Cis-effects on gene expression in the human prenatal brain associated with genetic risk for neuropsychiatric disorders

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Abstract

The majority of common risk alleles identified for neuropsychiatric disorders reside in non-coding regions of the genome and are therefore likely to impact gene regulation. However, the genes that are primarily affected and the nature and developmental timing of these effects remain unclear. Given the hypothesised role for early neurodevelopmental processes in these conditions, we here define genetic predictors of gene expression in the human fetal brain with which we perform transcriptome-wide association studies (TWASs) of attention deficit hyperactivity disorder (ADHD), autism spectrum disorder, bipolar disorder, major depressive disorder and schizophrenia. We identify prenatal *cis*-regulatory effects on 63 genes and 166 individual transcripts associated with genetic risk for these conditions. We observe pleiotropic effects of expression predictors for a number of genes and transcripts, including those of decreased *DDHD2* expression in association with risk for schizophrenia and bipolar disorder, increased expression of a *ST3GAL3* transcript with risk for schizophrenia and ADHD, and increased expression of an *XPNPEP3* transcript with risk for schizophrenia, bipolar disorder and major depression. For the protocadherin alpha cluster genes *PCDHA7* and *PCDHA8*, we find that predictors of low expression are associated with risk for major depressive disorder while those of higher expression are associated with risk for schizophrenia. Our findings support a role for altered gene regulation in the prenatal brain in susceptibility to various neuropsychiatric disorders and prioritize potential risk genes for further neurobiological investigation.

Introduction

Genome-wide association studies (GWAS) have successfully identified hundreds of high-confidence genetic risk loci for neuropsychiatric disorders [1-5]. However, the DNA variants exhibiting strongest evidence for association with these conditions are typically in non-coding sequence, making it difficult to predict the actual susceptibility genes. One possible solution is to use expression quantitative trait loci (eQTL) maps in relevant human tissues to determine whether identified risk variants are associated with altered expression of specific genes at these loci [e.g. 6]. An alternative approach is to first define the DNA variants associated with the *cis*-component of a gene's expression in a given tissue, and then use these to predict the relative expression of that gene in cases and controls from a much larger GWAS dataset [7, 8]. This latter design, known as a transcriptome-wide association study (TWAS), recognizes that a gene might be regulated by multiple *cis*-acting variants, involves a smaller number of independent tests than a typical GWAS, and allows direct interpretation of risk in terms of the potentially pathogenic mechanism of gene expression.

Prenatal brain development is hypothesised to be an important window of vulnerability for several neuropsychiatric disorders [9, 10], implying that regulatory effects of risk alleles might operate during this period. Indeed, common genetic risk variants for these conditions have been found to be enriched within epigenomic annotations indicative of active regulatory genomic sites in the fetal brain [11, 12]. Moreover, we have recently mapped eQTL operating in the human brain during the second trimester of gestation, showing these to be enriched among common risk variants for various neuropsychiatric disorders, and providing the means to link risk alleles with gene expression in the prenatal brain [13]. Here, we combine data from that study with large-scale GWAS data for neuropsychiatric disorders to identify, through TWAS, genes and individual transcripts where heritable *cis*-effects on their expression in the human fetal brain are associated with risk for

attention deficit hyperactivity disorder (ADHD), autism spectrum disorder (ASD), bipolar disorder, major depressive disorder and schizophrenia.

Methods

Datasets

Predictors of *cis*-regulated gene expression were derived from whole transcriptome RNA sequencing and genome-wide genotyping of brain tissue from 120 human fetuses aged 12 -19 post-conception weeks (for a full description of samples and data generation, see reference 13). We performed TWAS using genome-wide association summary statistics from recent large-scale studies of schizophrenia (40,675 cases, 64,643 controls) [1], major depressive disorder (135,458 cases, 344,901 controls) [2], ADHD (20,183 cases, 35,191 controls) [3], ASD (18,381 cases, 27,969 controls) [4] and bipolar disorder (20,352 cases and 31,358 controls) [5].

TWAS analysis using FUSION

Single nucleotide polymorphism (SNP)-weight predictors of Ensembl gene and transcript level *cis*-expression were generated from fetal brain RNA sequencing and genotype data using the FUSION pipeline (http://gusevlab.org/projects/fusion/). We restricted our analyses to genes and transcripts with significant evidence of *cis*-heritable expression at the default *P*-value ($P < 0.01$) in FUSION. GWAS summary statistics were prepared for use in FUSION using the munge_sumstats.py script in LD Score Regression (https://github.com/bulik/ldsc). The extended MHC region (GRCh37/hg19 coordinates chr6:28,477,797 - 33,448,354) was removed prior to analysis to avoid spurious associations driven by the linkage disequilibrium pattern in this region. TWAS was performed on autosomal chromosomes using the *FUSION.assoc.test.R* script with default parameters. A multiple-testing correction was applied to TWAS *P*-values using the Bonferroni method within each

phenotype. To compare our TWAS findings with GWAS risk loci, implicated genes and transcripts were mapped in relation to the genomic coordinates of the genome-wide significant loci from their corresponding GWAS (12 loci in ADHD [3], 5 loci in ASD [4], 30 loci in bipolar disorder [5], 44 loci in major depressive disorder [2], and 145 loci in schizophrenia [1]).

Conditional Analysis

Genes and transcripts achieving TWAS-wide significance (Bonferroni-corrected $P < 0.05$) for each disorder were assigned to loci using a +/-250kb window. Loci containing multiple implicated genes / transcripts were subjected to conditional analysis, implemented by the *FUSION.post_process.R* script, to determine statistically independent signals.

Data availability

The SNP weights for predictors of fetal brain gene- and transcript- level expression that were generated and analysed in the current study are available in a Figshare repository (https://doi.org/10.6084/m9.figshare.11637036). Individual RNA-Seq FASTQ files are available through the European Genome-phenome Archive (https://ega-archive.org) under study accession EGAS00001003214.

Results

Using FUSION software, we identified 1351 genes and 3985 individual Ensembl transcripts displaying significant *cis*-heritable expression ($P < 0.01$) in our reference panel of 120 fetal brains. We then determined genetic predictors of the *cis*-component of each gene / transcript's expression within

the reference panel and used these to impute the *cis*-genetic component of gene expression into GWAS summary data for five major neuropsychiatric disorders [1-5].

We identified 32 genes and 103 individual transcripts for which genetic *cis*-effects on fetal brain expression are associated with schizophrenia at a Bonferroni-corrected $P < 0.05$ (Supplementary Tables 1 and 2). Eleven of these genes and 34 of these transcripts are located within 16 of the 145 schizophrenia-associated loci identified in the corresponding GWAS [1]; a further 21 genes and 69 transcripts reside outside of these regions and were therefore not previously implicated. We excluded the major histocompatibility complex (MHC) on chromosome 6 from our analyses due to extensive linkage disequilibrium in the region, but note that we have previously implicated expression of *C4A* (as well as that of other genes at the MHC locus) in risk for schizophrenia in our previous analysis of these fetal brain gene expression data using summary data-based Mendelian randomization [13]. Of genes outside of the MHC region, most significant association was observed for predictors of increased expression of *SMDT1* ($P_{corrected} = 4.31 \times 10^{-7}$), encoding a mitochondrial calcium channel subunit. This association has not, to our knowledge, been reported in any schizophrenia TWAS performed using expression predictors from adult brain tissue and may therefore indicate a fetal-specific risk mechanism. Of the other 31 genes that we implicate in schizophrenia at Bonferroni-corrected significance, 20 are significantly associated with schizophrenia with the same direction of effect based on expression predictors from adult cerebral cortex [14] (Supplementary Table 1). These include increased expression of *AS3MT,* encoding *arsenite methyltransferase* (this study: $P_{corrected} = 7.55 \times 10^{-7}$; Gandal et al 2018 study: $P_{corrected} = 0.0001$), consistent with previous findings in adult and fetal brain using measures of allele-specific expression [15]. Of the 24 genes that we implicate that also have HUGO Gene Nomenclature Committee (HGNC) symbols, 6 (*SMDT1*, *WBP2NL*, *CSPG4P11*, *DDHD2*, *PCDHA2* and *ARL14EP*) are also reported to be differentially *cis*-regulated in association with schizophrenia genetic risk in a recent TWAS using a

distinct collection of human fetal brain samples [16], and all with the same direction of effect. We also note association with expression predictors for transcripts of genes that have been implicated in schizophrenia through splicing QTL in the adult [17, 18] or fetal brain [16], including transcripts of *APOPT1* ($P_{corrected}$ = 1.52 X 10^{-9}), *SDCCAG8* ($P_{corrected}$ = 4.6 X 10^{-8}) and *GNL3* ($P_{corrected}$ = 1.02 X 10^{-5}).

ADHD was found to be associated with fetal brain expression predictors for 3 genes and 4 individual transcripts at a Bonferroni-corrected $P < 0.05$ (Supplementary Tables 3 and 4). Only one of these genes (*ST3GAL3*; see below) resides within any of the 12 risk loci identified by the corresponding GWAS [3]. The strongest association at the gene level was with reduced expression of *COL28A1* ($P_{corrected}$ = 0.012), encoding a collagen that is predominantly expressed in developing neural tissue [19]. Consistent with this being a primarily early neurodevelopmental risk mechanism, a previous TWAS of ADHD using expression predictors from multiple adult brain regions found only nominally significant evidence for association with *COL28A1* expression in two brain areas (amygdala and cerebellum; Supplementary Table 3) [20]. The most significant association we observed for ADHD was with increased expression of a transcript of *ST3GAL3* ($P_{corrected}$ = 1.19 X 10^{-9}), a gene linked to intellectual disability [21] which has been reported to be differentially methylated at birth in association with later ADHD symptomatology [22]. The *ST3GAL3* gene has previously been implicated in ADHD through a TWAS analysis combining results across multiple adult brain regions [20]. The second most significant association with ADHD we observed was for predictors of increased expression of a transcript of the tyrosine kinase *TIE1* ($P_{corrected}$ 0.0047), again supported by TWAS in adult brain, where association between ADHD and predictors of higher *TIE1* expression in adult dorsolateral prefrontal cortex was reported [20].

We identified 17 genes and 29 transcripts for which predictors of c*is*-heritable expression in fetal brain were associated with ASD at a Bonferroni-corrected $P < 0.05$ (Supplementary Tables 5 and 6).

None of these genes or transcripts reside within the 5 loci exhibiting genome-wide significant association with ASD in the corresponding GWAS [4]. Consistent with our previous TWAS of ASD using expression weights derived from a subset of the present samples [23] and a recent TWAS using predictors derived from adult cerebral cortex [14], the majority of implicated genes are located within a common polymorphic inversion on chromosome 17q21, which is associated with differential *cis*-regulation of numerous genes in the fetal brain [13] . The gene within this inversion for which expression predictors from the adult brain showed strongest association with ASD is *LRRC37A* (supplementary Table 5), encoding a leucine-rich repeat-containing protein. Expression of *LRRC37A* in the fetal brain has also been implicated in intracranial volume [16] and the personality trait of neuroticism [13]. The only association with ASD we observed outside of this inversion was with decreased expression of a transcript of *TM2D2* ($P_{corrected}$ = 0.038), a protein-encoding gene of unknown function.

Bipolar disorder was found to be associated with fetal brain expression predictors for 8 genes and 19 transcripts at a Bonferroni-corrected $P < 0.05$ (Supplementary Tables 7 and 8). Five of these genes and 13 of these transcripts are located within 4 of the 30 loci associated with bipolar disorder in the corresponding GWAS [5]; a further 3 genes and 6 transcripts reside outside of these loci and therefore represent novel associations. Implicated protein-coding genes include *NMB*, encoding *neuromedin B* ($P_{corrected}$ = 0.0002), *LRRC57*, encoding *Leucine Rich Repeat Containing 57* ($P_{corrected}$ = 0.001) and *DDHD2* ($P_{corrected}$ = 0.013), encoding *DDHD Domain Containing 2*. *NMB* and *LRRC57* also show significant association with bipolar disorder in a previous TWAS based on expression predictors from adult cerebral cortex [14] (Supplementary Table 7); however, the association with *NMB* is in the opposite direction (i.e. increased, rather than decreased, expression in adult brain in association with risk for bipolar disorder), and neither observation is transcriptome-wide significant. The association between reduced *DDHD2* expression and bipolar disorder has previously been reported

based on gene expression predictors from the adult dorsolateral prefrontal cortex, cerebellum, pituitary and caudate [24]. Our most significant observation in fetal brain for bipolar disorder was for predictors of increased expression of a transcript of *PACS1* ($P_{corrected} = 3.8 \times 10^{-5}$), encoding *Phosphofurin Acidic Cluster Sorting Protein 1*, a gene in which rare mutations cause intellectual disability [25]. Predictors of increased expression of *PACS1* are also associated with bipolar disorder at the whole gene level in the adult cerebral cortex ($P = 0.000653$) [14], although this was not highlighted in that study as it did not survive Bonferroni correction.

We identified 3 genes and 11 individual transcripts with expression predictors associated with major depressive disorder at a Bonferroni-corrected $P < 0.05$ (Supplementary Tables 9 and 10). One of these genes and 7 transcripts map to 7 of the 44 independent genome-wide significant loci identified by the corresponding GWAS [2]. Most significant association at the gene level was with predictors of decreased expression of *IMMP1L*, encoding a mitochondrial protein ($P_{corrected} = 5.45 \times 10^{-5}$). We also observed association between depression and predictors of decreased expression of *PCDHA7* and *PCDHA8* ($P_{corrected} = 0.048$ and 0.011, respectively), encoding members of the protocadherin alpha cluster, which has been implicated in the formation and maintenance of complex neural circuits [26]. None of these genes were highlighted in a previous TWAS of major depressive disorder based on expression predictors from adult prefrontal cortex [2]. However, that previous study [2] did implicate adult brain expression of 3 genes in risk for major depressive disorder that we also find to be associated with the condition based on transcript-specific measures in the fetal brain; namely, *DENND1B* (this study $P_{corrected} = 0.0004$), *XPNPEP3* (this study $P_{corrected} = 0.004$) and *DLST* (this study $P_{corrected} = 0.014$).

For loci that contained, within a 500kb window, more than one gene or transcript implicated in each disorder, we performed conditional analyses to identify independent associations (Supplementary

table 11). In interpreting these data, we note that effects on genes / transcripts that are conditionally non-significant do not necessarily imply that they are unrelated to risk for these disorders, only that these effects are not independent of more significant effects on other genes / transcripts at these loci. However, for schizophrenia, we were able discern conditionally independent associations with transcripts of *MRM2* and *SNX8* at a locus on chromosome 7, transcripts of *C12ORF65*, *RSRC2* and *KNTC1* on chromosome 12, and the *SMDT1* and *WBP2NL* genes on chromosome 22. Schizophrenia was also associated with independent effects on alternative transcripts of *ST3GAL3*, *SDCCAG8*, *NCK1-DT* and *MARK3*, while bipolar disorder was associated with independent effects on *GOLGA2P7* and a transcript of *UBE2Q2P1* on chromosome 15, and transcripts of *CDK10* and *CHMP1A* at a locus on chromosome 16.

Cis-heritable effects on the expression of 8 genes and 13 transcripts were significantly associated with more than one neuropsychiatric condition (Fig. 1 and Fig. 2). For example, increased expression of the previously highlighted *ST3GAL3* transcript ENST00000489897 was associated with schizophrenia ($P_{corrected} = 0.013$) as well as ADHD ($P_{corrected} = 1.19 \times 10^{-9}$), while predictors of decreased expression of the *NMB* gene were associated with both schizophrenia ($P_{corrected} = 0.002$) and bipolar disorder ($P_{corrected} = 0.0002$). Predictors of increased expression of a transcript of *XPNPEP3*, encoding *X-Prolyl Aminopeptidase 3*, were significantly associated with major depressive disorder ($P_{corrected} = 0.0043$), bipolar disorder ($P_{corrected} = 0.0006$), and schizophrenia ($P_{corrected} = 0.0023$). Although only surviving Bonferroni correction for schizophrenia ($P_{corrected} = 2.7 \times 10^{-5}$) and bipolar disorder ($P_{corrected} = 0.013$), predictors of reduced expression of *DDHD2*, a brain triglyceride hydrolase associated with hereditary spastic paraplegia and intellectual disability [27], were significantly ($P < 0.05$) associated with all 5 tested neuropsychiatric conditions. Intriguingly, while predictors of low expression of the protocadherin genes *PCDHA7* and *PCDHA8* were associated with major depressive disorder ($P_{corrected} = 0.048$ and 0.011, respectively), predictors of higher expression

of these genes were associated with schizophrenia ($P_{corrected}$ = 0.019 and 0.0004, respectively). These genes reside at a locus on chromosome 5 where opposing effects on risk for schizophrenia and major depressive disorder have recently been reported [28].

Please insert Figures 1 and 2 around here

Discussion

An essential first step in translating GWAS findings into an understanding of molecular risk mechanisms for neuropsychiatric disorders is to elucidate the genes that are primarily affected, how they are functionally impacted and when (and where) these effects take place [29]. We have here applied TWAS methodology to genome-wide association summary statistics for 5 major neuropsychiatric disorders in order to identify genes that are differentially *cis*-regulated in the human second trimester fetal brain in association with genetic risk variation for these conditions. Our findings are consistent with the hypothesis that altered gene expression in the prenatal brain plays a role in the later development of neuropsychiatric disorders and nominate genes and individual gene transcripts for further neurobiological investigation.

While previous TWAS of neuropsychiatric disorders have largely focused on individual diagnoses using gene expression predictors derived from the adult brain [e.g. 18, 20], we have here performed TWAS on multiple neuropsychiatric conditions, based on gene expression in the fetal brain. This allowed us to identify genetic risk-associated differences in prenatal gene expression that are unique to, or shared by, neuropsychiatric diagnoses. Our finding of numerous prenatal gene expression differences associated with genetic risk for schizophrenia is consistent with the long-hypothesised early neurodevelopmental component to this condition [9, 30]. Although bipolar disorder is generally considered to be less neurodevelopmental in origin, we identified prenatal *cis*-regulatory effects on

a number of genes and transcripts associated with genetic risk for the condition, consistent with our previous finding that fetal brain eQTL are enriched within genetic risk variants for bipolar disorder as well as schizophrenia and ADHD [13]. Moreover, we observed multiple instances where schizophrenia-associated *cis*-effects on prenatal gene expression were shared with bipolar disorder, consistent with the high genetic correlation between the two conditions [31]. Apparent pleiotropic effects of prenatal *cis*-regulatory variation were also observed for schizophrenia and ADHD (affecting a transcript of *ST3GAL3*) and for schizophrenia, bipolar disorder and major depression (affecting a transcript of *XPNPEP3*). In the case of the protocadherin alpha cluster genes *PCDHA7* and *PCDHA8*, we observed significant opposing effects on their expression in fetal brain associated with genetic risk for schizophrenia and major depressive disorder. These findings may prove particularly important given recent evidence of intrinsic abnormalities in the expression of protocadherin alpha cluster genes in induced pluripotent stem cell-derived neurons from patients with these disorders [32, 33].

Our comparisons with TWAS findings from adult human brain suggest that some of the gene expression changes that we implicate in risk for neuropsychiatric disorders are specific to brain development, potentially reflecting genetic risk variants residing within development-specific regulatory elements (e.g. enhancers) [34]. However, many of the gene expression changes that we find to be associated with risk for neuropsychiatric disorders are also observed in adult brain, and these may therefore constitute ongoing risk mechanisms for these conditions. Experimental manipulations in model systems will be necessary to determine the relative impact of these gene expression changes at different developmental timepoints.

Around two-thirds of the genes (46 out of 63) and transcripts (111 out of 166) that we implicate in neuropsychiatric disorders at Bonferroni-corrected significance are located outside of known GWAS

risk loci, illustrating the strength of the TWAS approach in revealing novel associations. However, a limitation of the current study is that, due to the modest sample size of the fetal brain gene expression reference panel (N = 120), we could only define genetic expression predictors for a fraction of genes / transcripts that are likely to be variably *cis*-regulated in the prenatal human brain. Future increases in GWAS as well as expression reference sample sizes will likely yield many additional associations between gene expression in the developing human brain and genetic risk for neuropsychiatric disorders, which could form the basis for pathway and other tests of biological enrichment. These might include tests of expression enrichment in specific cell types of the developing human brain based on single cell transcriptomic data. It should also be noted that, while associations between *cis*-regulated gene expression and genetic risk are consistent with causal involvement of the affected gene in the disorder, additional genetic and functional investigations will be required for their substantiation as risk mechanisms for these conditions. In this endeavour, our delineation of directional *cis*-regulatory effects on specific gene transcripts should provide a focus for investigations and guide modelling in neural systems.

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Conflict of interest

The authors declare no conflict of interest in relation to this work.

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Legends to Figures

Figure 1. TWAS Z-scores across all 5 tested neuropsychiatric disorders for predictors of *cis*-heritable gene expression that are significantly associated with at least two conditions (gene-level analysis). A Bonferroni corrected $P < 0.05$ equates to a Z-score +/- 4.12. ADHD = attention deficit hyperactivity disorder; ASD = autism spectrum disorder; BD = bipolar disorder; MDD = major depressive disorder; SCZ = schizophrenia.

Figure 2. TWAS Z-scores across all 5 tested neuropsychiatric disorders for predictors of *cis*-heritable gene expression that are significantly associated with at least two conditions (transcript-level analysis). A Bonferroni corrected $P < 0.05$ equates to a Z-score +/- 4.36. The HUGO Gene Nomenclature Committee (HGNC) IDs for genes to which Ensembl transcripts are annotated are indicated on the left. ADHD = attention deficit hyperactivity disorder; ASD = autism spectrum disorder; BD = bipolar disorder; MDD = major depressive disorder; SCZ = schizophrenia.