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Integration of GWAS and brain eQTL identifies *FLOT1* as a risk gene for major depressive disorder

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Major depressive disorder (MDD) is the most prevalent mental disorder that affects more than 200 million people worldwide. Recent large-scale genome-wide association studies (GWAS) have identified multiple risk variants that show robust association with MDD. Nevertheless, how the identified risk variants confer risk of MDD remains largely unknown. To identify risk variants that are associated with gene expression in human brain and to identify genes whose expression change may contribute to the susceptibility of MDD, we systematically integrated the genetic associations from a large-scale MDD GWAS ($N = 480,359$) and brain expression quantitative trait loci (eQTL) data ($N = 494$) using a Bayesian statistical framework (*Sherlock*). *Sherlock* integrative analysis showed that *FLOT1* was significantly associated with MDD ($P = 6.02 \times 10^{-6}$), suggesting that risk variants may contribute to MDD susceptibility through affecting *FLOT1* expression. We further examined the expression level of *FLOT1* in MDD cases and controls and found that *FLOT1* was significantly upregulated in brains and peripheral blood of MDD cases compared with controls (European sample). Interestingly, we found that *FLOT1* expression was also significantly upregulated in peripheral blood of first-episode drug-naïve MDD cases compared with controls ($P = 1.01 \times 10^{-7}$, Chinese sample). Our study identified *FLOT1* as a novel MDD risk gene whose expression level may play a role in MDD. In addition, our findings also suggest that risk variants may confer risk of MDD through affecting expression of *FLOT1*. Further functional investigation of *FLOT1* may provide new insights for MDD pathogenesis.

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INTRODUCTION

MDD is a common mental disorder with complex etiology. The core symptoms of MDD include depressed mood, loss of interests, hopelessness, sleep disturbance and anxiety [1]. As the most prevalent mental disorder (the life time prevalence is about 15% [2, 3]), MDD affects over 200 million people worldwide [4]. In fact, MDD is the leading cause of disability worldwide and is a major contributor to the global burden of disease [5–7]. Though MDD becomes a major threat to global health and imposes great economic burden on society [8], the etiology of MDD remains largely unknown.

Accumulating evidence indicates that MDD is caused by a combination of genetic and environmental factors. Several environmental factors, including major life changes, stress, traumatic events, childhood abuse, and substance abuse were reported to have a role in MDD [9]. In addition to environmental factors, genetic factors also have pivotal role in MDD. The heritability of MDD is estimated around 40% [10], indicating the important role of genetic factors in MDD. To identify the genetic risk variants for MDD, numerous genetic studies were carried out in world populations [11–14]. Though several promising candidate genes (e.g., *SIRT1* and *LHPP*) have been reported [15], identification of MDD risk variants and genes remains a major challenge in

psychiatric genetics. For example, though genetic variants near *SIRT1* and *LHPP* were reported to be significantly associated with MDD in Chinese population [15], recent large-scale GWAS did not found significant association between these risk variants and MDD in European populations [16]. The frequency of the risk alleles of the identified risk variants (near *SIRT1* and *LHPP*) shows dramatic difference in Chinese and European populations (i.e., the risk alleles are common in Chinese population. However, the frequency of the risk alleles are very low in European population), suggesting the population heterogeneity of MDD risk variants.

In addition to population heterogeneity, the relative low heritability of MDD also impedes the identification of risk variants and genes. In 2012, the Major Depressive Disorder Working Group of the Psychiatric GWAS Consortium carried out a large-scale GWAS in European population [13]. Though more than 75,000 subjects were included in their final analysis, no genome-wide significant variants were identified. In 2016, Hyde et al. performed a larger GWAS (a total of 459,472 individuals were used) and successfully identified 15 risk loci for MDD [16]. In 2018, Wray et al. conducted a large-scale genome-wide association meta-analysis (135,458 cases and 344,901 controls were included) and identified 44 risk variants for MDD [17]. Recently, Howard et al. carried out the largest MDD GWAS meta-analysis (246,363 cases and 561,190

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controls were included) and reported 102 independent risk variants [18]. Though genetic study of MDD have made great achievements in recent years and over 100 genome-wide significant risk variants have been identified, how the reported risk variants affect MDD pathogenesis remains elusive. Considering the fact that most of identified MDD risk variants are located in non-coding region, it is likely that these risk variants exert their effects on MDD through affecting gene expression. To identify MDD risk variants that were associated with gene expression in human brain and to identify genes whose expression change may have a role in MDD, we carried out a Bayesian integrative analysis (*Sherlock*) through combining genetic associations from a large-scale GWAS of MDD and brain eQTL data. *Sherlock* integrative analysis identified 18 genes whose expression level may have a role in MDD. We further explored the expression level of these 18 genes and found that *FLOT1* was consistently upregulated in brain and peripheral blood of MDD cases compared with controls (European populations). Interestingly, we found that *FLOT1* was also significantly upregulated in peripheral blood of first-episode drug-naïve MDD cases compared with controls (Chinese populations). Our study identifies *FLOT1* as a novel risk gene for MDD and suggests that risk variants may contribute to MDD susceptibility through affecting *FLOT1* expression.

MATERIALS AND METHODS

MDD GWAS used in this study

The genetic associations from a large-scale MDD GWAS [17] were used in this study. Wray et al. performed a genome-wide association meta-analysis recently and identified 44 genome-wide significant risk variants for MDD [17]. Briefly, seven MDD cohorts were included (including PGC29 [13], deCODE, Generation Scotland [19, 20], GERA [21], iPSYCH [22], UK Biobank [23], and 23andme [16]) in study of Wray et al. and a total of 135,458 MDD cases and 344,901 controls were used in the final meta-analysis. Sample recruitment, diagnosis of MDD, genotyping, and statistical analyses can be found in individual cohort. We first downloaded the genome-wide associations from the PGC website (which includes GWAS summary statistics from a combined meta-analysis of PGC29 and five cohorts (deCODE, GenScotland, GERA, iPSYCH, and UK Biobank)). We then conducted a meta-analysis through combining the samples from above six cohorts and 23andme [14, 16]. Meta-analysis was performed as previously described [14] using PLINK [24] (the fixed-effect model was used).

Brain eQTL data

Previous studies have reported that the dorsolateral prefrontal cortex (DLPFC) may have pivotal roles in MDD [25, 26], suggesting that the DLPFC may represent a proper brain region to study the effects of MDD risk variants. We thus used the DLPFC to identify genes whose expression level were affected by the reported MDD risk variants. Brain expression quantitative trait loci (eQTL) from Ng et al. [27] were used in this study. Ng et al. created a multi-omic resource through using brain tissues from the DLPFC. In brief, 2093 individuals of European ancestry were collected by Ng et al. and genotyping was performed with the Illumina OmniQuad Express platform and the Affymetrix Genome-Wide Human SNP Array6.0. They then created an xQTL resources through integrating genotypes with omic data, including gene expression (measured with RNA sequencing, RNA Seq), DNA methylation, and histone acetylation. Expression (quantified with RNA Sequencing) and genotype data of 494 individuals were used to perform eQTL analysis using Spearman's rank correlation approach. More detailed information about brain tissue collection, genotyping, RNA quantification, quality control, and statistical analyses can be found in the original study [27].

Integration of MDD GWAS and brain eQTL using *Sherlock* integrative analysis

Most of the genome-wide significant MDD risk variants are located in non-coding region [17], implying that these variants may confer MDD risk through regulating gene expression. If expression level of a specific gene has a role in MDD, genetic variants that affect the expression of this gene are also likely to confer the risk of MDD. That is, risk variants contribute to MDD susceptibility through influencing gene expression. Thus, eQTL data may have important roles in dissecting genetic basis of MDD. To identify genes whose expression change may confer MDD risk, we integrated the single nucleotide polymorphism (SNP) associations from a large-scale MDD GWAS [17] and brain eQTL data from Ng et al. [27] using the *Sherlock* integrative analysis approach [28]. Briefly, *Sherlock* utilizes a Bayesian statistical framework to infer genes whose expression alternations influence the pathogenesis of complex diseases by using both *cis* (proximal to the gene) and *trans* (distal to the gene or on different chromosomes) genetic variations. For a given gene (whose expression level may have a role in MDD), usually there are several SNPs influence the expression level of this gene in a disease-related tissue (considering MDD is a mental disorder, brain may represent the most relevant tissue for exploring MDD pathogenesis). These expression-associated SNPs (hereafter referred to as eSNPs) may locate in different regions (e.g., promoter and enhancer regions) of the human genome and they may act synergistically to modulate the expression level of the given gene. Allelic differences (or genotype variation) at any of these eSNPs will affect gene expression level, and change of gene expression level eventually alter the disease susceptibility. Therefore, the eQTL of the given gene should overlap significantly with the risk loci of the disease.

Through integrating the GWAS associations and the eQTL data from a related tissue, *Sherlock* can identify genes whose expression change may have a role in disease risk. For a given gene, *Sherlock* calculates the Bayes factor for each eSNP individually and the sum of logarithm of Bayes factor (LBF) of each eSNP constitutes the total LBF score. The LBF score of a gene reflects the association strength between this gene and the disease. For example, a LBF of 5.0 means that the gene is more likely to be associated with the disease ($\exp(5.0) = \sim 148$ times) than no association. For each SNP, there are three scenarios: (1) If this SNP is significantly associated with gene expression and disease simultaneously, a positive LBF score will be given. (2) If this SNP is only associated with gene expression (but is not associated with disease), a negative LBF score will be assigned. (3) Disease-only associations (i.e., this SNP is only associated with disease, not associated with gene expression) have no effect to the score. The *P* thresholds for *cis* and *trans* association were set to 1.0×10^{-3} and 5.0×10^{-5} , respectively (as recommended by the default settings of *Sherlock*). And the *P* threshold for MDD GWAS associations was set to 1.0×10^{-3} . More detailed information about the principle of *Sherlock*, statistical model, LBF calculation can be found in the original paper [28].

Expression analysis of *FLOT1* in brains of MDD cases and controls *Sherlock* identify MDD-associated genes under the assumption that gene expression change may have a role in the pathogenesis of MDD. To further explore if the genes identified by *Sherlock* integrative analysis were dysregulated in MDD cases compared with controls, we compared the expression level of the genes identified by *Sherlock* in brains of MDD cases and controls using the expression data from the study of Labonte et al. [29]. Briefly, Labonte et al. conducted a transcriptome analysis using brain tissues of 26 MDD cases (13 males and 13 females) and 22 controls (13 males and 9 females). Through dividing the individuals into females and males, Labonte et al. identified transcriptional change in male and female MDD cases compared with control male and

female subjects. Tissues from six brain regions (including the dorsolateral prefrontal cortex (DLPFC), ventromedial prefrontal cortex (VMPFC), orbitofrontal cortex (OFC), ventral subiculum (VSub), anterior insula (AINS), and nucleus accumbens (NAC)) were included in the study of Labonte et al. and transcriptome was obtained using RNA sequencing. More detail information can be found in the original publication [29].

In addition to the DLPFC, previous studies also indicated the pivotal role of hippocampus in MDD [30–32]. Many studies repeatedly reported that MDD cases have smaller hippocampal volumes than healthy individuals [30, 31, 33], suggesting the involvement of hippocampus in MDD. Therefore, we explored the expression of genes identified by *Sherlock* integrative analysis in hippocampal tissues of MDD cases and controls using expression data from Duric et al. [34]. In brief, Duric et al. collected post-mortem hippocampal tissues (two subfields were collected, including the CA1 pyramidal cell layer and the dentate gyrus granule cell layer) from 21 MDD cases and 18 healthy controls. Genome-wide gene expression analysis was performed with the human whole-genome expression MI Ready microarrays (Microarray, Inc.). More detailed information about sample collection, tissue isolation, RNA extraction, and expression quantification can be found in the original paper.

Expression analysis of *FLOT1* in blood of MDD cases and controls (European sample)

In addition to genetic study and expression analysis in brains of MDD cases, gene expression analysis in blood is also a complementary approach to explore the potential role of a specific gene in disease [35, 36]. Furthermore, gene expression analysis in blood also provides a possibility to explore if *FLOT1* can be used as a potential biomarker for MDD diagnosis in future. We thus examined *FLOT1* expression in blood of MDD cases and controls using the expression data from Jansen et al. [37]. Briefly, Jansen et al. measured gene expression in peripheral blood of MDD cases and controls using a large-scale sample from the Netherlands Study of Depression and Anxiety. Three groups were used in the study of Jansen et al., including current MDD group ($N = 882$), remitted MDD group ($N = 635$), and control group ($N = 331$). The Composite Interview Diagnostic Instrument (version 2.1) [38] was used for MDD diagnosis and blood was drawn in the morning (8:30–9:30). RNA was extracted with Qiagen kit and gene expression was quantified the Affymetrix U219 arrays. More detailed information about MDD diagnosis, blood collection, RNA extraction, gene measurement, quality control, and statistical analyses can be found in the study of Jansen et al. [37].

Expression analysis of *FLOT1* in blood of first-episode drug-naïve MDD cases and controls (Chinese sample)

To further investigate if *FLOT1* was dysregulated in MDD cases, we quantified *FLOT1* expression in first-episode drug-naïve MDD cases and controls using real-time quantitative PCR (qPCR). Briefly, 50 first-episode drug-naïve MDD cases (22 males and 28 females, 35.22 ± 13.47 years) and 46 controls (23 males and 23 females, 39.59 ± 10.24 years) were recruited from the First people's hospital of Yunnan province. MDD cases were diagnosed by experienced psychiatrists using the DSM-5 criteria. The MDD patient must experience at least five following symptoms in two weeks: (1) Mood depression; (2) Loss of interest or pleasure; (3) Significant weight changes; (4) Insomnia or hypersomnia; (5) Psychomotor agitation or retardation; (6) Low energy or fatigue; (7) Feeling of worthless; (8) Difficulty of thinking and concentration; and (9) Thought of death repeatedly. Cases with substance abuse or other psychiatric disorders were excluded from this study. The 17-item Hamilton Rating Scale for Depression (HAM-D17) was used to assess the level of depression of MDD cases. The controls were also recruited from the First people's hospital of Yunnan province and subjects with physical diseases and mental disorders

were excluded. Informed consent was provided by all of the participants and this study was approved by Institutional Review Board (IRB) of Kunming institute of zoology, Chinese Academy of Sciences.

RNA was extracted from the peripheral blood using Trizol Reagent (Invitrogen, 10296028). The PrimeScript™ RT reagent Kit (Takara, RR047A) was used for reverse transcription and a total of 1 µg RNA was reverse transcribed according to the manufacturer's instruction. TB Green™ Premix Ex Taq™ II (Tli RNaseH Plus) (Takara, RR820A) was used to quantify the *FLOT1* expression level. The QuantStudio™12 K Flex (Applied Biosystems) instrument was used to conduct the real-time quantitative PCR (qPCR). The qPCR primers for *FLOT1* gene are as follows: Forward primer: 5'-CTCGAACAGCTCAAGTCCAAAAG-3'; Reverse primer: 5'-GCCATCTCGATCTCACTCAGTACT-3'. *ACTB* was used as the internal control and the qPCR primers of *ACTB* are as follows: Forward primer: 5'-CATGTACGTTGCTATCCAGGC-3'; Reverse primer: 5'-CTCCTTAATGTCACGCACGAT-3'. The expression of *FLOT1* in each individual was normalized to the expression of *ACTB*. The analysis of qPCR data was based on the Delta Ct (ΔCt) values and fold change (FC) was determined using $2^{-\Delta\Delta Ct}$ approach [39]. All reactions were run in triplicate and Student's *t*-test was used to test if *FLOT1* expression in MDD cases was significantly different from controls.

Expression analysis of *FLOT1* in different human tissues

To explore the expression profiling of *FLOT1* gene in different human tissues, we utilized the gene expression data from the GTEx (Genotype-Tissue Expression) project (v6p release) [40]. We downloaded the gene expression data (i.e., median gene expression values) of 53 human tissues [40]. The expression level of genes were quantified by RNA sequencing (read per kilobase per million (RPKM) value was used to reflect the expression level of each gene). More detailed information about tissue collection, RNA extraction, expression quantification and data processing can be found on the GTEx website (<http://gtexportal.org/>) and related publications (<https://gtexportal.org/home/publicationPage>).

Expression analysis of *FLOT1* in different cell types of the central nervous system

To explore the potential function of *FLOT1* in brain, we explored *FLOT1* expression in different cell types of the central nervous system using the data from Zhang et al. [41]. Zhang et al. isolated different cell types (including microglia/macrophage, astrocytes, oligodendrocytes, endothelial cells, and neurons) from the human brain and performed transcriptome analysis (using RNA sequencing method). We extracted the expression values (fragments per kilobase of transcript per million mapped reads (FPKM)) of *FLOT1* in different cell types and compared the expression level of *FLOT1* in different cell types. To compare if *FLOT1* has similar expression pattern in different cell types of human and mouse brains, we also explored the expression of *FLOT1* in different cell types of mouse brain using data from Cahoy et al. [42].

Temporal expression pattern of *FLOT1* in developing and adult human brains

To explore the potential role of *FLOT1* in the central nervous system, we examined the temporal expression pattern of *FLOT1* in developing and adult human brain. Two independent expression data sets were used in this study. The first expression dataset was from the BrainSpan (Atlas of the Developing Human Brain (<http://www.brainspan.org/>) [43]. Gene expression values (based on RNA sequencing) of *FLOT1* in the prefrontal cortex (PFC) ($N = 42$) was downloaded and transformed as previously described [44]. The transformed expression level was used to plot the temporal expression pattern of *FLOT1* in developing and adult human brain. The second expression dataset was from the study of Colantuoni et al. [45]. Colantuoni et al. explored the temporal dynamics of

gene expression in human prefrontal cortex using serial post-mortem brains from fetal to aging stages. The post-mortem brain tissues (PFC) of 267 normal (without neuropathological or neuropsychiatric diagnosis) subjects were included and transcriptome were measured with microarrays. More detail information about can be found in the original paper [45].

Association between rs2523593 and neuroticism

Neuroticism is an important risk factor for MDD [46, 47] and previous studies have showed significant genetic correlation between MDD and neuroticism [48, 49]. Our *Sherlock* integrative analysis showed that SNP rs2523593 may confer risk of MDD through affecting *FLOT1* expression. To test if rs2523593 is also associated with neuroticism, we examined the association between rs2523593 and neuroticism using the data from the study of Nagel et al. [50]. Nagel et al. carried out a large GWAS meta-analysis ($N = 449,484$) on neuroticism and identified over 100 independent significant risk loci ($P < 5 \times 10^{-8}$) for neuroticism. We extracted the association significance between rs2523593 and neuroticism using a subset sample ($N = 390,278$) from Nagel et al. [50].

RESULTS

Sherlock integrative analysis identifies 18 genes that showed significant association with MDD

To infer genes whose expression perturbations may confer risk of MDD, we used the *Sherlock* statistical inference method to integrate the SNP associations from a large-scale MDD GWAS [17] and brain eQTL [27]. *Sherlock* integrative analysis identified 18 genes whose expression change may contribute to MDD susceptibility ($FDR < 0.01$) (Table 1). These genes include *BTN3A3*, *CCHCR1*, *PPP1R18*, *GPANK1*, *CLIC1*, *ZNF193*, *NOTCH4*, *BTN3A2*, *AGER*, *VAR2*, *DDAH2*, *LY6G5B*, *C4B*, *HLA-C*, *HIST1H4C*, *FLOT1*, *MICA*, and *C4A*. For each gene, at least one SNP showed significant association with MDD and the expression of this gene simultaneously, suggesting that these genetic variants may confer MDD risk through affecting the expression of these genes. Of note, many of the risk genes identified by *Sherlock* are located in the MHC region, suggesting that genes in MHC region may have pivotal roles in the pathogenesis of MDD. The MDD risk SNPs that showed the most significant association with gene expression are rs13195402 (associated with expression of *BTN3A2*, eQTL $P = 5.01 \times 10^{-29}$) and rs2523593 (associated with expression of *HLA-C*, *C4A*, and *FLOT1*, eQTL $P = 7.25 \times 10^{-25}$, 4.98×10^{-19} and 1.37×10^{-15} , respectively).

Dysregulation of *FLOT1* in brains of MDD cases compared with controls

Sherlock integrative analysis identified 18 risk genes that showed significant association ($FDR < 0.01$) with MDD (Table 1). Of note, *Sherlock* identifies MDD risk genes under the assumption that expression change of the predicted genes (from *Sherlock* analysis) may have a role in MDD. We thus compared the expression level of the significant genes identified by *Sherlock* in brains of MDD cases and controls. As previous studies showed that the dorsolateral prefrontal cortex (DLPFC) and anterior insula (AINS) may have pivotal roles in MDD [51–53], we compared the expression level of the significant genes (i.e., genes listed in Table 1) in the DLPFC and AINS of MDD cases and controls using the expression data from Labonte et al. [29]. We first examined expression level of significant genes identified by *Sherlock* analysis in the DLPFC. Among the genes listed in Table 1, only two genes (*FLOT1* and *HLA-C*) showed significant difference ($P < 0.05$, uncorrected) in the DLPFC of MDD cases compared with controls (Fig. 1a, b). Of note, both *FLOT1* and *HLA-C* were significantly upregulated in the DLPFC of MDD cases compared with controls ($P = 0.040$ and 0.039 , respectively). To further validate the dysregulation of *FLOT1* and *HLA-C* in brains of MDD cases, we

compared the expression of *FLOT1* and *HLA-C* in the AINS. Again, we found that both *FLOT1* and *HLA-C* were significantly upregulated in the AINS of MDD cases compared with controls ($P = 0.046$ and 0.027 , respectively) (Fig. 1c, d). These expression results suggest that dysregulation of *FLOT1* and *HLA-C* may have a role in MDD.

Our above expression analysis suggested that dysregulation of *FLOT1* and *HLA-C* may have a role in MDD. To further verify the dysregulation of *FLOT1* and *HLA-C* in brains of MDD cases, we examined *FLOT1* and *HLA-C* expression using an independent expression dataset (i.e., expression data from Duric et al. [34]). We found that *HLA-C* expression was not significantly changed in brains of MDD cases compared with controls in the study of Duric et al. [34]. Intriguingly, we found that *FLOT1* showed a trend of significant upregulation in brains of MDD cases compared with controls (1.53 fold upregulation in MDD cases, $P = 0.07$) in the study of Duric et al. It should be noted that the P values of *FLOT1* and *HLA-C* were not corrected for multiple testing. After correcting for multiple testing (Bonferroni correction), these two genes did not show significant expression difference (corrected $P > 0.05$) in MDD cases and controls. As *FLOT1* showed consistent upregulation in studies of Labonte et al. [29] and Duric et al. [34], we used single-tailed Student's t -test to explore if *FLOT1* was significantly upregulated in MDD cases compared with controls. More work is need to elucidate if *FLOT1* and *HLA-C* were significantly dysregulated in MDD cases. Taken together, our *Sherlock* integrative analysis suggests that MDD-associated genetic variants may confer risk of MDD through affecting *FLOT1* expression. Consistent with this hypothesis, we found that *FLOT1* showed a trend of significant upregulation in MDD cases compared with controls, suggesting *FLOT1* dysregulation may have a pivotal role in MDD.

Dysregulation of *FLOT1* in peripheral blood of MDD cases compared with controls (European sample)

Expression of *FLOT1* was significantly upregulated in brain of MDD cases in study of Labonte et al. In addition, *FLOT1* also showed a trend of significant upregulation in brain of MDD cases in study of Duric et al. These expression data suggest that dysregulation of *FLOT1* may have a role in MDD. To further verify the dysregulation of *FLOT1* in MDD, we examined *FLOT1* expression in peripheral blood of MDD cases and controls using data from Jansen et al. [37]. Consistent with the significant upregulation in brains of MDD cases, we found that *FLOT1* was also significantly upregulated in peripheral blood of current MDD cases compared with controls ($P = 0.031$, 882 cases and 331 controls). Interestingly, we noticed that *FLOT1* was also significantly upregulated in peripheral blood of remitted MDD cases compared with controls ($P = 0.035$, 635 remitted cases and 331 controls). When current MDD cases and remitted cases were combined, *FLOT1* showed significant upregulation in current and remitted MDD cases compared controls ($P = 0.020$, 1,517 cases and 331 controls). Taken together, the significant upregulation of *FLOT1* in both brains and bloods of MDD cases suggests that dysregulation of *FLOT1* may have a role in MDD.

Dysregulation of *FLOT1* in first-episode drug-naive MDD cases compared with controls (Chinese sample)

FLOT1 showed a consistent significant upregulation in brains and peripheral blood of MDD cases compared with controls, implying that dysregulation of *FLOT1* may have a role in MDD. However, considering that the MDD cases recruited in study of Labonte et al. [29] and Jansen et al. [37] were not first-episode drug-naive patients, these studies could not exclude the potential effects of depression medications (e.g., using of antidepressants) on gene expression. We thus investigated *FLOT1* expression in first-episode drug-naive MDD cases and controls using qPCR. Consistent with the significant upregulation observed in brains and peripheral

Table 1. Top MDD-associated genes predicted by *Sherlock* integrative analysis

Gene	Gene position ^a	MHC	LBF ^b	<i>P</i> value ^c	Supporting SNP	Cis or Trans	GWAS <i>P</i> value ^d	eQTL <i>P</i> value ^e	FDR ^f
<i>BTN3A3</i>	chr6:26440700–26453643	No	7.86	5.02×10^{-7}	rs35627490 rs34150729	<i>Cis</i> <i>Cis</i>	4.43×10^{-7} 4.38×10^{-8}	2.65×10^{-4} 2.58×10^{-4}	<0.01
<i>CCHCR1</i>	chr6:31110216–31126015	Yes	5.50	5.02×10^{-7}	rs2523593	<i>Cis</i>	4.67×10^{-7}	3.02×10^{-12}	<0.01
<i>PPP1R18</i>	chr6:30644166–30655672	Yes	5.42	5.02×10^{-7}	rs2523593	<i>Cis</i>	4.67×10^{-7}	1.63×10^{-5}	<0.01
<i>GPANK1</i>	chr6:31629006–31634060	Yes	5.16	5.02×10^{-7}	rs2523593	<i>Cis</i>	4.67×10^{-7}	2.93×10^{-7}	<0.01
<i>CLIC1</i>	chr6:31698358–31707540	Yes	5.15	5.02×10^{-7}	rs2523593	<i>Cis</i>	4.67×10^{-7}	4.05×10^{-7}	<0.01
<i>ZNF193</i>	chr6:28192664–28201260	No	4.70	5.02×10^{-7}	rs13207689	<i>Cis</i>	4.74×10^{-8}	2.50×10^{-6}	<0.01
<i>NOTCH4</i>	chr6:32162620–32191844	Yes	4.60	5.02×10^{-7}	rs2523593	<i>Cis</i>	4.67×10^{-7}	4.13×10^{-7}	<0.01
<i>BTN3A2</i>	chr6:26365387–26378546	No	4.24	5.02×10^{-7}	rs13195402	<i>Cis</i>	4.79×10^{-9}	5.01×10^{-29}	<0.01
<i>AGER</i>	chr6:32148745–32152101	Yes	3.99	5.02×10^{-7}	rs2523593	<i>Cis</i>	4.67×10^{-7}	4.00×10^{-4}	<0.01
<i>VAR52</i>	chr6:30876019–30894236	Yes	3.78	5.02×10^{-7}	rs2523593	<i>Cis</i>	4.67×10^{-7}	1.16×10^{-11}	<0.01
<i>DDAH2</i>	chr6:31694815–31698394	Yes	3.77	5.02×10^{-7}	rs2523593	<i>Cis</i>	4.67×10^{-7}	3.67×10^{-4}	<0.01
<i>LY6G5B</i>	chr6:31637944–31641553	Yes	3.73	5.02×10^{-7}	rs2523593	<i>Cis</i>	4.67×10^{-7}	2.06×10^{-5}	<0.01
<i>C4B</i>	chr6:31982539–32003195	Yes	3.66	5.02×10^{-7}	rs2523593	<i>Cis</i>	4.67×10^{-7}	2.49×10^{-4}	<0.01
<i>HLA-C</i>	chr6:31236526–31239907	Yes	3.58	5.02×10^{-6}	rs2523593	<i>Cis</i>	4.67×10^{-7}	7.25×10^{-25}	<0.01
<i>HIST1H4C</i>	chr6:26104104–26104518	No	3.52	5.02×10^{-6}	rs34546498	<i>Cis</i>	4.05×10^{-7}	1.58×10^{-7}	<0.01
<i>FLOT1</i>	chr6:30695486–30710510	Yes	3.33	6.02×10^{-6}	rs28849176 rs2523593	<i>Cis</i> <i>Cis</i>	3.25×10^{-5} 4.67×10^{-7}	1.72×10^{-2} 1.37×10^{-15}	<0.01
<i>MICA</i>	chr6:31371356–31383092	Yes	3.33	6.02×10^{-6}	rs2523593	<i>Cis</i>	4.67×10^{-7}	6.66×10^{-12}	<0.01
<i>C4A</i>	chr6:31949801–31970458	Yes	3.21	6.02×10^{-6}	rs2523593	<i>Cis</i>	4.67×10^{-7}	4.98×10^{-19}	<0.01

FLOT1 was shown in bold

^agene locations were based on hg19

^bLBF, logarithm of Bayes factor. The LBF score of a gene reflects the association strength between this gene and MDD. For example, a LBF of 5.0 means that the gene is more likely to be associated with the disease ($\exp(5.0) = \sim 148$ times) than no association

^c*P* value from *Sherlock* integrative analysis. Larger LBF corresponds to smaller *P* value

^dGWAS *P* value indicates the association significance between this SNP and MDD

^eeQTL *P* value indicates the association significance between this SNP and gene expression in brain

^fFDR was corrected by Benjamini–Hochberg procedure

blood of MDD cases in European sample, we found that *FLOT1* expression was significantly elevated in first-episode drug-naïve MDD cases (Chinese sample) compared with controls in Chinese sample ($P = 1.01 \times 10^{-7}$, two-tailed Student's *t*-test) (Fig. 2). The significance was strengthened after three outlying MDD cases were removed ($P = 1.01 \times 10^{-10}$). Significant upregulation of *FLOT1* in peripheral blood of first-episode drug-naïve MDD cases provide further evidence that support the involvement of *FLOT1* in MDD. Collectively, the consistent upregulation of *FLOT1* observed in independent MDD cases supports that dysregulation of *FLOT1* may have a pivotal role in MDD. *Sherlock* integrative analysis and expression results suggest that genetic variants may confer risk of MDD through affecting *FLOT1* expression.

FLOT1 is widely expressed in diverse human tissues

Our *Sherlock* integrative analysis suggests that *FLOT1* is a MDD risk gene whose expression level may have a role in MDD. Consistent with this, we found that *FLOT1* was dysregulated in MDD cases compared controls. To further explore the potential function of *FLOT1*, we examined *FLOT1* expression in diverse human tissues using data from the GTEx. We found that *FLOT1* was widely expressed in different human tissues, with the highest expression level in whole blood (Fig. 3). This expression data implies that *FLOT1* may have a role in different human tissues.

FLOT1 is widely expressed in different cell types of the central nervous system

We further explored the expression of *FLOT1* in different cell types (including astrocytes, neurons, oligodendrocytes, and microglia cells) of the central nervous system. We found that *FLOT1* was

widely expressed in different cell types of the human brain (Fig. 4a). To compare if *FLOT1* has similar expression patterns in different cell types of the human and mouse brains, we examined *FLOT1* expression in different cell types of the mouse brain. Compared with the relatively low expression level observed in different cell types of the human brain, we found that the expression level of *Flot1* was high in different cell types of the mouse brain (Fig. 4b). Of note, we found that *Flot1* has the highest expression level in neurons and microglia cells of mouse brain (Fig. 4b). These expression data imply that *FLOT1* may have a role in the central nervous system.

Temporal expression pattern of FLOT1 in developing and adult human brains

We explored the expression pattern of *FLOT1* in developing and adult human brain using the expression data from the BrainSpan [43] and Colantuoni et al. [45]. We found that the expression of *FLOT1* was relatively low at early developmental stage in both data sets (Fig. 4c, d). However, *FLOT1* expression was gradually increased with the progress of development, and peaked at adult stage (Fig. 4c, d). These temporal expression pattern suggests that *FLOT1* may have different roles during brain development.

FLOT1 expression-associated SNP (rs2523593) showed significant association with neuroticism

Previous studies have revealed that MDD showed significant genetic correlation with neuroticism [17, 50], an important risk factor for MDD [46] and anxiety [54]. Our *Sherlock* integrative analysis indicated that rs2523593 was significantly associated with MDD and *FLOT1* expression simultaneously (Table 1), suggesting

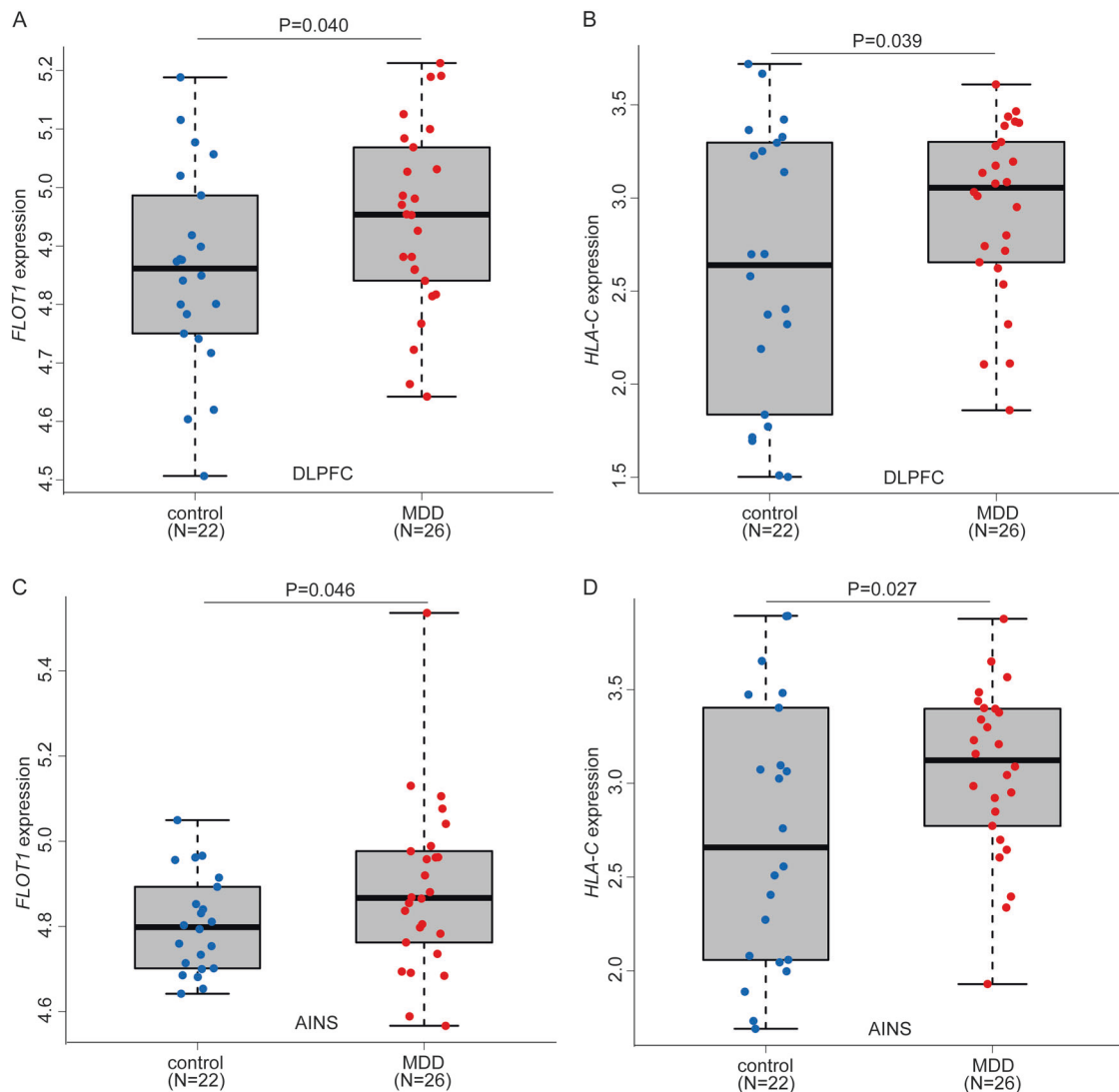


Fig. 1 Significant upregulation of *FLOT1* in brains of MDD cases compared with controls. **a, c** Compared with controls, *FLOT1* was significantly upregulated in the dorsolateral prefrontal cortex (DLPFC) and anterior insula (AINS) of MDD cases. **b, d** *HLA-C* was significantly upregulated in the dorsolateral prefrontal cortex (DLPFC) and anterior insula (AINS) of MDD cases. One-tailed Student's *t*-test was used to compare if the difference was significant ($P < 0.05$)

that SNP rs2523593 may confer risk of MDD through affecting *FLOT1* expression. Considering the significant genetic correlation between MDD and neuroticism, we hypothesized that rs2523593 may also be associated with neuroticism. We thus explored the association between rs2523593 and neuroticism using the data from the study of Nagel et al. [50]. We found that rs2523593 was also significantly associated with neuroticism ($P = 2.47 \times 10^{-5}$). Intriguingly, we found that the MDD risk-associated allele (i.e., T allele) also increases the risk of neuroticism. The association between rs2523593 and neuroticism provided additional evidence that supports rs2523593 may represent a promising risk SNP for MDD and neuroticism.

DISCUSSION

Recent large-scale GWAS have identified multiple risk variants that show strong association with MDD. Nevertheless, how the identified risk variants confer MDD risk remains largely unknown. Considering the fact that most of the identified MDD risk variants were located in non-coding region, it is possible that these MDD-associated variants confer risk of MDD through regulating gene

expression. To identify MDD risk genetic variants that were associated with gene expression in human brains and to prioritize genes whose expression change may have a role in MDD, we systematically integrated the genetic associations from the a large-scale MDD GWAS and brain eQTL using *Sherlock* integrative analysis. We identified 18 genes whose perturbations may have a role in MDD. Further expression analysis showed that *FLOT1* was consistently upregulated in brains and peripheral blood of MDD cases compared with controls (European sample). Interestingly, we found that *FLOT1* was significantly upregulated in first-episode drug-naïve MDD cases (Chinese sample). Our integrative analysis and gene expression results provide convergent lines of evidence that supports the potential involvement of *FLOT1* in MDD.

FLOT1 encodes Flotillin-1, a caveolae-associated integral membrane protein. The function of *FLOT1* has not been fully determined. However, previous studies have suggested that Flotillin-1 may have pivotal roles in the central nervous system [55, 56]. The protein sequence of *FLOT1* is highly conserved from *Drosophila* to humans [57], suggesting the important role of *FLOT1*. In addition, *FLOT1* was shown to be abundantly expressed in *Drosophila* brain [57], implying that *FLOT1* may have a role in

the central nervous system. Consistently, it has been reported that *FLOT1* is involved in neuronal differentiation, axon outgrowth, and regeneration [55]. These lines of evidence demonstrated the important role of *FLOT1* in the central nervous system.

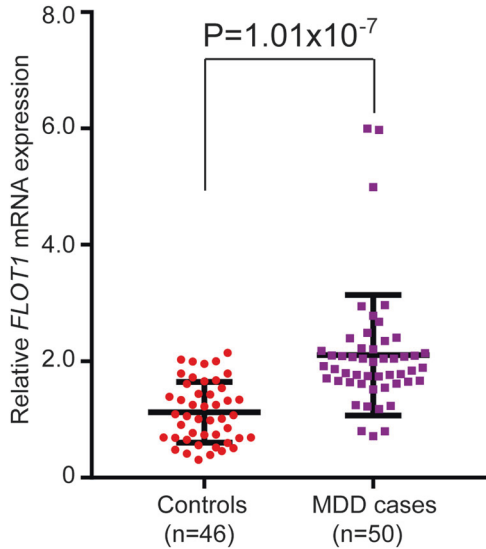


Fig. 2 Significant upregulation of *FLOT1* in peripheral blood of first-episode drug-naïve MDD cases compared with controls. Quantitative PCR showed that *FLOT1* was significantly upregulated ($P=1.01 \times 10^{-7}$) in peripheral blood of first-episode drug-naïve MDD cases compared with controls. Expression of *FLOT1* was quantified in 46 controls and 50 MDD cases. Two-tailed Student's *t*-test was used to compare if the difference was significant ($P < 0.05$)

Interestingly, Cremona et al. [56] showed that Flotillin-1 played an important role in the internalization of dopamine transporter (DAT) and the glial glutamate transporter EAAT2, members of two different neurotransmitter transporters families. In addition to dopamine transporter and the glial glutamate transporter, a recent study of Reisinger et al. revealed that Flotillin-1 modulates serotonergic neurotransmission through interacting with the serotonin transporter [58]. Considering the important role of serotonin transporter, dopamine transporter and glutamate transporter in MDD [59–63], it is possible that *FLOT1* may be involved in MDD through modulating the internalization of dopamine transporter and the glial glutamate transporter EAAT2. However, more work is needed to investigate the exact role of *FLOT1* in the pathogenesis of MDD.

In addition to playing important roles in the central nervous system, recent studies also revealed that *FLOT1* has important roles in cancer. Dysregulation of *FLOT1* expression was reported in different cancers, including epithelial ovarian tumors [64], transitional cell carcinoma [65], nasopharyngeal carcinoma [66], and renal cell carcinoma [67]. Moreover, studies also showed that *FLOT1* regulates migration, invasion, and proliferation of tumor cells [68, 69]. These studies demonstrated the important role of *FLOT1* in cancer. Intriguingly, it has been reported that depression is a risk factor for cancer prognosis [70]. Besides, previous studies also showed that prevalence of MDD in cancer patients was higher compared with the general population [71, 72].

Compared with the high expression level in mouse brain, we found that *FLOT1* expression was relatively low in different cell types of the human brain. These results suggest that *FLOT1* may have different roles in the human and mouse brain. In addition, these observations also implicate that we need to consider the difference of *FLOT1* expression pattern when we use transgenic (or knockout) mouse model to study the function of *Flot1* in the

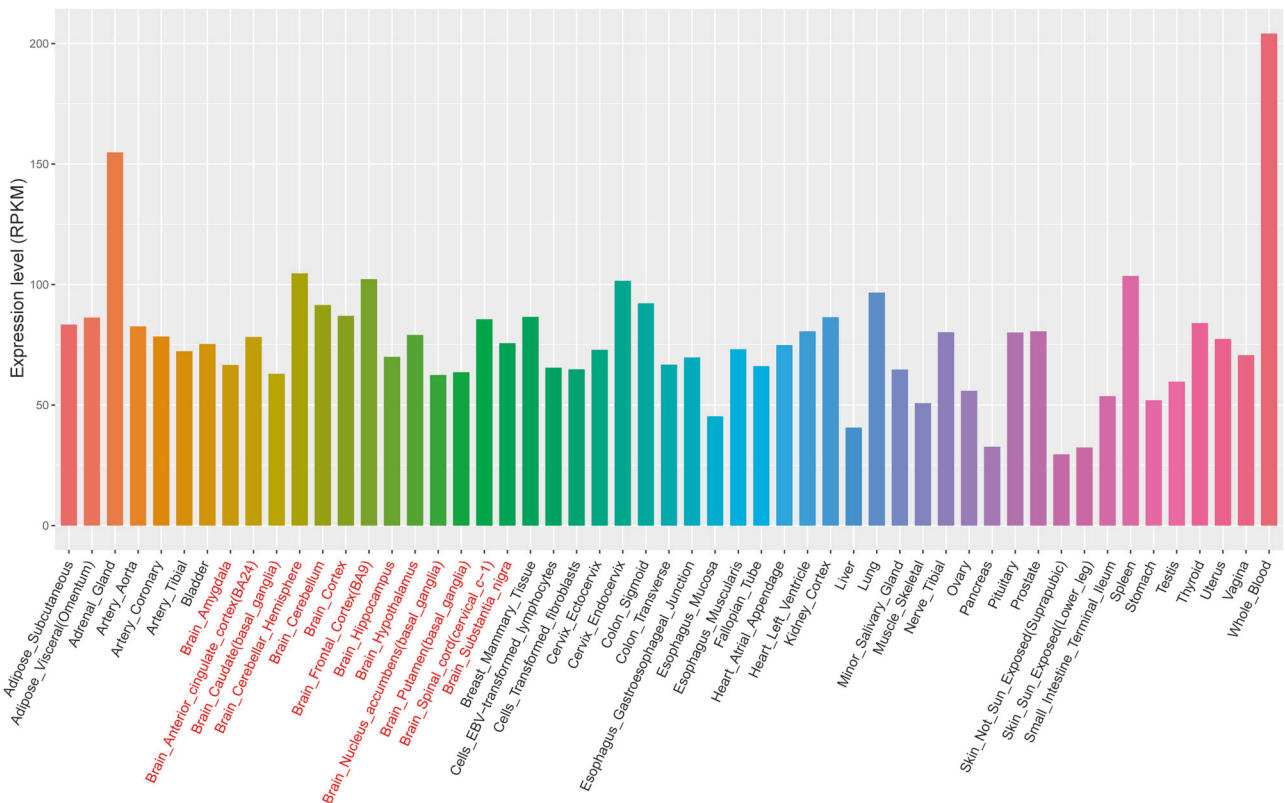


Fig. 3 *FLOT1* is widely expressed in diverse human tissues. RNA sequencing-based expression data from GTEx was used to explore *FLOT1* expression. *FLOT1* was abundantly expressed in different human tissues, with the highest expression level in blood

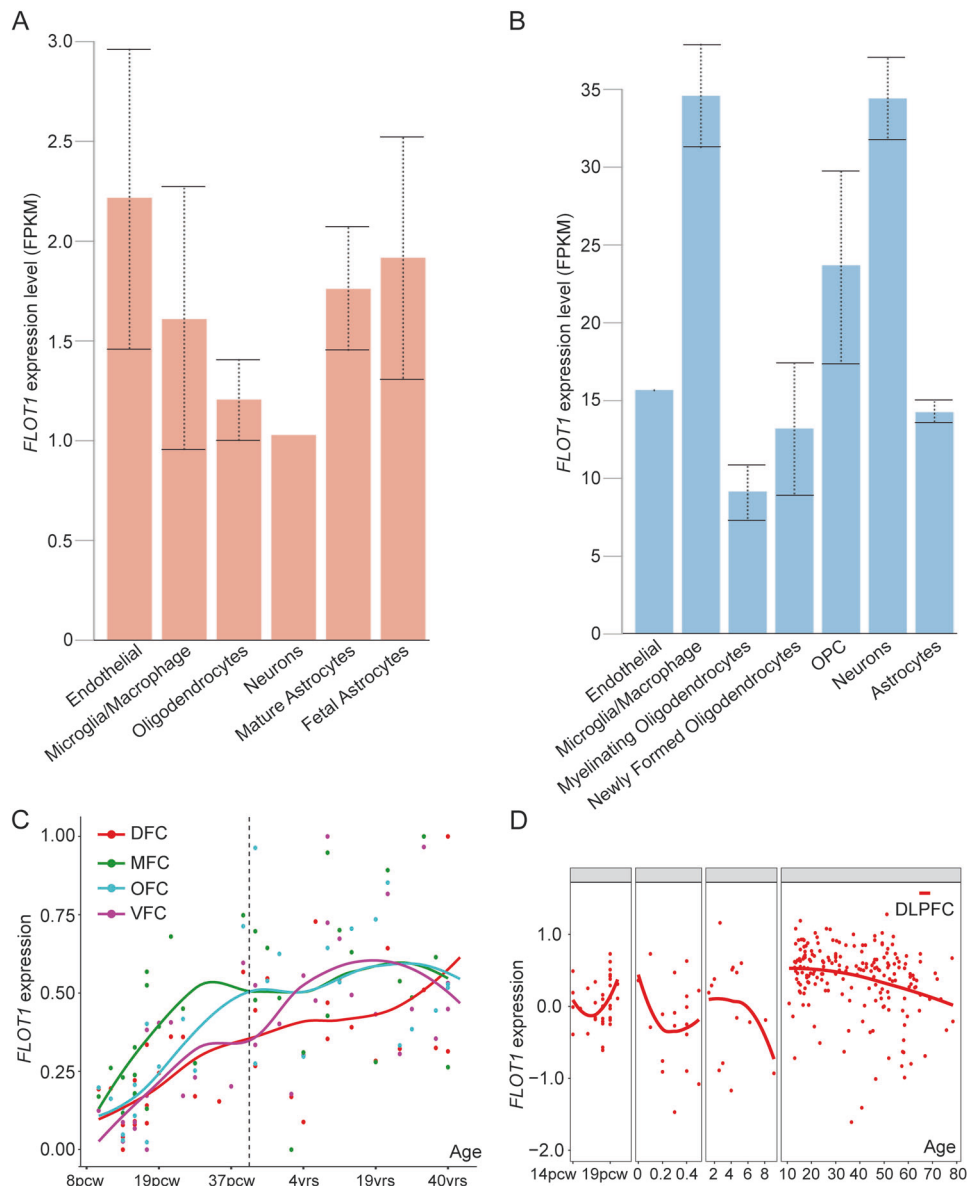


Fig. 4 Expression of *FLOT1* in different cell types of human and mouse brain. **a** *FLOT1* was expressed in different cell types (including neuron, microglia, oligodendrocytes, and astrocytes) of human brain. **b** *FLOT1* was abundantly expressed in different cell types of mouse brain, with the relatively high expression level in neurons and microglia cells. **c, d** Expression of *FLOT1* was relatively low at early developmental stage. With the progress of development, *FLOT1* expression was gradually increased, and peaked at adult stage. **c** Expression data from the BrainSpan was used for plotting. **d** Expression data from the BrainCloud was used for plotting

central nervous system (i.e., mouse model may not fully mimic (or reflect) the function of *FLOT1* in humans as this gene has different expression level in the human and mouse brain).

We found that the MDD risk SNP rs2523593 was also associated with neuroticism. Previous studies indicated that MDD has a high genetic correlation with neuroticism [48, 49] and individuals with higher score in neuroticism tend to be more susceptible to MDD [46, 73], suggesting that exploring the genetic basis of neuroticism may provide insights into etiology of MDD. Of note, we found that the association significance between rs2523593 and neuroticism was less significant than MDD. This could be due to the smaller sample size of neuroticism GWAS ($N=390,278$) compared with MDD GWAS ($N=480,359$). More work is needed to explore if rs2523593 is a risk variant for neuroticism.

Sherlock integrative analysis identified 18 MDD risk genes (Table 1) whose expression change may have a role in MDD. In

addition to *FLOT1*, following genes (including *NOTCH4*, *C4A* and *C4B*) may also be relevant to MDD from the aspect of biological function. Previous studies have indicated the important role of Notch signaling pathway in neuronal development and differentiation [74, 75]. Interestingly, it has been reported that *NOTCH4* is associated with schizophrenia [76], a severe psychiatric disorder. *C4A* and *C4B* encode complement C4-A and C4-B, respectively. A recent study revealed that C4 protein played an important role in synapse development and C4 genes (*C4A* and *C4B*) were associated with schizophrenia [77], suggesting the pivotal roles of *C4A* and *C4B* in the central nervous system. Collectively, these lines of evidence suggest that *NOTCH4*, *C4A* and *C4B* may also have a role in MDD. Nevertheless, more work is needed to explore the role of these genes in MDD.

There are several limitations of this study. First, though our *Sherlock* integrative analysis showed that genetic variants may

confer risk of MDD through regulating *FLOT1* expression. Due to the complexity of linkage disequilibrium and gene regulation, the causal (or functional) variants that regulate the expression of *FLOT1* and the exact regulatory mechanism remain elusive. Second, it is possible that *FLOT1* have a role in neurotransmission as previous studies have shown that Flotillin-1 played an important role in the internalization of dopamine transporter (DAT) and the glial glutamate transporter EAAT2. However, currently we still do not know the role of *FLOT1* dysregulation in MDD. Third, we examined the association between *FLOT1* and MDD in Chinese population by using the CONVERGE data (including 5303 MDD cases and 5337 controls) [15]. We found that rs2523593 was not significantly associated with MDD in Chinese population ($P = 0.70$). However, A SNP (rs115494986, which showed low level LD with rs2523593 ($r^2 = 0.20$)) near *FLOT1* is marginally associated with MDD in CONVERGE ($P = 0.029$). Further validation of the association between *FLOT1* and MDD in independent samples is needed. Fourth, our *Sherlock* integrative analysis identified 18 genes whose expression perturbation may have a role in MDD (Table 1). Nevertheless, we only focused on *FLOT1* in this study as expression of *FLOT1* was consistently upregulated in MDD cases compared with controls. Further work is needed to investigate if other genes predicted by *Sherlock* integrative analysis have a role in MDD.

In summary, our study provides convergent lines of evidence that support *FLOT1* is a new MDD risk gene. Our results suggested that MDD-associated variants may confer MDD risk through affecting *FLOT1* expression. The significant upregulation of *FLOT1* in MDD also suggests that *FLOT1* may be targeted in future as a potential marker for future therapeutics and diagnostics.

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ADDITIONAL INFORMATION

Competing interests: The authors declare that they have no competing interests.

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