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FKBP5 genetic variation: association with selective serotonin reuptake inhibitor treatment outcomes in major depressive disorder

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Abstract

Objectives—FKBP51 (51 kDa immunophilin) acts as a modulator of the glucocorticoid receptor and a negative regulator of the Akt pathway. Genetic variation in *FKBP5* plays a role in antidepressant response. The aim of this study was to comprehensively assess the role of genetic variation in *FKBP5*, identified by both Sanger and Next Generation DNA resequencing, as well as genome-wide single nucleotide polymorphisms (SNPs) associated with *FKBP5* expression in the response to the selective serotonin reuptake inhibitor (SSRI) treatment of major depressive disorder.

Methods—We identified 657 SNPs in *FKBP5* by Next Generation sequencing of 96 DNA samples from white patients, and 149 SNPs were selected for the genotyping together with 235 SNPs that were trans-associated with variation in *FKBP5* expression in lymphoblastoid cells. A total of 529 DNA samples from the Mayo Clinic PGRN-SSRI Pharmacogenomic trial for which genome-wide SNPs had already been obtained were genotyped for these 384 SNPs, and associations with treatment outcomes were determined. The most significant SNPs were genotyped using 96 DNA samples from white non-Hispanic patients of the NIMH-supported Sequenced Treatment Alternatives to Relieve Depression (STAR*D) study to attempt replication, followed by functional genomic studies.

Results—Genotype–phenotype association analysis indicated that rs352428 was associated with both 8-week treatment response in the Mayo study (odds ratio =0.49; $P = 0.003$) and 6-week

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Conflicts of interest

Dr Mrazek has developed intellectual property that has been licensed by AssureRx Health and subsequently incorporated into physician decision support software. He has also received research funding from AssureRx Health to create and maintain a bibliographic system designed to monitor the scientific literature. The rest of the authors report no biomedical financial interests or potential conflicts of interest.

response in the STAR*D replication study (odds ratio = 0.74; P=0.05). The electrophoresis mobility shift assay and the reporter gene assay confirmed the possible role of this SNP in transcription regulation.

Conclusion—This comprehensive *FKBP5* sequence study provides insight into the role of common genetic polymorphisms that might influence SSRI treatment outcomes in major depressive disorder patients.

Keywords

FKBP5; genotype–phenotype association; major depressive disorder; Next Generation DNA resequencing; selective serotonin reuptake inhibitor; single nucleotide polymorphism

Introduction

Major depressive disorder (MDD) is a common psychiatric disease with an estimated incidence of 16% in the general population of the USA [1]. Selective serotonin reuptake inhibitors (SSRIs) are one of the most widely prescribed classes of antidepressant drugs [2]. Clinical trials have shown large individual variation in SSRI treatment outcomes, with approximately half of the treated patients failing to benefit from therapy and many developing undesirable drug-related side effects [3]. *FKBP5* encodes the FKBP51 protein, a member of the family of large immunophilins [4]. Recently, we reported that FKBP51 acted as a scaffolding protein regulating Akt activity [5]. Activity of Akt has been shown to play a role in a variety of neuronal physiological functions [6–9]. Therefore, alterations in Akt activity might have implications in the development and treatment of psychiatric disorders [10–12].

In addition, it is known that the glucocorticoid receptor (GR) plays a role in stress-related psychiatric disorders, including MDD, probably by affecting the hypothalamic– pituitary–adrenal axis [13–15]. FKBP51 is also a cochaperon for GR maturation, modulating its sensitivity and, thus, playing a role in regulation of the stress response [16]. The GR can increase *FKBP5* transcription through intronic GR response elements. An increased FKBP51 level confers elevated GR resistance, completing an ultrashort negative feedback loop on GR sensitivity [17]. Because of the role of FKBP51 in the glucocorticoid pathway and in stress-related disease, previous studies have attempted to assess the role of genetic variation in *FKBP5* in MDD and in response to SSRI treatment. These studies reported that sequence variation in the *FKBP5* gene may be associated with risk for posttraumatic stress disorder, risk for recurrence of depression, and variation in response to antidepressant therapy [17–23]. *FKBP5* has also been reported to be associated with risk for attempted suicide and the occurrence of depressive episodes in bipolar patients [17–23]. Although these studies suggest that variation in the sequence or expression of *FKBP5* might be associated with variation in SSRI treatment outcome [5,18,19,24,25], none of them explored the full range of DNA variants present in the gene, and only one study by Binder *et al.* [18] suggested that one of the potential mechanisms by which those genetic variants might influence FKBP51 function is through their influence on protein levels. Therefore, the aim of the present study was to comprehensively investigate the role of genetic variation in *FKBP5*, identified by Next Generation DNA sequencing, followed by association studies carried out on depressed patients treated with SSRIs and functional characterization of selected single nucleotide polymorphism (SNPs). SNPs that were associated with SSRI treatment outcome were then genotyped in an independent patient cohort, the Sequenced Treatment Alternatives to Relieve Depression (STAR*D) patient cohort. Our results indicate that SNPs associated with *FKBP5* expression may be involved in its transcriptional regulation and, ultimately, modulation of clinical outcomes after SSRI therapy of patients with MDD.

Materials and methods

FKBP5 gene resequencing

Both Sanger and Next Generation sequencing were used to resequence *FKBP5* (primers listed in Supplementary Table 1, http://links.lww.com/FPC/A572), as described previously [26]. Sanger sequencing was used to resequence all exons, exon–intron splice junctions, and ~1000 bp of the 5′ and 3′ flanking regions using 96 DNA samples from lymphoblastoid cells generated from white American patients included in the 'Human Variation Panel' (HD100CAU; Coriell Institute, Camden, New Jersey, USA) [27]. Deep sequencing using an Illumina Next Generation sequencing platform (Genome Analyzer IIx; Illumina, San Diego, California, USA) was performed with the same DNA sample set to resequence a 160 kb genomic region on chromosome 6p21 that contained *FKBP5*. There was 99.8% sequence concordance between regions resequenced using both methods. For the small number of discordant genotypes, Sanger sequencing was repeated and discordant genotypes were replaced with Sanger sequencing results. The Next Generation sequencing results were also compared with Illumina and Affymetrix genome-wide SNP (Affymetrix, Santa Clara, California, USA) genotyping data obtained using the same DNA samples, and there was 98.4% concordance between these results. In this case, if there were discordant genotypes, the Illumina or Affymetrix genotype data were used. Sanger sequencing was also used to identify variations in *FKBP5* in the two other ethnic groups included in the 'Human Variation Panel', specifically DNA samples from 96 African Americans (AA) and 96 Han Chinese Americans (HCA; HD100AA and HD100CHI, respectively, Coriell Institute), with regard to exons, splice junctions, and 1000 bp of 5′ and 3′ flanking regions (Supplementary Table 2, http://links.lww.com/FPC/A572).

Expression quantitative trait loci analysis

We have also generated expression array and genomewide SNP data for 287 of the 'Human Variation Panel' lymphoblastoid cell lines (LCLs) [28,29]. The SNPs and expression array data have been deposited under the SuperSeries accession number GSE24277. Association analysis for expression and SNP data was carried out using Pearson's correlations, as described previously [29].

Study patients

DNA for our initial clinical SSRI study was obtained from 529 MDD patients treated with either citalopram or escitalopram in the Mayo Clinic Pharmacogenomics Research Network-Antidepressant Medication Pharmacogenomic Study (Mayo PGRN-AMPS), a study that has been described elsewhere [30]. Specifically, patients had to meet diagnostic criteria for MDD with a Hamilton Depression Rating Scale (Ham-D) score of 14 or higher at baseline to be enrolled. Fourteen of the patients were not white non-Hispanic (WNH) and were excluded from the analysis and three samples failed genotyping, resulting in 512 WNH patients in the final analyses. The design of the Mayo PGRN-AMPS trial was based on that of the large multicenter NIMH-supported STAR*D study, the largest MDD treatment-response study performed to date [31]. STAR*D was designed to assess which treatment strategies, and in what order, were most effective in depression management, always beginning with an SSRI, citalopram, a drug that was also used in the Mayo study. A total of 960 samples from the STAR*D study were used in our replication study. They were selected because they were from treatment-compliant WNH patients with initial Ham-D scores of 14 or higher at baseline, the entry criteria for our study.

Selective serotonin reuptake inhibitors outcome phenotypes

Treatment outcomes in both the Mayo PGRN-AMPS and the STAR*D studies were assessed using the 16-item Quick Inventory of Depressive Symptomatology-Clinician rating scores. Treatment outcome phenotypes that were analyzed during the association studies included 'response' (defined as ≥50% reduction in QIDS score from the beginning of treatment to the visit evaluated) and 'remission' (defined as a QIDS score of ≤5 at the last visit). The 'response' and 'remission' phenotypes used in this analysis were assessed at both 4 and 8 weeks after starting SSRI therapy for the Mayo PGRN-AMPS and at 4 and 6 weeks for the STAR*D study. The 'last visit' for 'response' or 'remission' phenotypes refers to an analysis of all patients enrolled in the study – that is both those who completed the full 8-week treatment regimen and those who dropped out before the 8-week time point. For 'last-visit' analyses, outcomes were defined based on the last observation carried forward. These analyses were not adjusted for time spent in study. Among the patients included in this study, 36% administered citalopram and 64% escitalopram. The drug to be administered was selected by the physician in consultation with the patient. When we compared the outcomes between patients treated with citalopram versus escitalopram, we found no statistically significant differences in remission or response rates between the two groups. Therefore, we did not carry out stratified analyses in the current study.

Single nucleotide polymorphism genotyping

To be included in the analysis of the Mayo PGRN-AMPS samples, SNPs identified from Next Generation sequencing had to have a minor allele frequency (MAF) of 1% or higher and had to pass the Illumina Golden Gate genotyping platform quality control criteria. A total of 149 SNPs of the 657 resequenced in *FKBP5* met those criteria and were selected for inclusion in the genotyping panel. We also genotyped 235 SNPs that were transgenome-wide associated with *FKBP5* expression [*FKBP5* expression Quantitative Trait Locus (eQTL)] in our 'Human Variation Panel' LCLs. Similar to the selection of SNPs identified from our resequencing effort, these SNPs also had to pass the Illumina Golden Gate genotyping quality control criteria. This final panel of 384 SNPs was used to genotype the 529 DNA samples using the Illumina BeadXpress platform (Illumina, San Diego, California, USA). These patient samples had also been subjected to genome-wide genotyping using the Illumina 610 BeadChip kit (Illumina) [32]. A TaqMan assay (Applied Biosystems, Foster City, California, USA) was used to perform replication genotyping using STAR*D DNA samples.

Statistical methods

After genotyping of 384 SNPs from our Mayo SSRI patient samples, quality control was also applied to remove SNPs that were significantly deviated from Hardy–Weinberg equilibrium, those with low call rate, which resulted in 340 SNPs remaining in the association analysis. We also removed three samples that failed genotyping. The effect of SNP genotypes on the binary phenotypes of 'response' and 'remission' in the Mayo PGRN-AMPS trial was assessed using logistic regression models adjusting for possible population stratification using four eigen vectors constructed from the genomewide SNP data [32,33]. The effect of each SNP adjusted for population stratification was tested using a likelihood ratio test. The relationship of the SNPs with percentage change in QIDS from baseline to last visit was assessed using Spearman's partial correlations and an *F*-test, in which the phenotype was adjusted for possible population stratification. To replicate Mayo PGRN-AMPS findings, the associations of SNPs of interest were also determined in the STAR*D patient population. Binary phenotypes of 'response' and 'remission' were tested using likelihood ratio tests. Finally, because association analysis of individual markers can be underpowered for rare markers, we used a novel test, the difference in MAF test, to determine associations of a set of markers with phenotypes. To test the association between

groups of SNPs in subregions of *FKBP5* and response and remission, variants were analyzed with sliding windows containing between 10 and 50 variants. Detailed statistical methods are described in the Supplementary methods.

Cell culture and transfections

Site-directed mutagenesis was performed to create variant constructs (Arg^{28}, Gln^{154}, and Phe^{437}), using a wildtype (WT) FKBP51 construct as a template, as described previously [34]. Primers are listed in Supplementary Table 3a, (http://links.lww.com/FPC/A572). HEK293T cells [American Type Culture Collection cells were used for transfections with the pIRES-GFP/Flag WT and variant constructs, as well as with the empty vector, using the Lipofectamine2000 protocol (Invitrogen; Life Technology, Grand Island, New York, USA)]. Green fluorescent protein was used to correct for transfection efficiency.

Electrophoresis mobility shift assay

Electrophoresis mobility shift assays (EMSAs) for the rs352428 SNP were performed with nuclear extracts from two glioblastoma cell lines, U-87MG and U251 (ATCC), as well as from a pool of lymphoblastoid cells from healthy individuals (Coriell Institute). Total protein concentrations were assayed using the Bradford method. EMSAs were performed using the LightShift Chemiluminescent EMSA Kit (Pierce, Rockford, Illinois, USA), as described previously [34]. Oligonucleotide sequences (sense and antisense) for WT and variant sequences of the rs352428 (G/A) SNP are listed in Supplementary Table 3b (http://links.lww.com/FPC/A572). For competitive assays, a 400-fold excess of unlabeled probe was added to the reaction mixture.

Reporter gene assay

A 231 bp region surrounding the rs352428 SNP was amplified using DNA isolated from LCLs containing WT or variant genotypes. Primer sequences are listed in Supplementary Table 3c (http://links.lww.com/FPC/A572). The PCR product was cloned into the pGL3-promoter vector (Promega, Madison, Wisconsin, USA). DNA sequences were verified by sequencing both strands. Vector without an insert was used as a control. Specifically, U-87MG and U251 cells were transfected with WT and variant constructs together with a pRL-TK DNA construct encoding Renilla luciferase, as a control for transfection efficiency. Luciferase activity was measured by a dual luciferase activity assay using a TD-20/20 Luminometer (Turner Designs, Sunnyvale, California, USA). Results are expressed as the ratio of firefly luciferase to Renilla luciferase light units, and all values are expressed as a percentage of the pGL3-promoter construct activity. All assays were performed in triplicate.

Results

Single nucleotide polymorphism selection for genotyping

Introduction—For genotyping, we selected a total of 384 SNPs by combining results from both *FKBP5* gene resequencing and *FKBP5* eQTL analysis in LCLs. The selection processes are represented in Fig. 1.

FKBP5 gene resequencing—Our *FKBP5* resequencing covered an area of 160 kb on chromosome 6 and identified 657 SNPs (Supplementary Table 4, http://links.lww.com/FPC/A572), as described in detail by Pelleymounter *et al.* [26]. The majority of the polymorphisms, including 44 indels (insertions/deletions), were located in introns, flanking regions, and untranslated regions. All but 18 SNPs (indicated in Supplementary Table 4, http://links.lww.com/FPC/A572) were in Hardy–Weinberg equilibrium ($P>0.05$). In total, 315 SNPs were novel as compared with data from the '1000 Genomes Project' (Phase 1 data

[35]) and/or dbSNP. A total of 316 SNPs had MAFs greater or equal to 1%. Sanger sequencing was also used to resequence *FKBP5* in 96 additional AAs and HCA DNA samples, respectively (Supplementary Table 2, http://links.lww.com/FPC/A572), resulting in the identification of another 29 novel SNPs, including three nonsynonymous (NS) SNPs (Gly22Arg in HCA, Arg154Gln in AA, and Val437Phe in AA), all with MAFs less than 5% (Supplementary Figure 1, http://links.lww.com/FPC/A572). Only 149 SNPs from *FKBP5* resequencing of HCA samples passed the Illumina genotyping criteria and were used for genotyping the Mayo PGRN-AMPS cohort.

FKBP5 expression Quantitative Trait Locus analysis in LCLs—To identify SNPs that might be associated with *FKBP5* expression through either *cis* or *trans* regulation, we carried out eQTL analysis for all 287 LCLs from all three ethnic groups. A total of 451 SNPs were associated with two of the three *FKBP5* Affymetrix expression probe sets (Affymetrix), 224856_at or 224840_at, with *P*-values less than 0.0001, as illustrated in the expression Manhattan plot in Fig. 2. The third *FKBP5* probe set, 224560_at, is not highly correlated with the other two probe sets; therefore, it was excluded from the analysis. Among the SNPs that were associated with *FKBP5* expression, 295 had not been genotyped previously in the Mayo PGRN-AMPS cohort using the genome-wide Illumina 610 Beadchip and, therefore, were included in our current genotyping study (black dots in Fig. 2). We selected the top 235 of the 295 genome-wide SNPs that passed the Illumina genotyping criteria to build a panel together with resequenced FKBP5 variants consisting of 384 SNPs for genotyping the Mayo PGRN-AMPS cohort.

Genotype–phenotype analysis of the Mayo Pharmacogenomics Research Network-Antidepressant Medication Pharmacogenomic Study patient samples

The panel consisting of 384 SNPs selected as outlined in Fig. 1a was genotyped in 529 Mayo PGRN-AMPS patient samples. This set represented 149 SNPs from *FKBP5* resequencing in addition to 235 *FKBP5* eQTL SNPs. In addition, because we had already performed the genome-wide association study (GWAS) genotyping for the Mayo PGRN-AMPS [32], on the basis of our LCL analysis, we were also able to include additional SNPs in *FKBP5* as well as those that were associated with *FKBP5* expression and were present on the GWAS platform (Fig. 2).

After quality control, genotype–phenotype association analyses for ‘response’ and ‘remission’ during SSRI therapy were carried out with 113 *FKBP5* resequenced SNPs, 14 additional *FKBP5* SNPs present on the GWAS platform, as well as *trans* FKBP5 eQTL SNPs ($P<10^{-4}$), including 227 genotyped in this study together with 127 SNPs present on the GWAS platform (Fig. 1b). We found that the *FKBP5* rs9380524 SNP (A allele) was associated with poor response at both the last visit [$P=0.0249$; odds ratio (OR)=0.65] and after 8 weeks of treatment ($P=0.0175$; OR=0.59) and that the 35758265 SNP (genomic location as no rs number has yet been assigned) was associated with percentage change in QIDS-C after the last visit ($P=0.042$; Table 1). In addition, 22 *FKBP5* SNPs, of which 21 were in the same haplotype block, were associated ($P<0.05$) with better remission at the last visit or after 8 weeks of treatment (OR>1; Table 1). Fifteen trans FKBP5 eQTL SNPs were associated with response at the last visit or after 8 weeks of treatment and/or percentage change in QIDS-C after treatment with *P*-value less than 0.05 (Table 2), and six SNPs were associated ($P<0.05$) with remission at the last visit or after 8 weeks of treatment (Table 2). These *FKBP5* eQTL SNPs were present in 14 different annotated genes, and three SNPs, rs235317, rs17818663, and rs4964463, had a *P*-value less than 0.05 for both phenotypes – that is, remission (last visit or 8 weeks) and response (last visit or 8 weeks). None of the associations were significant after correction for multiple testing.

Sequenced Treatment Alternatives to Relieve Depression replication

On the basis of the results of the initial genotype– phenotype association analysis, we selected six SNPs for inclusion in the replication study. These SNPs were genotyped in 960 WNH DNA samples from the STAR*D study after excluding noncompliant and low baseline Hamilton-D patients. Replication genotyping was followed by association analysis that included phenotypes similar to those included in the analysis of the Mayo PGRN-AMPS sample set. The six SNPs included the three SNPs in *FKBP5* that had the lowest association *P*-values for the response or remission phenotypes (Table 1), rs9380524 (*P*=0.0175 and OR=0.588 for response at 8 weeks), rs34866878 (*P*=0.0194 and OR=3.22 for remission at 8 weeks), and rs16878591 (*P*=0.0194 and OR=3.22 for remission at 8 weeks). rs34866878 and rs16878591 represented a large haplotype, within which 21 SNPs were associated with the remission phenotype (*P*<0.05). We chose two SNPs to represent this haplotype block for the replication genotyping panel because rs34866878 was a coding SNP located in the *FKBP5* exon 10 and rs16878591 was in strong linkage disequilibrium with other SNPs within that block (mean r^2=0.9186). Three SNPs trans-associated with *FKBP5* expression in LCLs with the lowest association *P*-values for SSRI outcomes were also included in the replication study. These three SNPs were rs4964463 (*P*=0.006 and OR=1.69) for remission at the last visit, rs352428 (*P*=0.002 and OR=0.49) for response at 8 weeks, and rs235317 (*P*=0.001 and OR 0.68) for remission at 8 weeks (Table 2). Among the six replication SNPs, only rs352428 had a replicated *P*-value of 0.05 (OR=0.74) for response at 6 weeks (Table 3). This SNP also showed a consistent direction of effect with OR less than 1 for both the PGRN-AMPS and STAR*D sample sets (Supplementary Figure 2, http://links.lww.com/FPC/A572). Supplementary Figure 2 (http://links.lww.com/FPC/A572) shows OR comparisons for the six SNPs selected for the replication study in the two samples sets, with an OR less than 1 indicating an association with poor response at 8/6 weeks of SSRI treatment. The sliding window method was also used to assess a combination of rare and common SNPs genotyped in *FKBP5* and their association with SSRI treatment outcomes (Supplementary Figure 3, http://links.lww.com/FPC/A572). However, no significant findings were observed (the lowest value was *P*=0.124 for remission at 8 weeks).

Functional characterization of rs352428

To characterize possible functional consequences of the rs352428 SNP (Table 3 and Supplementary Figure 2, http://links.lww.com/FPC/A572) that was located in an intragenic region on chromosome 8 between FZD3 (~58 kbp) and EXTL3 (~95 kbp), we performed a series of experiments. The TransFast in-silico transcription factor database suggested that transcription factor C/EBP bound to both A and G alleles for the SNP. However, transcription factor C/EBP bound only to the G allele. We then performed EMSA using two human brainderived cell lines, U-87MG and U251, and a pool of non-brain derived cell lines, lymphoblastoid cells. Fig. 3a shows that nuclear extract binding resulted in a ‘shift’ for the rs352428 variant nucleotide. This difference in nuclear protein binding between WT and variant oligonucleotides was present in all three cell lines. We next performed a reporter gene assay to determine the effect of the SNP on transcriptional activity. This region containing the variant SNP sequence resulted in a more than two-fold reduction in luciferase activity as compared with the WT sequence (Fig. 3b). There was also a reduction in luciferase activity when compared with the vector control. A significant decrease in luciferase activity for a construct carrying the rs352428 variant sequence, as compared with WT, might explain the negative correlation of this variant with *FKBP5* mRNA expression in LCLs (*R*= −0.267), observed during our association analysis. These results suggested not only that the region surrounding the rs352428 SNP is transcriptionally active, but also that the SNP could alter binding to transcription factors. In summary, these functional genomic studies identified and validated one region that potentially functions as a ‘silencer’. By

affecting the expression level of *FKBP5*, this regulatory region might also affect physiological and pharmacological function, in this case response to SSRIs.

Functional characterization of Gly22Arg, Arg154Gln, Val437Phe

Only three NS complementary single nucleotide polymorphisms (cSNPs) were observed during our Sanger resequencing study. Arg22 and Phe437 were observed only in HCA samples, with an MAF of 1%, and Gln154 only in the AA group, with a MAF of 2% (Supplementary Table 2, http://links.lww.com/FPC/A572). Because NS cSNPs have the potential to significantly alter protein function [36], we created expression constructs for WT and variant sequences for the NS cSNPs to determine their possible effect on protein function. Quantitative western blot analysis and quantitative real-time-PCR were performed to determine protein and mRNA expression levels (Supplementary Figure 4a and b, http://links.lww.com/FPC/A572). There were no statistically significant differences between WT and variant allozymes for levels of protein or mRNA expression.

Discussion

To our knowledge, this is the first application of in-depth Next Generation resequencing to study response to the treatment of MDD. The treatment of this disease remains challenging. Both environmental and genetic factors can contribute to MDD treatment outcomes. In the present study, we set out to comprehensively study how genetic variation in *FKBP5* and genome-wide SNPs associated with *FKBP5* expression might play a role in variation in treatment response for MDD. We chose to resequence *FKBP5* because of its involvement in the modulation of Akt activity [5], a pathway known to be important in many behavioral phenotypes and physiological functions in the brain [37–41]. In addition, *FKBP5* is a modulator of GR sensitivity [42], thus playing a role in the activity of the hypothalamic–pituitary–adrenal axis and, therefore, in the response to stress [43]. Moreover, several previous studies have implicated *FKBP5* in stress-related diseases and response to SSRIs [17–20,22, 23,44–52]. However, none of those studies took a comprehensive approach to identify genetic polymorphisms present in the *FKBP5* gene. We systematically resequenced *FKBP5* by both Next Generation and Sanger sequencing to identify SNPs present in the gene, followed by examining their association with SSRI treatment outcomes in depressed patients enrolled in a large clinical trial. SSRI treatment outcomes were assessed using QIDS and Ham-D scores.

Our resequencing study identified 657 SNPs in *FKBP5*, 362 of them novel (Supplementary Table 4, http://links.lww.com/FPC/A572). In addition, in AAs and HCAs, we identified 29 novel SNPs, including three NS cSNPs, by Sanger sequencing (Supplementary Table 2, http://links.lww.com/FPC/A572). In our study, we also took advantage of a genomic data-rich panel of 287 LCLs to identify SNPs that were associated with *FKBP5* gene expression and combined those SNPs with resequenced *FKBP5* SNPs to develop a panel that was used to genotype DNA from Mayo PGRN-AMPS patients treated with SSRIs [32]. Genotype–phenotype association studies for SSRI treatment outcomes in the Mayo PGRN-AMPS cohort patients revealed 24 SNPs within *FKBP5* and 19 SNPs trans-associated with *FKBP5* expression that were associated with SSRI treatment outcomes with *P*-values less than 0.05 (Tables 1 and 2). None of these SNPs were significant after correction for multiple testing. We also applied a sliding window analysis, an analysis that takes correction for multiple rare and common SNPs into account to test for association. It is worth mentioning that rs1360780, rs3800373, and rs4713916, SNPs that have previously been reported to be associated with SSRI treatment outcomes in MDD [19,24,25], were not significantly associated with any of the SSRI treatment phenotypes in our study. One of the reasons why we did not observe an association of rs4713916, an SNP reported in a recent meta-analysis to be associated with SSRI response [25], with any response phenotypes in our study could

be because of the discrepancies between the previous studies and Mayo PGRN-AMPS, including differences in baseline clinical characteristics of patients, which could contribute to our inability to replicate results from these studies.

In our replication study using STAR*D white non-Hispanic samples, only the *FKBP5* eQTL SNP, rs352428, was shown to be associated with response after 6 weeks (P=0.05; Table 3). Although the point of estimate of the effect size in the replication stage is weaker than the effect identified in the discovery stage, which is not unusual as discovery studies tend to have biased effect sizes because of what is known as 'winner's curse', the SNP showed the same OR trend in both studies (OR<1, Supplementary Figure 2, http://links.lww.com/FPC/A572), strongly indicating that it is associated with poor response at 8 or 6 weeks (Mayo PGRN-AMPS or STAR*D sample sets, respectively). rs352428 is an intergenic SNP that maps between *FZD3* and *EXTL3* on chromosome 8p21. Its chromosomal location has been described as a putative locus for the development of schizophrenia [53–55]. On the basis of the microarray database that is available for our LCLs, this SNP was associated with *FKBP5* expression with a P-value of 9.17×10^{-6} (R= −0.267) but was not associated with either *FZD3* or *EXTL3* expression. In addition, the region surrounding this SNP did not encode long noncoding RNA, as evaluated through an in-silico database search (http://www.lncrnadb.org) and through searching a reference catalog of human long noncoding RNA generated by Cabili *et al.* [56], nor did the region encode a validated miRNA. Our functional EMSA and reporter gene assays indicated that the region surrounding rs352428 was not only transcriptionally active but also showed a striking difference between the A and G allele signals (Fig. 3). The exact mechanisms by which this SNP might influence SSRI response through the regulation of *FKBP5* expression will require further studies. However, previous studies have also shown that SNPs resulting in alteration of *FKBP5* expression could contribute to the response to treatment in depressed patients, as reported by Binder *et al.* [18], who found that SNPs that increased the expression of *FKBP5* resulted in a good response to treatment. In our case, rs352428 caused a decreased transcriptional activity and low *FKBP5* expression and resulted in an association with poor response to SSRIs, an observation that is consistent with a previous finding.

The Mayo PGRN-AMPS trial was designed to mirror the initial phase of the STAR*D study using similar enrollment criteria and using the same SSRI drug (citalopram). However, several factors might contribute to differences between the two studies and, therefore, to the lack of reproducibility of results between the two. Particularly, MDD is often a chronic condition that tends to coexist with substantial psychiatric comorbidity and other medical conditions. Our observations highlight the challenges of performing psychiatric genomic research across studies and also suggest that functional genomic validation might provide a complementary strategy to help validate and characterize the functional consequences of any genomic markers identified [31]. In addition, we also acknowledge that we did not have a placebo arm in our Mayo designed trial; therefore, we could not exclude the possibility that the regulatory SNP is a prognostic factor for outcome. Therefore, to replicate our findings in other independent patient cohorts with similar clinical and demographic characteristics would be desired to better estimate the significance of our initial results.

Conclusion

Our study provides insight into the role of common genetic polymorphisms in *FKBP5* that might help predict SSRI treatment response in depression. Further, we evaluated both genetic variants in *FKBP5* itself and trans-SNPs associated with *FKBP5* expression and their effect on gene transcription. Finally, additional in-depth functional genomic studies are needed to determine their role in SSRI response mechanisms.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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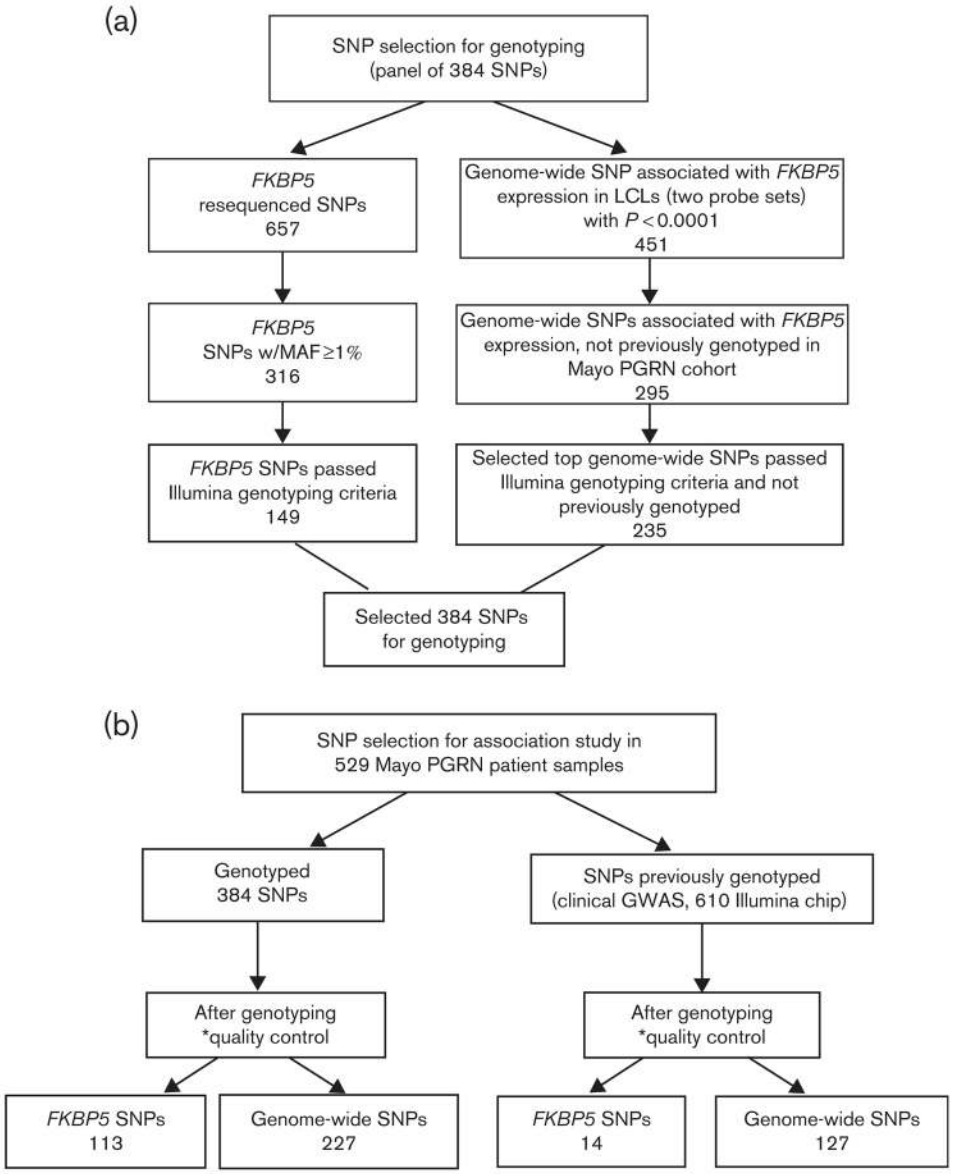

Fig. 1.

SNP selection for association analysis. The flow chart outlines the selection criteria for SNPs used for the genotyping and association studies. Genome-wide SNPs are *FKBP5* eQTLs. *SNP level quality control. SNPs selected for an association analysis had to meet the quality control measures: minor allele frequency >0.01, per SNP call rate>0.95, and Hardy–Weinberg equilibrium *P*-value>0.001. eQTLs, expression Quantitative Trait Locus; GWAS, genome-wide association study; LCLs, lymphoblastoid cell lines; MAF, minor allele frequency; Mayo PGRN, Mayo Clinic Pharmacogenomics Research Network; SNPs, single nucleotide polymorphisms.

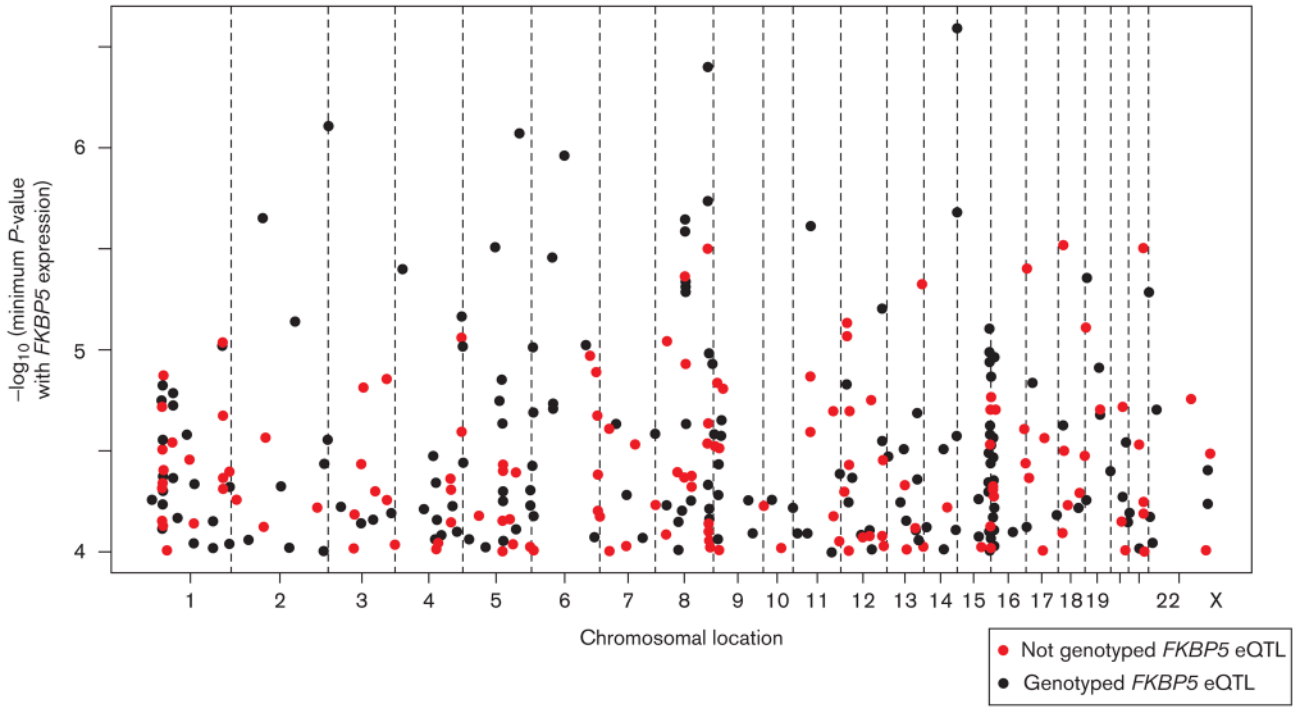

Fig. 2.

Graphical representation of the 'genome-wide' trans *FKBP5* expression Quantitative Trait Locus (eQTL) single nucleotide polymorphism (SNPs; $P<10^{-4}$). SNPs genotyped in this study are shown as red dots, whereas black dots were obtained from a genome-wide association study of these samples.

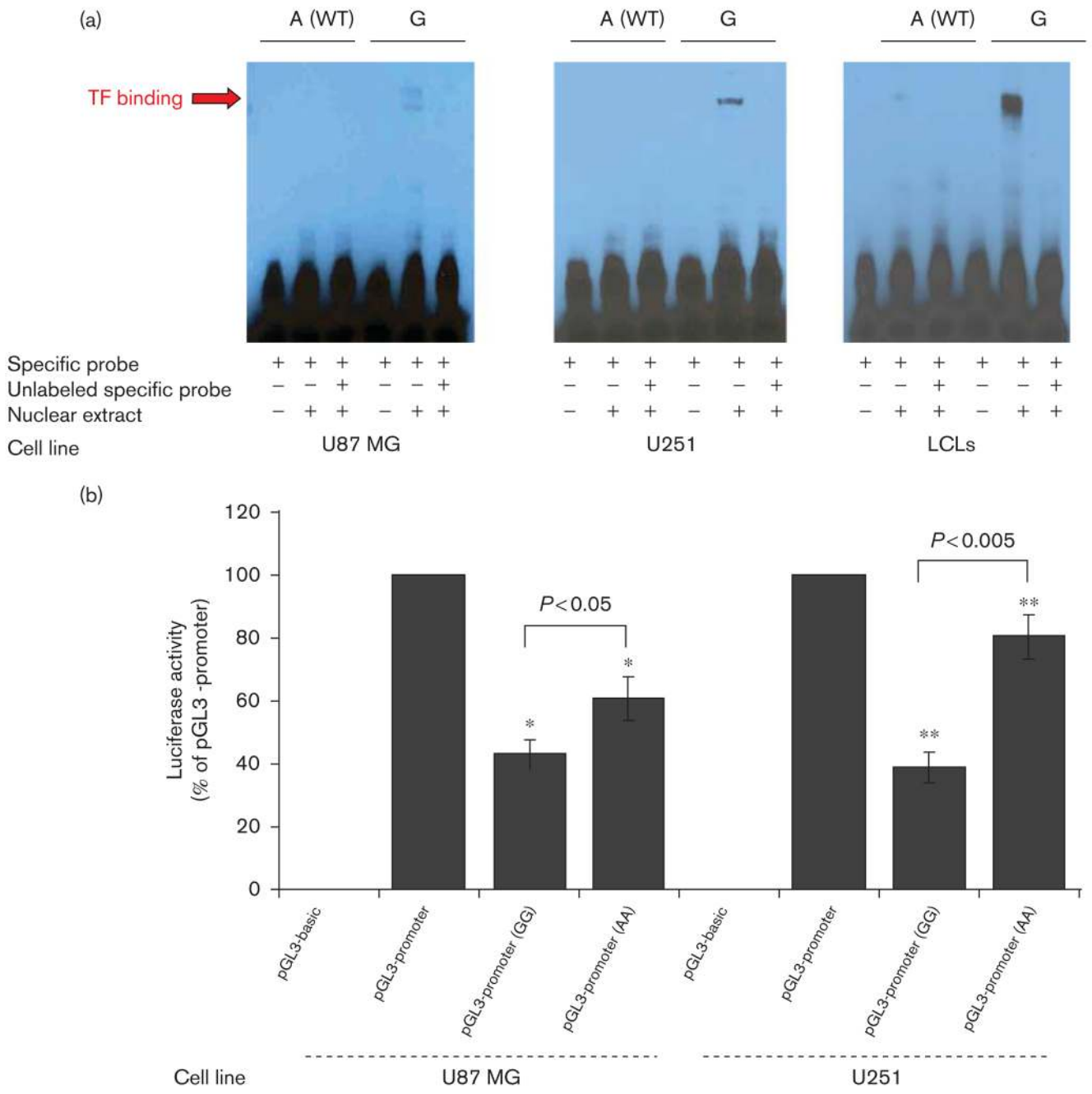

Fig. 3.

Functional characterization of the rs352428 single nucleotide polymorphism (SNP). (a) Electrophoretic mobility shift assays (EMSAs) with nuclear extract prepared from U87-MG, U251, and a pool from lymphoblastoid cells. A different binding pattern was observed between wild-type (WT) and variant sequences in each case. The arrow indicates the band that was observed with the variant but not the WT sequence. (b) Results from dual luciferase reporter gene assays performed in U87-MG and U251 glioblastoma cell lines. Error bars for each construct represent the average of relative luciferase activity calculated as a % of the pGL3-promoter construct activity obtained during six independent transfections (mean

±SEM). * and ** represent *T*-test *P*-values for comparing values of pGL3-promoter (AA) and pGL3-promoter (GG) activity. LCLs, lymphoblastoid cell lines; TF, transcription factor.

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Table 1

Top *FKBP5* SNP association results ($P < 0.05$) for response, remission, and percentage change in QIDS-C after SSRI treatment (on the basis of logistic regression adjustment for four eigen vectors)

			Response (last visit)		**Response (8 weeks)**		**Percentage change in QIDS**	
***FKBP5* SNP**	**Location**	**Position**	**OR (95% CI)[a]**	***P*-value[a]**	**OR (95% CI)[a]**	***P*-value[a]**	**Spearman[a]**	***P*-value[a]**
FKBP5 SNPs associated with the response outcome and percentage change in QIDS-C after SSRI treatment ($P < 0.05$)								
rs9380524	Intron 3	35589070	0.65 (0.44–0.95)	0.025	0.59 (0.38–0.91)	0.02	NS	NS
35758265	Intron 1	35650287	NS	NS	NS	NS	0.09	0.042
			Remission (last visit)		Remission (8 weeks only)			
FKBP5 SNP	Location	Position	OR (95% CI)[a]	*P*-value[a]	OR (95% CI)[a]	*P*-value[a]		
FKBP5 SNPs associated with the remission outcome after SSRI treatment ($P < 0.05$)								
35658181	Intron 8	35550203	2.34 (0.99–5.54)	0.05	3.22 (1.13–9.17)	0.02		
35660167	Intron 8	35552189	2.34 (0.99–5.54)	0.05	3.22 (1.13–9.17)	0.02		
35673999	Intron 5	35566021	2.34 (0.99–5.54)	0.05	3.22 (1.13–9.17)	0.02		
35678460	Intron 5	35570482	2.34 (0.99–5.54)	0.05	3.22 (1.13–9.17)	0.02		
35686829	Intron 5	35578851	2.34 (0.99–5.54)	0.05	3.22 (1.13–9.17)	0.02		
35689604	Intron 5	35581626	2.34 (0.99–5.54)	0.05	3.22 (1.13–9.17)	0.02		
rs11966198	Intron 5	35575656	2.34 (0.99–5.54)	0.05	3.22 (1.13–9.17)	0.02		
rs16878806	**Intron 5**	**35569119**	**2.34 (0.99–5.54)**	**0.05**	**3.22 (1.13–9.17)**	**0.02**		
rs16879318	Intron 3	35590391	2.34 (0.99–5.54)	0.05	3.22 (1.13–9.17)	0.02		
rs28675670	Intron 3	35601529	2.34 (0.99–5.54)	0.05	3.22 (1.13–9.17)	0.02		
rs34866878	**Exon 10**	**35544942**	**2.34 (0.99–5.54)**	**0.05**	**3.22 (1.13–9.17)**	**0.02**		
rs41270080	3 UTR	35542045	2.34 (0.99–5.54)	0.05	3.22 (1.13–9.17)	0.02		
rs45586932	Intron 10	35543995	NS	NS	3 (1.04–8.61)	0.03		
rs59320339	Intron 8	35550915	NS	NS	3 (1.04–8.61)	0.03		
rs16878591	Intron 8	35552627	NS	NS	3 (1.04–8.61)	0.03		
35692412	Intron 5	35584434	NS	NS	3 (1.04–8.61)	0.03		
35702644	Intron 3	35594666	NS	NS	3 (1.04–8.61)	0.03		
rs12110366	Intron 2	35610340	NS	NS	2.98 (1.04–8.56)	0.03		
35672403	Intron 6	35564425	NS	NS	3.21 (1.13–9.14)	0.03		
35751398	Intron 1	35643420	NS	NS	3.46 (1.09–11)	0.04		

			Response (last visit)		Response (8 weeks)		Percentage change in QIDS	
***FKBP5* SNP**	**Location**	**Position**	**OR (95% CI)[a]**	***P*-value[a]**	**OR (95% CI)[a]**	***P*-value[a]**	**Spearman[a]**	***P*-value[a]**
rs2092427	Intron 1	35622207	NS	NS	2.99 (1.04–8.61)	0.05		
35696209	Intron 3	35588231	NS	NS	3.22 (1.13–9.17)	0.02		

Highlighted in bold are the SNPs selected for replication study.

CI, confidence interval; OR, odds ratio; QIDS-C, Quick Inventory of Depressive Symptomatology-Clinician Rated; SNP, single nucleotide polymorphism; SSRI, selective serotonin reuptake inhibitor.

[a]Adjusted for population stratification.

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Table 2

Top *FKBP5* eQTL ($P < 0.05$) association results for response, remission, and percentage change in QIDS-C after SSRI treatment (on the basis of logistic regression adjustment for four eigen vectors)

				Response (last visit)		**Response (8 weeks)**		**% change in QIDS-C**	
rs SNP	**Chr.**	**Annotated gene**	**Position**	**OR (95% CI)**	***P*-value**	**OR (95% CI)**	***P*-value**	**Spearman**	***P*-value**
FKBP5 eQTLs associated with the response outcome and percent change in QIDS-C after SSRI treatment ($P < 0.05$)									
rs11045870	12	*SLCO1B1*	21371075	0.71 (0.51–0.98)	0.034	NS	NS	NA	NS
rs11045871	12	*SLCO1B1*	21372224	0.71 (0.51–0.98)	0.034	NS	NS	NA	NS
rs235317	**21**	***PTTG1P***	**46275047**	**0.76(0.58–0.99)**	**0.041**	**0.64 (0.47–0.87)**	**0.005**	NA	NS
rs7960384	12	–	21395908	0.72 (0.52–1.00)	0.049	NS	NS	NA	NS
rs4964463	12	*POLR3B*	106789188	1.58 (1.07–2.32)	0.021	1.64 (1.02–2.62)	0.040	0.080	0.006
rs4603275	11	*CNTN5*	98320413	0.73 (0.54–0.99)	0.041	NS	NS	NA	NS
rs6595125	5	*DTWD2*	117748700	1.31 (1.02–1.68)	0.038	1.38 (1.02–1.86)	0.038	NA	NS
rs7173166	15	*BNC1*	83934748	NS	NS	0.68 (0.50–0.94)	0.021	NA	NS
rs8027193	15	*HDGFRP3*	83879618	NS	NS	0.67 (0.51–0.97)	0.027	NA	NS
rs4341707	15	*BNC1*	83927857	NS	NS	0.71 (0.52–0.98)	0.039	NA	NS
rs17818663	9	–	13833407	NS	NS	0.65 (0.43–0.98)	0.043	NA	NS
rs352428	**8**	***FZD3***	**28478892**	**NS**	**NS**	**0.49 (0.32–0.76)**	**0.002**	**NA**	**NS**
rs10965529	9	*DMRTA1*	22943509	0.75 (0.58–0.98)	0.031	0.66 (0.48–0.90)	0.008	NA	NS
rs11078539	17	*PLD2*	4715977	NS	NS	NS	NS	– 0.103	0.020
rs2784113	1	*PTPRC*	198773703	NS	NS	0.71 (0.50–0.99)	0.046	NA	NS
				Remission (last visit)		Remission (8 weeks)			
rs SNP	Chr.	Annotated gene	Position	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value		
FKBP5 eQTLs associated with the remission outcome after SSRI treatment ($P < 0.05$)									
rs235317	**21**	***PTTG1P***	**46275047**	**0.74 (0.56–0.97)**	**0.029**	**0.68 (0.50–0.91)**	**0.001**		
rs17818663	9	–	13833407	NS	NS	0.64 (0.43–0.96)	0.030		
rs6502015	17	*TBCD*	80888164	NS	NS	1.41 (1.03–1.94)	0.033		
rs4964463	**12**	***POLR3B***	**106789188**	**1.69 (1.16–2.45)**	**0.006**	**1.67 (1.1–2.53)**	**0.016**		
rs11650232	17	*DLG4*	7088923	1.29 (1.00–1.67)	0.047	NS	NS		
rs1479957	3	*MAGI1*	65228662	NS	NS	1.65 (1.06–2.57)	0.026		

Highlighted in bold are the SNPs selected for replication study.

CI, confidence interval; eQTLs, expression quantitative trait locus; OR, odds ratio; QIDS-C, Quick Inventory of Depressive Symptomatology-Clinician Rated; SNP, single nucleotide polymorphism; SSRI, selective serotonin reuptake inhibitor.

Table 3

Replication study

	Last visit					**6 weeks**				
	MAF					**MAF**				
rs SNP	**Nonresponders**	**Responders**	***n***	**OR (95% CI)**	***P*-value**	**Nonresponders**	**Responders**	***n***	**OR (95% CI)**	***P*-value**
SNPs associated with the response outcome after SSRI treatment in STAR*D										
rs16878591	0.04	0.04	930	0.87 (0.58–1.31)	0.50	0.05	0.04	815	0.87 (0.57–1.32)	0.51
rs235317	0.32	0.32	933	0.98 (0.8–1.19)	0.80	0.32	0.32	816	1.00 (0.81–1.24)	0.97
rs34866878	0.03	0.02	917	0.76 (0.44–1.33)	0.33	0.03	0.02	802	0.78 (0.43–1.42)	0.42
rs352428	**0.13**	**0.10**	**928**	**0.77 (0.58–1.02)**	**0.07**	**0.14**	**0.10**	**812**	**0.74 (0.54–1.00)**	**0.05**
rs4964463	0.13	0.14	926	1.08 (0.82–1.41)	0.59	0.14	0.14	813	1.02 (0.76–1.35)	0.91
rs9380524	0.11	0.09	923	0.83 (0.62–1.11)	0.21	0.11	0.09	808	0.85 (0.62–1.18)	0.33
	Last visit					6 weeks				
	MAF					MAF				
rs SNP	Remitters	Nonremitters	*n*	OR (95% CI)	*P*-value	Remitters	Nonremitters	*n*	OR (95% CI)	*P*-value
SNPs associated with the remission outcome after SSRI treatment in STAR*D										
rs16878591	0.04	0.04	933	0.97 (0.64–1.47)	0.89	0.04	0.04	815	0.98 (0.64–1.49)	0.91
rs235317	0.31	0.33	936	1.10 (0.90–1.35)	0.35	0.31	0.33	816	1.11 (0.89–1.37)	0.36
rs34866878	0.03	0.02	920	0.57 (0.31–1.06)	0.06	0.03	0.02	802	0.61 (0.32–1.16)	0.12
rs352428	0.13	0.10	931	0.79 (0.59–1.07)	0.13	0.13	0.10	812	0.74 (0.54–1.02)	0.06
rs4964463	0.14	0.14	929	0.98 (0.75–1.28)	0.87	0.15	0.13	813	0.91 (0.68–1.21)	0.52
rs9380524	0.11	0.10	926	0.89 (0.66–1.21)	0.45	0.10	0.10	808	0.93 (0.67–1.28)	0.65

Highlighted in bold is the SNP that shows the lowest *P*-value for both last visit and 6 weeks response.

Selected SNPs were genotyped in STAR*D white non-Hispanic patient samples (excluding low baseline Ham-D patients) and associated with SSRI treatment outcomes, including response and remission.

CI, confidence interval; MAF, minor allele frequency; OR, odds ratio; SNP, single nucleotide polymorphism; SSRI, selective serotonin reuptake inhibitor; STAR*D, Sequenced Treatment Alternatives to Relieve Depression.