## **Expression-based mapping of SNPs**

**Overview**

We used both Transcriptome-wide Association Study (TWAS) (Gusev et al., 2016) and Summary-based Mendelian Randomisation (SMR) (Zhu et al., 2016) methods to infer differential gene expression associated with MDD based on the meta-analyzed summary statistics. Both methods test whether genetic variants associated with MDD are also associated with differential expression of nearby genes. TWAS and SMR have different limitations and are therefore complementary. TWAS considers the effect of multiple variants on gene expression and the GWAS phenotype, thereby increasing statistical power to detect associations, whereas SMR only considers the effect of each variant individually. However, TWAS requires multi-variant models predicting gene expression to have been generated in the genotype-expression dataset, which are not available in some cases. In contrast, SMR requires only expression quantitative trait (eQTL) summary statistics, enabling it to use a wider range of genotype-expression datasets, such as eQTL meta-analysis results from eQTLGen (Vosa et al., 2021) and MetaBrain (de Klein et al., 2021) consortia. For TWAS and SMR analysis, the European subset of the 1000 Genomes Phase 3 (N=503) was used as an LD reference.

**TWAS**

TWAS was performed based on a previous MDD TWAS (Dall'Aglio et al., 2021), using FUSION software with default settings. All gene expression panels were of European ancestry. Gene expression panels relating to the brain include dorsolateral prefrontal cortex (DLPFC) from PsychENCODE, differential expression and splicing in DLPFC from the CommonMind Consortium (CMC) and the 12 brain regions collected in the Genotype-Expression (GTEx) project. We also included panels capturing expression in pituitary, adrenal and thyroid tissues from GTEx, given prior evidence these tissues play a role in MDD (Varghese and Brown, 2001; Hage and Azar, 2012). Finally, we included panels capturing gene expression in blood from GTEx, the Netherlands Twin Registry (NTR) and the Young Finns Study (YFS) due to their increased sample size, the moderate correlation between cis-eQTLs across tissues (Consortium et al., 2017), and evidence that altered expression in blood could influence risk of MDD (Jansen et al., 2016; Miller and Raison, 2016). To distinguish associations for a gene captured by multiple panels, we refer to each panel-gene pair as *features*.

To account for multiple testing of genes across panels, we used the transcriptome-wide significance threshold previously estimated using a permutation procedure (Dall'Aglio *et al.*, 2021). The threshold for transcriptome-wide significance was p = 1.37×10−6. A more stringent significance threshold (α = .001; p= 3.69×10−8) was applied to distinguish high-confidence associations.

Colocalization of overlapping GWAS and gene expression associations was assessed using *coloc* (Giambartolomei et al., 2014) as implemented by FUSION. *coloc* is a Bayesian method that estimates the posterior probability that associations within a locus for two outcomes are driven by a shared causal variant (PP4).

Conditional analysis was performed using FUSION to determine whether associations within each locus were independent. FUSION also estimates the proportion of the GWAS association explained by the predicted expression of all features in the locus. Furthermore, TWAS-based fine mapping was carried out using FOCUS (Mancuso et al., 2019) to help identify which features were most likely causal for the association. FOCUS estimates the posterior inclusion probability (PIP) of each feature being causal within a region of association, using the sum of PIPs to define the default 90% credible set, a set of features likely to contain the causal feature.

**SMR**

SMR was run using eQTL meta-analysis summary statistics from European populations for blood from eQTLGen (Vosa *et al.*, 2021), and five nervous system tissues from MetaBrain (Basalganglia, Cerebellum, Cortex, Hippocampus and Spinal Cord) (de Klein *et al.*, 2021). SMR was run using default settings. The HEIDI test is performed alongside SMR to test for effect size heterogeneity between the GWAS and eQTL summary statistics, which would indicate that they are driven by different causal variants. The HEIDI test is a frequentist approach that is analogous to colocalization used to check for shared causal variants underlying TWAS associations.

**Inferring altered dorsolateral prefrontal cortex protein levels in MDD**

TWAS and SMR methods can also be applied to protein quantitative trait loci (pQTL) datasets, inferring whether genetic variation associated with MDD confer altered protein levels. Recently, pQTL data from the dorsolateral prefrontal cortex (DLPFC) has been prepared to perform proteome-wide association study (PWAS) (Wingo et al., 2021), using genotype-protein data from two datasets, referred to as ROSMAP and Banner et al. We followed the same procedure as the study originally performing PWAS, which included performing PWAS using both ROSMAP and Banner et al. panels, treating the larger ROSMAP panel as the discovery sample, and the Banner et al panel as a replication sample. Proteins were identified as statistically significant in ROSMAP if pFDR < 0.05 (correcting for all proteins tested) and considered replicated in Banner et al. if pFDR < 0.05 (correcting for number of proteins tested for replication). PWAS was performed using FUSION software with the in-built downstream colocalization analysis using *coloc* (PP4 > 0.8). As in the original PWAS, we used the HEIDI test within SMR to confirm evidence of colocalization based on the ROSMAP dataset (HEIDI p > 0.05).