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Interaction of genetic liability for attention deficit hyperactivity disorder (ADHD) and perinatal inflammation contributes to ADHD symptoms in children

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ABSTRACT

Objective: Genetic and environmental factors contribute to the development of Attention Deficit/Hyperactivity Disorder (ADHD). Perinatal inflammation is one of the promising environmental risk factors for ADHD, but the relationship between the genetic risk for ADHD and perinatal inflammation requires further examination.
Methods: A possible gene-environmental interaction between perinatal inflammation and ADHD polygenic risk score (ADHD-PRS) on ADHD symptoms was investigated in children aged 8–9 from the Hamamatsu Birth Cohort for Mothers and Children (N = 531). Perinatal inflammation was evaluated by the level of concentration of three cytokines assayed in umbilical cord blood. The genetic risk for ADHD was assessed by calculating ADHD-PRS for each individual using a previously collected genome-wide association study of ADHD.
Results: Perinatal inflammation (β [SE], 0.263 [0.017]; $P < 0.001$), ADHD-PRS (β [SE], 0.116[0.042]; $P = 0.006$), and an interaction between the two (β [SE], 0.031[0.011]; $P = 0.010$) were associated with ADHD symptoms. The association between perinatal inflammation and ADHD symptoms measured by ADHD-PRS was evident only in the two higher genetic risk groups (β [SE], 0.623[0.122]; $P < 0.001$ for the medium-high risk group; β [SE], 0.664[0.152]; $P < 0.001$ for the high-risk group).
Conclusion: Inflammation in the perinatal period both directly elevated ADHD symptoms and magnified the impact of genetic vulnerability on ADHD risk particularly among children aged 8–9 with genetically higher risk for ADHD.

Abbreviations: ADHD, attention deficit hyperactivity disorder; PRS, polygenic risk score; GWAS, genome-wide association study.

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1. Introduction

Attention Deficit/Hyperactivity Disorder (ADHD) is the most common neurodevelopmental disorder in childhood. While the heritability of ADHD is reported to be as high as 75%, both genetic and environmental factors play an important role in its development (Kim et al., 2020). It has been proposed that common genetic variants have a greater impact on the development of ADHD than rare ones and a recent large-scale, genome-wide association study (GWAS) of ADHD identified several genomic regions as being associated with ADHD (Demontis et al., 2019).

Polygenic risk scores (PRS) have been developed to explain the genetic liability of individuals for certain diseases or phenotypes using common genetic variants examined in GWAS (Rai et al., 2018). So far, a positive correlation between PRS for ADHD (henceforward ADHD-PRS) and ADHD symptoms has been reported, not only in diagnosed clinical cases (Hamshere et al., 2013), but also in the general population (Martin et al., 2014).

Although several environmental factors, such as maternal psychological stress, dietary intake, and adiposity (Faraone et al., 2021), are known risk factors for ADHD, recent evidence suggests that maternal inflammation during pregnancy is also associated with ADHD symptoms in offspring (Kim et al., 2020). Gustafasson et al. showed that maternal inflammation in the 3rd trimester of pregnancy, measured in plasma concentration of interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), and monocyte chemoattractant protein-1 (MCP-1, also known as C–C Motif Chemokine Ligand 2, or CCL2), is a promising marker of ADHD symptoms at age 6 (Gustafsson et al., 2020). The authors further speculated that maternal inflammation elevates the risk for ADHD in children by inducing neuroinflammation in their brain (Dunn et al., 2019). However, the potential interaction of perinatal inflammation and genetic liability for ADHD has not been explored to date.

Exploiting an ongoing longitudinal birth cohort study with information on perinatal inflammation measured by the concentration of three cytokines in umbilical cord blood (UCB) and ADHD symptoms measured by ADHD-RS at ages 8–9, we investigated 1) the association between inflammation and later ADHD symptoms and 2) the possible interaction effects of genetic liability (ADHD-PRS) and perinatal inflammation on later ADHD symptoms in children (N = 531).

2. Material and methods

2.1. Participants

Participants were from the Hamamatsu Birth Cohort for Mothers and Children (HBC Study) and included pairs of mothers and children (N = 531, 267 females and 264 males) born between December 2007 and June 2011. Recruitment procedures are fully described elsewhere (Takagai et al., 2016). The study procedures were approved by Hamamatsu University School of Medicine and the University Hospital Ethics Committee (research ID:17–037, 19–145 and 20–233), and written informed consent was obtained from each caregiver for his/her infant's participation. This study followed the STrengthening the Reporting of OBservational studies in Epidemiology (STROBE) reporting guidelines.

2.2. Measurement

2.2.1. Cytokines

Perinatal inflammation was assessed via plasma concentrations in UCB of three cytokines - interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α) and monocyte chemoattractant protein-1 (MCP-1). These measures were selected *a priori* as an index of inflammation induced by nuclear factor-kappa signaling (Liu et al., 2017), which is implicated in animal models of ADHD (Song et al., 2020) and a target pathway of pharmacotherapy of ADHD (Gur et al., 2021). These cytokines have previously been reported as elevated in children with neurodevelopmental and psychiatric disorders (Chang et al., 2020, 2021; ; Muller and Ackenheil, 1998). Ten ml of UCB were collected from 531 children immediately after delivery via venipuncture of the umbilical vein. The samples were kept at room temperature for 30 min after collection and then centrifuged at 3500 rpm for 10 min, divided in 200 μl aliquots and stored at −80 °C until analysis. The concentration of the three cytokines was measured by enzyme-linked immunosorbent assays (ELISA) according to the manufacturer's protocol. All samples were analyzed in triplicate, and the mean score was used for analysis.

2.2.2. Covariates

Covariates included were mother's body mass index (BMI) before delivery, diagnosis of diabetes during pregnancy and the mother's use of tobacco and/or alcohol during pregnancy.

2.2.3. ADHD symptoms

ADHD symptoms were ascertained by interview with the mothers when children were aged 8–9 using the Japanese version of the ADHD-Rating Scale (ADHD-RS), previously confirmed for its validity and reliability. The raw scores were converted to percentile scores using the scoring procedure provided in the manual. All psychological evaluations were conducted by trained psychologists with graduate degrees.

2.2.4. ADHD-PRS

Details of the ADHD-PRS calculation were described in our previous study (Takahashi et al., 2020; 2022). Briefly, genotyping was conducted using a Japonica array designed specifically for single nucleotide polymorphism (SNP) genotyping for a Japanese population (Kawai et al., 2015). SNPs and individuals were retained as follows: missing data for SNP <0.02, pairwise identify-by-descent (IBD) < 0.2, SNP Hardy Weinberg equilibrium of $p > 10^{-6}$ and minor allele frequency >0.01. Genotyping imputation was performed using BEAGLE 5.0 on the Japanese population in the 1000 Genome Project phase 3 reference panel (Browning et al., 2018). SNPs with an imputation INFO score <0.7 were excluded. We also excluded SNPs located within the MHC region because of high linkage equilibrium in this region. The number of SNPs analyzed for PRS was 5,606,655.

PRSice-2 was used to generate PRS according to the developers' protocol (Choi and O'Reilly, 2019). The summary GWAS data used to determine the PRS for ADHD were obtained from the Psychiatric Genomics Consortium (https://www.med.unc.edu/pgc/). To account for population stratification, we included 10 principal components (PCs). The PCs were calculated based on the pruned data with PLINK 1.9 (Chang et al., 2015). The criteria for SNP clumping were pairwise LD r^2 < 0.1 within a 1 Mb window. PRS scores were calculated with P value thresholds at 0.05 and standardized PRS scores (mean = 0 and standard deviation = 1) were used for the analyses.

2.2.5. Statistical analysis

Structural equation modeling (SEM) was used to investigate the association of perinatal inflammation and ADHD symptoms. A latent factor was created to represent perinatal inflammation with IL-6, TNF-α and MCP-1. ADHD-PRS was included in the secondary model and moderation analysis was conducted using an *nlcom* command to compute the indirect effects of the moderator variable (perinatal inflammation) and genetic liability (https://stats.idre.ucla.edu/stata/faq/how-can-i-do-moderated-mediation-in-stata/). Satorra-Bentler correction was used to correct for non-normality of the ADHD-RS percentile scores. Comparative fit index (CFI) and root mean squared error of the approximation (RMSEA) were used to evaluate the fitness of each model. Missing values of cytokines were tested for the missing completely at random (MCAR) assumption.

To explore the effect of perinatal inflammation as a function of severity of genetic liability for ADHD, the total sample was divided into four groups based on genetic risk: a high risk group with 0–20 percentile of ADHD-PRS, a medium-high risk group with 20-50- percentile of

ADHD-PRS, a medium-low risk group with 50–80 percentile of ADHD-PRS and a low risk group with 80–100 percentile of ADHD-PRS (Al Rifai et al., 2022; Hughes et al., 2021; Qassim et al., 2021). Regression analysis was used to calculate the standard coefficient of perinatal inflammation on ADHD symptoms among the different genetic risk groups. All analyses were conducted by Stata version 16.

2.2.6. Sensitivity analysis

Subjects with missing values in cytokines were excluded from the analysis. Satorra-Bentler correction does not allow imputation for missing values, so ADHD symptom severity was compared between subjects included in the analysis and those excluded.

3. Results

Characteristics of the 531 participants are summarized in Table 1. MCAR test shows missing values for cytokines (IL-6, TNFαand MCP-1) follows missing completely at random (Chi-square = 2.8949, df = 4, P = 0.5756). No statistically significant differences in ADHD symptoms are observed between subjects with or without missing values of any of the three cytokines (t = −0.9704, df = 4, P = 0.3321).

Fig. 1 shows the model including all covariates considered to be relevant to immune-related conditions in mothers. Perinatal inflammation was associated with elevated latent maternal immune-related functioning scores (β [SE], 0.083 [0.039]; P = 0.036) extracted by maternal BMI, diabetes and tobacco and alcohol use and perinatal inflammation was associated with increased ADHD symptoms (β [SE], 0.700 [0.257]; P = 0.007). Perinatal inflammation was associated with both inattention and hyperactivity symptoms (β [SE], 0.694 [0.252]; P = 0.006 for inattention; (β [SE], 0.696 [0.136]; P < 0.001 for hyperactivity).

Fig. 2 shows the model including ADHD-PRS. Both perinatal inflammation (β [SE] = 0.263[0.028]; P < 0.001) and ADHD-PRS (β [SE] = 0.116[0.042]; P = 0.006) lead to elevated ADHD symptoms in children. Similar results were obtained for inattention symptoms (β [SE] = 0.895[0.144], P < 0.001 for perinatal inflammation; β [SE] = 0.074 [0.035], P = 0.033 for ADHD-PRS) and hyperactivity symptoms (β [SE] = 0.966[0.148], P < 0.001 for perinatal inflammation; β [SE] = 0.090 [0.041], P = 0.027 for ADHS-PRS). Moderation analysis showed a positive interaction effect of perinatal inflammation and ADHD-PRS on ADHD symptoms (β [SE], 0.031[0.019]; P = 0.010). Interaction effects were consistent with inattention symptoms (β [SE] = 0.066[0.028], P = 0.022) and hyperactivity symptoms (β [SE] = 0.087[0.036], P = 0.015).

Fig. 3 shows that the effect of perinatal inflammation on ADHD symptoms varies by severity of genetic risk for ADHD. In the low and medium-low risk groups, perinatal inflammation does not increase ADHD symptoms (β [SE] = 0.029[0.500], P = 0.945 for the low risk group; β [SE] = −0.029[0.431], P = 0.946 for the medium-low risk group), whereas in the medium-high and high risk groups, it significantly increases ADHD symptoms (β [SE] = 0.611[0.181], P = 0.001 for the medium-high risk group; β [SE] = 0.651[0.274] and P = 0.018 for the high risk group).

Table 1
Sample Characteristics of children and their parents in the study.

	n (%)
Gender	
Male	264 (49.9)
Female	267 (50.1)
Ethnicity	
Japanese	531 (100)
Small for gestational age	
<10th percentile	30 (5.9)
10th-100th percentile	501 (94.1)
Paternal education	
<12 years	44 (8.5)
12 years and longer	487 (91.5)
Maternal education	
<12 years	28 (5.3)
12 years and longer	503(94.3)
	Mean (SD)
Birthweight (g)	2940.5 (438.8)
Gestational age at birth (weeks)	38.9 (1.6)
Paternal age at birth (years)	33.2 (5.8)
Maternal age at birth (years)	29.0 (5.8)
Household income (million JPY)	6.0 (2.8)
ADHD-RS percentile	
Hyperactivity	52.8 (25.8)
Inattention	50.4 (26.4)
Total	52.3 (29.3)
IQ (WISC-IV:FSIQ)	101.5(13.8)

Abbreviation: SD, Standard Deviation; JPY, Japanese Yen; ADHD-RS, Attention deficit hyperactivity disorder rating scale; IQ, intelligent quotient; WISC-IV, Wechsler Intelligent Scale for Children Version 4.

4. Discussion

The present study has two main findings. First, we demonstrate that perinatal inflammation is associated with increased ADHD symptoms scores in children 8–9 years old. Second, perinatal inflammation further magnifies the genetic liability of ADHD. This is the first study that has evaluated the inflammation profiles for offspring during the perinatal period on risk for elevated ADHD symptoms, while fully accounting for genetic liability. The study also expands the current knowledge regarding ADHD etiology by providing initial evidence that perinatal inflammation can magnify the genetic risk for ADHD symptoms later in childhood.

The present findings partially replicate the results of a previous study by Gustafsson (Gustafsson et al., 2020), which used serum from mothers in the 3rd trimester. The three selected cytokines, IL-6 and TNF-αand MCP-1, are well-established markers for nuclear-κappa signaling and have been reported to be implicated in the development of ADHD (Donfrancesco et al., 2016). Increased cytokine levels in the mother during pregnancy have been shown to activate inflammation in children, as can be seen in the increased levels of cytokines in UCB, the first inflammation marker in children (Ross et al., 2016). Animal models using maternal inflammation activation (MIA) suggest transmission of maternal inflammation to the fetus through changes in the placental barrier in the syncytiotrophoblast (STB) layer (Urakubo et al., 2001). As such, it is plausible to use maternal inflammation during pregnancy as a proxy of inflammation in offspring. Our study, however, demonstrated the direct effect of increased perinatal cytokines, instead of the mothers, in the development of ADHD in offspring. Although the exact role of the three separate cytokines in neurodevelopment remains to be elucidated, it is noteworthy that MCP-1 is identified as a risk factor for increased ADHD symptoms, since it is essential for the recruitment and activation of microglia, confirmed by a recent positron emission study that demonstrated activated microglia in the dorsolateral prefrontal cortex of ADHD participants (Yokokura et al., 2020).

The data in our study also show that both ADHD-PRS and perinatal inflammation increase ADHD symptoms in children, and that the interaction between the two has a significant effect, such that the impact of genetic liability on later ADHD symptoms is accelerated among children exposed a to greater level of inflammation in the perinatal period. It is notable that there was no direct association between ADHD-PRS and perinatal inflammation, suggesting that these two risk factors are independent. However, they act together to elevate ADHD symptoms in children, as observed in our findings that demonstrated perinatal inflammation significantly increases ADHD symptoms only in medium-high and high genetic risk groups. Taken together, these findings confirm that the interaction of perinatal inflammation and genetic risk for ADHD increases the severity of ADHD symptoms in children.

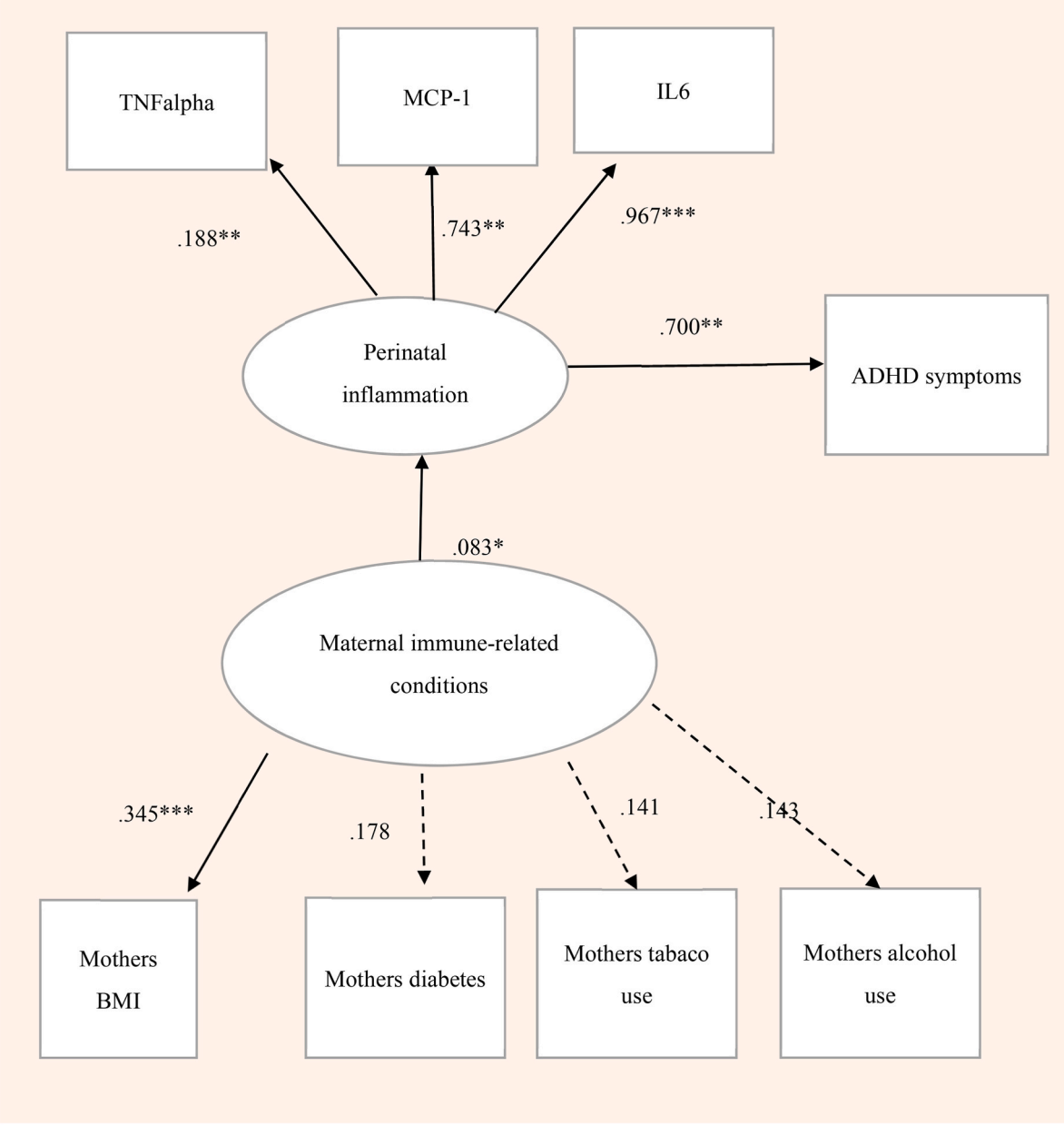

Fig. 1. Structural equation modeling of perinatal inflammation and ADHD symptoms in children including covariates.
Observed measures are represented by squares and latent variables are represented by circles. Single-headed arrows (path) define causal relationships between variables. Numbers represent the standard coefficient. CFI = 1.00, RMSEA<0.001, *P < 0.05, **P < 0.01, ***P < 0.001
Abbreviations: TNF, tumor necrosis factor-alpha; MCP-1, monocyte chemoattractant protein-1; IL-6, interleukin 6; ADHD, attention deficit hyperactivity disorder; CFI, comparative fit index; RMSEA, root mean squared error of the approximation (RMSEA).

Several potential mechanisms should be considered when explaining the interaction of the elevated perinatal inflammatory cytokines and the genetic risk for ADHD on children's ADHD symptoms. First, animals with maternal inflammation activation (MIA) by prenatal exposure to poly I:C have been used as an animal model for ADHD, and MIA has been reported to exaggerate hyperactivity and inattention in genetically modified mice by mutations in Nurr1, which is a transcription factor essential for normal dopaminergic development (Vuillermot et al., 2012). Second, the effect of perinatal inflammation on myelination should also be considered. Elevated cytokines have reportedly been associated with white matter injury (Leviton et al., 2011), which has been implicated in the etiology of ADHD from neuroimaging studies (Lesch, 2019). Interestingly, a recent, large-scale, genome-wide association study found that myelin-related genes, such as ST3GAL3, FOXP2, MEF2C, DUSP6, and SEMA6D, are associated with ADHD (Demontis et al., 2019). Therefore, it is fair to hypothesize that elevated perinatal cytokines induce abnormal myelin development in infants, which subsequently leads to increased ADHD symptoms in children with higher genetic risk for ADHD.

The present findings regarding the roles of perinatal inflammation in relation to ADHD risk in offspring also help to explain previous findings indicating increased risk for ADHD in association with several maternal factors, such as maternal obesity, tobacco and alcohol use, and diabetes (Kim et al., 2020), all of which are associated with high levels of inflammation. Although the importance of controlling these maternal risk factors for ADHD is well understood, a recent study by Usui et al. suggests that antioxidants reduce perinatal inflammation in the placenta of MIA models and may be an effective tool to reduce the severity of

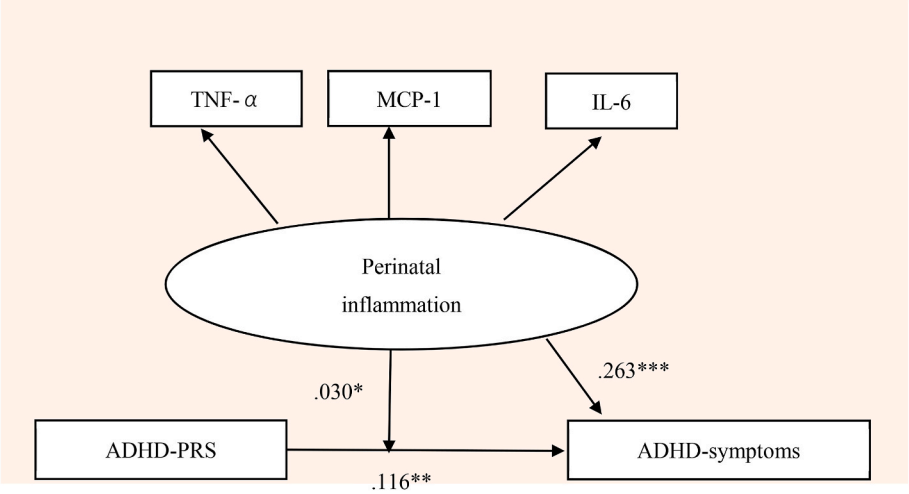

Fig. 2. Moderation analysis of perinatal inflammation, ADHD-PRS and ADHD symptoms in children.
Observed measures are represented by squares and latent variables are represented by circles. Single-headed arrows (path) define causal relationships between variables. Covariates and the paths from covariates to maternal distress were not shown to simplify the figure. Numbers represents the standard coefficient. Maternal immune-related conditions are not included in Fig. 2 because of the complexity of the figure. CFI = 1.00, RMSEA<0.001, *P < 0.05, **P < 0.01, ***P < 0.001.
Abbreviations: TNF, tumor necrosis factor-alpha; MCP-1, monocyte chemoattractant protein-1; IL-6, interleukin 6; ADHD-PRS, attention deficit hyperactivity disorder-polygenic risk score; ADHD, attention deficit hyperactivity disorder; CFI, comparative fit index; RMSEA, root mean squared error of the approximation (RMSEA).

ADHD symptoms in offspring (Usui et al., 2021). Furthermore, non-steroid anti-inflammatory agents are reported to reduce expression of pro-inflammatory cytokines, including IL-6 in the placenta, and ameliorate phenotypes resembling ADHD such as hyperactivity (Bronson and Bale, 2014). However, an epidemiological study examining the use of acetaminophen in mothers and risk for ADHD in their children came to a different conclusion, finding that maternal use of acetaminophen in pregnancy increased the risk of ADHD in children (Liew and Ernst, 2021). Additional data are required, therefore, to assess whether anti-inflammatory agents could be used to suppress perinatal inflammation and thereby decrease risk for ADHD. This is a promising direction to investigate.

There are limitations to this study. First, we were unable to include some important inflammation markers in the analysis. Substantial assays for CRP and IL-1β, for example, were beyond detection limits. However, we expect future studies that include other cytokines to strengthen our findings. Subjects with missing values of IL-6, TNF-α and MCP-1 were excluded from the analysis, but since there is no difference in ADHD symptoms between included and excluded subjects, we do not believe this affects the results. Second, it is not known whether perinatal inflammation continues throughout fetal and perinatal development, although maternal inflammation is proposed to increase cytokines even in UCB samples (Frascoli et al., 2018). While a replication study using independent samples is needed to establish the clinical utility of the three UCB cytokines studied here to predict subsequent risk of ADHD symptoms in children, our findings provide a possible modifiable target for identification of risk. If replicated, the results of this study would provide further clues regarding the biological mechanisms underlying ADHD, and potentially offer new directions for secondary prevention among children with high genetic vulnerability for ADHD.

5. Conclusion

Inflammation in the perinatal period both directly elevated ADHD symptoms and magnified the impact of genetic vulnerability on ADHD risk particularly among children aged 8–9 with genetically higher risk for ADHD.

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Role of the funder/sponsor

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Declaration of competing interest

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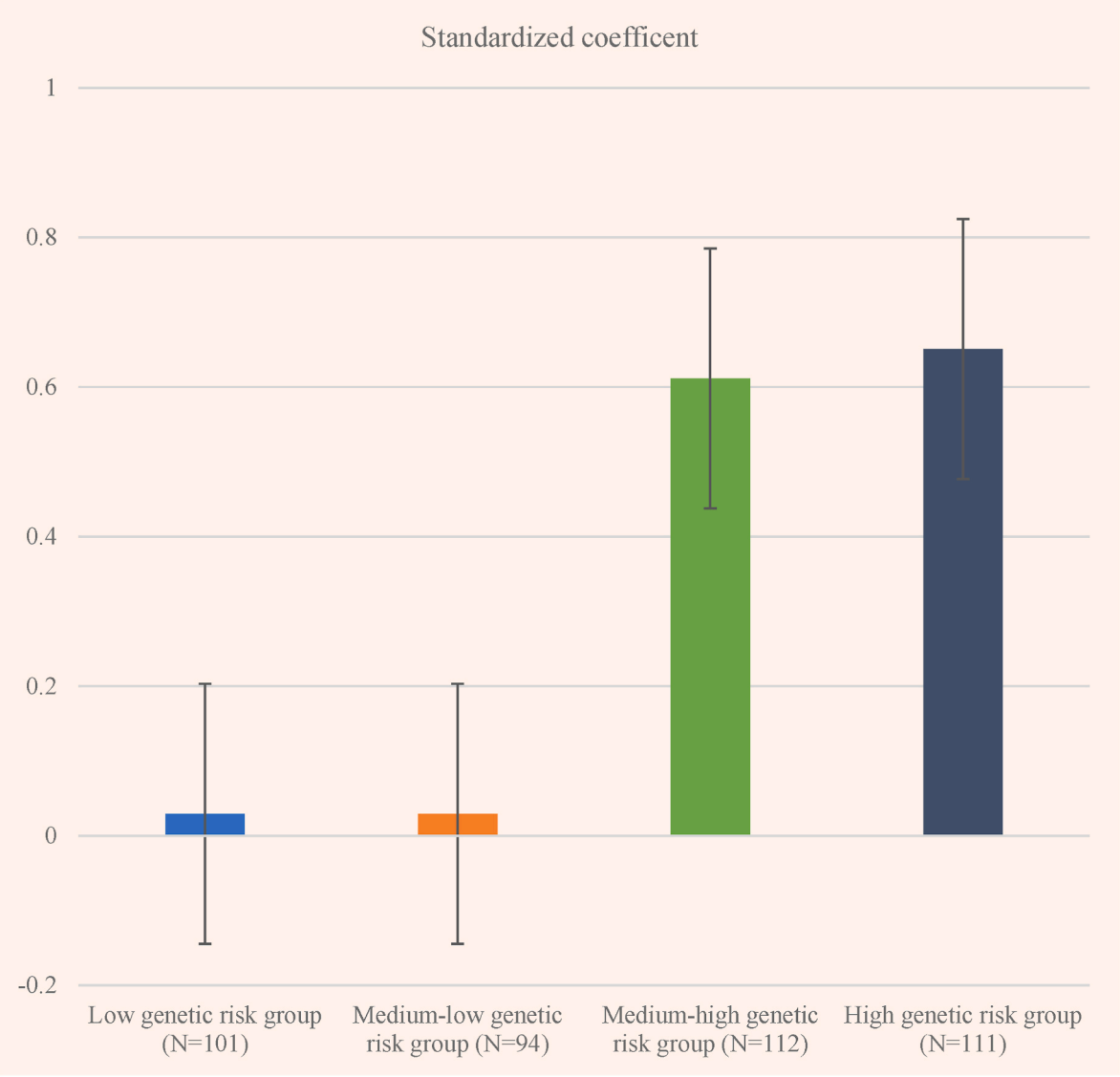

Fig. 3. Distinct effect of perinatal inflammation on ADHD symptoms among children with different levels of genetic risk for ADHD.
X axis represents standardized coefficients in regression analysis between perinatal inflammation and ADHD-RS percentile of children. Error bars represent standard error.
*P < 0.05, **P < 0.01, ***P < 0.001.
Abbreviations: ADHD-RS, attention deficit hyperactivity disorder rating scale.

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Data availability

The authors do not have permission to share data.

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