RELATIONSHIP BETWEEN FKBP5 POLYMORPHISMS AND DEPRESSION SYMPTOMS AMONG KIDNEY TRANSPLANT RECIPIENTS

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Background: Several polymorphisms in FK506 Binding Protein gene (FKBP5) and a history of child abuse have been shown to be associated with an increased risk for posttraumatic stress disorder (PTSD). It has also been demonstrated that the same polymorphisms of FKBP5 are associated with increased recurrence of depressive episodes and rapid response to antidepressant treatment. However, there are only limited numbers of studies replicating the polymorphisms as vulnerability factors for the development of mental illnesses, such as PTSD and depression after stressful life event, especially with a specific incidence, such as kidney transplant surgery.

Methods: A retrospective analysis was conducted using the electronic medical records of 131 adult kidney transplant recipients. Depression severity after kidney transplantation was measured by PHQ-9, and stored blood was genotyped for variants in the Serotonin Transporter (SLC6A4), Brain-Derived Neurotrophic Factor, Catecholamine-O-Methyltransferase, Corticotropin-Releasing Hormone Receptor, and FKBP5 genes. Spearman correlations were used to test for association between genetic variants and depression severity.

Results: The rare alleles at three out of four SNPs in FKBP5 (rs1360780, rs9296158, and rs9470080) were associated with increased PHQ-9 scores (P < .05), whereas the last FKBP5 SNP (rs3800373) showed a trend of association (P < .10). All four FKBP5 SNPs are in strong linkage disequilibrium. Although in a subgroup of Caucasian non–Hispanic subjects the association was not statistically significant, the direction of association was consistent with that observed in the entire sample as well as in previous studies. Polymorphisms in genes other than FKBP5 were not associated with PHQ-9 scores.

Conclusions: Polymorphisms in FKBP5 may be associated with higher depression scores in kidney transplant recipients.

Key words: depression; FKBP5; kidney transplant; stress; vulnerability

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INTRODUCTION

The psychiatric research community has been investing a substantial amount of effort in the investigation of interactions between genes and stressful environment with the aim of better understanding the pathophysiology of mental illnesses, such as depression and posttraumatic stress disorder (PTSD). The promoter region polymorphism (5HTTLPR) of the serotonin transporter gene (SLC6A4) is the most studied gene in this context. The original landmark research conducted by Caspi et al. has been replicated in many other studies in different contexts, such as different age groups (child, young adult, general adult, and elderly), different time frames, and various stressful environmental factors ranging from remote past history of child abuse to recent medical burdens, such as hip fracture, stroke, and acute myocardial infarction. Although there are many corroborating studies, a recent meta-analysis reported negative results of such Gene × Environment interaction for 5HTTLPR and stress. This suggested the need to investigate other candidate genes in addition to 5HTTLPR or SLC6A4 as vulnerability factors.

Other candidate genes that have been reported to show interactions between genotype and stressful environment include Brain-Derived Neurotrophic Factor (BDNF), Catecholamine-O-Methyltransferase (COMT), Corticotropin-Releasing Hormone Receptor (CRHR1), and FK506 Binding Protein (FKBP5). Among them, FKBP5 and CRHR1 are related to the hypothalamus–pituitary–adrenal (HPA) axis function, which emphasizes an important role of the HPA axis in Gene × Environment interaction and stress reactivity.

It has been demonstrated that several polymorphisms of FKBP5 are associated with increased recurrence of depressive episodes and rapid response to antidepressant treatment. With regard to the Gene × Environment interaction, subjects with the same polymorphisms in FKBP5 and a history of child abuse have been reported to be at an increased risk for PTSD; although when there was no history of child abuse, no difference in the risk of PTSD from any trauma, including trauma in later life, was observed across the genotype groups. Recently, another study showed a similar effect of Gene × Environment interaction between the same polymorphisms of FKBP5 gene and child abuse history associated with a risk of PTSD in an African American population. Also, the same polymorphisms showed an association with suicide risk either as a main effect or as a Gene × Environment interaction effect. These recent data support FKBP5 as a promising candidate gene for vulnerability to the development of mental illnesses, such as PTSD and depression, although further research is required to confirm these findings.

In addition to the problem of identifying promising candidate genes for risk of mental illnesses, such as PTSD and depression, another challenge in studying the interaction between a gene and stressful environmental factor stems from the heterogeneity of the environmental factors, especially if such events happened in the remote past. For example, child abuse history in the past may have different influence at the time of evaluation if assessed decades later, based on the additional environmental experience among individuals. From that standpoint, finding a population with a universal experience as their environmental factor is an important strategy. Medical conditions are often a source of stress, and organ transplant is definitely one of them with an ongoing stress due to the necessity for long-term care even after successful transplant surgery. There is also the stress of receiving an organ from another individual whether alive or deceased. Thus, certain patient population with specific medical condition would be good subjects to investigate the influence of relatively uniform stressful experience. For example, similar approach was used in a study recently published in J Neurovirology (PMID: 20726698), with a small HIV sample (N = 57) reporting marginal associations between depression symptom scales measured by Beck Depression Inventory and several FKBP5 SNPs.

Depression occurs frequently in patients following kidney transplant. In one study, the rate of depression after transplantation was as high as the matched subjects receiving dialysis. Depression after transplant has been associated with an increased risk of graft failure, return to dialysis, and death with a functioning graft. This makes kidney transplant recipients a good population for investigating the effect of candidate genes as depression vulnerability factors, given the relatively homogenous stressful life environment. However, to our knowledge, there is no previous research investigating this population to identify genetic risk factors for mental illness following transplant. The aim of this study was to identify genetic factors that relate to increased vulnerability to higher depression scores in relation to an index event—kidney transplant.

SUBJECTS AND METHODS

PARTICIPANTS

This study was approved by the Mayo Foundation Institutional Review Board. Most of the kidney recipients at Mayo Clinic, Rochester, provided informed consent for future use of their medical records and their stored biospecimen samples for research. They have been thoroughly evaluated both from the medical and psychiatric standpoint. The Patient Health Questionnaire (PHQ-9) depression questionnaires have been completed at the time of clinical evaluation visits since 2006. A retrospective chart review was conducted for 365 adult patients (age 19–82 years old, mean age 50.9 years old) who have

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received a kidney transplant during 2006 and 2007. One hundred and thirty-four subjects met the inclusion criteria: consent for use of their medical records for research, stored biospecimen samples available for research use including genotyping, and PHQ-9 scale scores postkidney transplant. Successful genotyping was possible in 131 patients who are included in the analysis.

GENOTYPING

Methods of genotyping. DNA extraction from the stored blood of the kidney transplant recipients was done by the column extraction method at the Biospecimens Accessioning Processing Laboratory at Mayo Clinic, Rochester. Genotyped polymorphisms included the promoter polymorphisms (5HTTLPR: “long” versus “short” allele), rs25331 (“long_A (LA)” versus “long_G (LG)” allele), and the intron 2 variable number tandem repeat polymorphism (Stin 2 VNTR) of the Serotonin Transporter gene (SLC6A4). Ten single nucleotide polymorphisms (SNPs) in other genes were also genotyped: BDNF (rs4680), COMT (rs6265), CRHR1 (rs110402, rs242924, rs7209436, and rs4792887), and FKBP5 (rs1360780, rs3800373, rs9296158, and rs9470080). For SLC6A4, the promoter region polymorphism and the intron 2 VNTR were genotyped using a combination of fragment analysis, sequencing, and restriction fragment length polymorphism analysis at the Psychogenomics Laboratory, Mayo Clinic, Rochester. All other SNPs were genotyped at the Mayo Clinic Genomic Shared Resources by TaqMan assay.

DEPRESSION SYMPTOM MEASUREMENT

As part of the routine clinical care, depression symptoms were measured after kidney transplant surgery using PHQ-9. The PHQ-9 was completed by patients in scannable forms that are imported automatically to the medical record. These forms were provided to the patient at each visit to the Transplant Center during the kidney transplant evaluation and at the 1-year posttransplant protocol visit.

DATA ANALYSIS

Data from 131 kidney transplant recipients that were successfully genotyped were used to assess the association between genetic variation and depression following kidney transplant. Before association testing, a test of departure from Hardy Weinberg Equilibrium was performed for each polymorphism. For tests of association between genotype and depression scores, posttransplant PHQ-9 score was the primary dependent variable. Pretransplant PHQ-9 was not assessed as an outcome or included as a covariate in analysis of posttransplant PHQ-9 scores, due to unavailability of pretransplant depression scores for a large proportion of subjects.

Kruskal–Wallis tests and Spearman correlations were used to examine the relationship between each genotype and posttransplant PHQ-9 depression score. The independent variables were the genotypes for each polymorphism, coded as 0, 1, 2 representing the number of minor alleles. The Kruskal–Wallis test provides a general test of genotype effects on PHQ-9 scores, whereas the Spearman correlation test treats the genotype (minor allele count) as a quantitative variable, and thus assumes that phenotypes of heterozygous individuals are intermediate between the two types of homozygotes. The Spearman correlation analysis, therefore, provides a trend test for allele effects. Age and sex were not associated with PHQ-9, and therefore were not used as covariates in the analysis. The variable representing days since transplant was also not associated with the quantitative PHQ-9 score, but there was a marginal evidence of association of a dichotomized PHQ-9 score (based on a cut-point of 5) with days since transplant (P = .08). Therefore, in addition to the Spearman correlation analyses to test for the association between genotypes and PHQ-9, we also performed exploratory regression analyses to assess the relationships between genotypes and PHQ-9, either with or without adjustment for days in the study. As the results of adjusted analyses did not differ substantially from the unadjusted analyses, these results are not shown.

In addition to separate analyses of each polymorphism, SLC6A4 5HTTLPR and rs25331 genotypes were analyzed together based on the tri-allelic system (L_A, L_G, S). Finally, for the three genes with multiple genotyped variants (SLC6A4, CRHR1, and FKBP5), haplotype analyses were performed using score tests proposed by Schaid et al.[26]

RESULTS

DEMOGRAPHIC CHARACTERISTICS OF KIDNEY TRANSPLANT RECIPIENTS

The 131 kidney transplant recipients included in this study ranged in age from 19 to 78 years old at the time of transplant, with an average age of 49.4; 67 (51.1 %) were male and 64 (48.9%) female. The sample was predominantly non-Hispanic Caucasian (90.7%). During the duration of the study, two kidney allografts failed due to causes other than patient death and two study subjects died with a functioning graft.

GENOTYPING

The SLC6A4 intron 2 VNTR was successfully genotyped for all 131 subjects, whereas the promoter variant genotyping (5HTTLPR and rs25331) failed in one subject. Due to insufficient DNA samples, 8 of the 131 subjects were not genotyped for the remaining 10 SNPs using TaqMan. After excluding those subjects, SNP rs9470080 had a call rate of 86%, whereas all other SNPs had call rates >97.5%. The median SNP call rate

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was 99%. No departures from Hardy–Weinberg Equilibrium were detected.

DEPRESSION SCALE MEASURED BY PHQ-9

The mean posttransplant PHQ-9 score was 4.9 (SD: 4.6, median: 4.0, range: 0–22). The PHQ-9 was given an average of 396 days after transplant (SD: 87, median: 372, range: 247–883). PHQ-9 total score for the nine items ranges from 0 to 27. Scores of 5, 10, 15, and 20 represent cut-points for mild, moderate, moderately severe and severe depression, respectively. Thus, the median PHQ-9 score of this sample is close to the cut-point for the mild level of PHQ-9 depression scale, indicating that a little more than half the subjects remain in the range of no depression, whereas the remaining subjects showed at least a mild level of depression.

RELATIONSHIP BETWEEN EACH GENOTYPE AND DEPRESSION SCALE

Analysis of the entire sample of subjects suggested that the FKBP5 rare alleles at three out of four SNPs in FKBP5 (rs1360780, rs9296158, and rs9470080) were associated with increased PHQ-9 scores (uncorrected \( P \leq .05 \)), whereas the last FKBP5 SNP (rs3800373) showed a trend of association (\( P < .10 \)) (Table 1). These four SNPs are in strong linkage disequilibrium (Fig. 1). Polymorphisms in genes other than FKBP5 were not associated with postkidney transplant PHQ-9 scores.

Further analysis of the subset of non-Hispanic Caucasian subjects did not confirm a significant association of FKBP5 SNPs with PHQ-9. However, a trend in the same direction was observed for all the FKBP5 SNPs, with the rare alleles being associated with higher PHQ-9 scores; for example, for rs9470080, whereas in the entire cohort the mean PHQ-9 scores were 3.9, 6.0, and 5.1 for genotypes CC, CT, and TT, respectively (\( P = .045 \)). For the subset of Caucasian patients, the mean scores in these three genotype groups were 3.8, 4.8, and 5.4 (\( P = .093 \)).

Analysis of the tri-allelic L\( _\text{O} \)/L\( _\text{C} \)/S SLC6A4 genotypes did not reveal a significant association with PHQ-9 scores. Haplotype analyses did not reveal significant global haplotype effects for SLC6A4, CRHR1, or FKBP5. However, consistent with our single-SNP analyses, the FKBP5 haplotype C-A-T-T (SNPs rs3800373, rs9296158, rs1360780, rs9470080) was marginally associated with higher PHQ-9 scores (simulation \( P \)-value = .049, not corrected for multiple haplotypes tested).

DISCUSSION

This is the first study investigating depression vulnerability genes in kidney transplant recipients. The role of FKBP5 as a mental illness susceptibility gene in the context of stress was previously reported.[11] However, there are only a limited number of studies replicating the polymorphisms as vulnerability factors for the development of psychiatric conditions, such as depression and suicide attempt after a stressful life event.[18–20]

The direction of the associations presented here is consistent with the previous report,[11] although the data is preliminary due to several limitations discussed below. The rare alleles at the investigated FKBP5 SNPs are associated with higher PHQ-9 in our study, and those were the SNPs shown to be associated with increased PTSD after multiple exposures to stressful life events in the original report.[11]

FKBP5 gene polymorphisms have been investigated in the context of various other aspects related to mental illnesses, such as depression,[15] PTSD,[11] and stress response.[27] The SNPs investigated here have also been shown to be associated with better response to antidepressants with different mechanism of action, including tricyclic antidepressants, selective serotonin reuptake inhibitors, and mirtazapine.[15] Although there is a recent report that failed to identify the FKBP5 polymorphisms related to antidepressants response,[28] these associations were replicated in several studies,[16,17] including the Sequenced Treatment Alternatives to Relieve Depression study. At the same time, those SNPs were associated with more depressive episodes in the original study.[15]

FKBP5 is a chaperon protein related to the glucocorticoid receptor complex. After glucocorticoid attaches to the receptor, FKBP5 is replaced by FKBP4, which enables the receptor complex to attach to DNA to initiate transcription.[11,29] Because of its function, response to HPA axis and its final product of glucocorticoid might be well affected by functional polymorphisms of FKBP5. As mentioned above, individual difference of HPA axis response after stress was shown to depend on the FKBP5 genotype,[27] which supports this possibility.

Of further interest, we note that FKBP5 is a protein originally identified for its role related to an immunosuppressant, FK506 (Prograf® also Tacrolimus or Fujimycin). FK506, or Tacrolimus, is widely used after an allogeneic solid organ transplant, including kidney transplant. In fact, almost all our study subjects were taking FK506 after kidney transplant. We aimed to measure the impact of several genetic variants in the context of the stressful medical burden of kidney transplantation, and observed an association with FKBP5 polymorphisms consistent with previous reports related to stress vulnerability. However, there is a possibility that the immunosuppressant medication, FK506, may have played a role in the mood status of the patients, and due to its functional polymorphism, different FKBP5 genotype groups might have had different PHQ-9 scores.

The reason why FKBP5 genotype can play a role as vulnerability factor to mental illness with exposure to stressful life event is not clear. FKBP5 may be related to genes associated with inflammatory factors, such as...
vascular endothelial growth factor (VEGF) and tumor necrosis factor (TNF)-. Chen et al. reported that the T allele of rs1360780 (one of the FKBP5 SNPs studied in this study) forms a haplotype with high inflammatory genotypes from VEGF and TNF-, which were, in turn, associated with acute and chronic rejection after kidney transplant.\[30\]

As shown in Figure 2, FKBP5 may have two clinically relevant features: one as a vulnerability factor and another as a factor associated with cytokines/proteins related to high inflammation. As shown in the original work by Binder et al. and recent reports, including this study, FKBP5 genotype is associated with the development of mental illness and related conditions, such as depression, PTSD, and suicide attempts.\[11,18–20\] Depression has been reported to be associated with HPA axis dysregulation,\[31,32\] which can interfere with transplant outcome, as almost always glucocorticoids are given with the posttransplant treatment. The role of cytokines in the pathophysiology of mental illness has also been reported.\[33\] and such high inflammatory status may lead to higher risk for rejection after

### Table 1. Univariate associations between posttransplant PHQ-9 total score and genotypes

<table>
<thead>
<tr>
<th>Variant</th>
<th>Genotype</th>
<th>Analysis of entire cohort</th>
<th>Analysis of Caucasian subset</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>N</td>
<td>Mean (SD)</td>
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<tr>
<td>SLC6A4</td>
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<td>44</td>
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<td></td>
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<td>5.0 ± 5.4</td>
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<td></td>
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<td>22</td>
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<td>10/12</td>
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<td>4.6 ± 4.5</td>
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<tr>
<td></td>
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<td></td>
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<td>AG</td>
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<td>GG</td>
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*Genotype P-values are based on the Kruskal–Wallis test of differences in PHQ-9 scores between genotype groups.

**Additive test P-values are based on Spearman correlations between PHQ-9 scores and genotypes coded as (0,1,2) representing the number of minor alleles.

***For the SLC6A4 Intron 2 VNTR, the rare 9-repeat alleles were grouped with the 10-repeat alleles.
transplant. These interrelationships suggest that if certain genotypes of \textit{FKBP5} have strong associations with high inflammatory factors, high inflammation itself can be a mechanism contributing to not only transplant rejection, but also to the development of psychiatric disorders, such as depression.

Also, when kidney transplant recipients are depressed, they may become nonadherent to immunosuppressants and other necessary medications, which can further lead to higher risk for rejection. Furthermore, if rejection happens that itself is an additional stressful event in the life of the transplant recipients, which can lead to more depression. In addition, treatment for an episode of rejection may require higher doses of corticosteroids, which are also known to affect mood. These complex interrelationships between vulnerability to mental illness, high inflammation, and transplant rejection require further investigation.

Other genes studied here (\textit{SLC6A4}, \textit{BDNF}, \textit{COMT}, and \textit{CRH1}) have also been reported to be associated with risk of depression upon exposure to stressful life events. However, the data presented here did not provide statistically significant evidence of such association. Apparently we did not genotype all possible SNPs in this study. For example, only one SNP was genotyped in \textit{BDNF} and only one SNP in \textit{COMT}, which could limit our findings because there might be associations with other polymorphisms of other genes studied. Understanding the reason for these negative findings requires careful consideration, and we acknowledge the following limitations. First, the sample size of this study is small, which limits the power to detect genotype effects. Second, due to limited availability of pretransplant PHQ-9 scores, there was no comparison of change in PHQ-9 (pre- to posttransplant), which could have provided insight into genetic contributors to changes in depression severity after transplant surgery.

![Figure 1. Linkage disequilibrium (LD) plot of the four SNPs in \textit{FKBP5} gene. They are in strong LD and the LD is depicted as r².](image)

![Figure 2. A hypothetical view of the different aspects of \textit{FKBP5} gene. One in the context of vulnerability for the increased risk of depression and PTSD upon exposure to a stressful life event (described in lower side of the figure) and another in the context of high inflammation, leading to the higher risk for rejection after organ transplant (described in upper side of the figure).](image)
Third, the depression scores measured by PHQ-9 do not necessarily indicate that subjects were suffering from specific mental illness, such as major depressive disorder. Given the low average PHQ-9 score (Ave = 4.9), the majority of scores were not in a range that suggests a diagnosis of major depressive disorder. The scale scores could be largely influenced by a subgroup of symptoms, other than depressed mood and/or anhedonia, including insomnia, fatigue, poor appetite, and low concentration, which could result from many medical conditions other than depression. For example, immunosuppressants can be the cause of increased fatigue leading to an increased PHQ-9 score. However, the medications that subjects were taking were not controlled or assessed in this preliminary study, although all patients were on immunosuppressants after transplant surgery.

Fourth, although we aimed to identify genetic factors that contribute to vulnerability and resiliency to stress, the phenomenon of stress response is very complex. Even the effect of the most well studied polymorphism, the serotonin transporter gene (SLC6A4, 5HTTLPR long versus short allele,[1,3–5] has been challenged by a recent meta-analysis with a negative result about its role in Gene × Environment interaction.[10] Criticisms of individual replication studies and the meta-analysis include the great variability in assessment of stressful life events. Here, to reduce the heterogeneity in types of stress and the complexity of assessing stress, we identified a group of subjects that had recently experienced the same stressful event (kidney transplant). However, it is worth noting that a successful kidney transplant can be a positive experience compared to the chronic treatment with dialysis that most kidney transplant recipients have gone through. Therefore, even in this cohort with a homogeneous experience, heterogeneous levels of stress may have been experienced.

Because there is a potential for false positives due to population stratification effects in the entire sample, we also conducted analysis for the non-Hispanic Caucasian subset. Results were consistent with those obtained for the entire sample but not significant, possibly because of inadequate power in this subset due to the even smaller sample size. Finally, the presented results were not corrected for multiple comparisons due to the nature of this preliminary study. Replication with larger sample size is needed for confirmation of the findings.

**CONCLUSION**

These preliminary data may support previous reports and suggest that polymorphisms in FKBPS may be associated with higher depression scores measured by PHQ-9 in kidney transplant recipients, although due to the limitations of this study discussed here, this result requires careful interpretation. Further research is needed to confirm this preliminary finding. If supported with a prospective study with larger sample size, these genotypes can be considered as one of many genetic factors associated with the individual differences for stress vulnerability and resiliency for mental illnesses, such as depression and PTSD. Identifying genetic factors related to vulnerability and resiliency to stressful life events could lead to a better understanding of the pathophysiology of mental illnesses, such as depression and PTSD. Determining who is at risk for depression following transplant could have important clinical implications resulting in treatment interventions that may ultimately lead to improved graft and patient survival.

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