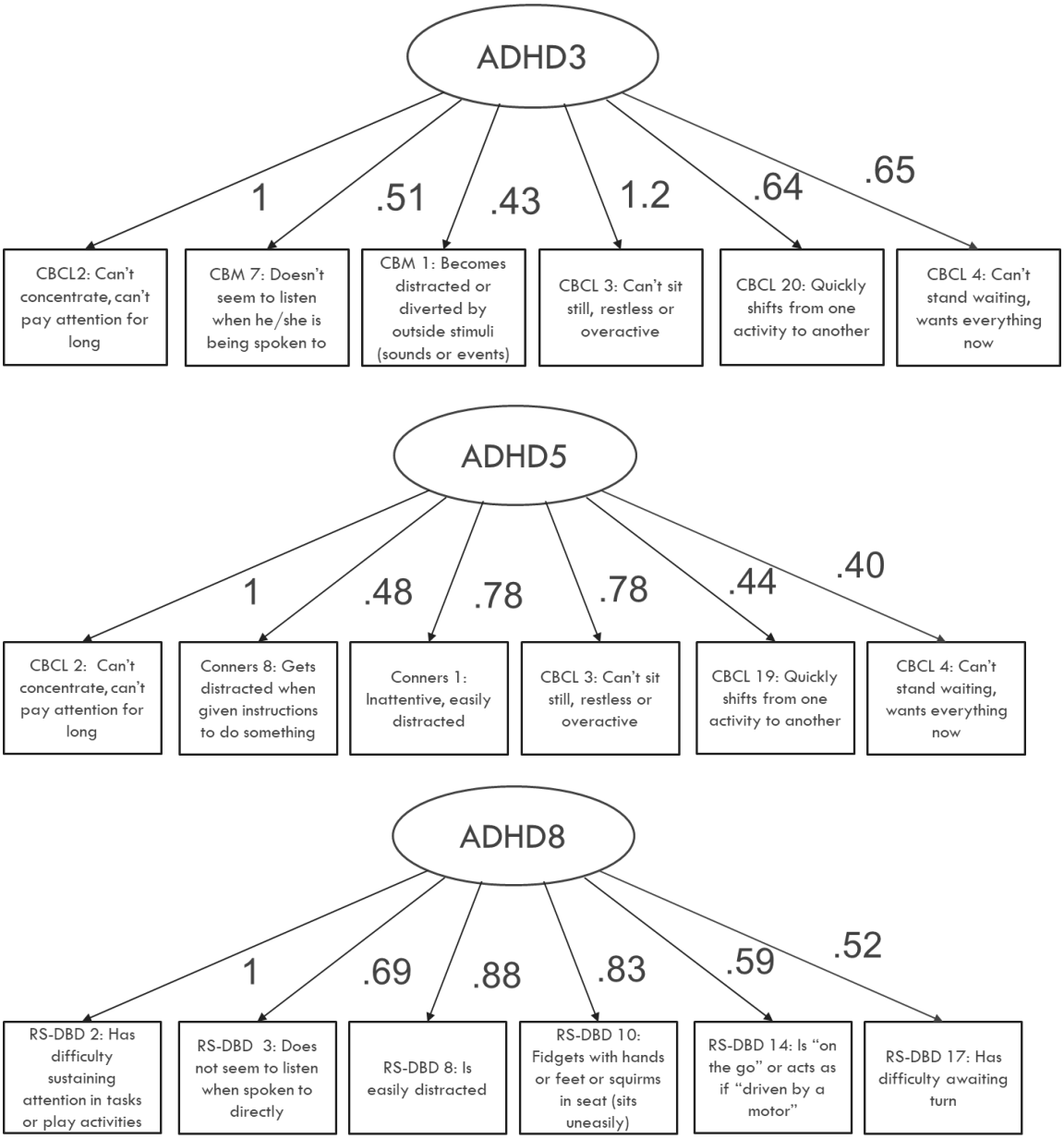


ADHD3 – ADHD-score at 3 years of age: ADHD5 – ADHD-score at 5 years; ADHD8 – ADHD-score at 8 years of age; PRS – polygenic risk score; SES – socioeconomic status; intercept – baseline; slope1 – change in ADHD score from 3 to 5 years: slope2 – change in ADHD score from 5 to 8 years.

Table 2. Demographic characteristics of the MoBa study population/HARVEST batch

	3 years, N (%)	**5 years, N (%)**	**8 years, N (%)**
Number of Trios	2 061	1 817	1 610
Offspring			
Females 1 606 (49.3 %)	1 015 (49.3)	890 (49.0)	788 (48.9)
Males 1 653 (50.7 %)	1 046 (50.8)	927 (51.0)	822 (51.1)
Maternal education at pregnancy			
Less than high school	25 (1.2)	26 1.4)	16 (1.0)
High school	609 (30.0)	525 (29.2)	439 (27.3)
4 years or more of college/university	1358 (66.5)	1208 (67.0)	1109 (69.3)
Missing education (n=58)	48 (2.3)	42 (2.4)	37 (2.4)
Total fiber intake (mean/SD, grams)	31.96/10.93	31.69/10.54	31.70/10.63
Trios included in the analyses (not missing maternal education)	*2 013*	*1 774*	*1 572*

Figure 3. Results of confirmatory factor analyses of ADHD scores at 3,5 and 8 years of age with loadings from each item used to calculate the score, all p<0.001



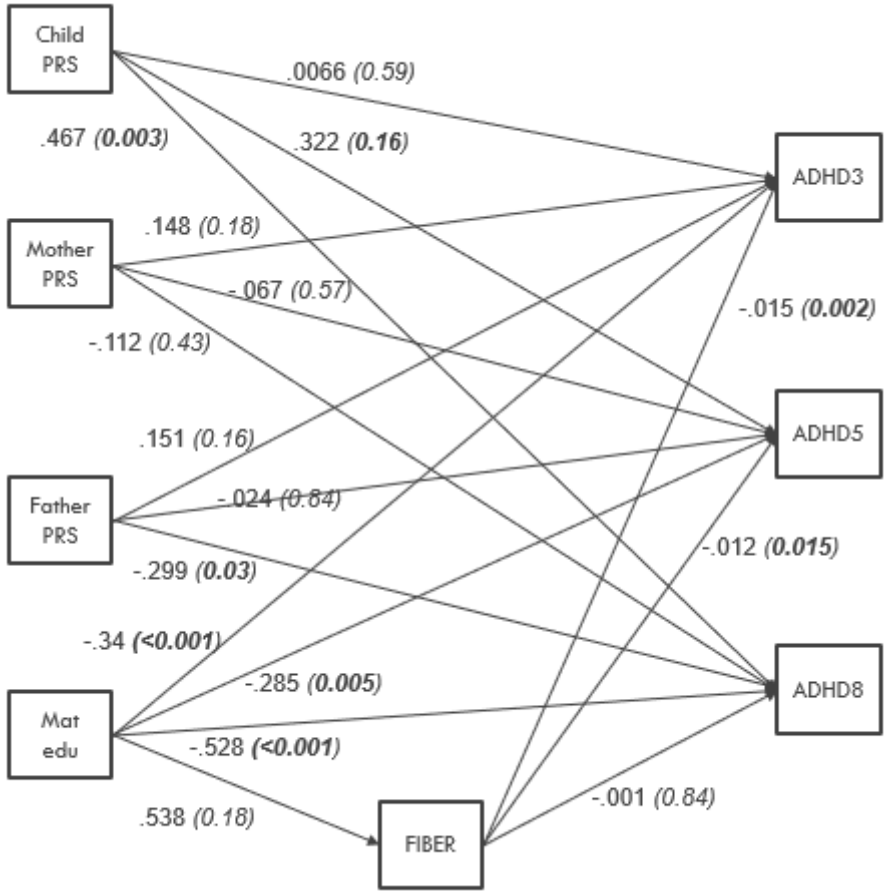
ADHD3 – ADHD-score at 3 years of age: ADHD5 – ADHD-score at 5 years; ADHD8 – ADHD-score at 8 years of age; CBCL – Child Behavior Checklist; CBM – Child Behaviuor and Manner; Conners - Conners Parent Rating Scale-Revised (CPRS-R (S)); RS-DBD - Parent/Teacher Rating Scale for Disruptive Behaviour Disorders (DSM-IV)

Table 3. SEM model 1: Statistical effects of genetics, maternal fiber intake during pregnancy, and maternal education on the overall level of ADHD symptoms in children at 3,5 and 8 years of age (HARVEST subsample, n=2,405)

	b	S.E.	*p*	R^2 %, (*p-value*)
ADHD3				**1.6 (*0.003*)**
Child PRS	0.066	0.123	*0.593*	
Mother PRS	0.148	0.109	*0.176*	
Father PRS	0.151	0.108	*0.164*	
FIBER	**-0.015**	**0.005**	***0.002***	
Maternal education	**-0.340**	**0.094**	***<0.001***	
ADHD5				**1.2 (*0.013*)**
Child PRS	**0.322**	**0.133**	***0.016***	
Mother PRS	-0.67	0.118	*0.571*	
Father PRS	-0.024	0.117	*0.838*	
FIBER	**-0.012**	**0.005**	***0.015***	
Maternal education	**-0.285**	**0.101**	***0.005***	
ADHD8				**1.8 (*0.005*)**
Child PRS	**0.467**	**0.157**	***0.003***	
Mother PRS	-0.112	0.141	*0.428*	
Father PRS	**-0.299**	**0.138**	***0.030***	
FIBER	-0.001	0.006	*0.842*	
Maternal education	**-0.528**	**0.122**	***<0.001***	

b - Unstandardized regression coefficient; S.E. – standard error of the mean; bold *p-values<0.05; R^2 – R-squared*

Figure 4. Diagram of SEM model 1: Statistical effects (regression coefficient (p-value)) of genetics and maternal fiber intake during pregnancy on the level of ADHD symptoms in children at 3,5 and 8 years of age, adjusted for maternal education

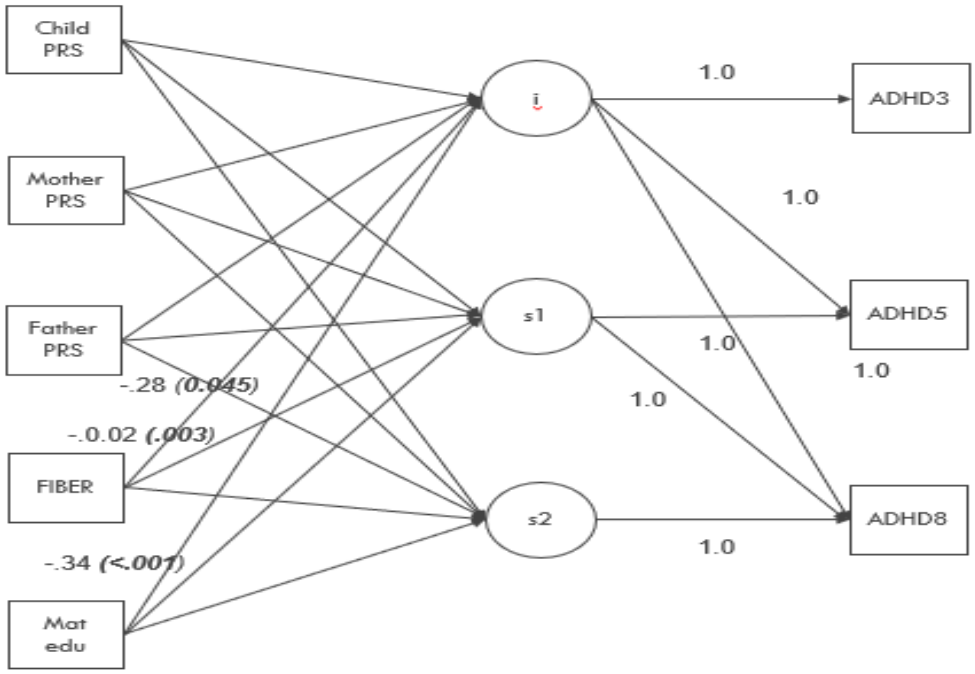


ADHD3 – ADHD-score at 3 years of age; ADHD5 – ADHD-score at 5 years; ADHD8 – ADHD-score at 8 years of age; PRS – polygenic risk score; FIBER – maternal fiber intake during pregnancy; Mat edu – maternal education as reported at delivery; p-values in brackets, bold = p<0.05

Table 4. SEM model 2: Effects of genetics and maternal fiber intake on the level of change over time in offspring's ADHD scores at 3,5 and 8 years of age, adjusted for maternal education (HARVEST subsample, n=2,405)

	b	**S.E.**	***p***	**R^2%, (*p-value*)**
	means			
Intercept				**1.6 (*0.003*)**
Child PRS	0.062	0.126	*0.621*	
Mother PRS	0.145	0.112	*0.196*	
Father PRS	0.156	0.111	*0.160*	
FIBER	**-0.015**	**0.005**	***0.003***	
Maternal education	**-0.342**	**0.096**	***<0.001***	
Slope 1				**1.2 (*0.013*)**
Child PRS	**0.257**	**0.134**	***0.055***	
Mother PRS	-0.217	0.120	*0.070*	
Father PRS	-0.179	0.118	*0.130*	
FIBER	**0.002**	**0.005**	***0.773***	
Maternal education	**0.053**	**0.103**	***0.606***	
Slope 2				**1.8 (*0.005*)**
Child PRS	**0.156**	**0.161**	***0.331***	
Mother PRS	-0.037	0.144	*0.800*	
Father PRS	**-0.283**	**0.141**	***0.045***	
FIBER	0.012	0.006	*0.054*	
Maternal education	**-0.243**	**0.125**	***0.062***	

Figure 5. Diagram of SEM model 2: Effects (regression coefficient (p-value)) of genetics and maternal fiber intake on the level of change over time in offspring's ADHD scores at 3,5 and 8 years of age, adjusted for maternal education.



ADHD3 – ADHD-score at 3 years of age; ADHD5 – ADHD-score at 5 years; ADHD8 – ADHD-score at 8 years of age; PRS – polygenic risk score; FIBER – maternal fiber intake during pregnancy; Mat edu – maternal education as reported at delivery; p-values in brackets, bold = p<0.05, I = intercept (baseline at ADHD3); s1=slope1, change between ADHD3 and ADHD5; s2=slope2, change between ADHD5 and ADHD8

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