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Short communication

Correlation of major depressive disorder symptoms with FKBP5 but not FKBP4 expression in human immunodeficiency virus–infected individuals

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Major depressive disorder (MDD) is a significant cause of morbidity in people living with the human immunodeficiency virus (HIV). FKBP5 is a candidate gene with single-nucleotide polymorphisms (SNPs) rs1360780 and rs3800373 associated with MDD. This gene product and its relative, FKBP4, physically associate with the glucocorticoid receptor whose function is implicated in MDD pathophysiology. Because these genes are expressed in blood and brain and elevated in HIV infection, we explored the relationship between gene expression, genotype, and MDD symptoms. Longitudinally followed subjects (*N* = 57) as part of the CNS HIV AntiRetroviral Effects Research study, with diagnosed MDD and who donated blood for genotyping and gene expression analysis, were assessed. Subjects donated blood on adjacent visits with and without meeting criteria for MDD episode. Changes in clinical parameters were compared changes in gene expression. Change in FKBP5 expression correlated with change in Beck Depression Inventory (BDI) for MDD → euthymic comparison in GG genotype of rs3800373 (*P* = .013) and TT carriers of rs1360780 (*P* = .02). In euthymic → MDD comparison, GG homozygous, FKBP5 expression correlated with more severe change in BDI. Change in FKBP4 expression did not correlate with changes in clinical or depression measurements. Higher FKBP5 expression correlated with greater symptom change for GG carriers of rs3800373. *Journal of NeuroVirology* (2010) **16,** 399–404.

Keywords: FKBP4; FKBP5; gene expression; genotyping; HIV; human immunodeficiency virus; major depression; major depressive disorder

Introduction

FKBP5, a candidate gene for major depressive disorder (MDD), is inducible by the stress-related hormones glucocorticoids (Binder *et al,* 2004). FKBP5 and its relative FKBP4 function to differentially regulate trafficking and signaling of hormone receptors (Wochnik *et al,* 2005). We previously showed that FKBP4 and FKBP5 were up-regulated in the frontal cortex of human immunodeficiency virus (HIV)-infected individuals (Tatro *et al,* 2009b). Taken together, along with the fact that the HIV-infected population is disproportionately affected by MDD (Bing *et al,* 2001), one mission of the neuroAIDS field is to understand whether and how HIV affects the brain that results in the frequency of MDD high in the era of antiretroviral (ARV) therapy and long-term survival with HIV infection. We reported some changes in gene expression in MDD of HIV subjects through microarray analysis in frontal cortex including somatostatin (Everall *et al,* 2006). We explored the relationship between FKBP gene expression

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in peripheral blood mononuclear cells and MDD symptoms in HIV-infected individuals on ARV. Here, we examined the longitudinal gene expression of both FKBP4 and FKBP5 in HIV-infected individuals diagnosed with MDD by comparing transcript levels during an episode of MDD to expression during the euthymic state as it relates to FKBP5 genotype.

Association between FKBP5 and MDD recurrence was originally discovered by genome-association study of three chromosome 6 genes related to glucocorticoid receptor (GR) function and the importance of FKBP5 for MDD were confirmed in the STAR*D cohort (Binder *et al,* 2004; Lekman *et al,* 2008). A report found increased risk of peritraumatic dissociation with rs1360780 minor allele (Koenen *et al,* 2005). Additionally, the minor allele, in which FKBP5 is hyperinduced by cortisol, is associated with the development of posttraumatic stress disorder (Binder *et al,* 20088). However, a more recent study reported no linkage between FKBP5 single-nucleotide polymorphisms (SNPs) and late-life MDD (Sarginson *et al,* 2010). The hyperinduction allele is thought to result in decreased negative feedback inhibition of cortisol release and therefore dysfunctionally prolonged physiologic stress response after stimuli (Binder, 2009). HIV-infected individuals are more likely to be hypercortisolemic and the infection is thought to, in part, disrupt the hypothalamic-pituitary-adrenal (HPA) axis (Chittiprol *et al,* 2007).

In this study, gene expression of FKBP5 correlated with change in depressive symptom severity in major depressive episode when HIV-positive subjects were homozygous for minor alleles of either FKBP5 SNPs rs1360780, an intronic SNP near enhancer and promoter elements, or rs3800373, in the 3′ untranslated region (UTR). Our results provide evidence that FKBP5 transcriptional dysregulation together with FKBP4 as its functional antagonist are implicated in biological features of MDD symptoms.

Methods

Ascertainment of cases

Cases were ascertained as part of the CNS HIV Antiretroviral Therapy Effects Research (CHARTER) Study (https://www.charterresource.ucsd.edu/images/M_images/x-sect_cohort_rep.pdf). Subjects were longitudinally assessed semiannually and neuromedical, behavioral, and laboratory data were obtained, including psychiatric assessment using the Beck Depression Inventory (BDI) (Beck and Brown, 1996) and the Composite International Diagnostic Interview (CIDI) (Kessler *et al,* 2003), which yield, current and lifetime psychiatric diagnosis based on *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition* (DSM-IV) criteria. A summary of parameters measured and methods is available at the CHARTER resource site (CHARTER Group, 2009). We chose subjects who met criteria for major depressive disorder in whom blood samples were available during both an episode of MDD and when euthymic at adjacent visit. All subjects included in this report tested negative for drugs of abuse and hepatis c virus antibodies at visits. Seventy-four percent were male (40:14 M:F ratio). Summary of clinical parameters at the two time points for subjects meeting these criteria are in Table 1. Note that global deficit score is a measure of neurocognitive impairment standardized at the HIV Neurobehavioral Research Center and is described by Carey *et al* (2004). All subjects gave informed consent as approved by the University of California San Diego Institutional Review Board.

The depression-remission patterns differed temporally among the subjects and could be divided into two cohorts: Cohort 1: ($N = 56$) MDD at first visit → euthymic at second visit; and Cohort 2: ($N = 30$) euthymic at first visit → MDD at second visit.

Gene expression and genotyping

Approximately 10 ml blood was drawn directly into EDTA-coated glass tubes and peripheral blood mononuclear cell RNA was isolated according to manufacturer's protocols (Paxgene, Valencia, CA). After reverse transcription using Superscript III enzyme (Invitrogen, Carlsbad, CA), Taqman quantitative polymerase chain reaction (PCR) was performed in 384-well format. Taqman primers were obtained from Applied Biosystems (Foster City, CA): for FKBP4, assay ID Hs00427038; for FKBP5, assay ID Hs00188025; for GAPDH, assay ID Hs02758991. Samples were analyzed in triplicate on the same plate to avoid batch-effect. Gene expression was quantified using $\Delta\Delta C_T$ method with glutaldehyde 3-phosphate dehydrogenase (GAPDH) as endogenous control. Taqman assays were run and analyzed at the

Table 1 Summary statistics of clinical parameters of subjects

Parameter	Median	Range	SD
Euthymic			
BDI	19	53	11.24
Plasma HIV	1.69	3.52	1.09
Number ARVs	3	5	1.2
GDS	0.33	3.43	0.76
CD4	490	1398	292
MDD			
BDI	25	42	9.67
Plasma HIV	1.69	3.56	1.28
Number ARVs	3	4	1.22
GDS	0.17	3.6	0.85
CD4	473	1057	251

Note. BDI = Beck's Depression Inventory; plasma HIV = log (plasma HIV RNA); ARVs = antiretroviral medications; GDS = global deficit score; CD4 = CD4+ T-cell count.

University of California San Diego Center for AIDS Research Genomics Core. Considering that genotype of FKBP5 is known to affect its transcript levels, we genotyped the rs1360780 and rs3800373 SNPs using Taqman allelic discrimination assays (Applied Biosystems) in each subject. We used multivariate regression analysis grouping by genotype to compare change in gene expression from euthymic → MDD and MDD → euthymic with changes in BDI.

Results

Effect of depression status on FKBP4 and FKBP5 gene expression

We considered that there would be differences between the two patterns (first MDD, then euthymic at 6-month follow-up, and vice versa), as the changes may either correlate with onset of major depression episode or with recovery and medication effects. Separating the sample into these two cohorts, there was a small to medium effect of depression status on gene expression for FKBP5 (Cohort 1: Cohen's $d = 0.23$, $P = .12$, Cohort 2: $d = 0.31$, $P = .09$) but no effect for FKBP4 (Cohort 1, $d = 0.06$, $P = .66$, Cohort 2: $d = 0.07$, $P = .78$) regardless of direction of change. In both cohorts, expression of FKBP5 was the same under the euthymic state. However, FKBP5 was elevated during MDD in Cohort 1, and decreased during MDD in Cohort 2, indicating a potential temporal difference in whether changes were due to onset or recovery.

Peripheral blood mononuclear cell (PBMC) RNA was available for analysis of a smaller subset of the subjects at a third, euthymic time point. In most cases, FKBP4 (81%) and FKBP5 (78%) RNA quantities were stable at two euthymic time points compared to the MDD time point, even though blood was drawn with 12 intervening months (see Figure S1; online Supplementary Materials, available at …). Further, other candidate genes were measured in a preliminary analysis, STAT1 and GADD45β, which had consistent levels at euthymic and MDD states (Figure S1) and so were not further analyzed. These observations help to rule out assay variability.

Effect of sex on FKBP4 and FKBP5 expression

To test for sex as a possible confounding variable, we segregated the subjects to compare change in gene expression and change in BDI by male versus female in both cohorts. In Cohort 2, (euthymic → MDD), the magnitude of change in FKBP4 in females was higher than males (difference = 0.35-fold, $P = .04$) and the magnitude of change in FKBP5 was higher in females than males (difference = 0.30-fold, $P = .02$). There was no difference with respect to BDI changes in Cohort 2. No statistically significant male/female differences were found in Cohort 1. These statistics are provided in Table S2 in the online Supplementary Materials.

Correlation of FKBP5 gene expression with BDI

Using a multivariate regression analysis comparing changes in euthymic and MDD gene expression versus changes in other clinical and laboratory parameters (listed in Table 1), separating by genotype, we found that change in FKBP5 expression correlated with change in BDI for MDD → euthymic comparison in GG genotype of rs3800373 ($P = .013$, Pearson's $R = .61$; Figure 1, *left*). Using this comparison, the same correlation was observed for rs1360780 ($P = .02$, $R = .56$; Figure S2). Because the SNPs were in linkage disequilibrium, we present the rs1360780 comparisons in Supplementary Materials. In euthymic → MDD comparison, GG homozygous FKBP5 expression correlated with more severe change in BDI (Figure 1, *right;* $P = .0499$, $R = .40$). In sum, higher gene expression correlated with

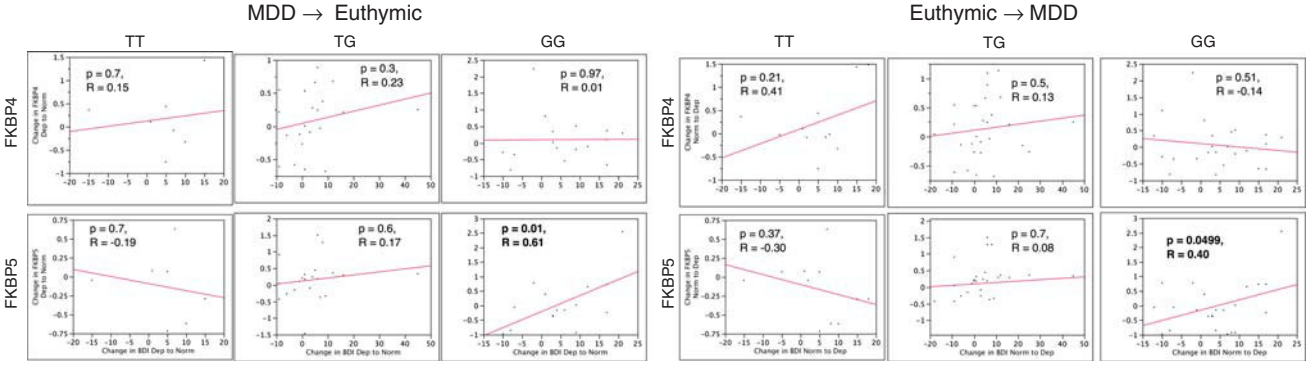

Figure 1 Correlation of Beck's Depression Inventory with FKBP4 and FKBP5 gene expression by rs3800373 genotype. After separating the subjects by FKBP5 genotype, correlation coefficients between change in BDI with change in gene expression from euthymic versus MDD status were calculated. For the euthymic → MDD group: as change in FKBP5 increased, BDI worsened. Correspondingly, for the MDD → euthymic group, increasing FKBP5 expression correlated with greater BDI improvement.

greater symptom change (Figure 1). Change in FKBP4 gene expression did not significantly correlate with changes in clinical or MDD mood measurements.

Conclusions

In this study we have observed a correlation of change in the severity of depressive mood, as measured by the BDI, with FKBP5 gene expression in individuals homozygous for GG of the FKBP5 SNP rs3800373. We suggest that this link of FKBP5 to BDI improvement in Cohort 1 is a component of the pathophysiological substrate of MDD. In this cohort, whose first visit was while they had MDD and the second when they were euthymic, the gene expression observations were more robust (P = .02 versus P = .049) and significant at both SNPs. Although both of the FKBP5 SNPs are in noncoding regions of the gene, the variants may affect translation efficiency and therefore the regulatory effectiveness of immunophilins on GR signaling. Our clinical and genetic results are correlational and animal or *in vitro* models of HIV to manipulate these genes would help determine causative roles in neurobehavioral symptoms we measure. Future work to analyze protein levels of these genes as in Binder *et al* (2004) should be carried out in a longitudinally followed HIV-positive cohort to further our understanding of the possible roles these genes play in MDD symptomatology.

Susceptibility to mood disorders is determined by interactions between genes and environment, heritability estimates range from 30% to 80% (Kendler, 1995). An important, but not sole, system that mediates long-term effects of stress on psychiatric disorders is the HPA system. Stress activates corticotropin release hormone (CRH) (Vale *et al,* 1981). In the central nervous system (CNS), CRH neurons innervate the locus coeruleus and activate the noradrenergic and sympathetic systems (Valentino *et al*, 1983). CRH regulates adrenal cortisol release as well as norepinephrine and epinephrine secretion (Tsatsanis *et al,* 2007). Glucocorticoids such as cortisol promote the fight and flight response in end organs mediated through the GR, which is critical for the negative-feedback loop terminating the response (Pariante and Miller, 2001). Hypercortisolemia and evasion from dexamethasone-induced cortisol suppression are physiologic features of MDD (Heuser *et al,* 1994). Because of hypercortisolemia observed in a significant number HIV-infected individuals (Chittiprol *et al,* 2007) and the increased risk of MDD in this population (Bing *et al,* 2001), we sought to determine if the GR-associated genes were implicated.

The proteins that are products of FKBP4 and FKBP5 associate physically with hormone receptors in many tissues, including peripheral blood mononuclear cells and the brain (Tatro *et al,* 2009b; Davies *et al,* 2002). FKBP4 gene product promotes fast nuclear translocation in neurons and FKBP5 gene product inhibits translocation of GR in the absence of hormone in neurons (Tatro *et al,* 2009a). Together, these genes regulate an ultrafast feedback loop within the cells. Our finding of a genotype-dependent correlation of FKBP5 mRNA expression with greater major depressive symptom recovery is in agreement with past studies (Kirchheiner *et al,* 2008), and we recapitulate this in HIV-infected individuals. FKBP5 may offer the future possibility of a biomarker to aid as a diagnostic or prognostic indicator for recovery of MDD in this population.

Appendix

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Galveston, Site: Benjamin Gelman, MD,PhD (PI); and Washington University, St Louis, Site: David Clifford, MD (PI), and Muhammad Al-Lozi, MD.

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Supplementary material available online

Figures S1, S2 and S3
Tables S1 and S2