Multi-ethnic, phenome-wide association of complement component 4 variation with psychiatric and brain developmental phenotypes in youth

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Research Article

Keywords: schizophrenia, psychosis, neuroimaging, genetics, gene expression, complement, brain

Posted Date: May 25th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1578682/v1

Version of Record: A version of this preprint was published at Genome Biology on March 7th, 2023. See the published version at https://doi.org/10.1186/s13059-023-02878-0.

Abstract

Background: Increased expression of the complement component 4A (*C4A*) gene is associated with greater lifetime risk of schizophrenia. In the brain, *C4A* is involved in synaptic pruning; yet, it remains unclear the extent to which upregulation of *C4A* alters brain development or is associated with risk for psychotic symptoms in childhood. Here, we performed a multi-ethnic phenome-wide association study in 7,789 children aged 9-12 years to examine the relationship between genetically-regulated expression (GREx) of *C4A*, childhood brain structure, cognition, and psychiatric symptoms.

Results: While *C4A* GREx was not related to childhood psychotic experiences, cognition, or global measures of brain structure, it was associated with a localized reduction in regional surface area (SA) of the entorhinal cortex. Furthermore, we show that reduced entorhinal cortex SA at 9-10 years predicts greater number and severity of psychosis-like events at 1-year and 2-year follow-up timepoints. We also demonstrate that the effects of *C4A* on the entorhinal cortex are independent of genome-wide polygenic risk for schizophrenia.

Conclusions: Our results suggest neurodevelopmental effects of *C4A* on childhood medial temporal lobe structure, which may serve as a biomarker for schizophrenia risk prior to symptom onset.

Background

Schizophrenia is a highly heritable neurodevelopmental disorder characterized by the presence of hallucinations, delusions, disorganized behavior, and an array of negative symptoms (e.g., flattened affect, reduced motivation) and cognitive deficits. While clinical diagnosis typically occurs in late adolescence or early adulthood, frank psychosis is often preceded by a prodromal period of weeks to years during which gradual changes in cognition, perception and motivation occur (1). An estimated 10% of youth between the ages of 9-18 experience subclinical psychosis-like experiences (PLEs), including atypical sensory experiences and delusional beliefs (2), which are associated with significantly greater odds of developing schizophrenia in adulthood (2,3). One proposed mechanism through which normative developmental processes may contribute to schizophrenia pathophysiology is through atypical brain development and refinement of synaptic connections across childhood and adolescence (4,5). A major goal of modern psychiatric research is to identify genetic and brain-based antecedents of prodromal psychosis, with the ultimate goal of facilitating early intervention and better treatment.

The strongest genetic association with schizophrenia is located in the major histocompatibility complex (MHC) on chromosome 6 – a region encoding for proteins critical to innate and adaptive immunity (6–10). Subsequent fine mapping of this region has revealed that a significant proportion of this signal can be attributed to an association with the complement component 4A (*C4A*) (11). The complement system plays a crucial role in the peripheral immune response (12), and in the brain also plays an active role in pruning, facilitating microglial-mediated engulfment of synapses during development (13–16). These findings have led to the hypothesis that overactivation of complement genes in the brain may contribute

to altered neural development and synaptic pruning, ultimately conferring risk for the phenotypic expression of schizophrenia symptoms.

The *C4* locus encodes for *C4A* and *C4B* genes, which show common but complex structural variation. Each human haplotype carries a range of *C4A* and/or *C4B* copies, present in either long or short genomic forms (i.e., *C4AL, C4AS, C4BL, C4BS*) depending on the presence of a human endogenous retroviral insertion (HERV) in intron 9(17). Higher *C4A* copy number is associated with greater risk for schizophrenia, proportional to its effects on *C4A* expression (11,18,19). *C4A* copy number also shows sex-dependent effects on schizophrenia risk, with stronger effects in males relative to females (18). Importantly, while recent neuroimaging work suggests that *C4A* also affects cognition and brain structure in the general adult population (20), no study to date has examined the impact of *C4A* on childhood psychosis-related symptoms or brain development.

Here, we performed a comprehensive, phenome-wide association between imputed gene expression at the *C4* locus and childhood psychiatric, behavioral, and brain structural phenotypes to elucidate the role of *C4* genetic variation on human development (**Fig. 1**). We imputed the genetically regulated component of expression (GREx) into a large, multi-ethnic sample of 9-10-year-old youth who participated in the Adolescent Brain Cognitive Development^{SM} (ABCD) Study. We then assessed whether *C4A* GREx was associated with psychosis-like experiences as well as longitudinal psychiatric and cognitive phenotypes. We find that *C4A* GREx is not broadly related to childhood psychiatric symptoms and cognition in 9-10 year old youth, but is associated with a reduction in entorhinal cortex surface area. Furthermore, we find that entorhinal cortex surface area at 9-10-years-of-age is predictive of the number and severity of psychotic-like events at 1- and 2-year follow-up visits. Together, this phenome-wide characterization of the developmental effects of *C4A* provides evidence for regional effects on temporal lobe structure, which may be an early biomarker for schizophrenia risk.

Results

Genetically regulated expression of C4A in the multi-ethnic ABCD youth cohort

Genetic and behavioral data were obtained from a community sample of sociodemographically and ethnically diverse youth who participated in the ABCD Study, an ongoing longitudinal investigation of child development in the United States (21). Following quality control, including removal of subjects with low *C4* imputation quality, the sample consisted of 9,638 multiethnic youth (1,694 African ancestry [AFR]; 5,829 European ancestry [EUR]; 2,115 Latinx ancestry; **Fig. 1A**). Imputation accuracy was confirmed by comparing the proportion of youth assigned to each common *C4* structural allele to rates previously reported in a large sample of 765 EUR and 250 AFR ancestry individuals, from which the imputation panel was derived (18) **(Supplementary Table 1)**. Subsequent analyses were restricted to 7,789 youth with the 5 most common *C4* structural haplotypes (1,327 AFR; 4,919 EUR; 1,543 Latinx; 47.7% female;

Supplementary Table 2), although in sensitivity analyses results were concordant across the full spectrum of haplotypes. *C4* structural haplotypes were then used to calculate GREx for *C4A* and *C4B* using previously described weights (11), the accuracy of which we independently validated using data from PsychENCODE (**Supplementary Fig. 1**). As expected, imputed expression values for *C4A* and *C4B* were negatively correlated (R = -0.25, p < 2.2e-16; **Supplementary Fig. 2**). To control for this, downstream statistical analyses included GREx for both *C4A* and *C4B*.

No widespread C4A associations with psychotic-like experiences or childhood cognition

Models testing associations between *C4A* GREx with rates and severity of PLEs were restricted to unrelated individuals who had complete data for the Prodromal Questionnaire Brief Version (22) (PQ-B) and the Kiddie Schedule for Affective Disorders and Schizophrenia (23) (KSADS-5) (N = 6,655; 1,168 AFR; 4,113 EUR; 1,374 Latinx). As previous research in clinical high-risk samples indicates that suspiciousness and unusual thought content best predict conversion to psychosis over a 2-year period (24), a binary prodromal psychosis score (PP_{bin}) was computed by categorizing youth who endorsed experiencing at least 3 PLEs reflecting suspicious or unusual thought content and significant distress on the PQ-B and whose parents indicated the presence of hallucinations or delusions on the KSADS-5 (N = 611 cases, N = 6,044 controls). We did not observe any significant associations between *C4A* GREx and psychosis symptoms (i.e., PQ-B_{sym}, PQ-B_{sev},PP_{bin}; **Fig. 2, Supplementary Fig. 3-5, Supplementary Table 3**). As previous work has identified sexually dimorphic effects of *C4A* on brain and behavior (18,19), we further examined a potential interaction between sex and *C4A* GREx – which was not significant for any of the psychosis variables tested (**Supplementary Fig. 6-8, Supplementary Table 3**).

In adults and schizophrenia patients, genetic variation within the *C4* locus has been associated with poorer cognition and memory (20,25–27). To assess whether similar effects are present in childhood, we performed a phenome-wide association study (PheWAS) using data from 145 cognitive and psychiatric phenotypes available from the ABCD dataset. In contrast to findings in adults, *C4A* GREx was not robustly associated with childhood cognition or psychiatric phenotypes (**Fig. 2, Supplementary Fig. 6-8; Supplementary Table S3**). The only association that surpassed correction for multiple testing in the multiethnic cohort was between *C4A* GREx and the UPPS-P Impulsive Behavior Scale (28) lack of premeditation/planning subscale (β = -0.05, 95% CI [-0.07, -0.04], P_{FDR} < 0.004), suggesting that youth with higher *C4A* GREx show less impulsivity and more careful thinking before taking action.

Additional PheWAS were performed using available data from follow-up timepoints 1 and 2 years later. There were no FDR-significant associations between *C4A* GREx and PheWAS variables at the 1- or 2-year follow-up (**Supplementary Fig. 9-14, Supplementary Table 4-5**).

Replicable effects of the C4 locus on entorhinal cortex structure

Our PheWAS results above indicate limited detectable effects of *C4* variation on cognitive or psychiatric outcomes in youth. However, in schizophrenia, subtle brain structural changes are known to precede clinical and behavior manifestations (29–31). To determine whether *C4A* affects childhood brain development, we next tested the association between GREx and measures of cortical thickness (CT), surface area (SA), and volume (VOL) across the whole-brain as well as within regional parcellations using the Desikan-Kiliany atlas (32).

In the multiethnic ABCD cohort, *C4A* GREx was associated with reduced regional SA of the entorhinal cortex (β = -10.45, 95% CI [-13.13, -7.76], P_{FDR} = 0.003; **Fig. 3A, Supplementary Table 6**). Furthermore, we found that the relationship between *C4A* GRExand entorhinal cortex was significant for both female and male youth (Female: β = -8.90, 95% CI [-12.46, -5.34], P = 0.01; Male: β = -11.6, 95% CI [-15.56, -7.63], P = 0.003; **Fig. 3B**). No other brain structure associations were observed after correction for multiple testing (**Supplementary Table 7-9)**.

To assess the robustness and reproducibility of the impact of *C4A* GREx on entorhinal cortex brain structure, we performed analogous analyses in 16,147 EUR-ancestry subjects from the UK Biobank. In the UK Biobank, *C4A* GREx was also significantly associated with entorhinal cortex SA, with the same direction of effect (Full sample: β = -5.01, 95% CI [-7.95, -2.06], P=0.00087; Female β = -5.47, 95% CI [-8.29, -0.85], P=0.02; Male β = -4.57, 95% CI [-10.10, -0.84], P=0.02; **Fig. 3C**).

The previous analyses show that *C4A* GREx is associated with a localized reduction in entorhinal cortex SA. In psychosis spectrum patients, size of the entorhinal cortex is a predictor of severity of delusions and negative symptoms (33,34). To assess this, we leveraged longitudinal psychosis data available from the ABCD 1- and 2-year follow-up visits. Due to differences in overall brain size between male and female youth, analyses were performed in each sex separately and focused on comparing youth in the top and bottom quartile for baseline entorhinal cortex SA (**Fig. 3D**). We observed a significant difference in both the number and severity of reported PLEs at the 1-year follow-up when comparing between youth in the top and bottom quartiles for entorhinal cortex SA; more specifically, female and male youth with smaller entorhinal cortex SA at baseline reported greater frequency of PLEs and more distressing PLEs 1-year later (Wilcoxon Rank Sum Tests r=0.05–0.09, P=0.0002–0.04; **Fig. 3E**, **Supplementary Table 10**). At the 2-year follow-up, female youth showed significant differences in PLEs as a function of baseline entorhinal cortex SA; here again, smaller entorhinal cortex SA at baseline was associated with greater frequency and severity of PLEs (r=0.11–0.12, P=0.0002; **Fig. 3E, Supplementary Table 10**).

Effects of C4A on the entorhinal cortex are independent of polygenic risk for schizophrenia

Schizophrenia is highly polygenic with thousands of genetic variants of small effect size acting additively to confer risk. To better understand the relationship between genetic risk for schizophrenia at

the *C4* locus and additive polygenic risk across the genome (outside the *C4* locus), we computed polygenic risk scores (PRS) in ABCD youth of EUR ancestry (N = 3,730 unrelated subjects) using the largest available genome-wide association study of schizophrenia (40,675 cases, 64,643 controls(10)), excluding variants in the MHC region. Similar to previous findings (35), we did not observe a significant association between schizophrenia PRS and psychotic symptoms in youth – nor was there an interaction between *C4A* GREx and schizophrenia PRS (Supplementary Table 11).

Finally, we tested whether schizophrenia PRS – or its interaction with *C4A* GREx – was a significant predictor of brain structure. Surprisingly, schizophrenia PRS showed no association with entorhinal cortex structure, nor was there an interaction with C4A GREx, indicating a dissociable effect of complement activation and schizophrenia polygenic burden on this brain structure (**Fig. 4, Supplementary Table 11-14**). To confirm this, the association between *C4A* GREx and entorhinal cortex SA remained significant after controlling for schizophrenia PRS (β_{C4A} = -8.88, 95% CI [-12.46, -5.29], P = 0.01). Together, these findings indicate that the effects of *C4A* GREx on entorhinal cortex SA are specific to the *C4* locus and are distinct from the effects of schizophrenia polygenic risk.

Discussion

Here, we report the first multi-ethnic, phenome-wide association study of complement component 4 (*C4*)variation with childhood cognitive, psychiatric and neuroimaging phenotypes. We find that genetically-regulated expression (GREx) of *C4A* is not associated with broad psychiatric symptoms or brain structure in 9-10 year old children, but rather shows localized and replicable effects on reduced regional surface area of the entorhinal cortex. Importantly, this *C4A*-mediated reduction in entorhinal cortex surface area at 9-10 years of age predicted higher rates and greater severity of psychosis-like experiences at follow-up time points 1 and 2 years later, indicating that the effects of *C4A* on temporal lobe structure may be a biomarker for schizophrenia risk prior to symptom onset (**Fig. 5**).

Despite having sufficient power to detect associations with small effect sizes, we find that predicted *C4A* expression is not robustly associated with psychiatric symptoms or cognition in 9-10-year-old youth. These null results are surprising given the strength of the association between *C4A* and schizophrenia risk (11). However, these negative findings comport with recent studies assessing the extent to which polygenic risk for schizophrenia is associated with broad psychiatric phenotypes in the ABCD cohort, which have found limited associations with fluid cognition and an index of binary psychosis severity in ABCD youth of European ancestry (36,37). Nevertheless, we identified a significant inverse association between predicted *C4A* expression and lack of planning on the UPPS-P Impulsive Behavior Scale (28). Higher scores on this subscale are indicative of a tendency not to plan ahead; thus, we find that *C4A* brain expression is related to less severe symptomatology. The effects of schizophrenia genetic risk at the *C4* locus and across the genome may exert more extensive effects on cognition and behavior during later developmental periods (e.g., adolescence and early adulthood), when clinically significant features of psychosis and schizophrenia are more likely to occur. We speculate that the developmental timing and tempo of synaptic pruning may be an important contributor to the emergence of psychiatric symptoms,

such that increased pruning during early childhood may be developmentally advantageous until reaching a threshold of 'over' pruning later in life.

In this demographically diverse multiethnic cohort, we observed a regionally localized association between *C4A* GREx and surface area of the entorhinal cortex. Our entorhinal cortex findings are consistent with the extant literature implicating medial temporal lobe structures in the pathophysiology of schizophrenia (38–40). Indeed, *in vivo* neuroimaging have consistently found atypical volume of medial temporal lobe structures in patients with psychotic disorders relative to controls (41–43) and several postmortem studies have found aberrant entorhinal cortex cytoarchitecture in SZ patients, suggestive of either atypical neuronal migration or altered pruning during neurodevelopment (44–48). Because the entorhinal cortex receives afferent projects from multiple cortical and subcortical brain regions and is the primary source of information to the hippocampus (49), disruptions to this region during development have the potential to affect both the integration of sensory information and memory (50–52). In-line with this hypothesis, previous work has shown that smaller entorhinal cortex volume is a predictor of cognition and negative/disorganized symptoms in psychosis spectrum youth (34). Similarly, we find that reduced entorhinal cortex surface area at 9-10 years-of-age predicts greater number and severity of psychosis-like events 1- and 2- years later. As brain volume is a function of surface area and cortical thickness, our findings of smaller surface area in youth with high genetic risk for schizophrenia at the *C4* locus help to more precisely identify potential morphometric biomarkers predictive of psychosis risk.

Our *surface area* specific findings in preadolescent youth contrast with adult neuroimaging studies, which have typically found more widespread reductions in *cortical thickness* in schizophrenia patients (53) and in clinically high-risk individuals who transition to psychosis (29). These contradictory findings may be explained by developmental differences in structural neuroimaging parameters. Recent neuroimaging studies have shown that cortical surface area continues to expand throughout the postnatal period and early childhood (54), while adolescence and early adulthood are characterized by nonlinear decreases in cortical thickness (55), likely driven by developmentally regulated alterations in cortical myelination and pruning. Thus, genetic risk for schizophrenia may have distinct effects on diverse aspects of brain structure at different developmental periods. Importantly, *C4A* cis-eQTLs (i.e., genomic loci that explain variation in gene expression) have been identified in the fetal brain (56), suggesting that the effects of *C4A* GREx on brain morphology may begin early in development. We therefore hypothesize that the observed effects of *C4A* GREXon cross-sectional entorhinal cortex surface area in children stem from early alterations in brain development and that future studies of the effects of *C4A* on brain development are likely to observe associations with morphological metrics in flux within a particular developmental window (e.g., adolescent cortical thickness).

Notably, the effects of predicted *C4A* GREx on entorhinal cortex surface area are independent and dissociable from genome-wide polygenic risk for schizophrenia. We provide several lines of evidence to support this conclusion. First, polygenic risk scores (PRS) trained using weights from the largest available GWAS of schizophrenia (10) failed to predict entorhinal cortex surface area in youth of European ancestry. Second, when examining the interaction between *C4A* GREx and PRS, we find no evidence that

schizophrenia PRS moderates the relationship between *C4A* GREx and entorhinal cortex surface area. And finally, the observed relationship between *C4A* GREx and entorhinal cortex surface area remains significant even when controlling for the effects of PRS. These findings indicate that the observed effects on entorhinal cortex surface area are exclusive to genetic risk for schizophrenia at the *C4* locus and are distinct from the effects of schizophrenia polygenic risk.

Conclusions

In sum, the current study reports the first multi-ethnic phenome-wide association of genetic variation with the *C4* locus in children. Our findings indicate that increased *C4A* brain expression in youth is associated with a localized reduction in the size of the entorhinal cortex that is independent of polygenic risk for schizophrenia. Overall, these data provide a mechanistic, genetic basis for the identification of brain-based biomarkers predictive of psychosis risk. Ultimately, a comprehensive understanding of the genetic and environmental precursors to psychosis will require longitudinal indices of brain development across the transition to adolescence and young adulthood, when clinically significant behavioral manifestations of psychiatric disorders often begin to emerge.

Methods

Participants

Data were obtained from the Adolescent Brain and Cognitive Development (ABCD) Study, an ongoing longitudinal investigation of brain and behavioral development in 11,878 children in the United States (U.S.). Youth were recruited primarily from schools within 21 catchment areas chosen to represent the ethnic and sociodemographic diversity of the larger U.S. population (21). Analyses were performed using de-identified phenotypic data from the baseline time point obtained from the ABCD 3.0 National Data Archive release (DOI 10.15154/1519007). Analyses were restricted to youth with available genetics data. See **Supplementary Table 1** for subject characteristics.

Genotyping and imputation

Genotyping for the ABCD dataset was performed at the Rutgers University Cell and DNA Repository using the Affymetrix NIDA Smokescreen Array (RUCDR). Quality control was performed by the RUCDR and the ABCD Data Analysis and Informatics Core (DAIC), and included filtering for call signals and rates, and processing through the Ricopili pipeline (57). Genotyping data was merged with data from the 1000 Genomes project and ancestry was assigned using the k-nearest neighbors classification with the first four genetic principal components.

C4 structural alleles were imputed for the ABCD dataset using Beagle4.1 (58) with the latest multiethnic *C4* imputation reference panel composed of 1,265 samples of diverse ancestry (18). *C4A* and *C4B* genetically regulated expression (GREx) was predicted using previously described expression weights(11).

Downstream analyses were restricted to samples of African (AFR), European (EUR), and Latinx ancestries as the imputation panel was composed of those individuals, and *C4* imputation accuracy (based on probabilistic dosage) was lower for Southeast Asian and East Asian. Individuals with average imputed probabilistic dosage <0.7 were excluded from subsequent analyses. To further ensure the use of high-quality imputed data, analyses were restricted to individuals with the five most common *C4* structural haplotypes (AL, AL-AL, AL-BL, AL-BS, BS) resulting in a final sample of 7,789 youth (see **Supplementary Table 2**).

For UK Biobank, chromosome 6 of the phased haplotype data (i.e. ukb_hap_chr6_v2.bgen) was used to impute *C4* structural alleles. Beagle4.1, with the multiethnic *C4* reference panel, was used for imputation, as was done for the ABCD dataset.

Polygenic risk prediction

Polygenic risk scores (PRS) were calculated for EUR subjects. Genotype imputation was performed on the Michigan Imputation Server (59) using the TOPMed reference panel. Quality control included filtering for genotyping rate >0.99, sample missingness <0.01, Hardy-Weinberg Equilibrium $p>1\times10^{-6}$, minor allele frequency >1%, and imputation score >0.8. GCTA v1.93.31 (60) was used to compute the genetic relationship matrix; a relatedness cutoff of 0.05 was applied. Genetic variants in the major histocompatibility complex (MHC) locus were excluded prior to PRS calculation. PRS were generated using summary statistics from the largest available genome-wide association study of schizophrenia (10) (40,675 cases, 64,643 controls) in PRS-CS (61), applying the LD reference panel from the 1000 Genomes Project phase 3 samples and the recommended global shrinkage parameter for highly polygenic traits (phi=1e-2). Analyses assessing the interaction between *C4* expression and PRS were restricted to youth with common *C4* structural haplotypes (AL, AL-AL, AL-BL, AL-BS, BS), resulting in a final sample of 3,730 EUR youth (female N = 1,737; male N = 1,993) and 7,715,663 genetic variants.

Neuroimaging data acquisition and preprocessing

Details of the ABCD magnetic resonance imaging (MRI) protocol have been described previously (62). Briefly, imaging data were collected on either a Siemens Prisma, Phillips, or GE 750 3T scanner using 32 channel head or 64 channel head/neck coils. T1-weighted scan parameters were as follows: Siemens - matrix size 256x256, 176 slices, FOV 256x256, resolution (mm) 1x1x1, TR 2,500ms, TE 2.88ms, flip angle 8°, total scan time 7:12; Phillips - matrix size 256x256, 225 slices, FOV 256x240, resolution (mm) 1x1x1, TR 6.31ms, TE 2.9ms, flip angle 8°, total scan time 5:38; GE - matrix size 256x256, 208 slices, FOV 256x256, resolution (mm) 1x1x1, TR 2,500ms, TE 2ms, flip angle 8°, total scan time 6:09.

Tabulated data representing T1-weighted measurements of subcortical volume (VOL), cortical thickness (CT), and surface area (SA) as derived from the Desikan parcellation atlas(32) in FreeSurfer v5.3 (http://surfer.nmr.mgh.harvard.edu/) were downloaded from the NDA. ABCD recommended imaging inclusion criteria were followed to ensure inclusion of high-quality MRI scans, this excluded participants

with serious MR findings and participants whose T1-weighted images or FreeSurfer parcellations failed to pass ABCD DAIC quality control. Regional MRI metrics were averaged across hemispheres.

Phenotypic data

Psychotic experiences were assessed with the Prodromal Questionnaire Brief Version (22) (PQ-B) and the Kiddie Schedule for Affective Disorders and Schizophrenia (23) (KSADS-5). The PQ-B is a 21-item child-report questionnaire designed to measure the presence and severity of psychotic-like experiences (PLEs) in childhood. PQ-B symptom scores ($PQ\text{-}B_{sym}$) were calculated as the total number of endorsed PLEs; severity score ($PQ\text{-}B_{sev}$) was calculated as the child-reported level of distress (range 1-5) across all endorsed items. In line with previous research indicating that unusual thought content and suspiciousness are most predictive of psychosis risk(24), a binary score (PP_{bin}) was developed by defining prodromal psychosis "cases" as youth who endorsed experiencing at least 3 PLEs reflecting suspicious or unusual thought content (i.e., PQ-B items 1, 4, 5, 7, 8, 11, 12, 13-18), significant distress related to these experiences (i.e., PQ-B distress scores > 6 across items 1, 4, 5, 7, 8, 11, 12, 13-18), and whose parents indicated the child experienced hallucinations, delusions, and associated psychotic symptoms on the KSADS-5 assessment; youth not meeting these criteria served as the "control" group. Cognitive and psychiatric variables from the reported *C4* phenome-wide association study (PheWAS) can be found in **Supplementary Table 3**.

Rates and severity of childhood PLEs were non-normally distributed, with the majority of youth reporting zero or few PLE symptoms ($PQ\text{-}B_{sym}$; M = 2.55, SD = 3.52) and endorsing low-levels of PLE severity ($PQ\text{-}B_{sev}$; M = 6.09, SD = 10.39; **Figure 1B**).

Statistical analyses

Generalized linear models with gamma family and log link were run using the *glm* package in R version 3.6.3 to test the association between *C4* GREx and continuous measures of psychotic experiences (i.e., $PQ\text{-}B_{sym,}$ $PQ\text{-}B_{sev}$), as these variables demonstrated a right-skewed distribution. The association between *C4* GREx and PP_{bin} was tested via logistic regression with binomial family using the *glm* package in R. Fixed effects included predicted *C4A* and *C4B* GREx, age, socioeconomic status (SES; taken as the average of parent education and income at the baseline time point), four genetic principal components, sex, and site ID. Statistical models run in related individuals using family ID as a random effect failed to converge; thus, analyses were restricted to one randomly selected subject from each family.

Models testing multivariable phenome-wide associations were also restricted to one individual per family. For normally distributed variables, linear models were fitted, while for non-normally distributed variables, generalized linear models with gamma distribution and log link were fitted. *C4A* and *C4B* GREx, age, SES, four genetic principal components, sex, and site ID were specified as fixed effects. Benjamini-Hochberg False Discovery Rate (FDR) correction was used to account for the number of variables tested at each

timepoint (i.e., baseline, 1-year follow-up, 2-year follow-up. The significance of covariates of interest was assessed using the likelihood ratio test.

To test the association between *C4* GREx and neuroimaging indices of brain volume, cortical thickness, and surface area, linear mixed models were run via the *lme4* package in R. *C4A* and *C4B* GREx, age, four genetic principal components, SES, and sex were entered as fixed effects; MRI device serial number and family ID were entered as random effects. To control for global brain effects, whole brain volume, mean cortical thickness, or mean surface area were also included as covariates for respective models. The significance of covariates of interest was assessed using the likelihood ratio test, and FDR correction was used to account for the number of variables tested within each imaging measure (i.e., VOL, CT, SA).

Sex-differences in the effects of *C4* GREx on brain and behavioral phenotypes were tested by including an interaction term between *C4A* and *C4B* GREx and sex in statistical models. Follow-up analyses were performed in each sex separately.

The total number of participants in each analysis (i.e., those with complete PQ-$B_{sym,}$ PQ-B_{sev}, PP_{bin}, phenotype, and/or brain-imaging data) are provided in **Supplementary Tables**.

Power analyses were conducted in G*Power (63) to determine the range of detectable effects with the current sample size. With an estimated N of 6,500, alpha of 0.05, 1 tested predictor, and 11 covariates, we have 95% power to detect effects (Cohen's f^2) as small as 0.002, equivalent to a Cohen's d of 0.004. As previous research in adults suggests that we can expect to see a significant association between *C4A* GREXand cognitive measures at Cohen's $f^2 = 0.056$ (20), power calculations indicate that our study is sufficiently powered to detect small but statistically significant effects, equivalent to a Cohen's d of 0.004.

Plots were generated using R packages *ggpubr*, *ggplot*, and *ggesg*. PheWAS results were plotted in R by modifying publicly available code from Dr. Yoonjung Joo (https://rpubs.com/helloyjjoo/pheWAS_connectome).

Replication in the UK Biobank

The UK Biobank resource was accessed through application number 38029. Brain imaging data was acquired for 18,361 unrelated individuals with European ancestry. Haplotypes at the *C4* locus were imputed as described for the ABCD dataset. After further restricting to individuals with the five most common *C4* haplotypes (AL, AL-AL, AL-BL, AL-BS, BS), a total of 16,147 individuals remained in the final analyses. A linear model was then fitted between entorhinal cortex surface area and *C4A* GREx, using *C4B* GREx, sex, age at scan, average cortex surface area, location of head in scanner (Data fields 25756 to 25759), first four genotype PCs and scan sites as covariates. A null model was also fitted with only covariates but not *C4A* GREx. Statistical significance of the association was evaluated by performing a likelihood ratio test. The analysis was performed in females (N = 8,357) and males (N = 7,790) separately, without using sex as a covariate.

Validation of expression weights for *C4* genes in PsychENCODE

Previously harmonized genotype array and frontal cortex RNA-seq data from PsychENCODE (19,64) consisted of uniformly processed data from six studies: BipSeq, LIBD_szControl, CMC_HBCC, CommonMind, BrainGVEX, and UCLA-ASD. We imputed *C4* alleles in six studies from PsychENCODE separately in the same manner as the ABCD dataset using Beagle4.1 with the latest multiethnic *C4* imputation reference panel. This analysis consisted of 340 AFR, 863 EUR, and 50 Latinx samples. *C4* expression was then predicted using previously described expression weights(11), which was associated with observed *C4* gene expression (**Supplementary Fig. 1**). Observed *C4* gene expression was strongly associated with corresponding predicted gene expression, except for *C4B* gene expression in AFR samples ($P > 0.05$).

Declarations

Availability of data and materials

The datasets supporting the conclusions of this article in the National Data Archive, (doi: 10.15154/1523041, https://nda.nih.gov/abcd) and with application to the UK Biobank (https://www.ukbiobank.ac.uk/). The code used to perform statistical analyses are available at: https://github.com/leamhernandez/C4A-ABCD.

The authors declare that they have no competing interests.

Funding

This work was supported by the National Institute of Mental Health (R01MH121521 to MJG; R01MH123922 to MJG; P50HD103557 to MJG, R01MH107250 and U01MH081902 to CEB; K00MH119663 to LMH; R01MH120025 to CCF; T32MH073526 and F30MH125523 to MK), SFARI Bridge to Independence Award to MJG, National Center for Advancing Translational Science (UL1TR001881 to GDH), National Institute of General Medical Sciences (T32GM008042 to MK), UCLA Friends of the Semel Institute Research Scholar Award to GDH, Karen Seykora NARSAD Young Investigator Grant from the Brain & Behavior Research Foundation to GDH, and the Harvey L. and Maud C. Sorensen Foundation Fellowship to GDH.

Data used in the preparation of this article were obtained from the Adolescent Brain Cognitive Development^{SM} (ABCD) Study (https://abcdstudy.org), held in the NIMH Data Archive (NDA). This is a multisite, longitudinal study designed to recruit more than 10,000 children age 9-10 and follow them over 10 years into early adulthood. The ABCD Study® is supported by the National Institutes of Health and additional federal partners under award numbers U01DA041048, U01DA050989, U01DA051016, U01DA041022, U01DA051018, U01DA051037, U01DA050987, U01DA041174, U01DA041106, U01DA041117, U01DA041028, U01DA041134, U01DA050988, U01DA051039, U01DA041156, U01DA041025, U01DA041120, U01DA051038, U01DA041148, U01DA041093, U01DA041089,

U24DA041123, U24DA041147. A full list of supporters is available at https://abcdstudy.org/federal-partners.html. A listing of participating sites and a complete listing of the study investigators can be found at https://abcdstudy.org/consortium_members/. ABCD consortium investigators designed and implemented the study and/or provided data but did not necessarily participate in analysis or writing of this report. This manuscript reflects the views of the authors and may not reflect the opinions or views of the NIH or ABCD consortium investigators.

Authors' contributions

This study was designed by LMH and MJG. SYB, CCF, and WKT contributed to ongoing project design and management of the Adolescent Brain Cognitive Development Study. LMH performed data analysis, with support from MK, PZ, CCF, WKT, and MJG. LMH, MK, PZ, RAI, GH, RL, DS, SYB, CCF, CEB, WKT, and MJG contributed to data interpretation. LMH, MK, PZ, and MJG wrote the manuscript, with critical input from all authors.

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Figures

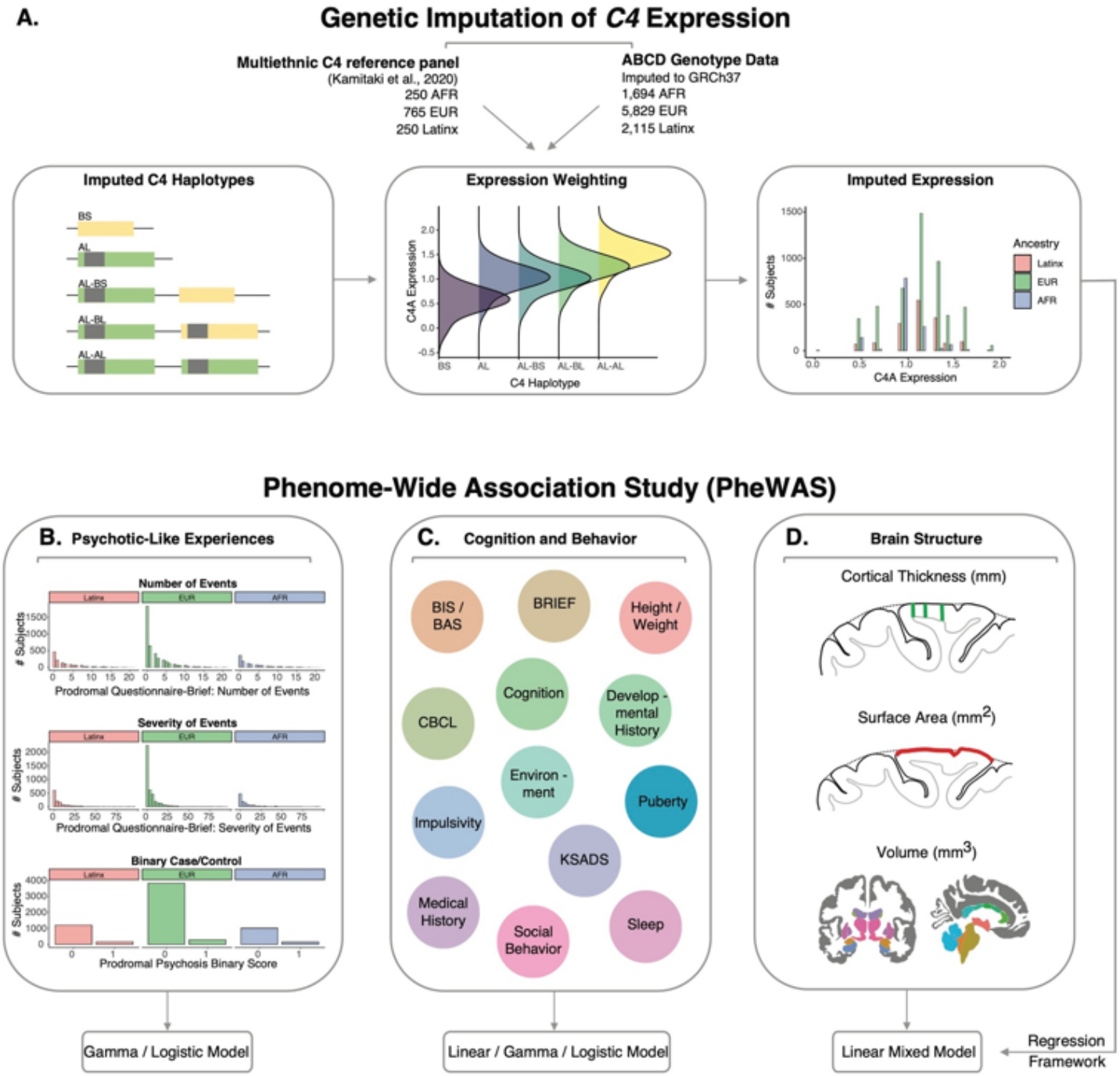

Figure 1

Study design. **A)** Individual-level genotype data from ABCD subjects was used to impute *C4* structural alleles using a multiethnic reference panel (Kamitaki et al., 2020). *C4A* and *C4B* brain GREx was calculated using previously described weights (Sekar et al., 2016). **B)** Generalized linear models were used to test for associations between *C4A* GREx with psychotic-like experiences (PLE) in ABCD Youth. Associations were examined in the whole multi-ethnic cohort, as well as within ancestry- and sex-specific subgroups. **C)** We performed a phenome-wide association study (PheWAS) to assess associations between *C4A* GREx and a host of developmental, cognitive and behavioral phenotypes (Methods). **D)**

Linear mixed-effects models tested the association between predicted *C4A* GREx and neuroimaging measures of cortical thickness, surface area, and volume.

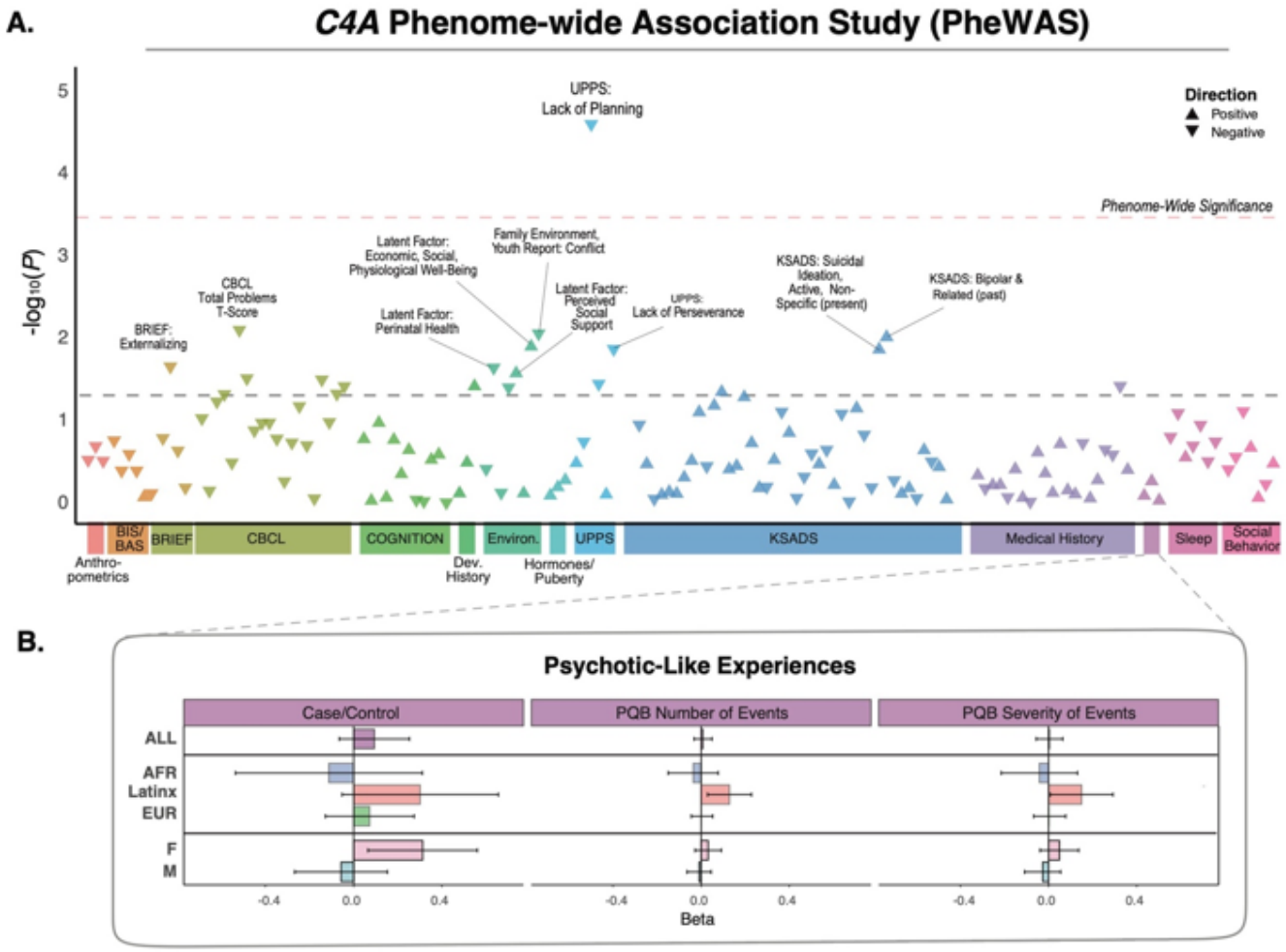

Figure 2

Phenome-wide association between *C4A* GREx and cognitive, behavioral phenotypes in the ABCD cohort. A) The relationship between *C4A* GREx and 145 phenotypes was tested in the multiethnic cohort (N's per phenotype ~ 6,000). Increased predicted expression of *C4A* was associated with lower scores on the UPPS-P Impulsive Behavior Scale (Lynam, 2013) lack of premeditation/planning subscale (FDR-corrected P=0.004). Phenotypes are grouped into broad categories by color. Negative or positive associations with *C4A* GREx are indicated by the direction of arrows. The FDR-based threshold for phenome-wide significance is indicated by the red dashed line. **B)** Association (absolute β ± standard error) between *C4A* GREx and each of the three psychosis variables investigated (binary case/control, PQ-B number PQ-B severity) in the multiethnic cohort (i.e., "ALL" youth [as in top panel]), as well as within each ancestry and sex separately. See **Supplementary Table 3** for regression model summary statistics. Results by ancestry and sex are shown in **Supplementary Fig. 3-8**.

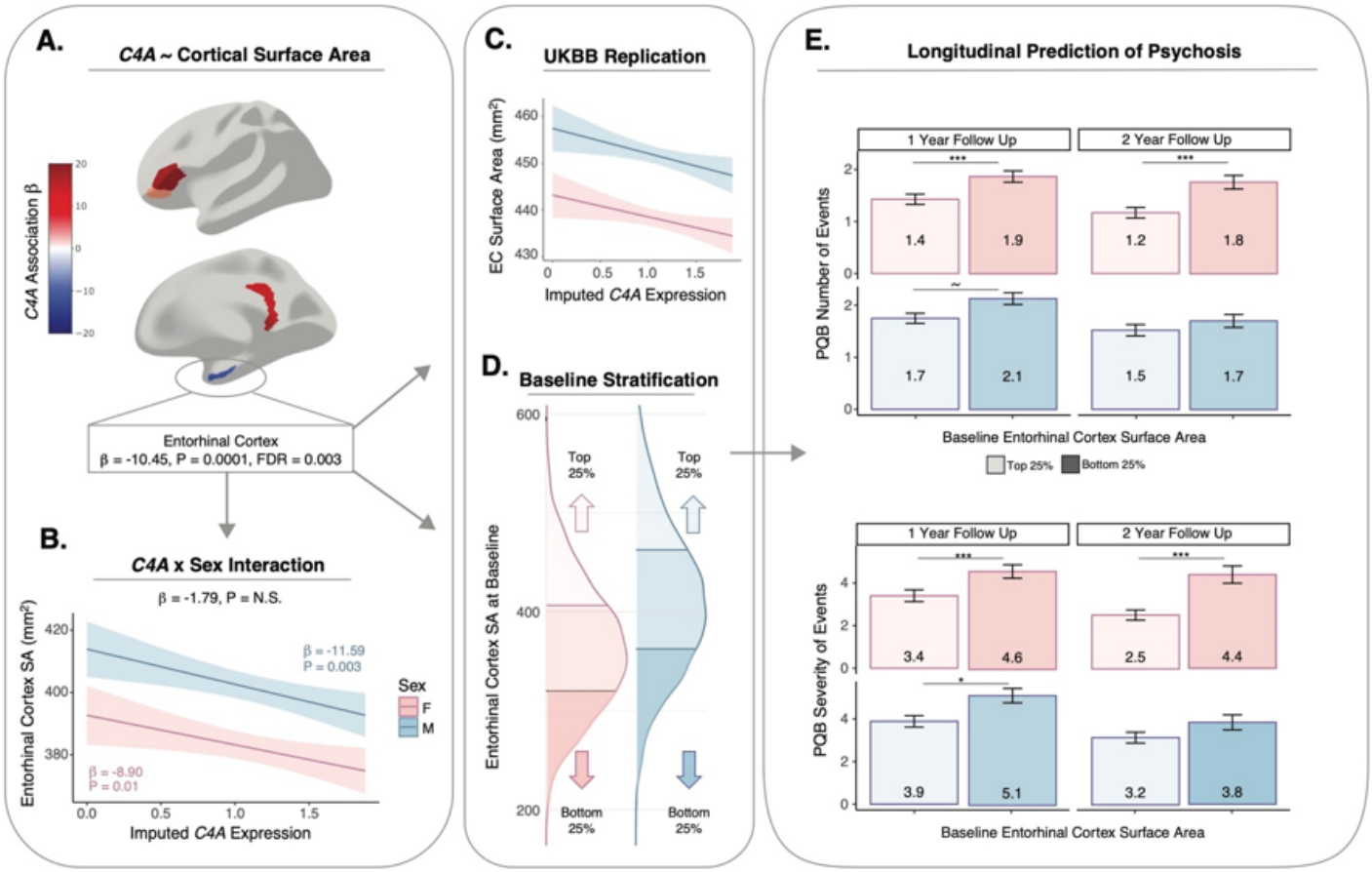

Figure 3

***C4A* GREx is associated with entorhinal cortex surface area and predicts longitudinal psychosis symptoms. A)** Linear regression analyses were performed in the multiethnic cohort to test the association between *C4A* GREx and regional brain structure (N's with brain imaging data passing quality control ~ 6,500). Brain regions demonstrating a nominally significant association between *C4A* GREx and surface area (mm^2) are shown in color (P<0.05); only the association with the entorhinal cortex survived FDR correction. Results for all brain regions are provided in **Supplementary Table 6-9. B)** Female and male youth showed a significant effect of *C4A* GREx on reduced entorhinal cortex surface area. **C)** Youth falling in the top and bottom quartiles in terms of baseline entorhinal surface area were identified. **D)** Smaller entorhinal cortex surface area (bottom quartile) at baseline predicted greater number and severity of psychosis-like experiences at the 1- and 2-year follow-up timepoints. The mean number and severity of PLEs are shown inside each bar plot. ~P=0.05, *P<0.05, **P<0.01, ***P<0.001.

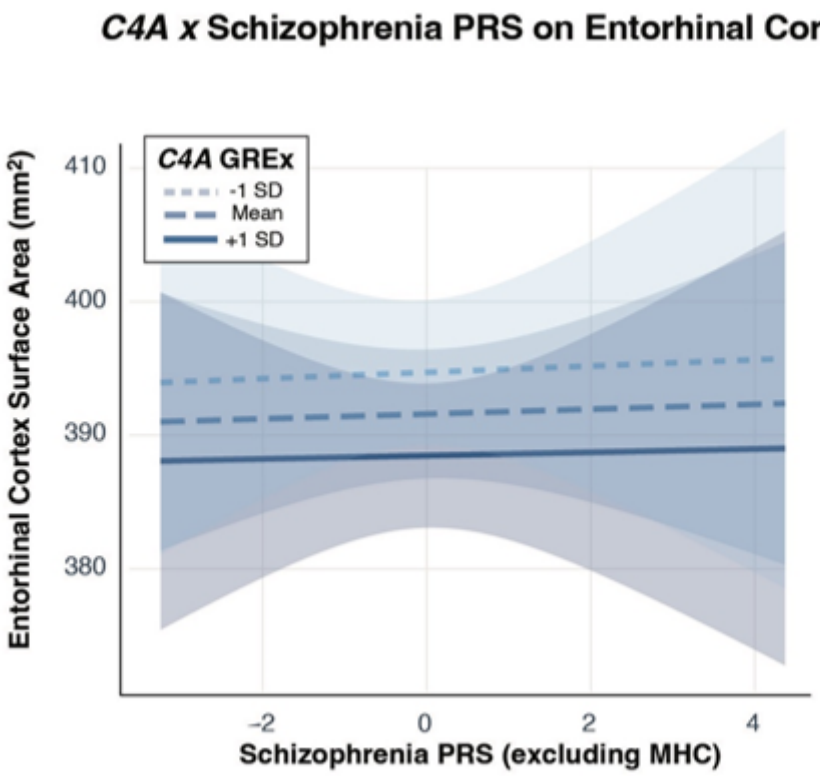

Figure 4

Interactions between *C4A* GREx and polygenic risk for schizophrenia. The interaction between *C4A* GREx and schizophrenia PRS was not a significant predictor of entorhinal cortex surface area in 9-10-year-old youth of European ancestry (N=3,349); higher *C4A* GREx was associated with smaller entorhinal cortex surface area across all levels of PRS.

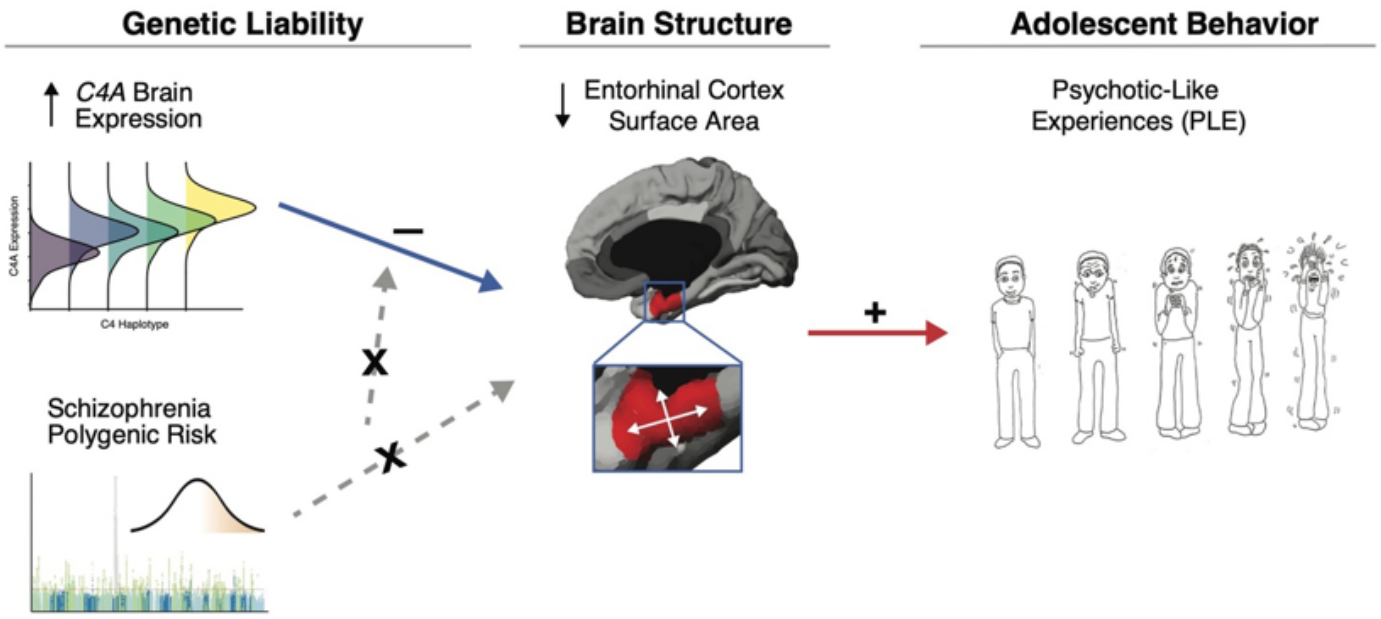

Figure 5

Model for the effects of *C4A* brain expression on childhood brain structure. The effects of increased *C4A* GREx on reduced entorhinal cortex volume in childhood are dissociated from the effects of genome-wide polygenic risk for schizophrenia. Reduced entorhinal cortex surface area in childhood is associated with higher rates of youth-reported psychotic-like experiences (PLE's) in early adolescence, as well as greater severity of events. Note: Image under "Psychotic-Like Experiences" taken from the Prodromal Questionnaire Brief Version (PQ-B) to assess the severity of distress associated with psychotic-like events.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementary.Information.docx
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