

Prefrontal cortex VAMP1 gene network moderates the effect of the early environment on cognitive flexibility in children

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Abstract

During development, genetic and environmental factors interact to modify specific phenotypes. Both in humans and in animal models, early adversities influence cognitive flexibility, an important brain function related to behavioral adaptation to variations in the environment. Abnormalities in cognitive functions are related to changes in synaptic connectivity in the prefrontal cortex (PFC), and altered levels of synaptic proteins. We investigated if individual variations in the expression of a network of genes co-expressed with the synaptic protein VAMP1 in the prefrontal cortex moderate the effect of early environmental quality on the performance of children in cognitive flexibility tasks. Genes overexpressed in early childhood and co-expressed with the VAMP1 gene in the PFC were selected for study. SNPs from these genes (post-clumping) were compiled in an expression-based polygenic score (PFC-ePRS-VAMP1). We evaluated cognitive performance of the 4 years-old children in two cohorts using similar cognitive flexibility tasks. In the first cohort (MAVAN) we utilized two CANTAB tasks: (a) the Intra-/Extra-dimensional Set Shift (IED) task, and (b) the Spatial Working Memory (SWM) task. In the second cohort, GUSTO, we used the Dimensional Change Card Sort (DCCS) task. The results show that in 4 years-old children, the PFC-ePRS-VAMP1 network moderates responsiveness to the effects of early adversities on the performance in attentional flexibility tests. The same result was observed for a spatial working memory task. Compared to attentional flexibility, reversal learning showed opposite effects of the environment, as moderated by the ePRS. A parallel ICA analysis was performed to identify relationships between whole-brain voxel based gray matter density and SNPs that comprise the PFC-ePRS-VAMP1. The early environment predicts differences in gray matter content in regions such as prefrontal and temporal cortices, significantly associated with a genetic component related to Wnt signaling pathways. Our data suggest that a network of genes co-expressed with VAMP1 in the PFC moderates the influence of early environment on cognitive function in children.

Keywords: spatial short-term memory; MAVAN; GUSTO; synaptic proteins; neuroplasticity; neuroimaging.

Introduction

James McGaugh was one of the first neuroscientists to point to the important influence of stress hormones on memory consolidation (McGaugh, Gold, Van Buskirk, & Haycock, 1975). He and others considered that hormones released by stressful experiences could enhance memory consolidation, indicating particularly the hormones epinephrine and glucocorticoids as memory modulators (McGaugh & Roozendaal, 2009). It was suggested that several brain regions work in synergy to assure that emotionally significant experiences are well-remembered, and this could prepare the organism for future experiences by inducing long-term behavioral changes (Bahtiyar, Karaca, Henckens, & Roozendaal, 2020; McGaugh, 2013). However, whereas moderate acute stress may facilitate later retrieval, chronic stress can impair cognitive functions, depending on factors such as type, duration and intensity of the stressor, and type of cognitive function evaluated.

One of the brain structures impacted by stress exposure is the prefrontal cortex (PFC), which mediates multiple behavioral responses, including planning, accounting for the demands of a changing environment. Many studies, both in animals and humans, have described stress-induced effects upon executive functions related to this area, such as working memory, cognitive flexibility, and attentional set-shifting (e.g., (Bondi, Rodriguez, Gould, Frazer, & Morilak, 2008; Jett, Bulin, Hatherall, McCartney, & Morilak, 2017; Conor Liston et al., 2006; Merz & McCall, 2011). In these studies, different types of chronic stressors were shown to be able to affect specific prefrontal cognitive functions: for example, several studies reported that chronic stress disrupts extradimensional set-shifting (Jett et al., 2017; Conor Liston et al., 2006; Moench, Breach, & Wellman, 2020; Nikiforuk & Popik, 2014; Xu et al., 2016; Zhang, Shao, Wang, Xie, & Wang, 2017), while physical stressors, such as chronic intermittent cold stress, may affect reversal learning (Bondi et al., 2008; Danet, Lapiz-Bluhm, & Morilak, 2010; Lapiz-Bluhm, Soto-Piña, Hensler, & Morilak, 2009). The inability to adapt to an unanticipated environment by changing behavioral strategies may have detrimental consequences for an organism (Clark, Cools, & Robbins, 2004; Graybeal, Kiselycznyk, & Holmes, 2012; Tremblay & Schultz, 1999; Wellman, Bollinger, & Moench, 2020), and this is why the study of stress-induced disruption of PFC functions is a subject of increasing interest.

Stress effects on behavior and executive functions are associated with altered plasticity in specific sub-regions of the PFC, such as, for example, changes in the arborization or the spine density at the apical dendrites of pyramidal neurons, particularly in the mPFC (Cerqueira et al., 2005; Cook & Wellman, 2004; Dias-Ferreira et al., 2009; Conor Liston et al., 2006; Michelsen et al., 2007; Radley et al., 2006; Silva-Gomez, Rojas, Juarez, & Flores, 2003), and effects upon the morphology and imbalanced activation patterns of the mPFC have also been reported in stressed humans (Soares et al., 2012). Consistently, it is known that experiences during sensitive periods of development - periods of maturation and intense plasticity - have a long-term impact on brain function (Folha et al., 2017; Lazzaretti et al., 2018; Sarabdjitsingh, Loi, Joëls, Dijkhuizen, & Van Der Toorn, 2017). One of the best studied examples of early life stress-induced brain function alteration is the life-long modified control of stress-related hormones release (McGowan et al., 2009; Weaver et al., 2004).

Studies both in humans (Harms, Shannon Bowen, Hanson, & Pollak, 2018; Romero-Martínez, Lila, Catalá-Miñana, Williams, & Moya-Albiol, 2013) and in animal models (Baudin et al., 2012; Lazzaretti et al., 2018; Xu et al., 2016) have shown that early adversities have long-term influence upon cognitive flexibility. The high vulnerability of PFC to the effects of early-life stress

(Chocyk, Majcher-Maślanka, Dudys, Przyborowska, & Wędzony, 2013; Sarabdjitsingh et al., 2017) might be explained by the fact that this is one of the last brain regions to mature during development (Fuster, 2015; Hoops & Flores, 2017; Reynolds et al., 2018). It is reasonable to expect that early adversities may lead to reductions in PFC-based cognitive functions, which include the impairment of children's ability to adapt to different conditions in their environment through adequate stress and emotional responses (Hostinar, Stellern, Schaefer, Carlson, & Gunnar, 2012).

Notwithstanding the above-mentioned effects on cognitive flexibility, several other lines of evidence suggest that the quality of the early life environment induces changes in synaptic plasticity within the PFC (Folha et al., 2017; Lazzaretti et al., 2018; Lesuis, Lucassen, & Krugers, 2019; Xu et al., 2016), which might underlie the distinct cognitive-behavioral responses verified. The Soluble N-ethylmaleimide-sensitive factor Attachment Protein Receptor (SNARE) complex, for instance, which is involved in synaptic plasticity by enabling membrane fusion during neurotransmitter release (Cupertino et al., 2016), is a strong target candidate. SNARE is composed by three distinct proteins, all with an important role in neurodevelopment (Cupertino et al., 2016; Halim et al., 2003), and one of them, VAMP 1 (vesicular associated membrane protein 1), is a highly conserved synaptic molecule in evolution (Südhof, Baumert, Perin, & Jahn, 1989). Certain pathologies which result in abnormal cognitive functions, such as attention and/or working memory deficits, were also shown to have altered synaptic connectivity in the PFC, and among them, altered VAMP levels have been observed (Halim et al., 2003).

Our general hypothesis is that the early interaction between the environment and the genetic background results in changes in brain plasticity and neurotransmission, which may influence cognitive flexibility, among other functions. In recent years, studies covering the interface between genetics and behavioral outcomes have made use of polygenic risk scores (PRS) in order to detect vulnerability to neurological or psychiatric diseases (Guo et al., 2018; Mistry, Escott-Price, Florio, Smith, & Zammit, 2019; Morgan et al., 2017; Palk, Dalvie, De Vries, Martin, & Stein, 2019), and recent reports have focused on gene networks integrated by genes related to certain characteristics (Hari Dass et al., 2019; Miguel et al., 2019). These latter reports propose the use of biologically-informed genetic scores, which could aggregate a potential array of genes co-expressed with a known gene relevant to the outcome under question (expression-based polygenic risk score or ePRS), operating in a specific brain region. In this way, variations in the ePRS could be interpreted as reflecting variations in the function of the network. This analysis includes individual variations and allows to compare behavioral outcomes according to the environment.

We thus hypothesize that VAMP1 and its entourage of co-expressed proteins constitutes an interesting heuristic guide to investigate PFC-mediated behavioral-cognitive changes putatively based on stress-induced local plasticity changes, including those resulting from stressful events in early life. We therefore investigated whether individual variations in the expression of a gene network co-expressed with the gene encoding for the synaptic protein VAMP1 in the prefrontal cortex is somehow correlated with the causal effect of early life stress on the performance of children in cognitive flexibility tasks, spatial working memory, as well as brain gray matter density in childhood, analyzing data from two different cohorts.

Materials and methods

Subjects.

Discovery sample: MAVAN is a community-based birth cohort study of Canadian women and their offspring. Pregnant women aged 18 years and above were recruited in Montreal (Quebec) and Hamilton (Ontario), Canada. Eligibility criteria for mothers included singleton gestation, and

fluency in French or English. Severe chronic illness, placenta previa, and history of incompetent cervix, impending delivery, or a fetus/infant affected by a major anomaly or born at a gestational age of less than 37 weeks or had birth weight less than 2,000 g were exclusion criteria. 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. The study was conducted in accordance with the rules and regulations of the University ethics committees and informed consent was obtained from all participants. A total of 132 children had the necessary data (information on early life adversities/buffering conditions, genotype, and the score on at least one of the cognitive flexibility tasks at 48m), and this sample is described in Table 1. From these children, 106 completed stage 7 of IED task at 48 months and 103 completed stage 8 and were included in the behavioral analyses related to these stages. One hundred twenty-nine children performed the SWM task (4 boxes) at this age. A total of 43 children had complete data (genotype, information on early life adversities/buffering conditions, and imaging data) and were included in the imaging study.

Replication Sample: Growing Up in Singapore Towards Healthy Outcomes (GUSTO)(Soh et al., 2014): Pregnant women aged 18 years and above were recruited at the National University Hospital and KK Women's and Children's Hospital from Singapore. The pregnant women included Chinese, Malay, or Indian ethnicity with homogeneous parental ethnic background. Mothers receiving chemotherapy, psychotropic drugs or who had type I diabetes mellitus were excluded. The study was approved by the National Healthcare Group Domain Specific Review Board (NHG DSRB) and the Sing Health Centralized Institutional Review Board (CIRB). Informed written consent was obtained from each participant. A total of 140 children had complete data (genotype, information on early life adversities/buffering conditions, age, and scores on a cognitive flexibility task at 54 months of age) and were included in the study.

Demographic information for MAVAN and GUSTO cohorts is provided in Table 1.

Procedures

Genotyping:

In MAVAN, autosomal SNPs were genotyped using genome-wide platforms (PsychArray/PsychChip, Illumina) according to the manufacturer's guidelines with 200 ng of genomic DNA derived from buccal epithelial cells. Participant samples with a low call rate ($< 90\%$) were removed, as well as SNPs with a low call rate ($< 95\%$), a minor allele frequency less than 5% or a low p-value on Hardy-Weinberg equilibrium test ($p < 10^{-40}$), resulting in 242,211 SNPs. PLINK 1.951 (Purcell et al., 2007) was used for quality control procedures. The remaining SNPs were submitted for imputation to Sanger Imputation Service using a Haplotype Reference Consortium (release 1.1) panel (McCarthy et al., 2016) and post-imputation quality control, resulting in 20,790,893 SNPs with imputation accuracy greater than 0.80. We recoded imputed dosage genotypes to hard-called genotypes using posterior genotype probability greater than 0.90. Polygenic scores were calculated based on hard-called genotypes

In GUSTO, genomic DNA was extracted from frozen umbilical cord specimens. Samples were genotyped on Illumina Omni Express Exome arrays, following the manufacturer's instructions

(Expression Analysis Inc). SNPs with call rates < 95%, minor allele frequencies < 5% and Hardy-Weinberg equilibrium of $p \leq 10^{-6}$ were removed. Variants discordant with their respective subpopulation in the 1000 Genomes (1000 Genomes Project Consortium, 2015) reference panel were removed. More precisely, variants with different allele coding than 1000 Genomes Project as well as SNPs with frequencies that differ more than the threshold specified for the reference population (Chinese: EAS with a threshold of 0.20; Malay: EAS with a threshold of 0.30; Indian: SAS with a threshold of 0.20) were excluded. The resulting data were pre-phased using SHAPEIT v2.837 with family trio information. The genotyping data were then sent to Sanger Imputation Service (<https://www.sanger.ac.uk/tool/sanger-imputation-service/>) for imputation, choosing 1000 Genomes Project Phase 3 as the reference panel and imputed using the Positional Burrows Wheeler Transform (PBWT) algorithm (Durbin, 2014).

Population structure

The population structure of the MAVAN and GUSTO cohorts were evaluated using principal component analysis (Patterson, Price, & Reich, 2006) of all autosomal SNPs that passed quality control. Genotyped SNPs with high linkage disequilibrium ($r^2 < 0.20$) were pruned with a sliding window of 50 kilobases in increments of 5 SNPs using PLINK 1.9. We performed principal component analysis using SMARTPCA on this pruned data set and generated a scree plot. Based on the inspection of the scree plot, the first 3 principal components were the most informative of population structure and were included in all analyses as covariates.

Construction of the PFC-VAMP1 gene network

Discovery sample

Genes co-expressed with VAMP1 in the PFC and ePRS calculation

The expression-based polygenic risk score was created considering genes co-expressed with the *VAMP1* gene (ePRS- *VAMP1*) in the PFC, according to the protocol described previously (Silveira et al., 2017). This ePRS was generated using several resources: (a) GeneNetwork (<http://genenetwork.org>) (b) BrainSpan (<http://www.brainspan.org>), (c) NCBI Variation Viewer (<https://www.ncbi.nlm.nih.gov/variation/view>) and (d) GTEx (<https://www.gtexportal.org/home>).

The ePRS was constructed in several stages: (i) GeneNetwork (<http://genenetwork.org>) was used to generate a list of genes co-expressed with *Vamp1* in the PFC in mice. Only genes with absolute value of the co-expression correlation r higher or equal to 0.5 were kept; (ii) Brainspan (<http://www.brainspan.org>) was used to identify transcripts from this list with enrichment within the human PFC from the last gestational weeks until mid-childhood (25 to 38 post-conception weeks and first 5 years of life). We selected autosomal transcripts that were differentially expressed in PFC at 1.5-fold during this period of development as compared to adult samples (<http://brainspan.org>; Miller et al., 2014) due to our interest in gene networks with higher activity in the period considered for the environmental index. The resulting list consisted of 33 genes. (iii) Based on their functional annotation in the National Center for Biotechnology Information, U.S. National Library of Medicine (NCBI Variation Viewer), using GRCh37.p13, we gathered all the existing SNPs from these genes, and merged this list with SNPs from the Genotype-Tissue Expression (GTEx) project (Lonsdale et al., 2013) data in human PFC, and from our study sample. The list of common SNPs was subjected to linkage disequilibrium clumping ($r < 0.25$). It resulted in 276 independent functional SNPs that (iv) were weighed using the SNP-gene expression association slope from the GTEx. Number of effect alleles at a given cis-SNP was weighed by the estimated effect of the genotype on gene expression from the GTEx. The sum of these values over the total number of SNPs provided the PFC-ePRS-*VAMP1* score. Our final list of genes included

32 genes (one of the original genes had no common SNPs when considering NCBI, GTEx and the samples) (Supplementary Table S1). The standardized PFC-ePRS-*VAMP1* score was used as a continuous variable.

Specificity of the findings: In order to test the specificity of the PFC-ePRS-*VAMP1* score, we also investigated the ability of other scores to moderate the effect of early life environment on the outcome (cognitive flexibility): (i) We calculated a score considering genes co-expressed with the *VAMP2* gene in prefrontal cortex (PFC-ePRS-*VAMP2*). *VAMP2* is also a protein of the VAMP family which is highly expressed in PFC (Uhlén et al., 2015) (<http://www.proteinatlas.org>); (ii) We calculated the score considering genes co-expressed with the *VAMP1* gene in a different brain region, the hippocampus (HPC-ePRS- *VAMP1*). These scores were calculated with the same premise and using the same methods described above for the PFC-ePRS-*VAMP1*. The final list of co-expressed genes included 184 genes for PFC-ePRS-*VAMP2* (Supplementary Table S2), and 92 genes for HPC-ePRS-*VAMP1* (Supplementary Table S3). These scores were tested for the interaction ePRS x environmental index on cognitive flexibility, as was the PFC-ePRS-*VAMP1*.

Replication sample.

In order to replicate and test the generalizability of our findings in a different cohort, we conducted the same analyses in the GUSTO cohort. For that, ePRS was created following the same steps as above, starting from the 33 genes.

Characterization of early life environment

Discovery sample.

Environmental score: The following tools were used to calculate a early life environmental index combining adversity and positive experiences in MAVAN: The *Health and well-being questionnaire* is a composite of validated short versions of multiple measures (Kramer, Goulet, et al., 2001): a) a subscale from the Daily Hassles is used to measure how often, and to what degree, the woman has lacked money for basic needs such as food, heating and electricity, since the beginning of pregnancy (Kanner, Coyne, Schaefer, & Lazarus, 1981); b) The Marital Strain Scale of Pearlin and Schooler is used to assess chronic stress with the romantic partner (Pearlin & Schooler, 1978); c) The Abuse Assessment Screen is used to assess conjugal violence. This five-item instrument assesses the frequency, severity, perpetrator, and body sites of injury (Newberger et al., 1992; Parker, McFarlane, Soeken, Torres, & Campbell, 1993); d) Questions about anxiety during pregnancy (Lobel & Dunkel-Schetter, 1990; Lobel, Dunkel-Schetter, & Scrimshaw, 1992). *Smoking during pregnancy* was simply scored as a binary outcome. *Household gross income* was assessed according to the Institut de la statistique du Québec (Daveluy & Québec, 2001). *Birth weight and gestational age* were assessed using birth records obtained directly from the birthing unit. Birth weight percentiles were calculated using the Canadian reference (Kramer, Platt, et al., 2001). Besides, the following tools were also used: *Child Health Questionnaire:* Includes questions on acute and chronic conditions, as well as hospitalizations (Plante, Courtemanche, & Groseilliers, 2002). *Attachment:* The Preschool Separation – Reunion Procedure (PSRP) was applied at 36 months. The PSRP is a modified and developmentally appropriate version of the Ainsworth Strange Situation used to measure attachment security in preschool-aged children (Cassidy & Marvin, 1992; Moss, Bureau, Cyr, Mongeau, & St-Laurent, 2004). The task consists of a baseline interaction, followed by two separation and reunion episodes lasting 5 minutes; scoring was based on video coding (reliability $k=0.83$). Four categories were assessed: secure, ambivalent, avoidant and disorganized. *Family Assessment Device (FAD):* The FAD is a 60-item self-report instrument developed to assess the six dimensions of the family functioning outlined in

the McMaster Model of Family Functioning (Epstein, Baldwin, & Bishop, 1983). The first six scales assess problem solving, communication, roles, affective responsiveness, affective involvement and behavior control. A general functioning scale assesses overall health-pathology (Kabacoff, Miller, Bishop, Epstein, & Keitner, 1990). *Breastfeeding Questionnaire*: This is a maternal self-report that queries the age of the baby (in weeks) when she or he was fed for the first time with something other than breast milk and the age of the baby (in weeks) when mothers stopped nursing (or giving breast milk). Women were provided the option of responding that they never breastfed or provided breast milk to their baby (Adedinsewo et al., 2014). See Table 2 for factors included in the environmental score. A combined score was calculated, giving negative points to adverse exposures and positive points to supportive/enriching events, and therefore ranging from more negative (adverse environment) to more positive values (positive scenarios).

Replication sample.

In GUSTO, the tools applied for the calculation of these scores were similar to MAVAN (see description above), except for the absence of the following components: attachment, domestic violence, lack of money, pregnancy anxiety, and marital strains information (see Table 2).

Behavioral outcomes

Discovery sample.

Intra-/Extra-dimensional Set Shift (IED) (Downes et al., 1989) The IED task from the CANTAB battery comprises rule acquisition and reversal (set shifting) throughout nine stages with increasing difficulty. There are two dimensions used in the task (color-filled shapes, introduced in the first stage, and white lines, introduced in the third stage). In the first 7 stages, shape remains the relevant dimension. At stage 6 new stimuli are presented, still with the same 2 dimensions, and subjects are required to learn which of the new exemplar is correct (intra-dimensional shift). An extra-dimensional shift occurs at stage 8, when subjects are required to shift attention to the previously irrelevant dimension (i.e., lines). At stages 2, 5, and 7, contingencies are reversed, so that the previously incorrect stimulus is now correct (reversal learning). We focused on the sum of errors for stages 2, 5 and 7 as a measure of reversal learning, and on the errors during stage 6 and stage 8 as the measures of attentional flexibility (intra and extradimensional shifts, respectively). This task was applied in MAVAN children at the age of 48 months (mean±S.D.: 4.10±0.08 years).

Spatial Working Memory (SWM): This is a self-ordered task, also from the CANTAB battery, which measures the ability to retain information and manipulate items in working memory, being related to frontal lobe executive function (Owen, Downes, Sahakian, Polkey, & Robbins, 1990). Participants were shown a group of boxes on a computer screen. They were asked to search for a token hidden beneath one of the boxes by touching a box to discover whether or not a token was there. After the token was found, the procedure was repeated, and the participants were told that a token would never be hidden beneath the same box. Thus, participants needed to remember which of the boxes had previously hidden a token within a set of trials. Performance on the test was evaluated by quantifying number of (a) between errors (i.e., revisiting a box in which a token had been found in a previous trial); (b) within errors (i.e., searching a box already found to be empty during a single trial); (c) double errors (errors categorized as both between and within errors); and (d) total errors (i.e., between errors + within errors – double errors).

Replication sample.

For the evaluation in the replication cohort (GUSTO), we used the *Dimensional Change Card Sort (DCCS)*. The DCCS is test of executive functioning and, like the attentional flexibility in IED, measures the ability to learn and apply new rules while refraining from using old rules (Zelazo,

2006). DCCS was applied at 54 months, as described in Pang et al. (Pang et al., 2020). In this test, children were asked to sort pictures according to color and then subsequently by spatial orientation or emotional expression. The order in which the children received the two versions of the test was counterbalanced. In the present study, we used the non-emotional condition, in order to maintain comparability of the dataset to the discovery sample. The test was administered on a computer with press pad, using E-Prime version 2.0. Children were first asked to sort bivalent pictures according to one rule for an entire block of 5 trials, and then according to a different rule for an entire block of 5 trials. One of the rules included “sort by color”, and the other rule was to sort by spatial orientation. That is, children saw neutral colored faces that were either upside down or right side up. An additional block was added for children who passed the simpler conditions. In this last “mixed trials” block consisting of 30 trials, an instruction word and sound cue preceded each trial and indicated the rule for sorting the test stimuli in that trial. We considered the ability of the children to perform (% of accuracy) in the mixed trials block, since it was more relevant to replicate the findings in the extradimensional shift for the discovery sample.

Gray matter volume

Parallel-independent component analysis (p-ICA):

Structural MRI acquisition was performed when children were 8-11 years-old. High-resolution T1-weighted images for the whole brain were acquired using a 3T trio Siemens scanner available at Cerebral Imaging Center, Douglas Mental Health Institute (Montreal, Canada) and a GE MR750 Discovery 3T MRI scanner at the Imaging Research Centre, St Joseph’s Healthcare (Hamilton, Canada). The following parameters were used: 1 mm isotropic 3D MPRAGE, sagittal acquisition, 256 x 256 mm matrix, TR=2300ms, TE=4ms, FA=9degrees (Montreal); a 3D inversion recovery-prepped, T1-weighted anatomical data set, fSPGR, axial acquisition, TE/TR/flip angle = 3.22/10.308/9, 512 x 512 matrix with 1mm slice thickness and 24cm FOV (Hamilton). Computational Anatomy Toolbox (CAT12) from the Statistical Parametric Mapping software (SPM12; Wellcome Department of Cognitive Neurology, London, UK) was used to process the T1-weighted images. In the preprocessing step, the images were normalized and segmented into gray matter and white matter. After a high-dimensional Diffeomorphic Anatomical Registration Through Exponentiated Lie Algebra (DARTEL) normalization, a smoothing process was applied using 8mm full width half maximum kernel.

A multivariate parallel independent component analysis (pICA) was applied to identify relationships between two different data modalities (SNPs and brain gray matter density) in a data-driven manner (Khadka et al., 2016). This analysis estimates the maximum independent components within each data modality separately while also maximizing the association between modalities using an entropy term based on information theory (Liu & Calhoun, 2014; Pearlson, Calhoun, & Liu, 2015). We sought the relationship between the SNP based PFC-ePRS-*VAMP1* (or genotype * GTEX gene expression slope at each SNP that compose the PFC-ePRS-*VAMP1* score) and the voxel based gray matter in the whole brain. Weighted SNPs were aggregated with the most significant principal components from population stratification for adjustment (ethnicity). T1 gray matter weighted images were adjusted by age and sex using the R package RNifti (<https://cran.r-project.org/web/packages/RNifti>). The early environment defined the groups for comparison (21 children high environmental score, meaning low adversity or high buffering environment, 22 children low environmental score, meaning more adversity). The Fusion ICA Toolbox (<http://mialab.mrn.org/software/fit/>) within MATLAB® R2014 was used to run the analysis. The number of independent components estimated using minimum description length

criteria (Calhoun, Adali, Pearlson, & Pekar, 2001; Pearlson et al., 2015) was 8 for MRI data. SNP dimensionality inside the toolbox was used for the genetic modality resulting in 16 estimated independent components. Components for both modalities were converted to z-scores and a threshold at $|Z| > 3.5$ was used to identify significant brain regions and $|Z| > 2.5$ for SNPs that contributed the most for the component overall pattern. Significant components associations p-value was adjusted for multiple comparison (128 comparisons) by Bonferroni. To identify the anatomical classification of brain areas included in the significant MRI components, Talairach coordinates were used (Talairach & Tournoux, 1988.). The SNPs from the significant genetic components were analyzed using Metacore® and Panther (<http://pantherdb.org/>), to identify associated underlying biological processes. Loading coefficients, which describe the presence of the identified component across subjects (Liu et al., 2012), were extracted for each component, modality and subject. The mean subject-specific loading coefficients of these components between children from high and low early environment index groups were compared using Student's t-test.

Characterization of the prefrontal *VAMPI* co-expression network

Gene ontology (GO) enrichment analysis: Enrichment analysis of the genes comprising PFC-ePRS-*VAMPI* was performed using Metacore® (<https://portal.genego.com>) for molecular functions.

Gene interaction network analysis: GeneMANIA (<https://genemania.org/>; (Warde-Farley et al., 2010) was used to analyze functional interactions between the genes from our list of PFC-*VAMPI* co-expressed genes. The topological properties (Neves de Oliveira, Dalmaz, & Zeidán-Chuliá, 2018; Yu, Kim, Sprecher, Trifonov, & Gerstein, 2007) of the network (such as *degrees* and *betweenness*) were considered using Cytoscape software (Saito et al., 2012). The data were retrieved from the GeneMania database and the network constructed in Cytoscape. *Degrees* are calculated by summing the number of adjacent nodes, giving information about the local topology, while *betweenness* considers the number of shortest paths linking two nodes and passing through a node n. Nodes with degrees higher than +1SD above the mean were considered hubs, and nodes with betweenness higher than +1SD above the mean were considered bottlenecks (Neves de Oliveira et al., 2018), and were identified as central nodes within the network.

In order to obtain insight into possible biological mechanisms related to this gene network, we used the *FUMA* (Watanabe, Taskesen, Van Bochoven, & Posthuma, 2017) (<http://fuma.ctglab.nl>) GENE2FUNC process. Age specific expression patterns based on Brainspan (Miller et al., 2014) for each gene were visualized as a heatmap. Overrepresentation in sets of differentially expressed genes (DEG; sets of genes which are more (or less) expressed in a specific age in brain compared to other ages) was also obtained. The proportions of overlapping genes, against gene sets obtained from MsigDB and WikiPathways, enrichment P-value, and which input genes are overlapping with the present gene list were also visualized using FUMA (Watanabe et al., 2017).

Statistical Analysis

The data were analyzed using the IBM Statistical Package for the Social Sciences (SPSS) version 24.0 (Armonk, NY: IBM Corp.) and R (R Core Team, 2014).

Descriptive statistics: Baseline characteristics of the samples according to ePRS categories (median split) are shown in Table 1. Low and high ePRS groups were compared using Student's t-test for independent samples for means and Chi-square test for percentages (for categorical variables). In the main (MAVAN) cohort, differences between low and high ePRS groups were

not statistically significant for birth weight, gestational age at birth or maternal age at birth, socio-economic status, or environmental index. In the replication (GUSTO) cohort, no differences were observed in these parameters, except for birth weight. Therefore, we included birth weight as one of the covariates in the model. Student's t-test was also applied to compare mean subject-specific loading coefficients between children from high and low environment score in p-ICA.

Analysis: For all behavioral outcomes in both main and replication cohorts, we applied linear regression analysis to examine the effect of interaction between genetic scores (PFC-ePRS-*VAMPI*, as well as PFC-ePRS-*VAMP2* and HPC-ePRS-*VAMPI* when testing the specificity of the score) and the environmental index on cognitive flexibility and spatial working memory. Simple slope analysis was conducted to analyze the post-hoc differences between low and high ePRS groups. In the replication cohort, one subject was identified as an influential point and was excluded from the analyses. All analyses were adjusted for sex, age, three genetic PCs, and site for MAVAN cohort.

Results

PFC-ePRS-VAMPI score created from co-expressed genes in the PFC moderates the effect of the environmental index on cognitive flexibility and spatial working memory in 4 years-old children

A Poisson regression showed a significant effect of ePRS and environmental index interaction in predicting reversal learning ability (evaluated as the sum of errors in the reversal stages of IED task, stages 2, 5, and 7) in MAVAN 4 years-old children ($\beta = -0.12$, $p = 0.003$; see Figure 1A). To investigate the specific effects in the low and high ePRS groups we performed simple slopes analysis, that showed that the effect of environmental index on the number of errors in reversal learning stages was larger with the increase of PFC-ePRS-*VAMPI*: for low ePRS score (-1 SD) the estimated effect of the environmental score on the outcome was $\beta=0.11$, $p = 0.06$ and for the high ePRS score (+1 SD) the estimated effect was $\beta=-0.13$, $p = 0.01$. This suggests that children from the high ePRS group had less ability to show reversal memory when exposed to an adverse environment.

There was also a significant interaction effect between ePRS and environmental index in predicting attentional flexibility in the extradimensional set shift (evaluated as the number of errors in the 8th stage, where extradimensional shift occurs in the IED task) ($\beta = 0.04$, $p = 0.03$; Figure 1B). A simple slopes analysis showed that the effect of environmental index on the number of errors in attentional flexibility increased with the decrease of PFC-ePRS-*VAMPI*: for low ePRS score (-1 SD); $\beta=-0.05$, $p = 0.08$ and for the high ePRS score (+1 SD) $\beta=0.03$, $p = 0.16$. Therefore, attentional flexibility for extradimensional shift may be influenced by the quality of the environment, and individual differences are expected according to the ePRS. No significant differences were observed for the interaction between environmental index and PFC-ePRS-*VAMPI* in predicting errors in the intra-dimensional shift ($\beta=-0.12$, $p=0.14$).

A linear regression analysis of the performance in the spatial working memory task (SWM) showed a significant effect of ePRS x environmental index interaction on total errors in the 4 boxes stage of the task (Figure 1C; $\beta = 0.34$, $p = 0.02$). Simple slope analysis showed that the effect of environmental index on the number of Total errors changes with the PFC-ePRS-*VAMPI*: for low ePRS score (-1 SD); $\beta=-0.33$, $p = 0.11$ and for the high ePRS score (+1 SD) $\beta=0.32$, $p = 0.08$. This suggests that this ePRS influences the way the individual responds to the environment, when evaluating spatial working memory at this age.

Pearson correlations to check interrelationships between performances in the distinct CANTAB tests were performed. No correlations were found between (i) spatial working memory

(total errors) and IED (errors in stage 8) (-0.00259), (ii) spatial working memory and reversals (0.0425), or IED (stage 8) and reversals in the IED test (-0.102).

In order to test the region-specificity of the PFC-ePRS-*VAMP1* findings, we calculated ePRS scores considering genes co-expressed with the *VAMP1* gene in the hippocampus (HPC-ePRS-*VAMP1*); we tested the protein-specificity considering genes co-expressed with the gene coding for VAMP2 (*VAMP2*) in the prefrontal cortex (PFC-ePRS-*VAMP2*). No significant interaction effects between ePRS (hippocampal ePRS-*VAMP1* score and prefrontal ePRS-*VAMP2* score) and environmental index were observed on all the outcomes. More specifically, linear regression analysis for reversal learning (errors in stages 2, 5, and 7 of the IED task) including HPC-ePRS-*VAMP1*, $\beta=-0.07$, $p=0.21$, and including PFC-ePRS-*VAMP2*, $\beta=-0.03$, $p=0.47$; for attentional flexibility (errors in stage 6 and stage 8 of the IED task), for HPC-ePRS-*VAMP1*, $\beta=0.004$, $p=0.97$ for errors in the 6th stage, and $\beta=0.02$, $p=0.37$ for errors in the 8th stage, and for PFC-ePRS-*VAMP2*, $\beta=0.007$, $p=0.94$ for errors in the 6th stage, and $\beta=0.004$, $p=0.82$ for errors in the 8th stage; and for total errors in the SWM task, for HPC-ePRS-*VAMP1*, $\beta=-0.24$, $p=0.12$, and for PFC-ePRS-*VAMP2*, $\beta=0.11$, $p=0.44$.

Replication sample

We then aimed at replicating our findings, exploring whether the PFC-ePRS-*VAMP1* would interact with early life environmental index to predict attentional flexibility in children in the GUSTO cohort (at 54 months of age). A linear regression analysis was conducted to investigate if percentage of accuracy in the orientation mixed trials of the DCCS task, which involves attentional flexibility, is associated with the ePRS x environment interaction. A significant interaction effect between PFC-ePRS-*VAMP1* and environmental index was observed (Figure 2; $\beta = -0.622$, $p = 0.05$). As was observed for the MAVAN cohort, accuracy decreases with increased adversity in the lower ePRS group. A simple slopes analysis showed that the effect of environmental index on the accuracy changes with the PFC-ePRS-*VAMP1*: for low ePRS score (-1 SD); $\beta=1.00$, $p = 0.03$; and for the high ePRS score (+1 SD) $\beta=-0.183$, $p = 0.68$. Since this sample had shown some effect of high or low ePRS on birth weight, we repeated the analysis adjusting the model for birth weight (using birth weight as a covariate). The same results were obtained (an interaction between PFC-ePRS-*VAMP1* and environmental index was observed: $\beta = -0.624$, $p = 0.05$). Simple slopes analysis also remained similar: for low ePRS score (-1 SD); $\beta=1.03$, $p = 0.03$; and for the high ePRS score (+1 SD) $\beta=-0.15$, $p = 0.73$.

Characterization of the prefrontal VAMP1 co-expression network

Figure 3A shows the connected network (related mainly by coexpression of the genes). Data were retrieved from the Genemania database and the network constructed in Cytoscape. Centralities measures are displayed in Supplementary Table S4. RFNG, IGFBP4, and SGTA had high centrality values for both degrees and betweenness. *Enrichment analyses* of the genes comprising the PFC-ePRS-*VAMP1* were performed using Metacore® (<https://portal.genego.com>) for molecular functions (Figure 3B), and point to the Wnt signaling pathway and actin monomer binding, which is involved in synaptic assembly and neurotransmission. Figure 3C shows overlapping genes from this network, against gene sets obtained using FUMA (Watanabe et al., 2017); many of the gene sets related to the genes in our ePRS are related to inflammation.

Figure 4 displays a heatmap (Figure 4A) of age-specific expression patterns based on Brainspan (Miller et al., 2014) and obtained from FUMA for each gene, and sets of differentially expressed genes (DEG; Figure 4B), whose expression is up or down regulated in the brain

according to the age. It may be observed the presence of sets of genes up-regulated during infancy and childhood. This result suggests distinct co-expression patterns of these genes in adulthood.

MRI Gray matter density and the PFC-ePRS-VAMP1

A multivariate p-ICA identified imaging/genetic significant associations, correlating regional gray matter density and SNP-based PFC-ePRS-*VAMP1* data. Significant relationships were found between three phenotype-genotype component pairs (Table 3). When comparing the mean loading coefficients of these components between children from high and low environmental index groups using Student's t-test, we observed statistically significant differences for MRI components 4 ($t = -3.388$, $p = 0.001$) and 7 ($t = -2.149$; $p = 0.037$) and for genetic component 11 ($t = 2.556$, $p = 0.014$). This suggests that the quality of the environment influences gray matter density and SNP-based PFC-ePRS-*VAMP1* relationship in these components. Since MRI component 4 and genetic component 11 association was significant after multiple comparison correction by Bonferroni and both had a significant difference in its loading coefficient according to the environment, it was of primary interest in the present study, and it will be further considered. Figure 5 illustrates the relationship for the chosen linked components (genetic component 11 and MRI component 4). Active regions are listed in Supplementary Table S5. Many regional variations contribute to this component, located in distinct portions of PFC, parietal cortex, cingulate gyrus, and temporal gyrus.

In the genetic component 11, from the 281 SNPs used, we found 7 significant SNPs with contribution weights above 2.5 (Supplementary Figure S1). An enrichment analysis pointed to GO processes Wnt-signaling (adjusted $p = 0.046$) and extracellular matrix-cell signaling (adjusted $p = 0.046$). The genes related to these SNPs are also significantly enriched in a gene set of the Hallmarck P53 pathway (adjusted $p = 0.016$).

Discussion

In this study we considered individual differences in the expression of a gene network that might explain some observed susceptibilities to environmental changes, and the influence of these factors on cognitive flexibility in children. A scheme of potential effects is illustrated in Figure 6. We observed interactions between the quality of the environment in which the individual was raised and the PFC-ePRS-*VAMP1* score on attentional flexibility in the extra-dimensional shift, spatial working memory, and reversal learning in young children. Our results are in line with reports from the literature, that show that these functions are dissociated (e.g., Birrell & Brown, 2000; Conor Liston et al., 2006; McAlonan & Brown, 2003; Rogers, Andrews, Grasby, Brooks, & Robbins, 2000), to such an extent that early environment interacted with the genetic score distinctly when considering different modalities of executive functions. We also observed an association between extracted data components of this gene network and data from gray matter density components.

Regarding the effect of the early environment, the present findings are consistent with previous studies, both in animal models and in humans, that have pointed to the susceptibility of cognitive flexibility to the milieu in which the individual is raised (Harms et al., 2018; Lazzaretti et al., 2018; Romero-Martínez et al., 2013; Sakhai, Saxton, & Francis, 2016; Xu et al., 2016). Our gene network was built around a select set of genes co-expressed with VAMP1, a SNARE complex protein, whose functionality in human cortex has been related to some cognitive abilities (Ramos-Miguel et al., 2018). In addition, early stress leads to altered synaptic morphology and function (Arcego et al., 2016; Folha et al., 2017; Lazzaretti et al., 2018; Lesuis et al., 2019; Xu et al., 2016;

Zimmermann, Richardson, & Baker, 2019), and changes in synaptic connectivity in the PFC have also been observed concomitantly with altered working memory or attention (e.g., Halim et al., 2003; Hansel & Mato, 2013; Morris et al., 2016).

Four years-old children from MAVAN cohort showed a performance somehow better in the CANTAB tasks than that reported by Luciana and Nelson (Luciana & Nelson, 1998), who studied children with different ages. This could be due to differences in the sample, but possibly another factor to consider would be the fact that our study is more recent, and children have become more used to perform tests in computer screens in the last decades. We also used the site of data collection (Hamilton or Montreal) as a variable in the regression analysis, and no effect was observed comparing these two sites.

An interesting, yet somehow expected finding was that the quality of the early environment had opposite effects upon reversal learning and attentional flexibility in terms of *PFC-ePRS-VAMPI* effect as a moderator. These two functions have distinct requirements since they operate at different moments of cognitive information processing: While attentional flexibility demands the ability to shift attention to a distinct stimulus, reversal learning requires the individual to be able to make a context discrimination and, then, learn that the opposite becomes true.

These functions are also mediated by different subregions of the PFC. Several lines of evidence derived from lesion and pharmacological studies in animals, as well as lesion and imaging studies in humans, support the idea that distinct regions of the PFC mediate different forms of cognitive flexibility (Birrell & Brown, 2000; Bissonette et al., 2008; Dias, Robbins, & Roberts, 1996; Floresco, Block, & Maric, 2008; Manes et al., 2002; McAlonan & Brown, 2003; Morris et al., 2016; Rogers et al., 2000; Rygula, Walker, Clarke, Robbins, & Roberts, 2010). It is suggested that the medial prefrontal cortex (mPFC) in rodents (Birrell & Brown, 2000; Bissonette et al., 2008; Floresco et al., 2008), or the dorsolateral PFC (dlPFC) in primates (Dias et al., 1996; Manes et al., 2002; Morris et al., 2016; Nakahara, Hayashi, Konishi, & Miyashita, 2002; Rogers et al., 2000), has a role in shifting between attentional sets or strategies. Regarding working memory, studies have also implicated parts of the mPFC, such as the prelimbic and infralimbic cortices in this function in rodents (Gisquet-Verrier & Delatour, 2006; Schwabe, Enkel, Klein, Schütte, & Koch, 2004), and the lateral PFC in humans (Manes et al., 2002; Müller, Machado, & Knight, 2002; Owen et al., 1990). On the other hand, the orbitofrontal cortex (OFC) mediates reversal learning. Lesions or pharmacological inactivation of the OFC impair reversal learning in rodents (Bissonette et al., 2008; Hervig, Piilgaard, Božič, Alsiö, & Robbins, 2020; McAlonan & Brown, 2003; Schoenbaum, Setlow, Saddoris, & Gallagher, 2006). Similar results are found in non-human primates (Dias et al., 1996; Rygula et al., 2010), and in humans (Hornak et al., 2004), and the importance of OFC for reversal learning is also shown through event-related fMRI (Hampshire & Owen, 2006). In addition, OFC lesions do not impair extradimensional shifting (Manes et al., 2002; McAlonan & Brown, 2003; but see Chase, Tait, & Brown, 2012), and conversely lesions in the medial PFC do not impair reversal learning (Birrell & Brown, 2000; Floresco et al., 2008). In humans, OFC and dlPFC also show distinct patterns of activation during performance in cognitive tasks (see Moghaddam & Homayoun, 2008 for a review). Therefore, a functional dissociation is observed between these regions.

This functional dissociation is also observed when considering stress effects on cognitive flexibility, as we pointed in the Introduction section. For example, in animal models, chronic stress impairs working memory (Barsegyan, Mackenzie, Kurose, McGaugh, & Roozendaal, 2010; Cerqueira et al., 2005; Mika et al., 2012; Mizoguchi et al., 2000), and extradimensional set-shifting (Bondi et al., 2008; Jett et al., 2017; Conor Liston et al., 2006; Moench et al., 2020; Mohamed et

al., 2020; Naegeli, O'Connor, Banerjee, & Morilak, 2013; Nikiforuk & Popik, 2014; Xu et al., 2016; Zhang et al., 2017), while reversal learning is preserved. However, some physical stressors may affect reversal learning (Bondi et al., 2008; Danet et al., 2010; Lapiz-Bluhm et al., 2009). In humans, four weeks of psychosocial stress changed the connectivity of dlPFC, and impaired attentional set-shifting (Liston, McEwen, & Casey, 2009), but not reversal learning. It has been suggested that chronic stress turns strategies towards habitual responding (Soares et al., 2012).

With regard to the opposing effects of the early environment on these functions according to the genetic scores, some studies in animal models have also pointed to distinct effects of stress according to the structure and function analyzed and according to the previous stress history. For example, (Liston et al., 2006) showed that repeated restraint stress in rats induced contrasting effects on mPFC and OFC apical dendrites: a retraction of apical dendrites in the mPFC was observed, with a corresponding selective impairment of extradimensional attentional set-shifting, while reversal learning was not affected, and an increased dendritic arborization was observed in the OFC (Liston et al., 2006). These contrasting changes in mPFC and OFC neurons morphology after stress have also been observed in their firing properties (Moghaddam & Homayoun, 2008), further suggesting that mPFC and OFC functions are differently modulated by stress exposure. In addition, Graybeal et al (2011) found that under some conditions, stress can facilitate reversal learning. Another study (Snyder, Hill-Smith, Lucki, & Valentino, 2015) has reported a dissociation of the effect of corticotropin-releasing hormone (CRH), a neuropeptide whose levels in adulthood may be affected by early adversities (Carpenter et al., 2004). CRH may facilitate set-shifting or reversal learning, depending on the stress history of the animals. The authors suggest a potential for stress history to modulate cognitive processing according to variations in CRH neurotransmission. The lack of correlation in the performance on these two tasks in our sample agrees with the literature, also suggesting that the interaction between PFC-VAMP-1 gene network and early adversity affects more than one executive function domain.

In our pICA analysis, we observed that genetic component 11 was significantly associated with MRI component 4, and that the mean loading coefficients of these components between children from high and low environmental index groups was distinct. In other words, adversity is linked to differences in processes like Wnt-signaling and extracellular matrix-cell signaling, particularly in frontal, temporal and parietal regions. It is interesting that many of the brain regions from this component, for example the superior and medial frontal gyrus (Brodmann Areas or BA 6, 10), as well as the superior parietal lobule (BA 7), show altered cerebral blood flow while subjects are performing an ID/ED shift learning task (Rogers et al., 2000). Other regions from this component such as the cingulate cortex (BA 24), demonstrate altered blood flow when the individuals are performing a task of reversal learning relative to ID shift learning (Rogers et al., 2000). Finally regions such as the medial frontal gyrus and the inferior parietal lobule show altered blood flow when the subject is performing extradimensional shifts (Nakahara et al., 2002; Rogers et al., 2000). Some of the regions positively associated with this genetic component have also been related to performance in the DCCS task, including some temporal regions (Pan, Sawyer, McDonough, Slotpole, & Gansler, 2018). These areas are mostly related to cognitive flexibility, working memory, attention and decision making (Fuster, 2015), but some of them are also related to language, as has already been pointed out in neuroimaging studies considering the performance in this type of task (Pan et al., 2018). In sum, the brain regions identified in our anatomo-functional analysis are highly involved in the behavioral outcomes investigated in our study.

MRI studies were done in middle childhood, a late critical phase of brain growth in which brain circuitry is finely tuned to support the operations of the coming adult brain (Caviness Jr,

Kennedy, Richelme, Rademacher, & Filipek, 1996). This phase is associated with significant developmental gains and a shift toward adult level functional capacities in memory (Townsend, Richmond, Vogel-Farley, & Thomas, 2010), and response inhibition (Nichelli, Scala, Vago, Riva, & Bulgheroni, 2005; Velanova, Wheeler, & Luna, 2008). The fact that environment affected associations between genetic components of this ePRS and brain morphology at this stage suggests that environment effects, observed earlier on neuropsychological tests, may leave modifications in brain morphology at this later childhood phase.

Concerning the performance variation in the ability to shift attention, it has been proposed that it could result from alterations in the working memory (Pantelis et al., 2009). In the present study, no correlations were found between these performances, in agreement with other studies (Friedman et al., 2006; Robbins et al., 1998).

The molecular functions enriched in the ePRS-*VAMP1* network were especially related to Wnt signaling pathways and actin monomer binding. Wnt signaling pathways (canonical and noncanonical) are related to synaptic assembly and neurotransmission, through regulation of transcription, actin polymerization, and calcium signaling (Inestrosa & Varela-Nallar, 2014; Lie et al., 2005), and these pathways have also been reported to be important in cell proliferation, neurogenesis, and mitochondrial biogenesis (Inestrosa & Varela-Nallar, 2014; Lie et al., 2005). It should be pointed that synaptic plasticity is essential for cognitive function and for biological adaptation to changes in the environment. It involves reorganization of the cytoskeleton and synthesis of new proteins (Ehlers, 2003; Ma et al., 2017). In addition, genetic variations in Wnt signaling pathways have been related to ADHD (Grünblatt et al., 2019), and to spatial learning (Tabatadze et al., 2012). Neuroprotective effects of Wnt signaling on cognitive function in animal models of Alzheimer's disease have been reported (Cisternas et al., 2019; Oliva, Vargas, & Inestrosa, 2013), and chronic stress-induced reductions in Wnt signaling in the PFC in rats has been associated with impairment in cognitive flexibility (Mohamed et al., 2020).

Among the hubs and bottlenecks detected in the network, RFNG codes for O-Fucosylpeptide 3-Beta-N-Acetylglucosaminyltransferase. This transferase modulates NOTCH1 activity (Moloney et al., 2000), which is involved in several functions, including vascular permeability (Miloudi et al., 2019) and expression of synaptic vesicle proteins (Hayashi et al., 2016). Two other nodes with high centrality indexes are the insulin-like growth factor binding protein 4 (IGFBP4), and ITGA7, that codes for integrin subunit alpha 7. Integrins mediate cell membrane interactions with the extracellular matrix, and are present at high levels at synapses, where they help shaping synapse structure and plasticity (Park & Goda, 2016). Therefore, these three central nodes code for proteins that modify plasticity.

When comparing overlapping genes from PFC-ePRS-VAMP1 network with other gene sets (using FUMA (Watanabe et al., 2017)), we observed that many of the gene sets related to the present ePRS network are from studies involving inflammation. Early life stress has been shown to predict peripheral inflammation later in life (Mondelli & Vernon, 2019; O'Connor et al., 2020; Reid et al., 2020), and Nusslock and Miller (Nusslock & Miller, 2016) have proposed a neuro-immune network hypothesis, in which early adversity would sensitize immune cells, propagating inflammation, that could spread to brain structures. That was particularly interesting, since studies both in animals (Culley et al., 2014) and humans (Lasselin et al., 2016) have related inflammation and cognitive flexibility, some of them using the same task used in the present study. Also, performance in the intra/extra-dimensional set shift (IED) test was assessed in obese and non-obese human subjects, and it was observed that low-grade inflammation was an important contributor to explain attentional flexibility performance observed in obese subjects (Lasselin et

al., 2016). In rats, the attentional set-shifting task was used to evaluate the contribution of systemic inflammation to attention impairment, and it was also observed that inflammation affects reversal learning and attentional shifts (Culley et al., 2014).

In conclusion, the network of genes whose expression was enriched in early life and that were coexpressed with *VAMP1* in the PFC seems to be acting as a moderator of early environment quality influence on cognitive flexibility and spatial working memory, both functions in which the PFC is strongly involved. These effects were verified in young children, and this finding was replicated in a different cohort, besides testing the network specificity. pICA analysis pointed to associations between genetic components of this ePRS and gray matter density in regions related to performance in the tasks observed, suggesting that the gray matter density in some of these MRI components is moderated by the quality of the environment. Overall, this study may shed light on how the quality of early environment exerts a long-lasting influence on cognition, and the individual differences in their susceptibility to changes in the environment. Also, new experimental approaches derived from these paradigms might produce empirical support for the observed effects as well as explanation for their mechanisms.

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Authors contributions

CD and PPS conceived the study, participated in its design, and wrote the manuscript. BB participated in the study design, the imaging analysis and wrote the manuscript. RBS, GBCH, and MSK performed acquisition of data on neuroimaging and genotyping. SP contributed by calculating ePRSs for both cohorts. IP and ZW analyzed and interpreted the data. DMA conducted enrichment analysis, made figures, and participated in the interpretation of the data. RMSL made topological analysis and participated in the discussion. EJMF made important methodological contributions and participated in the discussion. JAQ revised important intellectual content. MM was the main person in charge for both cohorts, obtained funding, supervised the research, and reviewed the article in the neurobiological and technological aspects. All authors provided feedback and revision of the final manuscript.

The authors have no competing interests to declare.

References

- 1000 Genomes Project Consortium, Auton, A., Brooks, L.D., Durbin, R.M., Garrison, E.P., Kang, H.M., Korbel, J.O., . . . Abecasis, G.R. (2015) A global reference for human genetic variation. *Nature*, 526(7571), 68-74.
- Adedinsewo, D. A., Fleming, A. S., Steiner, M., Meaney, M. J., Girard, A. W., & team, M. (2014). Maternal anxiety and breastfeeding: findings from the MAVAN (Maternal Adversity, Vulnerability and Neurodevelopment) Study. *Journal of Human Lactation*, 30(1), 102-109.
- Arcego, D. M., Krolow, R., Lampert, C., Toniazzo, A. P., Berlitz, C., Lazzaretti, C., . . . Dalmaz, C. (2016). Early life adversities or high fat diet intake reduce cognitive function and alter BDNF signaling in adult rats: Interplay of these factors changes these effects. *International Journal of Developmental Neuroscience*, 50, 16-25.
- Bahtiyar, S., Karaca, K. G., Henckens, M. J., & Roozendaal, B. (2020). Norepinephrine and glucocorticoid effects on the brain mechanisms underlying memory accuracy and generalization. *Molecular and Cellular Neuroscience*, 103537.
- Barsegyan, A., Mackenzie, S. M., Kurose, B. D., McGaugh, J. L., & Roozendaal, B. (2010). Glucocorticoids in the prefrontal cortex enhance memory consolidation and impair working memory by a common neural mechanism. *Proceedings of the National Academy of Sciences*, 107(38), 16655-16660.
- Baudin, A., Blot, K., Verney, C., Estevez, L., Santamaria, J., Gressens, P., . . . Naudon, L. (2012). Maternal deprivation induces deficits in temporal memory and cognitive flexibility and exaggerates synaptic plasticity in the rat medial prefrontal cortex. *Neurobiology of learning and memory*, 98(3), 207-214.
- Birrell, J. M., & Brown, V. J. (2000). Medial frontal cortex mediates perceptual attentional set shifting in the rat. *Journal of Neuroscience*, 20(11), 4320-4324.
- Bissonette, G. B., Martins, G. J., Franz, T. M., Harper, E. S., Schoenbaum, G., & Powell, E. M. (2008). Double dissociation of the effects of medial and orbital prefrontal cortical lesions on attentional and affective shifts in mice. *Journal of Neuroscience*, 28(44), 11124-11130.
- Bondi, C. O., Rodriguez, G., Gould, G. G., Frazer, A., & Morilak, D. A. (2008). Chronic unpredictable stress induces a cognitive deficit and anxiety-like behavior in rats that is prevented by chronic antidepressant drug treatment. *Neuropsychopharmacology*, 33(2), 320-331.
- Calhoun, V. D., Adali, T., Pearlson, G. D., & Pekar, J. J. (2001). A method for making group inferences from functional MRI data using independent component analysis. *Human brain mapping*, 14(3), 140-151.
- Carpenter, L. L., Tyrka, A. R., McDougle, C. J., Malison, R. T., Owens, M. J., Nemeroff, C. B., & Price, L. H. (2004). Cerebrospinal fluid corticotropin-releasing factor and perceived early-life stress in depressed patients and healthy control subjects. *Neuropsychopharmacology*, 29(4), 777-784.
- Cassidy, J., & Marvin, R. (1992). Attachment organization in 2½ to 4½ year olds: Coding manual. *Unpublished coding manual, University of Virginia, Charlottesville, VA, USA*.
- Caviness Jr, V., Kennedy, D., Richelme, C., Rademacher, J., & Filipek, P. (1996). The human brain age 7–11 years: a volumetric analysis based on magnetic resonance images. *Cerebral cortex*, 6(5), 726-736.

- Cerqueira, J. J., Pêgo, J. M., Taipa, R., Bessa, J. M., Almeida, O. F., & Sousa, N. (2005). Morphological correlates of corticosteroid-induced changes in prefrontal cortex-dependent behaviors. *Journal of Neuroscience*, 25(34), 7792-7800.
- Chase, E. A., Tait, D. S., & Brown, V. J. (2012). Lesions of the orbital prefrontal cortex impair the formation of attentional set in rats. *European Journal of Neuroscience*, 36(3), 2368-2375.
- Chocyk, A., Majcher-Maślanka, I., Dudys, D., Przyborowska, A., & Wędzony, K. (2013). Impact of early-life stress on the medial prefrontal cortex functions—A search for the pathomechanisms of anxiety and mood disorders. *Pharmacological Reports*, 65(6), 1462-1470.
- Cisternas, P., Zolezzi, J. M., Martinez, M., Torres, V. I., Wong, G. W., & Inestrosa, N. C. (2019). Wnt-induced activation of glucose metabolism mediates the in vivo neuroprotective roles of Wnt signaling in Alzheimer disease. *Journal of neurochemistry*, 149(1), 54-72.
- Clark, L., Cools, R., & Robbins, T. (2004). The neuropsychology of ventral prefrontal cortex: decision-making and reversal learning. *Brain and cognition*, 55(1), 41-53.
- Consortium, G. P. (2015). A global reference for human genetic variation. *Nature*, 526(7571), 68.
- Cook, S. C., & Wellman, C. L. (2004). Chronic stress alters dendritic morphology in rat medial prefrontal cortex. *Journal of neurobiology*, 60(2), 236-248.
- Culley, D. J., Snayd, M., Baxter, M. G., Xie, Z., Lee, I. H., Rudolph, J., . . . Crosby, G. (2014). Systemic inflammation impairs attention and cognitive flexibility but not associative learning in aged rats: possible implications for delirium. *Frontiers in aging neuroscience*, 6, 107.
- Cupertino, R. B., Kappel, D. B., Bandeira, C. E., Schuch, J. B., da Silva, B. S., Müller, D., . . . Mota, N. R. (2016). SNARE complex in developmental psychiatry: neurotransmitter exocytosis and beyond. *Journal of Neural Transmission*, 123(8), 867-883.
- Danet, M., Lapiz-Bluhm, S., & Morilak, D. A. (2010). A cognitive deficit induced in rats by chronic intermittent cold stress is reversed by chronic antidepressant treatment. *International Journal of Neuropsychopharmacology*, 13(8), 997-1009.
- Daveluy, C., & Québec, I. d. l. s. d. (2001). *Enquête sociale et de santé 1998*: Institut de la statistique du Québec.
- Dias-Ferreira, E., Sousa, J. C., Melo, I., Morgado, P., Mesquita, A. R., Cerqueira, J. J., . . . Sousa, N. (2009). Chronic stress causes frontostriatal reorganization and affects decision-making. *Science*, 325(5940), 621-625.
- Dias, R., Robbins, T. W., & Roberts, A. C. (1996). Dissociation in prefrontal cortex of affective and attentional shifts. *Nature*, 380(6569), 69-72.
- Downes, J. J., Roberts, A., Sahakian, B., Evenden, J., Morris, R. G., & Robbins, T. (1989). Impaired extra-dimensional shift performance in medicated and unmedicated Parkinson's disease: evidence for a specific attentional dysfunction. *Neuropsychologia*, 27(11-12), 1329-1343.
- Durbin, R. (2014). Efficient haplotype matching and storage using the positional Burrows–Wheeler transform (PBWT). *Bioinformatics*, 30(9), 1266-1272.
- Ehlers, M. D. (2003). Activity level controls postsynaptic composition and signaling via the ubiquitin-proteasome system. *Nature neuroscience*, 6(3), 231-242.

- Epstein, N. B., Baldwin, L. M., & Bishop, D. S. (1983). The McMaster family assessment device. *Journal of marital and family therapy*, 9(2), 171-180.
- Floresco, S. B., Block, A. E., & Maric, T. (2008). Inactivation of the medial prefrontal cortex of the rat impairs strategy set-shifting, but not reversal learning, using a novel, automated procedure. *Behavioural brain research*, 190(1), 85-96.
- Folha, O. A. d. A. C., Bahia, C. P., de Aguiar, G. P. S., Herculano, A. M., Coelho, N. L. G., de Sousa, M. B. C., . . . Pereira, A. (2017). Effect of chronic stress during adolescence in prefrontal cortex structure and function. *Behavioural brain research*, 326, 44-51.
- Friedman, N. P., Miyake, A., Corley, R. P., Young, S. E., DeFries, J. C., & Hewitt, J. K. (2006). Not all executive functions are related to intelligence. *Psychological science*, 17(2), 172-179.
- Fuster, J. (2015). *The prefrontal cortex*: Academic Press.
- Gisquet-Verrier, P., & Delatour, B. (2006). The role of the rat prelimbic/infralimbic cortex in working memory: not involved in the short-term maintenance but in monitoring and processing functions. *Neuroscience*, 141(2), 585-596.
- Graybeal, C., Feyder, M., Schulman, E., Saksida, L. M., Bussey, T. J., Brigman, J. L., & Holmes, A. (2011). Paradoxical reversal learning enhancement by stress or prefrontal cortical damage: rescue with BDNF. *Nature neuroscience*, 14(12), 1507-1509.
- Graybeal, C., Kiselycznyk, C., & Holmes, A. (2012). Stress-induced impairments in prefrontal-mediated behaviors and the role of the N-methyl-D-aspartate receptor. *Neuroscience*, 211, 28-38.
- Grünblatt, E., Nemoda, Z., Werling, A. M., Roth, A., Angyal, N., Tarnok, Z., . . . Hebebrand, J. (2019). The involvement of the canonical Wnt-signaling receptor LRP5 and LRP6 gene variants with ADHD and sexual dimorphism: Association study and meta-analysis. *American Journal of Medical Genetics Part B: Neuropsychiatric Genetics*, 180(6), 365-376.
- Guo, W., Machado-Vieira, R., Mathew, S., Murrough, J. W., Charney, D. S., Grunebaum, M., . . . Henter, I. (2018). Exploratory genome-wide association analysis of response to ketamine and a polygenic analysis of response to scopolamine in depression. *Translational psychiatry*, 8(1), 1-8.
- Halim, N., Weickert, C., McClintock, B., Hyde, T., Weinberger, D., Kleinman, J., & Lipska, B. (2003). Presynaptic proteins in the prefrontal cortex of patients with schizophrenia and rats with abnormal prefrontal development. *Molecular psychiatry*, 8(9), 797-810.
- Hampshire, A., & Owen, A. M. (2006). Fractionating attentional control using event-related fMRI. *Cerebral cortex*, 16(12), 1679-1689.
- Hansel, D., & Mato, G. (2013). Short-term plasticity explains irregular persistent activity in working memory tasks. *Journal of Neuroscience*, 33(1), 133-149.
- Hari Dass, S. A., McCracken, K., Pokhvisneva, I., Chen, L. M., Garg, E., Nguyen, T. T. T., . . . Silveira, P. (2019). A biologically-informed polygenic score identifies endophenotypes and clinical conditions associated with the insulin receptor function on specific brain regions. *EBioMedicine*, 42, 13.
- Harms, M. B., Shannon Bowen, K. E., Hanson, J. L., & Pollak, S. D. (2018). Instrumental learning and cognitive flexibility processes are impaired in children exposed to early life stress. *Developmental Science*, 21(4), e12596.

- Hayashi, Y., Nishimune, H., Hozumi, K., Saga, Y., Harada, A., Yuzaki, M., . . . Tomita, T. (2016). A novel non-canonical Notch signaling regulates expression of synaptic vesicle proteins in excitatory neurons. *Scientific reports*, 6, 23969.
- Hervig, M. E., Piilgaard, L., Božič, T., Alsiö, J., & Robbins, T. W. (2020). Glutamatergic and serotonergic modulation of rat medial and lateral orbitofrontal cortex in visual serial reversal learning. *Psychology & Neuroscience*, 13(3), 438.
- Hoops, D., & Flores, C. (2017). Making dopamine connections in adolescence. *Trends in neurosciences*, 40(12), 709-719.
- Hornak, J., O'doherty, J., Bramham, J., Rolls, E., Morris, R., Bullock, P., & Polkey, C. (2004). Reward-related reversal learning after surgical excisions in orbito-frontal or dorsolateral prefrontal cortex in humans. *Journal of cognitive neuroscience*, 16(3), 463-478.
- Hostinar, C. E., Stellern, S. A., Schaefer, C., Carlson, S. M., & Gunnar, M. R. (2012). Associations between early life adversity and executive function in children adopted internationally from orphanages. *Proceedings of the National Academy of Sciences*, 109(Supplement 2), 17208-17212.
- Inestrosa, N. C., & Varela-Nallar, L. (2014). Wnt signaling in the nervous system and in Alzheimer's disease. *Journal of molecular cell biology*, 6(1), 64-74.
- Jett, J. D., Bulin, S. E., Hatherall, L. C., McCartney, C. M., & Morilak, D. A. (2017). Deficits in cognitive flexibility induced by chronic unpredictable stress are associated with impaired glutamate neurotransmission in the rat medial prefrontal cortex. *Neuroscience*, 346, 284-297.
- Kabacoff, R. I., Miller, I. W., Bishop, D. S., Epstein, N. B., & Keitner, G. I. (1990). A psychometric study of the McMaster Family Assessment Device in psychiatric, medical, and nonclinical samples. *Journal of family psychology*, 3(4), 431.
- Kanner, A. D., Coyne, J. C., Schaefer, C., & Lazarus, R. S. (1981). Comparison of two modes of stress measurement: Daily hassles and uplifts versus major life events. *Journal of behavioral medicine*, 4(1), 1-39.
- Khadka, S., Pearlson, G. D., Calhoun, V. D., Liu, J., Gelernter, J., Bessette, K. L., & Stevens, M. C. (2016). Multivariate imaging genetics study of MRI gray matter volume and SNPs reveals biological pathways correlated with brain structural differences in attention deficit hyperactivity disorder. *Frontiers in Psychiatry*, 7, 128.
- Kramer, M. S., Goulet, L., Lydon, J., Séguin, L., McNamara, H., Dassa, C., . . . Genest, J. (2001). Socio-economic disparities in preterm birth: causal pathways and mechanisms. *Paediatric and perinatal epidemiology*, 15, 104-123.
- Kramer, M. S., Platt, R. W., Wen, S. W., Joseph, K., Allen, A., Abrahamowicz, M., . . . System, F. I. H. S. G. o. t. C. P. S. (2001). A new and improved population-based Canadian reference for birth weight for gestational age. *Pediatrics*, 108(2), e35-e35.
- Lapiz-Bluhm, M. D. S., Soto-Piña, A. E., Hensler, J. G., & Morilak, D. A. (2009). Chronic intermittent cold stress and serotonin depletion induce deficits of reversal learning in an attentional set-shifting test in rats. *Psychopharmacology*, 202(1), 329-341.
- Lasselin, J., Magne, E., Beau, C., Aubert, A., Dexpert, S., Carrez, J., . . . Capuron, L. (2016). Low-grade inflammation is a major contributor of impaired attentional set shifting in obese subjects. *Brain, behavior, and immunity*, 58, 63-68.
- Lazzaretti, C., Kincheski, G. C., Pandolfo, P., Krolow, R., Toniazzi, A. P., Arcego, D. M., . . . Bertolini, D. (2018). Neonatal handling impairs intradimensional shift and alters

- plasticity markers in the medial prefrontal cortex of adult rats. *Physiology & behavior*, 197, 29-36.
- Lesuis, S. L., Lucassen, P. J., & Krugers, H. J. (2019). Early life stress impairs fear memory and synaptic plasticity; a potential role for GluN2B. *Neuropharmacology*, 149, 195-203.
- Lie, D.-C., Colamarino, S. A., Song, H.-J., Désiré, L., Mira, H., Consiglio, A., . . . Dearie, A. R. (2005). Wnt signalling regulates adult hippocampal neurogenesis. *Nature*, 437(7063), 1370-1375.
- Liston, C., McEwen, B. S., & Casey, B. (2009). Psychosocial stress reversibly disrupts prefrontal processing and attentional control. *Proceedings of the National Academy of Sciences*, 106(3), 912-917.
- Liston, C., Miller, M. M., Goldwater, D. S., Radley, J. J., Rocher, A. B., Hof, P. R., . . . McEwen, B. S. (2006). Stress-induced alterations in prefrontal cortical dendritic morphology predict selective impairments in perceptual attentional set-shifting. *Journal of Neuroscience*, 26(30), 7870-7874.
- Liu, J., & Calhoun, V. D. (2014). A review of multivariate analyses in imaging genetics. *Frontiers in neuroinformatics*, 8, 29.
- Liu, J., Ghassemi, M. M., Michael, A. M., Boutte, D., Wells, W., Perrone-Bizzozero, N., . . . Potkin, S. G. (2012). An ICA with reference approach in identification of genetic variation and associated brain networks. *Frontiers in human neuroscience*, 6, 21.
- Lobel, M., & Dunkel-Schetter, C. (1990). Conceptualizing stress to study effects on health: Environmental, perceptual, and emotional components. *Anxiety Research*, 3(3), 213-230.
- Lobel, M., Dunkel-Schetter, C., & Scrimshaw, S. C. (1992). Prenatal maternal stress and prematurity: a prospective study of socioeconomically disadvantaged women. *Health psychology*, 11(1), 32.
- Lonsdale, J., Thomas, J., Salvatore, M., Phillips, R., Lo, E., Shad, S., . . . Young, N. (2013). The genotype-tissue expression (GTEx) project. *Nature genetics*, 45(6), 580-585.
- Luciana, M., & Nelson, C. A. (1998). The functional emergence of prefrontally-guided working memory systems in four-to eight-year-old children. *Neuropsychologia*, 36(3), 273-293.
- Ma, Q., Ruan, H., Peng, L., Zhang, M., Gack, M. U., & Yao, W.-D. (2017). Proteasome-independent polyubiquitin linkage regulates synapse scaffolding, efficacy, and plasticity. *Proceedings of the National Academy of Sciences*, 114(41), E8760-E8769.
- Manes, F., Sahakian, B., Clark, L., Rogers, R., Antoun, N., Aitken, M., & Robbins, T. (2002). Decision-making processes following damage to the prefrontal cortex. *Brain*, 125(3), 624-639.
- McAlonan, K., & Brown, V. J. (2003). Orbital prefrontal cortex mediates reversal learning and not attentional set shifting in the rat. *Behavioural brain research*, 146(1-2), 97-103.
- McCarthy, S., Das, S., Kretzschmar, W., Delaneau, O., Wood, A. R., Teumer, A., . . . Sharp, K. (2016). A reference panel of 64,976 haplotypes for genotype imputation. *Nature genetics*, 48(10), 1279-1283.
- McGaugh, J. L. (2013). Making lasting memories: Remembering the significant. *Proceedings of the National Academy of Sciences*, 110(Supplement 2), 10402-10407.
- McGaugh, J. L., Gold, P. E., Van Buskirk, R., & Haycock, J. (1975). Modulating influences of hormones and catecholamines on memory storage processes. In *Progress in brain research* (Vol. 42, pp. 151-162): Elsevier.

- McGaugh, J. L., & Roozendaal, B. (2009). Drug enhancement of memory consolidation: historical perspective and neurobiological implications. *Psychopharmacology*, 202(1-3), 3-14.
- McGowan, P. O., Sasaki, A., D'alessio, A. C., Dymov, S., Labonté, B., Szyf, M., . . . Meaney, M. J. (2009). Epigenetic regulation of the glucocorticoid receptor in human brain associates with childhood abuse. *Nature neuroscience*, 12(3), 342-348.
- Merz, E. C., & McCall, R. B. (2011). Parent ratings of executive functioning in children adopted from psychosocially depriving institutions. *Journal of Child Psychology and Psychiatry*, 52(5), 537-546.
- Michelsen, K. A., van den Hove, D. L., Schmitz, C., Segers, O., Prickaerts, J., & Steinbusch, H. W. (2007). Prenatal stress and subsequent exposure to chronic mild stress influence dendritic spine density and morphology in the rat medial prefrontal cortex. *BMC neuroscience*, 8(1), 1-8.
- Miguel, P. M., Pereira, L. O., Barth, B., de Mendonça Filho, E. J., Pokhvisneva, I., Nguyen, T. T., . . . Gallant, H. (2019). Prefrontal cortex dopamine transporter gene network moderates the effect of perinatal hypoxic-ischemic conditions on cognitive flexibility and brain gray matter density in children. *Biological psychiatry*, 86(8), 621-630.
- Mika, A., Mazur, G. J., Hoffman, A. N., Talboom, J. S., Bimonte-Nelson, H. A., Sanabria, F., & Conrad, C. D. (2012). Chronic stress impairs prefrontal cortex-dependent response inhibition and spatial working memory. *Behavioral neuroscience*, 126(5), 605.
- Miller, J. A., Ding, S.-L., Sunkin, S. M., Smith, K. A., Ng, L., Szafer, A., . . . Aiona, K. (2014). Transcriptional landscape of the prenatal human brain. *Nature*, 508(7495), 199-206.
- Miloudi, K., Oubaha, M., Ménard, C., Dejda, A., Guber, V., Cagnone, G., . . . Binet, F. (2019). NOTCH1 signaling induces pathological vascular permeability in diabetic retinopathy. *Proceedings of the National Academy of Sciences*, 116(10), 4538-4547.
- Mistry, S., Escott-Price, V., Florio, A. D., Smith, D. J., & Zammit, S. (2019). Genetic risk for bipolar disorder and psychopathology from childhood to early adulthood. *Journal of affective disorders*, 246, 633-639.
- Mizoguchi, K., Yuzurihara, M., Ishige, A., Sasaki, H., Chui, D.-H., & Tabira, T. (2000). Chronic stress induces impairment of spatial working memory because of prefrontal dopaminergic dysfunction. *Journal of Neuroscience*, 20(4), 1568-1574.
- Moench, K. M., Breach, M. R., & Wellman, C. L. (2020). Prior stress followed by a novel stress challenge results in sex-specific deficits in behavioral flexibility and changes in gene expression in rat medial prefrontal cortex. *Hormones and behavior*, 117, 104615.
- Moghaddam, B., & Homayoun, H. (2008). Divergent plasticity of prefrontal cortex networks. *Neuropsychopharmacology*, 33(1), 42-55.
- Mohamed, A. M., Habib, M. Z., Ebeid, M. A., Abdelraouf, S. M., El Faramawy, Y., Aboul-Fotouh, S., & Magdy, Y. (2020). Amisulpride alleviates chronic mild stress-induced cognitive deficits: Role of prefrontal cortex microglia and Wnt/ β -catenin pathway. *European Journal of Pharmacology*, 885, 173411.
- Moloney, D. J., Panin, V. M., Johnston, S. H., Chen, J., Shao, L., Wilson, R., . . . Haltiwanger, R. S. (2000). Fringe is a glycosyltransferase that modifies Notch. *Nature*, 406(6794), 369-375.
- Mondelli, V., & Vernon, A. C. (2019). From early adversities to immune activation in psychiatric disorders: the role of the sympathetic nervous system. *Clinical & Experimental Immunology*, 197(3), 319-328.

- Morgan, A. R., Touchard, S., O'Hagan, C., Sims, R., Majounie, E., Escott-Price, V., . . . Morgan, B. P. (2017). The correlation between inflammatory biomarkers and polygenic risk score in Alzheimer's disease. *Journal of Alzheimer's Disease*, 56(1), 25-36.
- Morris, L. S., Kundu, P., Dowell, N., Mechelmans, D. J., Favre, P., Irvine, M. A., . . . Harrison, N. A. (2016). Fronto-striatal organization: defining functional and microstructural substrates of behavioural flexibility. *cortex*, 74, 118-133.
- Moss, E., Bureau, J.-F., Cyr, C., Mongeau, C., & St-Laurent, D. (2004). Correlates of attachment at age 3: construct validity of the preschool attachment classification system. *Developmental psychology*, 40(3), 323.
- Müller, N. G., Machado, L., & Knight, R. T. (2002). Contributions of subregions of the prefrontal cortex to working memory: evidence from brain lesions in humans. *Journal of cognitive neuroscience*, 14(5), 673-686.
- Naegeli, K. J., O'Connor, J. A., Banerjee, P., & Morilak, D. A. (2013). Effects of milnacipran on cognitive flexibility following chronic stress in rats. *European Journal of Pharmacology*, 703(1-3), 62-66.
- Nakahara, K., Hayashi, T., Konishi, S., & Miyashita, Y. (2002). Functional MRI of macaque monkeys performing a cognitive set-shifting task. *Science*, 295(5559), 1532-1536.
- Neves de Oliveira, B.-H., Dalmaz, C., & Zeidán-Chuliá, F. (2018). Network-based identification of altered stem cell pluripotency and calcium signaling pathways in metastatic melanoma. *Medical Sciences*, 6(1), 23.
- Newberger, E. H., Barkan, S. E., Lieberman, E. S., McCormick, M. C., Yllo, K., Gary, L. T., & Schechter, S. (1992). Abuse of pregnant women and adverse birth outcome: current knowledge and implications for practice. *Jama*, 267(17), 2370-2372.
- Nichelli, F., Scala, G., Vago, C., Riva, D., & Bulgheroni, S. (2005). Age-related trends in Stroop and conflicting motor response task findings. *Child Neuropsychology*, 11(5), 431-443.
- Nikiforuk, A., & Popik, P. (2014). Ketamine prevents stress-induced cognitive inflexibility in rats. *Psychoneuroendocrinology*, 40, 119-122.
- Nusslock, R., & Miller, G. E. (2016). Early-life adversity and physical and emotional health across the lifespan: a neuroimmune network hypothesis. *Biological psychiatry*, 80(1), 23-32.
- O'Connor, T. G., Willoughby, M. T., Moynihan, J. A., Messing, S., Sefair, A. V., Carnahan, J., . . . Caserta, M. T. (2020). Early childhood risk exposures and inflammation in early adolescence. *Brain, behavior, and immunity*, 86, 22-29.
- Oliva, C. A., Vargas, J. Y., & Inestrosa, N. C. (2013). Wnts in adult brain: from synaptic plasticity to cognitive deficiencies. *Frontiers in cellular neuroscience*, 7, 224.
- Owen, A. M., Downes, J. J., Sahakian, B. J., Polkey, C. E., & Robbins, T. W. (1990). Planning and spatial working memory following frontal lobe lesions in man. *Neuropsychologia*, 28(10), 1021-1034.
- Palk, A. C., Dalvie, S., De Vries, J., Martin, A. R., & Stein, D. J. (2019). Potential use of clinical polygenic risk scores in psychiatry—ethical implications and communicating high polygenic risk. *Philosophy, Ethics, and Humanities in Medicine*, 14(1), 1-12.
- Pan, J., Sawyer, K., McDonough, E., Slotpole, L., & Gansler, D. (2018). Cognitive, neuroanatomical, and genetic predictors of executive function in healthy children and adolescents. *Developmental neuropsychology*, 43(7), 535-550.

- Pang, W. W., Tan, P. T., Cai, S., Fok, D., Chua, M. C., Lim, S. B., . . . Yap, F. (2020). Nutrients or nursing? Understanding how breast milk feeding affects child cognition. *European journal of nutrition*, 59(2), 609-619.
- Pantelis, C., Wood, S. J., Proffitt, T. M., Testa, R., Mahony, K., Brewer, W. J., . . . McGorry, P. D. (2009). Attentional set-shifting ability in first-episode and established schizophrenia: relationship to working memory. *Schizophrenia research*, 112(1-3), 104-113.
- Park, Y. K., & Goda, Y. (2016). Integrins in synapse regulation. *Nature Reviews Neuroscience*, 17(12), 745-756.
- Parker, B., McFarlane, J., Soeken, K., Torres, S., & Campbell, D. (1993). Physical and emotional abuse in pregnancy: a comparison of adult and teenage women. *Nursing Research*.
- Patterson, N., Price, A. L., & Reich, D. (2006). Population structure and eigenanalysis. *PLoS genet*, 2(12), e190.
- Pearlin, L., & Schooler, C. (1978). The structure of coping J Health Soc. *Behav.* 19 (1): 2, 21.
- Pearlson, G. D., Calhoun, V. D., & Liu, J. (2015). An introductory review of parallel independent component analysis (p-ICA) and a guide to applying p-ICA to genetic data and imaging phenotypes to identify disease-associated biological pathways and systems in common complex disorders. *Frontiers in genetics*, 6, 276.
- Plante, N., Courtemanche, R., & Groseilliers, L. (2002). Survey description and methodology-part II—statistical methodology—logitudinal aspects of 1998 to 2000 rounds. *Québec Longitudinal Study of Child Development (QLSCD 1998-2002)—From Birth to*, 29.
- Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M. A., Bender, D., . . . Daly, M. J. (2007). PLINK: a tool set for whole-genome association and population-based linkage analyses. *The American journal of human genetics*, 81(3), 559-575.
- R, C. T. (2014). *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing.
- Radley, J. J., Rocher, A. B., Miller, M., Janssen, W. G., Liston, C., Hof, P. R., . . . Morrison, J. H. (2006). Repeated stress induces dendritic spine loss in the rat medial prefrontal cortex. *Cerebral cortex*, 16(3), 313-320.
- Ramos-Miguel, A., Jones, A. A., Sawada, K., Barr, A. M., Bayer, T. A., Falkai, P., . . . Honer, W. G. (2018). Frontotemporal dysregulation of the SNARE protein interactome is associated with faster cognitive decline in old age. *Neurobiology of disease*, 114, 31-44.
- Reid, B. M., Doom, J. R., Argote, R. B., Correa-Burrows, P., Lozoff, B., Blanco, E., & Gahagan, S. (2020). Pathways to inflammation in adolescence through early adversity, childhood depressive symptoms, and body mass index: a prospective longitudinal study of Chilean infants. *Brain, behavior, and immunity*, 86, 4-13.
- Reynolds, L. M., Pokinko, M., Torres-Berrio, A., Cuesta, S., Lambert, L. C., Pellitero, E. D. C., . . . Kolb, B. (2018). DCC receptors drive prefrontal cortex maturation by determining dopamine axon targeting in adolescence. *Biological psychiatry*, 83(2), 181-192.
- Robbins, T. W., James, M., Owen, A. M., Sahakian, B. J., Lawrence, A. D., McInnes, L., & RABBITT, P. M. (1998). A study of performance on tests from the CANTAB battery sensitive to frontal lobe dysfunction in a large sample of normal volunteers: Implications for theories of executive functioning and cognitive aging. *Journal of the International Neuropsychological Society*, 4(5), 474-490.
- Rogers, R. D., Andrews, T., Grasby, P., Brooks, D., & Robbins, T. (2000). Contrasting cortical and subcortical activations produced by attentional-set shifting and reversal learning in humans. *Journal of cognitive neuroscience*, 12(1), 142-162.

- Romero-Martínez, Á., Lila, M., Catalá-Miñana, A., Williams, R. K., & Moya-Albiol, L. (2013). The contribution of childhood parental rejection and early androgen exposure to impairments in socio-cognitive skills in intimate partner violence perpetrators with high alcohol consumption. *International journal of environmental research and public health*, 10(8), 3753-3770.
- Rygula, R., Walker, S. C., Clarke, H. F., Robbins, T. W., & Roberts, A. C. (2010). Differential contributions of the primate ventrolateral prefrontal and orbitofrontal cortex to serial reversal learning. *Journal of Neuroscience*, 30(43), 14552-14559.
- Saito, R., Smoot, M. E., Ono, K., Ruschinski, J., Wang, P.-L., Lotia, S., . . . Ideker, T. (2012). A travel guide to Cytoscape plugins. *Nature methods*, 9(11), 1069.
- Sakhai, S. A., Saxton, K., & Francis, D. D. (2016). The influence of early maternal care on perceptual attentional set shifting and stress reactivity in adult rats. *Developmental psychobiology*, 58(1), 39-51.
- Sarabdjitsingh, R. A., Loi, M., Joëls, M., Dijkhuizen, R. M., & Van Der Toorn, A. (2017). Early life stress-induced alterations in rat brain structures measured with high resolution MRI. *PLoS One*, 12(9), e0185061.
- Schoenbaum, G., Setlow, B., Saddoris, M. P., & Gallagher, M. (2006). Encoding changes in orbitofrontal cortex in reversal-impaired aged rats. *Journal of neurophysiology*, 95(3), 1509-1517.
- Schwabe, K., Enkel, T., Klein, S., Schütte, M., & Koch, M. (2004). Effects of neonatal lesions of the medial prefrontal cortex on adult rat behaviour. *Behavioural brain research*, 153(1), 21-34.
- Silva-Gomez, A. B., Rojas, D., Juarez, I., & Flores, G. (2003). Decreased dendritic spine density on prefrontal cortical and hippocampal pyramidal neurons in postweaning social isolation rats. *Brain research*, 983(1-2), 128-136.
- Silveira, P. P., Pokhvisneva, I., Parent, C., Cai, S., Rema, A. S. S., Broekman, B. F. P., . . . Meaney, M. J. (2017). Cumulative prenatal exposure to adversity reveals associations with a broad range of neurodevelopmental outcomes that are moderated by a novel, biologically informed polygenetic score based on the serotonin transporter solute carrier family C6, member 4 (SLC6A4) gene expression. *Development psychopathology*, 29(5), 1601-1617.
- Snyder, K. P., Hill-Smith, T. E., Lucki, I., & Valentino, R. J. (2015). Corticotropin-releasing factor in the rat dorsal raphe nucleus promotes different forms of behavioral flexibility depending on social stress history. *Neuropsychopharmacology*, 40(11), 2517-2525.
- Soares, J. M., Sampaio, A., Ferreira, L. M., Santos, N., Marques, F., Palha, J. A., . . . Sousa, N. (2012). Stress-induced changes in human decision-making are reversible. *Translational psychiatry*, 2(7), e131-e131.
- Soh, S.-E., Tint, M. T., Gluckman, P. D., Godfrey, K. M., Rifkin-Graboi, A., Chan, Y. H., . . . Chong, Y.-S. (2014). Cohort profile: Growing Up in Singapore Towards healthy Outcomes (GUSTO) birth cohort study. *International journal of epidemiology*, 43(5), 1401-1409.
- Südhof, T. C., Baumert, M., Perin, M. S., & Jahn, R. (1989). A synaptic vesicle membrane protein is conserved from mammals to *Drosophila*. *Neuron*, 2(5), 1475-1481.
- Tabatadze, N., Tomas, C., McGonigal, R., Lin, B., Schook, A., & Routtenberg, A. (2012). Wnt transmembrane signaling and long-term spatial memory. *Hippocampus*, 22(6), 1228-1241.

- Talairach, J., & Tournoux, P. (1988.). A coplanar stereotaxic atlas of the human brain: An approach to medical cerebral imaging. In: Thieme Medical Publishers, Inc., New York.
- Townsend, E. L., Richmond, J. L., Vogel-Farley, V. K., & Thomas, K. (2010). Medial temporal lobe memory in childhood: Developmental transitions. *Developmental Science*, 13(5), 738-751.
- Tremblay, L., & Schultz, W. (1999). Relative reward preference in primate orbitofrontal cortex. *Nature*, 398(6729), 704-708.
- Uhlén, M., Fagerberg, L., Hallström, B., Lindskog, C., Oksvold, P., Mardinoglu, A., . . . Kampf, C. (2015). Proteomics. Tissue-based map of the human proteome. *Science*, 347, 1260419-1260419.
- Velanova, K., Wheeler, M. E., & Luna, B. (2008). Maturation changes in anterior cingulate and frontoparietal recruitment support the development of error processing and inhibitory control. *Cerebral cortex*, 18(11), 2505-2522.
- Warde-Farley, D., Donaldson, S. L., Comes, O., Zuberi, K., Badrawi, R., Chao, P., . . . Lopes, C. T. (2010). The GeneMANIA prediction server: biological network integration for gene prioritization and predicting gene function. *Nucleic acids research*, 38(suppl_2), W214-W220.
- Watanabe, K., Taskesen, E., Van Bochoven, A., & Posthuma, D. (2017). Functional mapping and annotation of genetic associations with FUMA. *Nature communications*, 8(1), 1-11.
- Weaver, I. C. G., Cervoni, N., Champagne, F. A., D'Alessio, A. C., Sharma, S., Seckl, J. R., . . . Meaney, M. J. (2004). Epigenetic programming by maternal behavior. *Nature neuroscience*, 7(8), 847.
- Wellman, C. L., Bollinger, J. L., & Moench, K. M. (2020). Effects of stress on the structure and function of the medial prefrontal cortex: Insights from animal models. *International review of neurobiology*, 150, 129-153.
- Xu, H., Zhang, Y., Zhang, F., Yuan, S.-n., Shao, F., & Wang, W. (2016). Effects of duloxetine treatment on cognitive flexibility and BDNF expression in the mPFC of adult male mice exposed to social stress during adolescence. *Frontiers in Molecular Neuroscience*, 9, 95.
- Yu, H., Kim, P. M., Sprecher, E., Trifonov, V., & Gerstein, M. (2007). The importance of bottlenecks in protein networks: correlation with gene essentiality and expression dynamics. *PLoS Comput Biol*, 3(4), e59.
- Zelazo, P. D. (2006). The Dimensional Change Card Sort (DCCS): A method of assessing executive function in children. *Nature protocols*, 1(1), 297-301.
- Zhang, Y., Shao, F., Wang, Q., Xie, X., & Wang, W. (2017). Neuroplastic correlates in the mPFC underlying the impairment of stress-coping ability and cognitive flexibility in adult rats exposed to chronic mild stress during adolescence. *Neural plasticity*, 2017.
- Zimmermann, K. S., Richardson, R., & Baker, K. D. (2019). Maturation changes in prefrontal and amygdala circuits in adolescence: implications for understanding fear inhibition during a vulnerable period of development. *Brain Sciences*, 9(3), 65.

Table 1: Description of the baseline characteristics of the MAVAN and GUSTO samples according to high and low PFC-ePRS-*VAMP1*.

Cohorts	MAVAN (discovery sample)			GUSTO (replication sample)		
Characteristics	Low ePRS (n = 66)	High ePRS (n = 66)	P-value	Low ePRS (n = 70)	High ePRS (n = 69)	P-value
<i>Males (%)</i>	34 (51.5%)	30 (45.5%)	0.6	32 (45.7%)	28 (40.6%)	0.66
<i>Maternal age at birth (years)</i>	31.3 $\pm$ 4.33	30.1 $\pm$ 4.88	0.2	32.02 $\pm$ 4.52	30.79 $\pm$ 6.0	0.20
<i>Full weeks of gestation</i>	39.2 $\pm$ 1.11	39.2 $\pm$ 1.3	0.8	38.92 $\pm$ 1.34	38.99 $\pm$ 1.04	0.67
<i>Birth weight (in grams)</i>	3356 $\pm$ 459	3264 $\pm$ 452	0.2	3207 $\pm$ 412	3057 $\pm$ 425	0.03
<i>Low SES</i>	17 (26.6%)	15 (25%)	0.8	7 (10.0%)	9 (13.0%)	0.71
<i>Early life environmental score</i>	1.97 (2.17)	1.47 (2.27)	0.2	1.11 $\pm$ 1.53	0.96 $\pm$ 1.58	0.55
<i>IED Total Errors Stage 8</i>	23.8 (8.77)	24.4 (7.68)	0.7			
<i>IED Total Errors Stages 2 +5 +7</i>	8.67 (7.67)	8.45 (8.83)	0.9			
<i>SWM Total Errors 4 boxes</i>	6.28 (2.97)	5.95 (3.51)	0.6			
<i>DCCS</i>				19.83 (6.11)	18.62 (5.71)	0.23

Data are expressed as means ($\pm$ standard deviations) or number of participants (percentages). Differences between low and high ePRS groups were not significant for all variables shown (Student's t-test for means and chi-square test for percentages). Low socioeconomic status (SES) in MAVAN: maternal education is high school or less and/or monthly income under low cut off bound according to Statistics Canada (<https://www150.statcan.gc.ca/n1/en/catalogue/75F0002M2006004>). Low socioeconomic status (SES) in GUSTO: maternal education attained primary school and/or monthly income <\$2000. For environmental score, see text.

Table 2. Components included in Adversity and positive early life environment scores. The final scores ranged from negative (more adverse) to positive (buffer) values. Presence of each component (described in each bullet) yielded 1 point, and the scores represent the summation of points (see text for further details).

Score	Component	
	MAVAN	GUSTO
Adversity	Hospitalization in the first 6 months of life	Hospitalization in the first 6 months of life
	Birth size percentile below 10 th percentile or above 90 th percentile	Birth size percentile below 10 th percentile or above 90 th percentile
	Gestational age below or equal to 37 weeks	Gestational age below or equal to 37 weeks
	Smoking during pregnancy	Smoking during pregnancy
	Household total gross income below 30,000\$	Household monthly income below SG\$2000
	Lack of money score above 7	
	Presence of domestic violence (physical or sexual)	
	Disorganized attachment	
	Poor family function (score greater or equal 2.00)	Poor family function (score greater or equal 2.17)
	Marital strain score above 3.32	
Positive	Pregnancy anxiety greater than 1.95	
	Birth size percentile greater or equal to 40% and below or equal to 70%	Birth size percentile greater or equal to 40% and below or equal to 70%
	Gestational age between 39-40 weeks	Gestational age between 39-40 weeks
	Household total gross income 80,000\$ and above	Household monthly income above SG\$6000
	Still breastfeeding at 3 months	Still breastfeeding at 3 months
	Secure attachment	
	Good family function (score below 1.15)	Good family function (score below 1.35)
	Marital strains below 1.45	

Table 3. Significant MRI-genetic components association.

Genetic component	Image (MRI) component	Correlation	t value	p-value	Bonferroni corrected p-value
6	3	0.696	6.2149	2.14e-07	2.7392e-05
11	4	-0.653	-5.526	2.0381e-06	0.0002608768
14	7	-0.63891	5.317	4.0123 e-06	0.0005135744

Figure 1. Interactions between PFC-ePRS-*VAMP1* and the environmental quality affecting cognitive flexibility and working memory (4 years-old MAVAN children). **A.** A Poisson regression analysis showed a significant effect of ePRS and environmental index interaction in predicting reversal learning ability (evaluated as the sum of errors in the reversal stages of IED task) in MAVAN 4 years-old children ($\beta = -0.12$, $p = 0.003$). The effect of environmental index on the number of errors in reversal learning was larger with the increase of *PFC-ePRS-VAMP1* (for the high ePRS score (+1 SD) $\beta = -0.13$, $p = 0.01$; simple slopes analysis). **B.** There was a significant interaction between ePRS and environmental index in predicting attentional flexibility in the extradimensional set shift (evaluated as the number of errors in the 8th stage of the IED task) ($\beta = 0.04$, $p = 0.03$). **C.** Errors in the spatial working memory task: A significant effect of ePRS and environmental index interaction for total errors in the 4 boxes stage of the task ($\beta = 0.34$, $p = 0.02$).

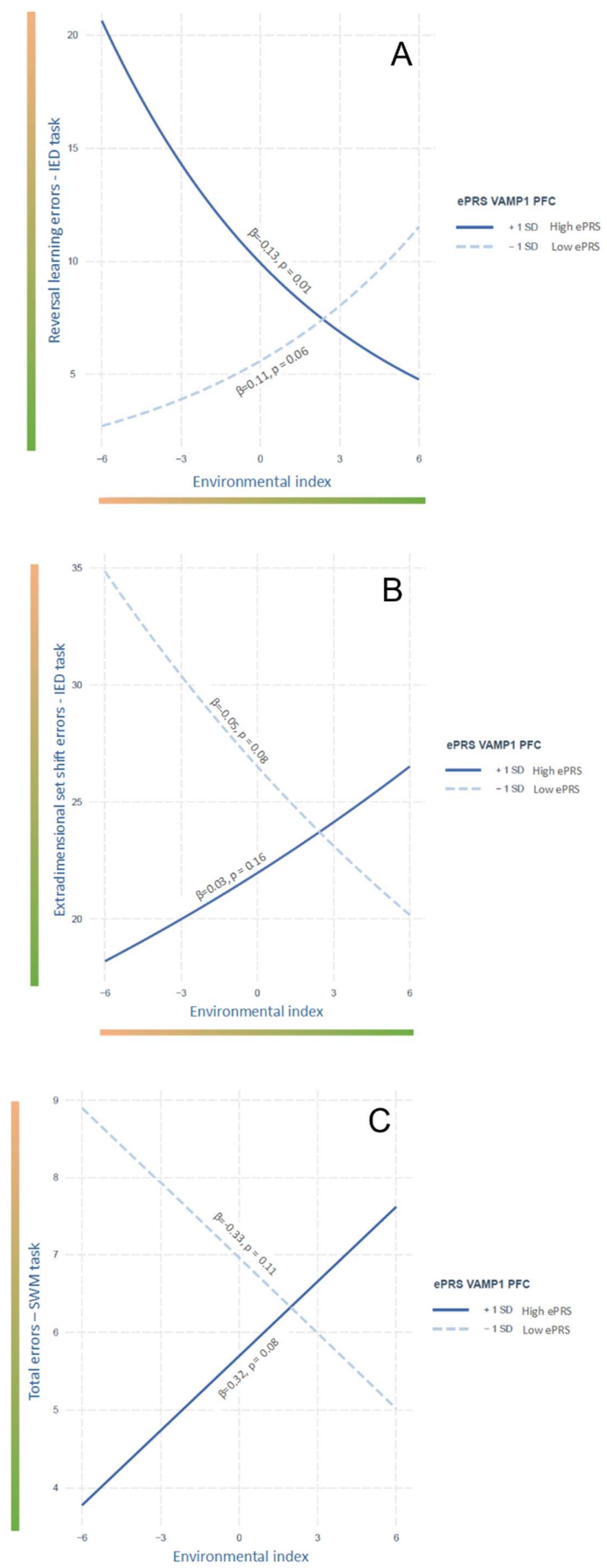


Figure 2. Interaction between PFC-ePRS-*VAMP1* and the environmental quality affecting cognitive flexibility (4.5 years-old GUSTO children). Results show percentage of accuracy in the orientation mixed trials of the DCCS task ($\beta = -0.622$, $p = 0.05$). The effect of environmental index on the accuracy was larger with the decrease of *PFC-ePRS-VAMP1* (for low ePRS score (-1 SD); $\beta=1.00$, $p = 0.03$).

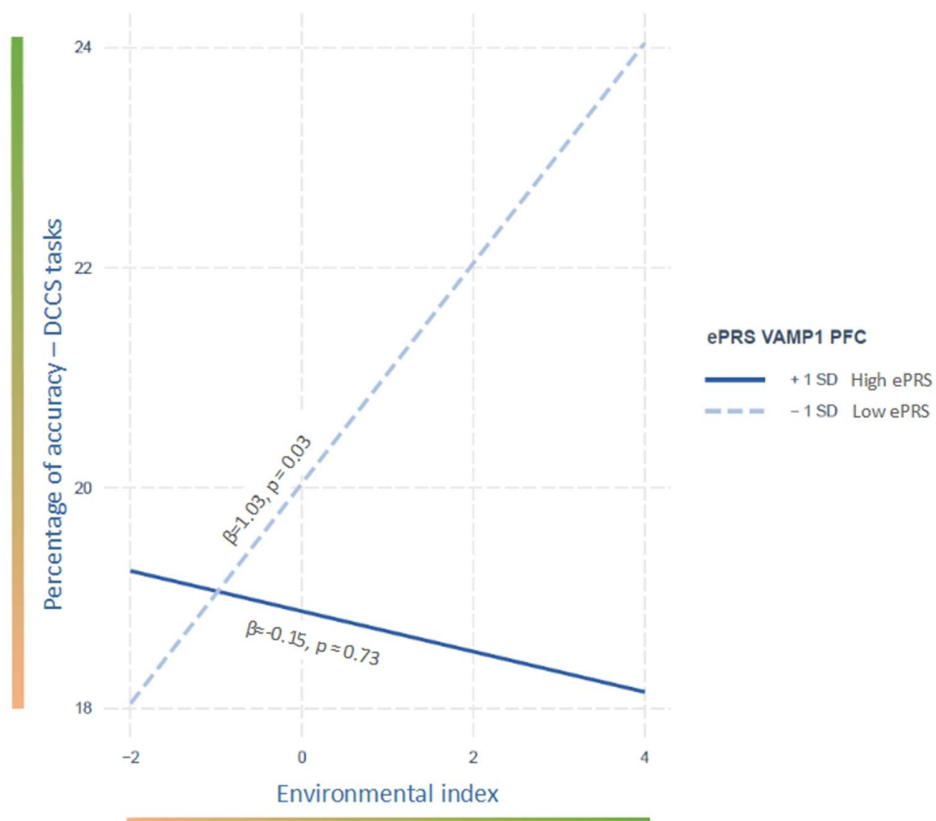


Figure 3. Characterization of the PFC-ePRS-*VAMP1* network. **A.** Connected network. Data retrieved from Genemania, and the network constructed in Cytoscape. Edges: gray for co-expression; red for co-localization. Sizes of the nodes are related to degrees (centrality parameter), so that the hubs are bigger. **B.** Enrichment analysis of the genes co-expressed with VAMP1 in PFC using Metacore®. The ten top molecular functions are shown. **C.** Overlapping genes from this network, against gene sets for immunological signatures obtained using FUMA (<https://fuma.ctglab.nl/>).

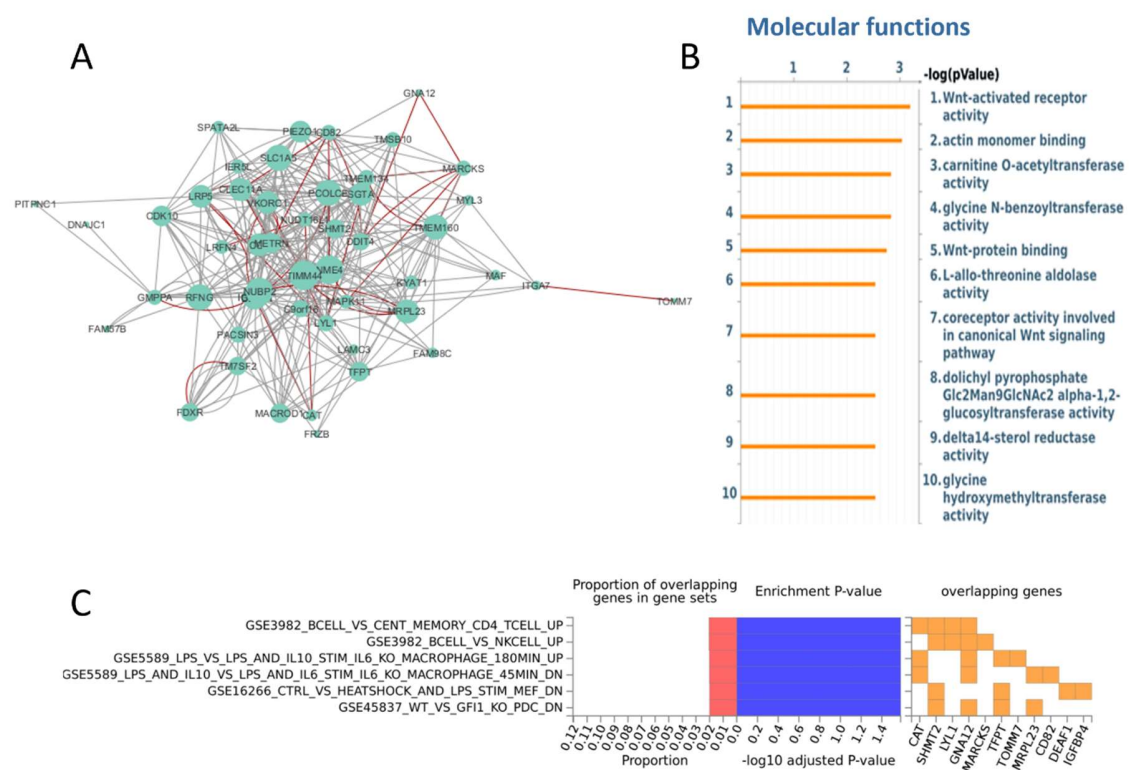


Figure 4. Age-specific expression patterns of the genes composing the PFC-ePRS-*VAMPI* network. **A.** Heatmap of expression pattern for each gene (based on Brainspan and obtained from FUMA). **B.** Sets of differentially expressed genes (DEG), up or down regulated in brain according to the age. Periods in this figure and Ns are as follows: Early prenatal (8-12 pcw, N=5), Early mid-prenatal (13-18 pcw, N=7), Late mid-prenatal (19-24 pcw, N=4), Late prenatal (25-39 pcw, N=3), Early infancy (Birth-5 months, N=2), Late infancy (6-18 months, N=3), Early childhood (19 months – 5 years, N=4), Late childhood (6-11 years, N=3), Adolescence (12-19 years, N=4), Young adulthood (20-37 years, N=5), Middle adulthood (~40 years, N=2); (<http://help.brain-map.org/display/devhumanbrain/Documentation>, Donors and samples).

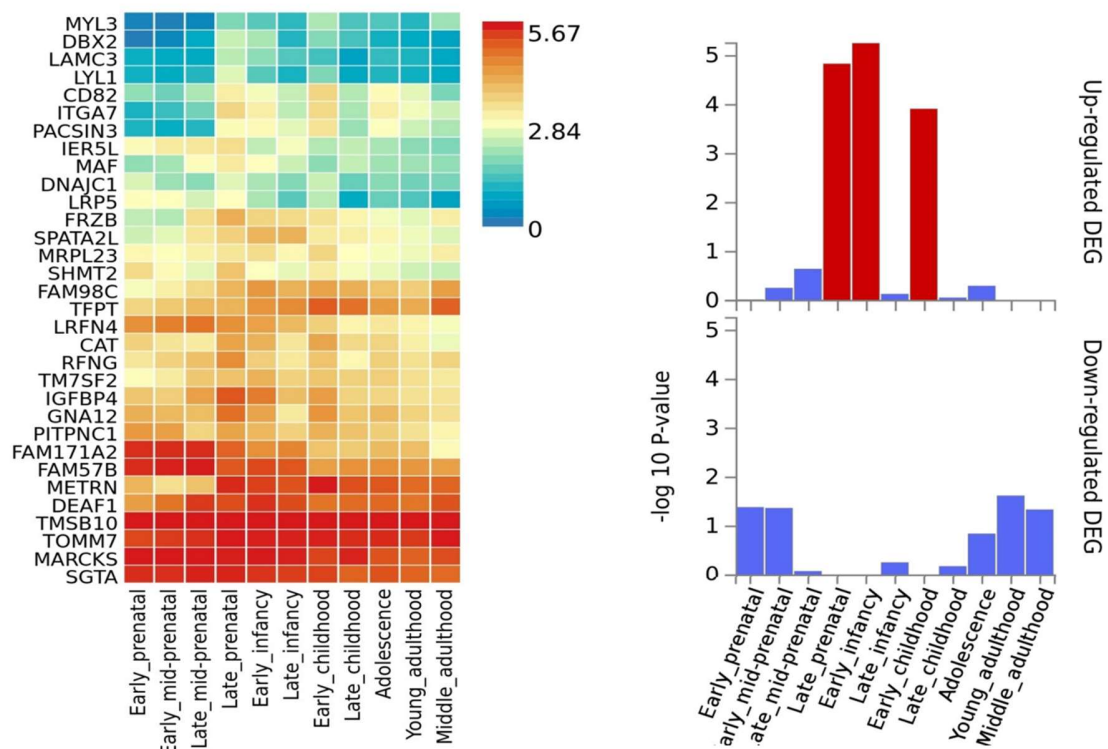


Figure 5. Relationship for genetic component 11 and MRI component 4. Upper panel (A) shows loading coefficients for gene component 11 (in blue) and MRI component 4 (in brown) for each subject in high and low environmental scores groups. (B) shows z scores for each inputted SNP for genetic component 11. (C) shows regional variations in gray matter density contributing to MRI component 4.

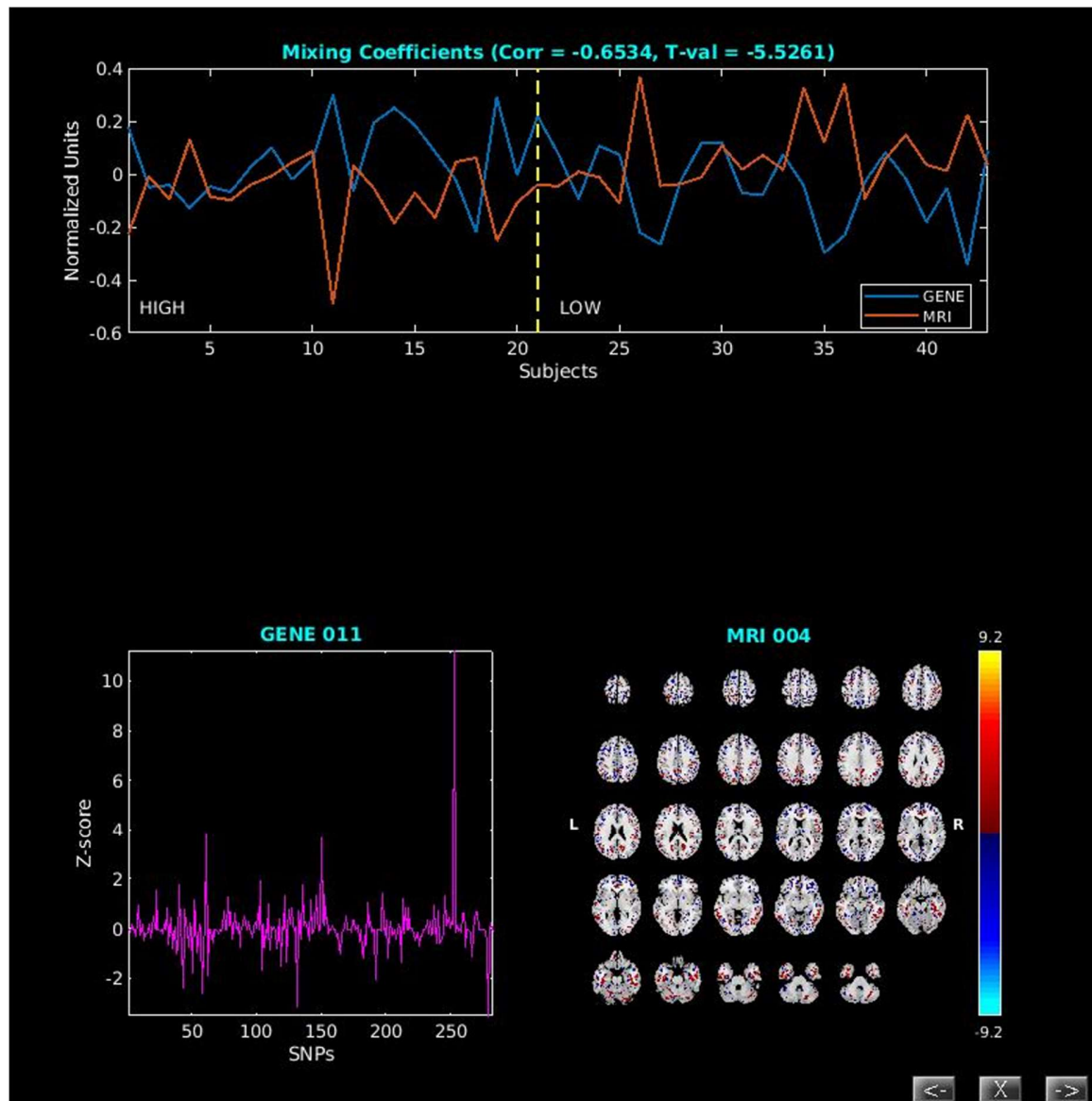


Figure 6. Summary of the findings. Expression-based PFC-ePRS-*VAMP1* moderates the effects of environmental quality on cognitive flexibility and working memory. The PFC-ePRS-*VAMP1* network is enriched in biological processes such as Wnt signaling and actin monomer binding, and the genes in this network show overlap with gene sets from studies related to inflammation. A parallel-ICA study showed that genetic component 11 was significantly associated with MRI component 4, and that the mean loading coefficients of these components between children from high and low environmental index groups was distinct. SNPs from this MRI component are enriched in processes related to Wnt signaling, P53 pathway, and integrin signaling.

Figure 6

