Archival Report

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Prefrontal Cortex Dopamine Transporter Gene Network Moderates the Effect of Perinatal Hypoxic-Ischemic Conditions on Cognitive Flexibility and Brain Gray Matter Density in Children

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ABSTRACT

BACKGROUND: Genetic polymorphisms of the dopamine transporter gene (*DAT1*) and perinatal complications associated with poor oxygenation are risk factors for attentional problems in childhood and may show interactive effects.

METHODS: We created a novel expression-based polygenic risk score (ePRS) reflecting variations in the function of the *DAT1* gene network (ePRS-*DAT1*) in the prefrontal cortex and explored the effects of its interaction with perinatal hypoxic-ischemic–associated conditions on cognitive flexibility and brain gray matter density in healthy children from two birth cohorts—MAVAN from Canada (n = 139 boys and girls) and GUSTO from Singapore (n = 312 boys and girls).

RESULTS: A history of exposure to several perinatal hypoxic-ischemic–associated conditions was associated with impaired cognitive flexibility only in the high-ePRS group, suggesting that variation in the prefrontal cortex expression of genes involved in dopamine reuptake is associated with differences in this behavior. Interestingly, this result was observed in both ethnically distinct birth cohorts. Additionally, parallel independent component analysis (MAVAN cohort, n = 40 children) demonstrated relationships between single nucleotide polymorphism–based ePRS and gray matter density in areas involved in executive (cortical regions) and integrative (bilateral thalamus and putamen) functions, and these relationships differ in children from high and low exposure to hypoxic-ischemic–associated conditions.

CONCLUSIONS: These findings reveal that the impact of conditions associated with hypoxia-ischemia on brain development and executive functions is moderated by genotypes associated with dopamine signaling in the prefrontal cortex. We discuss the potential impact of innovative genomic and environmental measures for the identification of children at high risk for impaired executive functions.

Keywords: ADHD, Cognitive flexibility, *DAT1*, Dopamine transporter gene, Hypoxic-ischemic conditions, Parallel independent component analysis

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The dopamine transporter (DAT) is a transmembrane protein responsible for the reuptake of dopamine (DA) from the synaptic cleft into the presynaptic neuron, which thereby terminates DA signaling (1,2). The DAT regulates the strength and duration of dopaminergic transmission, a role that is revealed by the effects of many DAT-targeted pharmacological therapies, such as methylphenidate, that improve DA dysfunction in attention-deficit/hyperactivity disorder (ADHD) (3–5). Although DAT is abundant in the striatum and sparse in the prefrontal cortex (PFC) (6,7), several studies show that low doses of methylphenidate (dosages that are usually more effective in treating attentional impairments than hyperactivity) preferentially increase DA release in the PFC (8–11). The PFC is highly involved in executive functions—a set of cognitive processes comprising cognitive flexibility and planning—that are typically impaired in children with ADHD (12–14).

The DAT gene (*DAT1*, also known as *SLC6A3*), which is located in chromosome 5p15.3, is one of the most studied genes in ADHD (15). A 40-bp variable number of tandem repeats polymorphism and single nucleotide polymorphisms

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(SNPs), for example rs2652511, were identified as risk factors for ADHD (16–18). Although we acknowledge the contribution of studies focusing on a single candidate polymorphism, genes act not in isolation but in concert with other genes in molecular pathways. The principle of gene networks considers that gene expression is coregulated by other genes, and consequently genes involved in the same network are expected to have similar expression profiles (19). Analyzing genomic data through gene sets defined by functional pathways represents a potentially powerful and biologically oriented link between genotypes and phenotypes (20).

Although genetic factors contribute substantially to the etiology of ADHD, there is considerable evidence for the influence of environmental factors (21). Getahun *et al.* (22) identified a direct relationship between hypoxic-ischemic–associated conditions (HICs) in utero and the later development of ADHD. Preeclampsia, Apgar score <7 at 1 or 5 minutes, breech or transverse presentations, prolapsed or nuchal cord, and elective cesarean births were all significantly associated with ADHD (23,24). Using an animal model of perinatal hypoxia-ischemia, we demonstrated cognitive inflexibility using the attentional set-shifting task, a task comparable to the Intra-Extra/Dimensional (IED) set shift in humans. Additionally, we showed that the cognitive inflexibility in this model was correlated to PFC atrophy and dopaminergic dysregulation also in the PFC, reflecting the profile reported in ADHD individuals (25).

Our rodent study suggests that perinatal hypoxia-ischemia associates with both impaired cognitive flexibility and altered PFC DA signaling. Considering that the literature has linked *DAT1* polymorphisms and ADHD, we hypothesized that a genomic measure based on a PFC-specific *DAT1* gene network would moderate the impact of perinatal HICs on cognitive flexibility in children. To test this hypothesis, we constructed an expression-based polygenic risk score (ePRS) that reflects the function of a PFC *DAT1* gene network (ePRS-*DAT1*/PFC) and analyzed its interaction with perinatal HICs on cognitive flexibility performance and brain gray matter density in healthy children.

METHODS AND MATERIALS

Participants

We used data from two prospective birth cohorts, one based in Canada [Maternal Adversity, Vulnerability and Neurodevelopment (MAVAN) (26)] and the other from Singapore [Growing Up in Singapore Towards Healthy Outcomes (GUSTO) (27)].

Main Cohort (MAVAN). MAVAN is a community-based, birth-cohort study of Canadian mothers and their offspring. Pregnant women aged 18 years and above were recruited in Montréal (Quebec) and Hamilton (Ontario), Canada. Approval for the MAVAN project was obtained from McGill University, Université de Montréal, Royal Victoria Hospital, Jewish General Hospital, Centre hospitalier de l'Université de Montréal, Hôpital Maisonneuve-Rosemount, St Joseph's Hospital, and McMaster University. A total of 139 children of both sexes had complete data (birth records, genotype, and cognitive flexibility task at 6 years of age) and were included in the study. Of this sample, 40 participants had brain magnetic resonance imaging (MRI) used for the parallel independent component analysis (p-ICA) (see the subsection "Parallel Independent Component Analysis").

Replication Cohort (GUSTO). Pregnant women $\geq$18 years of age were recruited at the National University Hospital and KK Women's and Children's Hospital in Singapore. The cohort included women of Chinese, Malay, or Indian ethnicity with homogeneous parental ethnic background that allowed us to extend the analysis to include Southeast Asian ethnic groups (28). The study was approved by the National Healthcare Group Domain Specific Review Board and the Sing Health Centralized Institutional Review Board. Informed consent was obtained from each participating adult. A total of 312 children had complete data (birth records, genotype, and cognitive flexibility task at 4.5 years of age) and were included in the study.

PFC *DAT1* Coexpressed Genes and ePRS

The ePRS was created considering genes coexpressed with the DA transporter gene (ePRS-*DAT1*) in the PFC, according to the protocol previously described by Silveira *et al.* (28,29) (Supplemental Figure S1). The genetic score was created using data from the 1) GeneNetwork (http://genenetwork.org), 2) BrainSpan (http://www.brainspan.org), 3) National Center for Biotechnology Information Variation Viewer (https://www.ncbi.nlm.nih.gov/variation/view), and 4) Genotype-Tissue Expression (GTEx) (https://www.gtexportal.org/home). A full explanation and the final list of coexpressed genes included in the ePRS (Supplemental Table S1) are described in the Supplement. The final score in both cohorts was categorized into "low ePRS" or "high ePRS" using a median split for the behavioral analysis.

Perinatal HICs Score

We aggregated information reported by Getahun *et al.* (22) and Linnet *et al.* (30) and compiled by Smith *et al.* (31), which provided the list of nine variables to consider in the score: 1) Apgar score at 1 minute <7 (32), 2) respiratory distress, 3) fetal dystocia, 4) occurrence of umbilical cord prolapse, 5) placental abruption, 6) breech or transverse birth presentation, 7) neonatal resuscitation, 8) maternal smoking during pregnancy, and 9) birth weight ratio (observed birth weight/mean populational weight adjusted by sex and gestational age) (33,34). Each one of these variables was categorized into absent or present condition (Supplemental Table S2).

A principal component analysis was performed on these variables using tetrachoric correlations, extracting one component. Two variables (maternal smoking during pregnancy and birth weight ratio) did not exhibit significant component loadings and were excluded. We reapplied a principal component analysis to the remaining variables to compute the perinatal HICs score according to the method proposed by Distefano *et al.* (35). This method aims to maximize validity by producing scores that are highly correlated with the underlying component in order to obtain unbiased

estimates (35). A higher absolute value of the loading is indicative of a larger contribution of the corresponding variable to the component score (see Supplemental Table S2 and Supplemental Figure S2).

The HICs score was either used as a continuous variable (for the behavioral outcomes) or categorized into low or high HICs score groups using a median split (for the p-ICA). Low HICs score indicates minimal exposure to HICs in the perinatal period, and a high HICs score suggests a history of exposure to several HICs.

Behavioral Outcomes

IED Set Shift. The IED set shift task comprises rule acquisition and reversal throughout nine stages with increasing difficulty. There are two dimensions used in the task (color-filled shapes and white lines), and in the first seven stages, shape remains the relevant dimension. An extra-dimensional shift occurs in stage 8, where the white lines are the relevant dimension for a correct response (36) (Supplemental Figure S3A). This task is part of the CANTAB battery and was performed by MAVAN children at 72 months of age. We focused specifically on stage 8 (extra-dimensional shift), which measures cognitive flexibility.

Dimensional Change Card Sort. The Dimensional Change Card Sort (DCCS), like the IED task, measures the ability to shift between two dimensions, but it is more readily completed by younger children. For this reason, we used this task to assess the cognitive flexibility of children at 54 months of age in the GUSTO cohort. In the standard version of the DCCS task, children are shown cards with two dimensions: different colors (red vs. blue) and shapes (rabbit vs. boat). In the first stage (preswitch), children must sort the cards by the color dimension independently of the shape presented on the cards. In the following stage (postswitch), the rule changes and children must sort the cards by the shape dimension and ignore the first rule, requiring attentional flexibility (37) (Supplemental Figure S3B).

Gray Matter Density

Parallel Independent Component Analysis. Structural MRI acquisition and data preparation were conducted prior to the p-ICA (see the Supplement). A multivariate p-ICA was applied to identify relationships between clusters of interrelated SNPs and brain gray matter information in a data-driven manner (38). We sought to find the relationship between the SNP-based ePRS-*DAT1* (or genotype × GTEx gene expression slope at each SNP comprised by the ePRS-*DAT1*) and the voxel-based gray matter in the whole brain (full description in the Supplement), instead of investigating the relationship between the crude genotype and the gray matter-voxel-based measures. The groups for comparison (20 children with high HIC score and 20 children with low HIC score) were defined by the perinatal environment aggregated with population stratification (ethnicity) for adjustment. Loading coefficients, which describe the presence of the identified component across participants (39), were extracted for each component, modality, and participant. The mean participant-specific loading coefficients of these components between children from high- and low-HICs-score groups was compared using Student's *t* test.

Validation of the Prefrontal *DAT1* Coexpression Network

Gene Expression Levels at Different Time Points. We used BrainSpan data to analyze the correlation between the expression levels of all genes included in the ePRS-*DAT1* in the human PFC at different time points: perinatal, childhood, and adulthood. Thus, we can investigate whether the same pattern of coexpression is maintained through the life course. The analyses were performed in R (https://www.r-project.org) using the heatmaply package (40).

Gene Ontology Enrichment Analysis. Enrichment analysis for functional ontologies of the genes included in ePRS-*DAT1* was performed using Metacore (https://portal.genego.com).

Protein–Protein Interaction Network Analysis. The STRING database (https://string-db.org) was used to analyze functional interactions between the corresponding protein from our list of *DAT1* coexpressed genes (269 genes) and the same top 269 genes associated with SNPs from the genome-wide association study (GWAS) for ADHD (41). We compared the mean number of interactions of the top 20 most interactive proteins of each dataset (ePRS-*DAT1* and GWAS-ADHD).

Comparison With Another PFC ePRS. As the creation of the ePRS-*DAT1* was highly informed by the main action of a pharmacological agent (e.g., methylphenidate), we created a control PFC ePRS with the same premise and using the same methods. The control ePRS had the serotonin transporter solute carrier family 6 member 4 (*SLC6A4*) as the target (ePRS-*SLC6A4*) considering the action of serotonin reuptake inhibitors (e.g., fluoxetine). This choice is interesting because methylphenidate also acts on the serotonin transporter, but with much less affinity than that for the DAT (42,43).

Statistical Analysis

Data were analyzed using the SPSS version 20.0 (SPSS Inc., Chicago, IL) and R. Significance levels for all measures were set at $\alpha = .05$. Student's *t* test was performed to compare 1) the mean number of protein interactions between the ePRS and GWAS-ADHD, 2) the continuous data of the sample baseline characteristics between low and high ePRS, and 3) the mean subject-specific loading coefficients between high- and low-HICs-score groups (p-ICA). χ^2 tests were performed to analyze the categorical variables of the sample baseline characteristics. Linear regressions were used to examine the effect of interaction between the genetic score (median split: low and high ePRSs) and the perinatal HICs (continuous variable: HICs) on the behavioral outcomes (IED and DCCS tasks). The ePRSs and HICs scores were included as main factors along with covariates of sex and population stratification. Additionally, the preswitch performance was included as covariate for the DCCS task. Simple

slope analyses were conducted to analyze the post hoc differences.

RESULTS

Behavioral Outcomes

In both MAVAN and GUSTO datasets, children with high and low genetic scores on ePRS-*DAT1* do not differ in the main confounding variables, which were chosen based on the literature (see Table 1). We considered well-established variables that affect child neurodevelopment as possible confounders: birth weight and gestational age (44–46), maternal age (47), socioeconomic status (48), and maternal education level (49).

IED Task (Stage 8, Extra-Dimensional Shift). In the IED task, we observed a significant ePRS × HICs interaction in predicting the latency to respond ($\beta = 32489.1$, $p < .001$) at stage 8 of the task. The simple slopes analysis showed that the high-ePRS group demonstrated worse outcomes (higher latency to respond) as HICs score increased ($\beta = 29002.6$, $p < .05$) (Figure 1A). No significant interactions were seen for number of trials ($\beta = 3.92$, $p = .14$) or errors ($\beta = 1.89$, $p = .29$). Results for other IED stages are shown in Supplemental Table S3.

DCCS Task (Postswitch Phase). We then replicated the ePRS-*DAT1*/PFC × HICs interaction effect in the GUSTO cohort. The significant ePRS-*DAT1* × HICs score interaction was observed for total accuracy ($\beta = -.46$, $p < .05$) and number of commission errors ($\beta = .44$, $p < .05$) in the postswitch phase. Higher HICs was associated with lower accuracy ($\beta = -.50$, $p < .001$) and higher commission errors ($\beta = .44$, $p < .01$) only in the high-ePRS group (Figure 1B and 1C, respectively). The adjusted/unadjusted analysis for IED stage 8 in MAVAN and DCCS in GUSTO for the main effect model and for the model including the interaction term are described in Supplemental Table S4.

Specificity of the *DAT1* Gene Network. We then analyzed the specificity of our findings in relation to the *DAT1* gene network. We used the same ePRS bioinformatic process (Supplemental Figure S1) to create an ePRS from genes that are coexpressed with the *SLC6A4* gene in the PFC. Despite the fact that ePRS-*SLC6A4* also formed a cohesive gene network (Supplemental Figure S4), there were no significant interactions between this genetic score and the HICs score on IED outcomes (number of trials, $\hat{\beta} = -1074.52$, $p = .11$; number of errors, $\hat{\beta} = -576.50$, $p = .20$; and latency, $\hat{\beta} = 87428.9$, $p = .97$).

Gray Matter Density

The p-ICA identified highly significant relationships between regional gray matter volume and SNP-based ePRS-*DAT1* data on 1) the genetic component 2 and MRI component 1 ($r = -.77$, $p = 5.953 \times 10^{-9}$); 2) genetic component 5 and MRI component 7 ($r = .69$, $p = 7.1487 \times 10^{-7}$); and 3) genetic component 12 and MRI component 4 ($r = -.61$, $p = 2.1291 \times 10^{-5}$). When comparing the mean loading coefficients of these components between children from high- and low-HICs-score groups by Student's *t* test, we found statistically significant differences in the pair genetic component 2 and MRI component 1 (Figure 2), suggesting that the relationship between ePRS-*DAT1* and gray matter volume in these brain regions is moderated by the neonatal environmental condition. Genetic component 5 was also significantly different between the groups. The pair MRI component 7 did not reach significance ($p = .069$), although it is clear on Figure 2 that groups have opposite directions in loading coefficients. For the other relationship between genetic component 12 and MRI component 4, no differences between groups were observed.

To define the significant SNPs in each component, we used a threshold of higher than 2.5 and lower than −2.5. In component 2, we found 78 significant SNPs, and the enrichment analysis demonstrated that these SNPs are involved especially in positive regulation of long-term synaptic potentiation (false discovery rate [FDR]–adjusted $q = 3.635 \times 10^{-6}$), astrocyte activation (FDR-adjusted $q = 6.529 \times 10^{-6}$), dopaminergic transmission (FDR-adjusted $q = 2.710 \times 10^{-5}$), and gamma-aminobutyric acidergic transmission (FDR-adjusted $q = 1.867 \times 10^{-6}$). Additionally, these SNPs were enriched for diseases including anxiety disorders (FDR-adjusted $q = 9.243 \times 10^{-5}$), schizophrenia (FDR-adjusted $q = 3.269 \times 10^{-3}$), and dementia (FDR-adjusted $q = 9.835 \times 10^{-3}$). This group of SNPs was related to differential gray matter density in areas of the putamen and thalamus (MRI component 1). In

Table 1. Description of the Baseline Characteristics of the MAVAN and GUSTO Samples

	MAVAN			GUSTO		
Sample Characteristic	Low ePRS, *n* = 67	High ePRS, *n* = 72	*p* Value	Low ePRS, *n* = 150	High ePRS, *n* = 162	*p* Value
Male Participants, *n* (%)	38 (56.7)	35 (48.6)	.339	62 (41.3)	82 (50.6)	.100
Maternal Age at Birth, Years	31.25 ± 5.18	31.82 ± 4.19	.075	31.45 ± 5.21	31.62 ± 5.18	.772
Full Weeks of Gestation	39.09 ± 1.28	38.78 ± 1.14	.248	38.60 ± 1.22	38.33 ± 1.33	.324
Birth Weight, Grams	3404.68 ± 429.13	3366.11 ± 440.65	.732	3131.65 ± 411.48	3067.46 ± 420.42	.741
HICs Score	0.04 ± 1.07	−0.14 ± 0.81	.179	0.04 ± 1.04	−0.04 ± 0.96	.473
Low SES[a], *n* (%)	14 (23.3)	8 (14.3)	.214	28 (19.4)	21 (13.6)	.176

Data are expressed as mean ± SD unless otherwise noted.

Differences between low- and high-ePRS groups were not significant for all variables shown (Student's *t* test for means and χ^2 test for percentages).

ePRS, expression-based polygenic risk score; GUSTO, Growing Up in Singapore Towards Healthy Outcomes; HICs, hypoxic-ischemic–associated conditions; MAVAN, Maternal Adversity, Vulnerability and Neurodevelopment study; SES, socioeconomic status.

[a]Low SES in MAVAN: Maternal education attained high school or less, or monthly income under low bound from the cutoff proposed by Statistics Canada (71). Low SES in GUSTO: Maternal education attained primary school or monthly income <$2000.

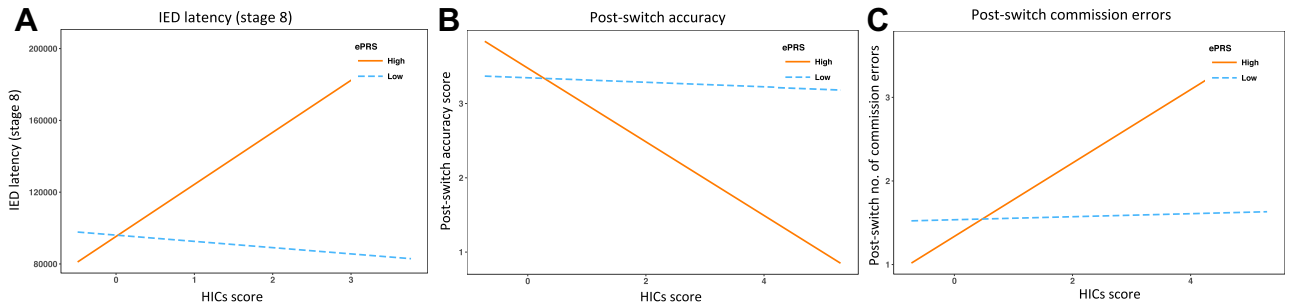

Figure 1. Cognitive flexibility performance **(A)** in the Intra-Extra/Dimensional (IED) set shift task in children in the Maternal Adversity, Vulnerability and Neurodevelopment study and **(B and C)** in the Dimensional Change Card Sort task in children in the Growing Up in Singapore Towards Healthy Outcomes study. **(A)** A higher hypoxic-ischemic–associated conditions (HICs) score was associated to longer latency to respond only in the high expression-based polygenic risk score (ePRS) group in the IED task. **(B and C)** A higher HICs score was associated with lower accuracy and higher number of commission errors only in the high-ePRS group in the Dimensional Change Card Sort task. Analysis comprised linear regression followed by simple slope analysis.

genetic component 5, we found 77 significant SNPs that were involved in nervous system development (FDR-adjusted $q = 7.617 \times 10^{-4}$), neurogenesis (FDR-adjusted $q = 7.365 \times 10^{-4}$), and neuron migration (FDR-adjusted $q = 6.755 \times 10^{-4}$). This component was related to differential gray matter in cortical regions (MRI component 7).

Validation of the *DAT1* Coexpression Network

We used BrainSpan data to correlate the PFC expression levels of all genes included in the ePRS during the perinatal period. We observed two large clusters of highly coexpressed genes specifically at this developmental period (Figure 3A). These findings confirmed the coexpression network from the genes included in the ePRS-*DAT1* in the PFC. For the developmental trajectory analysis, we kept the same order of the genes that composed the perinatal correlation matrix to visualize whether the clusters would be consistent throughout development. We observed that the general pattern of coexpression was generally maintained during the life course (Figure 3B and 3C). We analyzed *DAT1* expression by age in different human brain regions using Human Brain Transcriptome data (http://hbatlas.org) (50) and observed stable gene expression throughout development, with very similar levels of expression in the neocortex and striatum (Supplemental Figure S5).

The gene ontology enrichment analysis showed several statistically significant enrichment processes, functions, and cellular localizations, and we focused on the top 10 significant

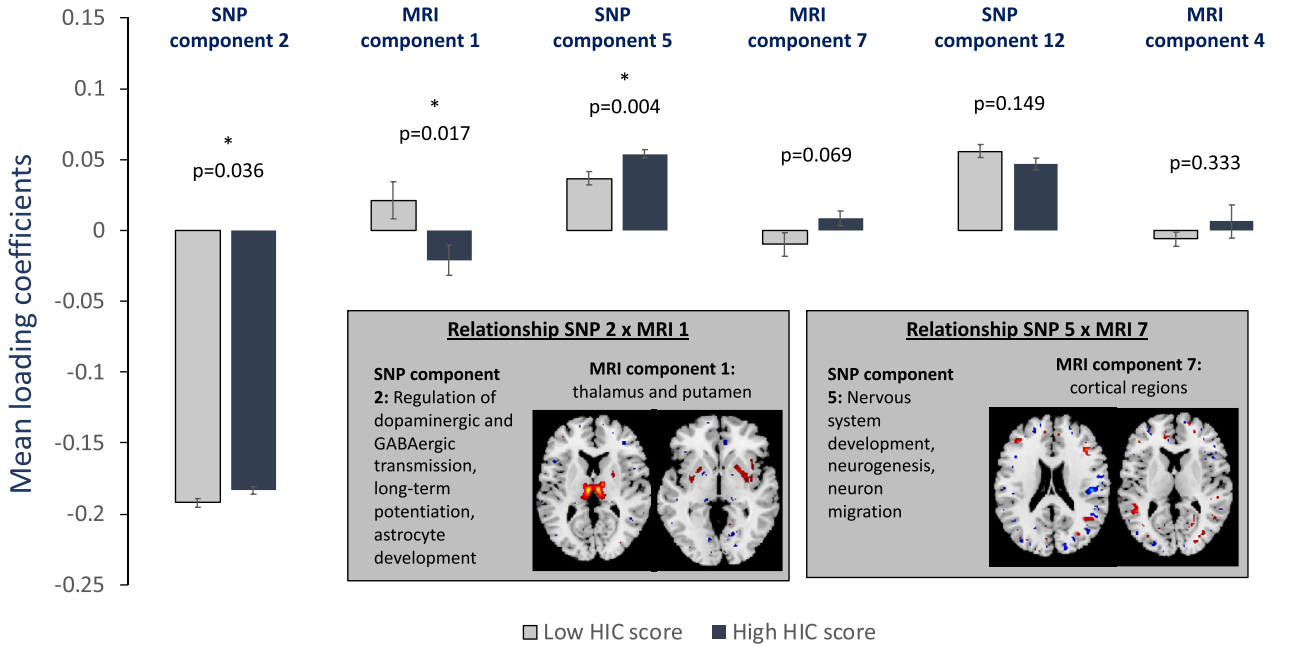

Figure 2. A bar plot of the mean loading coefficients of brain magnetic resonance imaging (MRI) component and genetic component. Student's *t* test was performed to compare loading coefficients means between groups. *Group differences among children with low and high hypoxic-ischemic–associated condition (HIC) scores. GABAergic, gamma-aminobutyric acidergic; SNP, single nucleotide polymorphism.

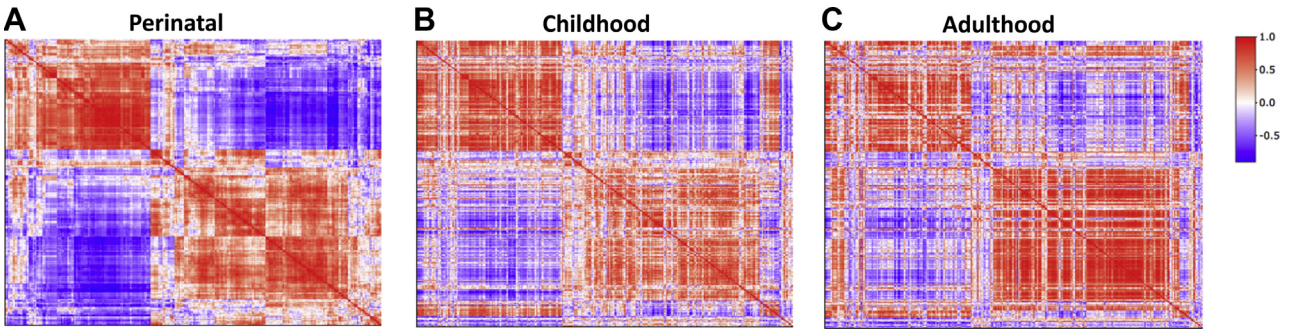

Figure 3. Heat map of *DAT1*-related genes (expression levels) correlation throughout development in the human prefrontal cortex. Genes from the same expression quantification tend to cluster together and could be visualized in red (positive correlation). A blue pattern indicates a negative correlation. In this analysis, the arrangement in clusters considering similar gene expression are shown in the **(A)** perinatal, **(B)** childhood, and **(C)** adulthood stages. The perinatal period ranges from 24 weeks post conception to 4 months of age (n = 5); childhood is defined as 1–12 years of age (n = 8) and adulthood as 20–40 years of age (n = 7).

results (Supplemental Figure S6). This analysis revealed gene ontology processes involving neurodevelopmental processes and cell signaling, among others. Molecular functions were enriched for protein binding (FDR-adjusted $q = 8.71 \times 10^{-11}$) and transmembrane receptor tyrosine kinase activity (FDR-adjusted $q = 1.28 \times 10^{-\times}$). Gene ontology localizations were enriched for extracellular space, cytoplasm, adherens junction, and cell junction.

The protein–protein network analysis is depicted in Figure 4, demonstrating the protein network resulting from the ePRS-*DAT1* (Figure 4A), and the top genes (comparable size) from the 2017 ADHD GWAS (41) (Figure 4B). Analyzing the top 20 most interactive proteins, we found a significantly higher number of interactions in our *DAT1* network compared with those in the GWAS-ADHD dataset ($p < .0001$, mean *DAT1* = 11.65 ± 5.41, mean GWAS-ADHD = 2.8 ± 1.64), suggesting that the ePRS-*DAT1* represents a more cohesive gene network.

DISCUSSION

We used a novel informatics approach to show that the association between HICs and executive function in childhood is moderated by genetic variants in a PFC-specific *DAT1* gene network. A composite measure of perinatal HICs was related to cognitive flexibility only for children with a genetic background reflecting higher PFC activity of the DAT machinery (high ePRS-*DAT1*). This result was observed in two ethnically distinct birth cohorts: MAVAN (Caucasians from Canada) and GUSTO (Southeast Asians from Singapore). The SNP-based ePRS-*DAT1* also moderated the relation between perinatal HICs and gray matter density in areas involved in executive (cortical regions) and integrative (bilateral thalamus and putamen) functions.

The DA system is implicated in the regulation of cognitive flexibility. In clinical trials, systemic administration of an antagonist of the DA receptor D_2 impaired the attentional set-shifting performance (51), whereas methylphenidate administration improved performance (52). One of the trigger points for DA system dysfunction seems to be the DAT, and several studies reveal associations between polymorphisms in the *DAT1* gene and a higher risk for attentional problems (17,18,53–55). Our findings extend studies focusing on single variants to show that a *DAT1* PFC expression–based gene network moderates the impact of perinatal conditions known to increase the risk for ADHD on executive function in childhood. These findings are consistent with the position that the analysis of gene sets defined by functional pathways is a promising approach for investigating the relationship between genotypes and phenotypes (20).

We validated our *DAT1* network using several approaches. We used databases that included gene expression levels in human PFC to demonstrate that a high proportion of the *DAT1*-related genes have similar expression levels in the perinatal period, confirming that the coexpression patterns within this gene network go beyond the coexpression with *DAT1* only, but they form several clusters of coexpressed genes. Clusters of coexpressed genes are generally maintained throughout development, suggesting that this network is also operative at later ages. Protein network analysis resulting from the ePRS-*DAT1* shows that the *DAT1* network represents a more cohesive protein network with significantly more connections than the protein network resulting from the same number of top genes from the most recent GWAS for ADHD (41). An ePRS based on PFC *SLC6A4* coexpression produced a gene network that was not associated with the cognitive flexibility performance in children, emphasizing the specificity of the ePRS technique.

The ePRS method is a robust approach that goes beyond finding associations between scattered genetic variants and phenotypes and captures information about the whole gene network, and its function, in specific brain regions (28,29). Our enrichment analysis for the *DAT1* network included nervous system development, which is in agreement with the choice of genes overexpressed during fetal and early postnatal periods when we filtered using BrainSpan data. Enrichment for many extracellular localizations, but also the cytoplasmic part, adherens, and cell junction, is aligned with the reported function of DAT mediating the transport of extracellular DA to the intracellular space (56,57). Multiple intracellular and extracellular signaling pathways have been implicated in the regulation of DAT function, and its expression is modulated through internalization and recycling from the cell surface (58).

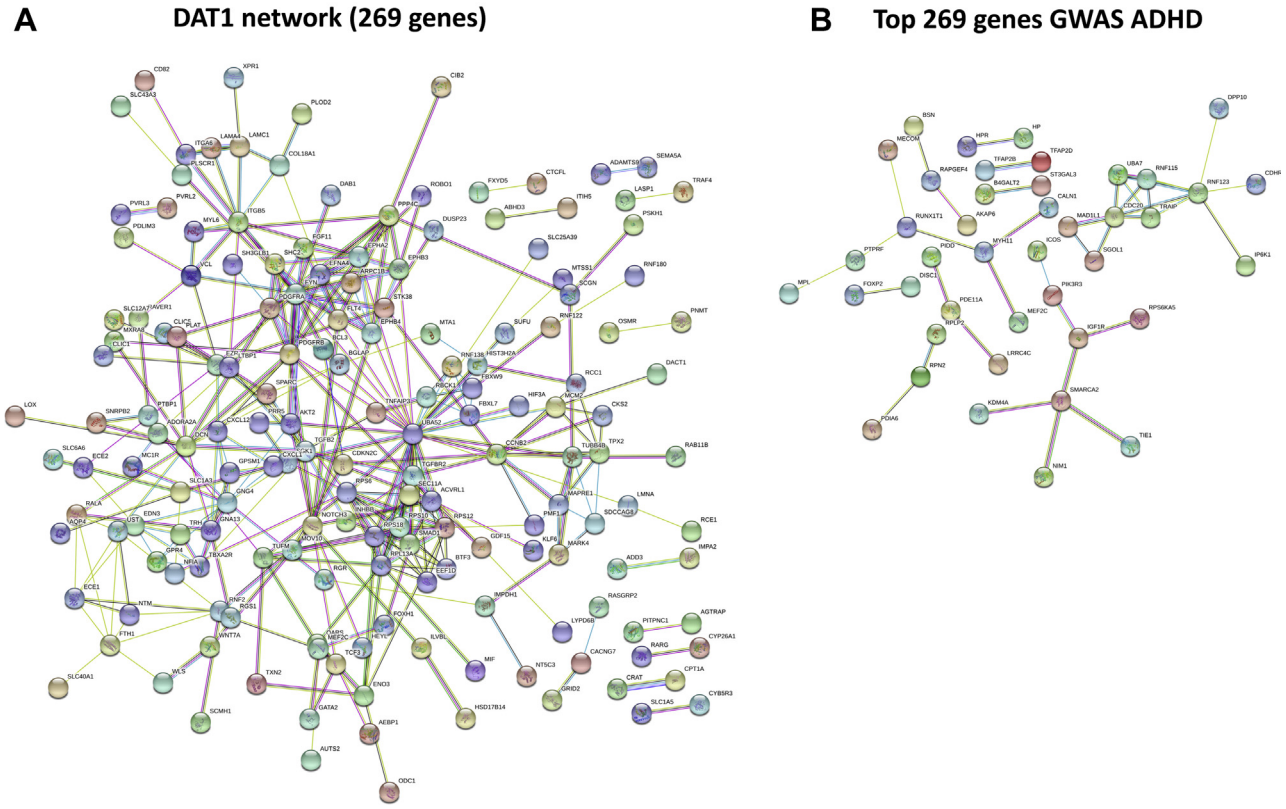

Figure 4. Protein networks **(A)** from the genes featured in the expression-based polygenic risk score reflecting variations in the function of the *DAT1* gene network and **(B)** a comparable number of the top genes from the attention-deficit/hyperactivity disorder (ADHD) genome-wide association study (GWAS) (41). The expression-based polygenic risk score reflecting variations in the function of the *DAT1* gene network contains proteins with a higher number of connections ($p < .0001$; Student's *t* test).

Moreover, DAT is the main target site for psychostimulant drugs, and thus several binding functions observed in the enrichment analysis provide a valid representation of the *DAT1* gene network function.

Several factors, such as birth asphyxia, preeclampsia, respiratory distress syndrome, low Apgar score, and prolapsed or nuchal cord, were significantly associated with ADHD (22–24). Considering that different, intercorrelated conditions could influence perinatal oxygenation levels, we aimed to create a novel cumulate index of perinatal adversity, the HICs score. Smith *et al.* (31) have proposed that accounting for multiple ischemia-hypoxia–related obstetric complications during pregnancy and birth may provide a more accurate measure of ischemia-hypoxia exposure in community-based samples. These authors recommend creating a weighted summary score of perinatal risk factors, allowing for the severity of ischemia-hypoxia exposure to be measured on a continuum, an approach we adopted for the current study. Other studies had used a similar approach, and investigators observed that perinatal health risk increased inattention and hyperactivity or impulsivity later in life (59–61). A strength of our prospective study is that we used birth cohorts with extensive and detailed data including gestational and birth records to create a reliable perinatal score without reliance on self-report retrospective questionnaires.

Differential exposure to perinatal HICs modifies the relationships between the SNP-based ePRS-*DAT1* and gray matter volume in areas involved in information processing (cortical regions, thalamus, and putamen). Our previous work using the rat model of hypoxia-ischemia demonstrates smaller total brain and gray matter volumes in areas such as the cerebral cortex and striatum, in addition to attentional impairments observed in adult animals (62). Experimental studies indicate that perinatal hypoxia-ischemia induces lasting changes in dopaminergic neurotransmission depending on the severity and duration of the hypoxic insult (25). Neuropathological and in vivo imaging studies in humans indicate that dopamine synthesis capacity is reduced in participants exposed to high HICs, and this is positively related to brain atrophy (63,64). Such reduced dopamine synthesis capacity could even worsen the strength and duration of dopamine transmission in general and especially in the high-ePRS-*DAT1* group. In clinical studies, smaller thalamus and frontal and parietal cortices were associated with lower attentional and executive functions in adolescents and adults born preterm or with low birth weight (65–67). Additionally, frontostriatothalamic circuitry has been implicated in ADHD pathophysiology in several studies (68–70). Considering that, we can infer that differences in gray matter density of the described structures appear to contribute to the impaired cognitive

flexibility in our study, with perinatal HICs modulating the relationship between the genetic background and gray matter volumes.

One limitation of the study is the smaller sample size for the neuroimaging analysis; hence, these results require replication. Independent replication and the use of a falsification approach (another pathway) are strengths of this study, which with other efforts such as preregistration can help avoid type I errors in future studies using our methodology.

We demonstrated that the gene network associated with PFC DAT interacts with the history of exposure to perinatal HICs, impairing cognitive flexibility and modifying the relationship between genetics and gray matter density. DA neurotransmission in the PFC is an essential moderator of the effects of perinatal adversity on attentional outcomes and brain development and define children's endophenotype and risk for attentional disturbances. We innovate by proposing new ways of integrating genotype data and perinatal history of HICs to predict cognitive flexibility in community cohorts, which may inform practices for early detection of vulnerability to poor academic performance. The proposed research approach could be important for the study of other DA-related gene networks and psychiatric disorders (71). Considering that we cannot modify our genetic predisposition for certain traits or disease, we highlight the significance of preventive measures to improve intrauterine and intrapartum health, avoiding disturbances in the fragile fetal developing brain.

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ARTICLE INFORMATION

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