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ORIGINAL ARTICLE

Differential Expression of Immunophilins FKBP51 and FKBP52 in the Frontal Cortex of HIV-Infected Patients with Major Depressive Disorder

Erick T. Tatro · Ian P. Everall · Eliezer Masliah · Britta J. Hult · Ginger Lucero · Gursharan Chana · Virawudh Soontornniyomkij · Cristian L. Achim · HNRC

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Abstract Patients infected with human immunodeficiency virus (HIV) have a higher risk of developing major depressive disorder (MDD) than the general population. Immunophilins FKBP51 and FKBP52 are expressed in cortical neurons and regulate the function of the glucocorticoid receptor (GR). Previous reports have shown that genetic variants in the *FKBP5* gene encoding FKBP51 are linked to psychiatric disorders. We sought to determine whether immunophilins are upregulated in HIV infection. To determine whether FKBP52 and FKBP51 are associated with MDD and/or HIV, we compared protein and gene expression in autopsy tissues from the frontal cortical gray matter. The study cases were divided into five groups: control, MDD, MDD with psychosis, HIV^+, and HIV^+ with MDD. Gene expression and protein levels were determined by real-time PCR and Western blot analysis of fresh frozen tissues. Genotyping of previously published alleles of the *FKBP5* gene was also performed. We found correlation of upregulation of both immunophilins in the HIV-infected groups. In the HIV^+ population with MDD, *FKBP4* expression is significantly higher while *FKBP5* is more variable. After analyzing the *FKBP5* gene for single nucleotide polymorphisms, we found that rs3800373 CC genotype is more frequent in the MDD and MDD/Psychosis groups. We hypothesized that the levels of FKBP51, as modulator of the nuclear translocation of GR, would be lower in MDD. Instead, an increase in FKBP51 at both the transcript (*FKBP5*) and protein level correlated with MDD. Increased *FKBP4* expression of correlated to HIV^+MDD but not to HIV without MDD.

Keywords HIV · immunophilins · major depressive disorder · glucocorticoid receptor

E. T. Tatro · I. P. Everall · B. J. Hult · G. Lucero · G. Chana · V. Soontornniyomkij · C. L. Achim (✉)
Department of Psychiatry-0603,
University of California at San Diego,
BSB#2066, 9500 Gilman Drive, La Jolla,
San Diego, CA 92093-0603, USA
e-mail: cachim@ucsd.edu

E. Masliah
Department of Neurosciences,
University of California at San Diego,
San Diego, CA, USA

E. Masliah · C. L. Achim
Department of Pathology, University of California at San Diego,
San Diego, CA, USA

Introduction

Immunophilins are a class of chaperone and adapter proteins widely expressed in most tissues, particularly abundant in the brain, specifically in neurons (Steiner et al. 1992; Sinars et al. 2003). FKBP52 and FKBP51 are the products of the *FKBP4* and *FKBP5* genes, respectively. FKBP52 functions as an adapter protein within the glucocorticoid receptor (GR) and HSP90 complex that links the hormone-bound receptor complex to the molecular motor dynein allowing for shuttling of the activated receptor complex to the nucleus (Wochnik et al. 2005). In contrast to FKBP52, the FK506-binding domain of FKBP51 lacks isomerase function, but is shown to bind the GR through the tetratricopeptide repeat domains and therefore functions as a competitive inhibitor to formation of the GR-FKBP52–dynein complex (Denny et al. 2000; Westberry et al. 2006). Sapolsky's glucocorticoid-cascade hypothesis proposes a feed-forward mechanism of decreased feedback inhibition with increased sensitivity of the brain to cortisol (Sapolsky and Plotsky 1990). The action

of the GR in the brain may be central to the molecular mechanism whereby the hypothalamo–pituitary–adrenal axis feedback loop is dysregulated in hypercortisolemia observed in depression (Sapolsky and Plotsky 1990; Garcia et al. 2004). Therefore, the modulation of GR signaling in the cortex of the brain may be a level of regulation that could affect the susceptibility of neurons to lose synaptic and dendritic density found in hypercortisolemia and depression (Cotter et al. 2002a, b).

Single nucleotide polymorphisms (SNPs) in the *FKBP5* gene have been associated with MDD, posttraumatic stress disorder, treatment response to drugs, and delayed recovery from psychosocial stress in healthy adults (Binder et al. 2004, 2008; Ising et al. 2008; Lekman et al. 2008). The specific molecular consequences of these SNPs are not known. Binder et al. identified two polymorphisms that link to decreased FKBP51 protein levels in the blood (Binder et al. 2004). The same polymorphisms in *FKBP5* were linked with peritraumatic dissociation in medically injured children (Koenen et al. 2005). However, one prospective study failed to definitively assign the designated SNPs' associations with MDD and affective disorder (Gawlik et al. 2006). *FKBP5* may play a role only in some depression cases, with other genes involved in the glucocorticoid system responsible for disturbances of the HPA axis during depressive episodes.

Since the prevalence of major depressive disorder (MDD) is significantly elevated in individuals infected with HIV, we sought to determine whether there was a relationship between HIV infection, MDD, and the expression of immunophilins in the frontal cortex (Reiter 2000; Valente 2003; Porche and Willis 2006). We have previously showed an increase of the related immunophilin FKBP12 in the deep gray matter of HIV patients, which we hypothesized to be a protective response (Christeff et al. 1997; Bing et al. 2001; Valente 2003; Porche and Willis 2006).

Materials and methods

Demographic information

Autopsy fresh frozen brain tissues were kindly provided by the Stanley Foundation and the California NeuroAIDS Tissue Network (CNTN). Twelve cases from each group were studied with an age range of 24–63 years. The cases were divided into five study groups: control, MDD, MDD with psychosis, HIV^+, and HIV^+ with MDD (Table 1). The cause of death in the control group was mainly cardiac arrest, while in the depressed groups, the cause of death was predominantly suicide. The cause of death for the two HIV groups was AIDS-associated complications. The postmortem interval (PMI) was generally within 24 h (the average in the CNTN cohort was approximately 12 and 35 h for the Stanley Foundation cohort). One subject in the HIV/MDD group was diagnosed with HIV-associated dementia (HAD), no. 49, and two subjects in the HIV group were diagnosed with minor cognitive and motor deficit (MCMD), subjects no. 41 and no. 45.

FKBP4 and *FKBP5* gene expression

For RNA isolation, 100 mg was homogenized in ice-cold TRIzol reagent (Invitrogen) following manufacturer's instructions. RNA quality was assessed by the A260/A280 ratio and by agarose electrophoresis. CDNA synthesis was performed using 1 μg of RNA using the SuperScript III kit from Invitrogen. For quantitative PCR, 40 ng cDNA per reaction was used, and FAM-labeled 20× prevalidated probes were purchased from Applied Biosystems. For *FKBP5*, assay Hs00188025_m1; for *FKBP4*, assay Hs00427038_g1; and for *GAPDH*, assay Hs02758991_g was used. TaqMan master mix (2×) purchased from Applied Biosystems was used in 20 μL reactions on 96 well plates, and assays were performed at the University of California San Diego Center for AIDS Research Genomics Core. Gene expression is reported as fold control versus the median of the control group using the ΔΔ-CT method comparing to housekeeping gene *GAPDH* whereby $\Delta CT = CT_{GAPDH} - CT_{Gene}$, $\Delta\Delta CT = \Delta CT_{Control} - \Delta CT_{Disease}$, and $Fold - Control = 2^{-\Delta\Delta CT}$. A test for normality showed that not all groups are distributed in a Normal curve; therefore, nonparametric statistical analyses were performed, and Kruskal–Wallace rank sums test and Dunn's multiple comparison were done using Graphpad Prism software to compare the groups.

FKBP52 and FKBP51 protein analysis

For protein extraction, 100 mg of frozen brain tissue was cut and homogenized in 0.9 mL ice-cold lysis buffer consisting of 50 mM Tris–HCl, 1% sodium dodecyl sulfate, 150 mM NaCl, 1 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, 1 μg/mL aprotinin, 1 mM sodium Na_3VO_4, pH 7.4. Five micrograms of total protein from each case was run in 10% polyacrylamide gels and transferred to polyvinylfluoridine membranes. Western blotting analysis for immunophilins FKBP52 and FKBP51 was performed using primary antibodies incubated overnight at 4°C: mouse anti-FKBP52 (Stressgen SRA1400) at 1:5,000 and rabbit anti-FKBP51 (Abcam ab2901) at 1:1,000. Secondary antibodies were horseradish peroxidase-conjugated donkey anti-mouse and donkey anti-rabbit (Jackson Immunoresearch), respectively, at 1:5,000. The chemiluminescence signal was developed according to manufacturer's protocols (Perkin Elmer NEL103001) and quantified using densitometric analysis with Image J software; changes are expressed as fold actin and normalized fold actin.

Table 1 Demographic description of study groups

Patient ID	Age	Sex	PMI	pH	Cause of death
Control					
1	48	M	12	6.51	Cardiac
2	24	M	17	6.6	MVA
3	50	F	35	6.31	Cardiac
4	44	M	27	6.82	AAP
5	35	M	31	6.59	MVA
6	63	M	40	6.91	Cardiac
7	50	M	11	6.5	Cardiac
8	63	M	37	6.5	Cardiac
9	34	M	9	6.56	MVA
10	56	F	29	6.78	Cardiac
11	56	F	31	6.66	Cardiac
12	39	F	24	6.88	Cardiac
Mean	46.8		25.3	6.64	
MDD					
13	32	F	19	6.8	Suicide
14	47	F	25	6.88	Suicide
15	56	M	38	6.59	Suicide
16	33	M	25	6.86	Suicide
17	45	F	29	6.9	Cardiac
18	24	M	21	6.61	Suicide
19	56	F	15	6.59	Suicide
20	44	M	24	6.52	Cardiac
21	34	M	24	6.79	Suicide
22	53	M	21	6.64	Cardiac
23	45	M	29	6.75	Suicide
24	45	F	13	6.58	Suicide
Mean	42.8		23.6	6.71	
MDD psychosis					
25	48	F	24	6.36	Suicide
26	40	M	52	6.48	OD
27	28	M	26	6.7	Suicide
28	28	F	40	6.68	Suicide
29	62	M	65	6.57	Suicide
30	28	F	40	6.68	Suicide
31	32	F	19	6.7	Suicide
32	63	M	31	6.6	Suicide
33	51	F	36	6.3	Unknown
34	40	F	49	6.72	Suicide
35	35	M	36	6.6	Suicide
36	36	F	32	6.74	PE
Mean	*41.5*		*35.8*	*6.59*	
HIV+					
38	45	M	12		AIDS
39	57	M	12		AIDS
40	35	M	5		AIDS
41	55	M	5		AIDS
42	57	M	12		AIDS
43	54	M	12		AIDS
44	49	M	10		AIDS
45	37	F	1		AIDS
46	40	M	10		AIDS
47	47	F	12		AIDS
48	46	M	12		AIDS
Mean	*46.6*		*8.8*		
HIV^{+}MDD					
49	43	M	12		AIDS
50	43	M	31		AIDS
51	34	M	10		AIDS
52	55	M	120		AIDS
53	59	M	12		AIDS
54	54	M	11		AIDS
55	35	M	10		AIDS
56	38	M	7		AIDS
57	38	M	10		AIDS
58	39	M	12		AIDS
59	34	M	4		AIDS
Mean	*44.7*		*15.2*		

PMI postmortem interval, *MVA* motor vehicle accident, *AAP* acute alcohol poisoning, *OD* drug overdose, *PE* pulmonary embolism, *AIDS* (complications due to) acquired immunodeficiency syndrome

FKBP5 genotyping of patient cohort

Genotyping of previously published alleles of the *FKBP5* gene implicated in mood disorders was performed. Genomic DNA from the patients was isolated from the tissue following manufacturer's instructions of the DNeasy kit (Qiagen no. 69504). Genotyping was performed using 20 ng DNA following manufacturer's protocols of the SNP Genotyping Assay from Applied Biosystems: an A/C substitution in the 3′ untranslated region of *FKBP5* gene, SNP rs3800373, (Applied Biosystems Assay ID C_27489960_10), and a C/T substitution in Intron 1 of the *FKBP5* gene, SNP rs1360780 (Applied Biosystems Assay ID C_8852038_10). A five by three table was constructed of the possible genotypes with the five test groups and Chi-square analysis compared the expected genotype frequencies with observed. Expected frequencies are determined by genotype frequency in North American Caucasian population deposited in NCBI SNP database; for rs3800373 record ID ss2334697 and for rs1360780, record ID ss4777328 was used (Haga et al. 2002). Two by five tables were constructed and allelic frequencies and Chi-square analysis compared the expected versus observed frequencies.

Results

Immunophilin gene expression

In order to determine whether age, PMI, and brain pH were possible confounders, tests for correlation were performed. Since gene expression is calculated as $\text{Fold} - \text{Change} = 2^{-\Delta\Delta CT}$, a value of 0.5 corresponds to a

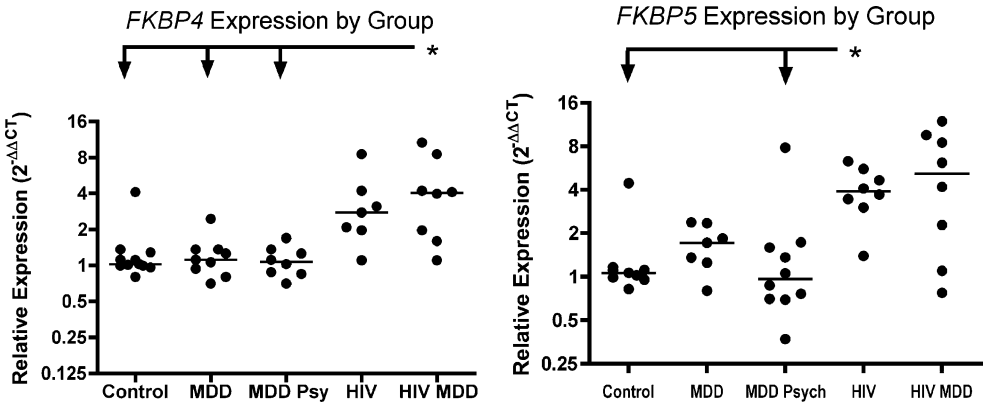

Fig. 1 FKBP4 and FKBP5 expression is increased in HIV and HIV with MDD. Gene expression in the cortical gray matter was determined by qPCR using the ΔΔCT method and normalizing the gene of interest to GAPDH expression. Fold-Control is plotted for each of the study groups; *circles* represent individual patients, and *bars* represent median within the group. Kruskal–Wallace rank sums test with Dunn's multiple comparison tested for significant difference among the groups (*$p<0.05$)

twofold decrease; therefore, a Log_2 scale for the *y*-axis is used so that 0.5 and 2 are equidistant from baseline; regression analyses for correlation account for this. Expression was plotted versus age, PMI, and brain pH, and Pearson's test for correlation was performed. For all three characteristics, there were no correlations with gene expression, $R<0.50$.

In examining Table 1, one can see that the HIV^+ specimen obtained from the CNTN had more males. The HIV-negative groups obtained from the Stanley Foundation had statistically indistinguishable male/female compositions. *t* tests comparing expression in non-HIV males to expression in non-HIV females of the two immunophilin genes found no difference between the sexes with respect to immunophilin expression, showing 95% confidence intervals interquartile ranges. Since there was no difference between male and female expression of *FKBP4* ($p=0.179$) and *FKBP5* ($p=0.716$), sex is unlikely to be a confounder to the analysis.

In order to represent the natural variation in the population at large, fold control was calculated against the median ΔCT value from the control group. Therefore, half of the controls appear above and half below 1. As shown in Fig. 1, the majority of patients in the two HIV^+ populations showed higher expression of *FKBP4* and *FKBP5* above the control median. HIV^+ patients exhibited a fold increase of 3.4±2.3 (mean ± 95% confidence interval), and HIV^+MDD patients exhibited a fold increase of 4.5±2.9 for the *FKBP4* gene. HIV^+ patients showed 4.0±2.3-fold increase over control, and HIV^+MDD patients showed 5.6±3.5-fold increase over control for the *FKBP5* gene. In the HIV^+MDD population, *FKBP5* was more variable, ranging from 0.7- to 12.0-fold change, while the HIV^+ ranged from 1.4- to 6.3-fold increase. In MDD, MDD/Psyc, HIV, and HIV/MDD, gene expression of both immunophilins is more variable. In the HIV^-MDD group, the trend is toward increased expression of *FKBP5* with median expression 1.7-fold higher than control; however, no change is apparent for *FKBP4* in the HIV^-MDD population. For the *FKBP4* gene, HIV^+ patients exhibited a fold increase of 3.2± 2.6 (mean ± standard deviation), and HIV^+MDD patients exhibited a fold increase of 3.1±3.3. For the *FKBP5* gene, HIV^+ patients showed 13.5±15.8-fold increase over control, and HIV^+MDD patients showed 9.6±12.7-fold increase over control. In the HIV^+MDD population, *FKBP5* was more variable in the patient populations, ranging from 0.7- to 44.3-fold change in HIV^+MDD and in HIV^+ from 1.4- to 50-fold increase. In the HIV-negative MDD group,

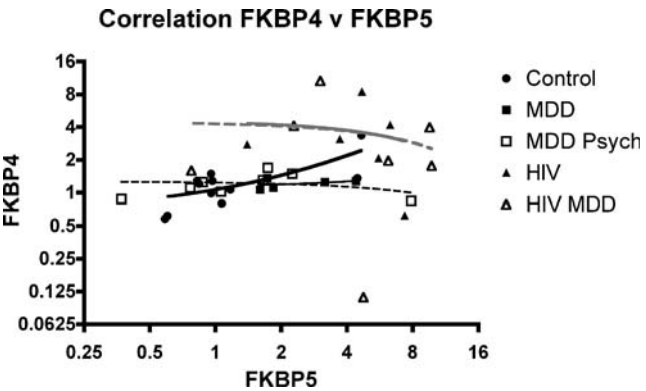

Fig. 2 Correlation of FKBP4 and FKBP5 expression in frontal cortex separated by group. Gene expression from the frontal cortex of patients listed in Table 1 is determined by qPCR using the ΔΔCT method and normalizing the gene of interest to *GAPDH*. Fold-Control is plotted for each of the study group, plotting the *FKBP4* expression on the *y*-axis and *FKBP5* expression on the *x*-axis for each individual patient. For the control (*circles*, *thick line*) group, Pearson's test for correlation showed a correlation between *FKBP4* and *FKBP5* ($R=0.745$). For the MDD (*filled squares*, *black thin line*) and MDD/Psych (*empty squares*, *black dashed line*) groups, *FKBP4* and *FKBP5* were not correlated ($R=0.389$ and -0.287). For both HIV (*filled triangles*, *gray thin line*) and HIV/MDD (*empty triangles*, *gray dashed line*), there was no correlation ($R=-0.178$ and -0.203, respectively)

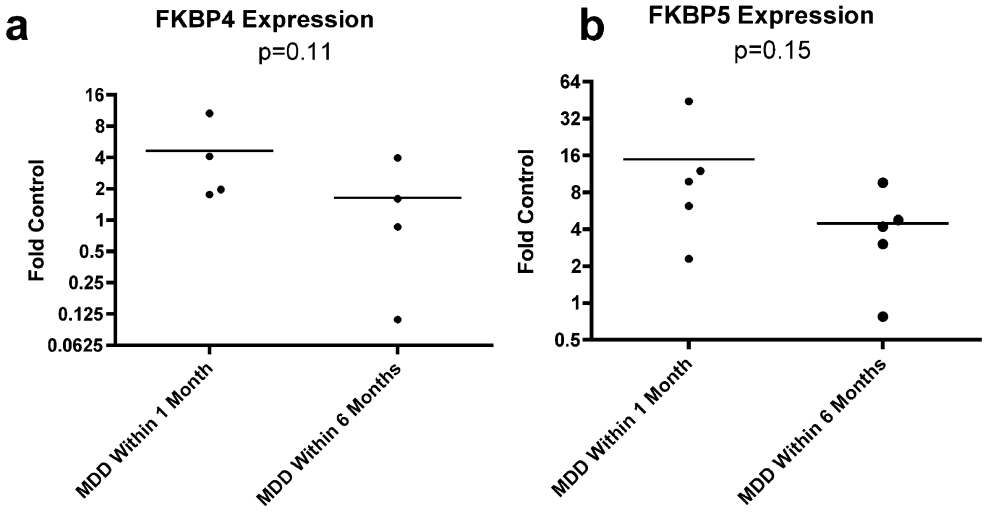

Fig. 3 Expression of *FKBP4* (**a**) and *FKBP5* (**b**) in the CNTN groups separated by most recent episode. Gene expression from frontal cortex of patients for whom MDD clinical data were available is determined by qPCR using the ΔΔCT method and normalizing the gene of interest to *GAPDH* expression. Patients are divided based on MDD episode within 1 month or within 6 months and Fold-Control plotted; *circles* represent individuals, and *bars* represent median. Student's *t* test comparing the two groups' expression showed a nonsignificant trend based on most recent MDD episode for expression of *FKBP4* $p=0.11$ (**a**) or *FKBP5* $p=0.15$ (**b**)

the trend is toward increased expression of *FKBP5* with median expression 2.4¬-fold higher than control; however, no change is apparent for *FKBP4* in this group. In comparing HIV^+ to HIV^-, both *FKBP4* and *FKBP5* genes are expressed at higher levels.

One subject was diagnosed with HAD, no. 49, and had the lowest expression of *FKBP4* in the HIV/MDD group, 0.14-fold of control and an increase in *FKBP5*, 4.6-fold of control. Two subjects in the HIV group were diagnosed with MCMD; subjects no. 41 and no. 45. Patient no. 41 had the highest *FKBP4* expression in the HIV group, 8.5-fold for *FKBP4* and 4.6-fold for *FKBP5* over control. Patient no. 45 was 2.7- and 1.4-fold over control for *FKBP4* and *FKBP5*, respectively. It is interesting that the patients with cognitive impairment were outliers in some respects; the only patient with HAD correlated with the lowest *FKBP4* expressor in the HIV/MDD group, and one patient with MCMD correlated to the highest *FKBP4* expression in the HIV group. These three subjects were not outliers in their groups with respect to *FKBP5* expression.

We also hypothesized that there would be a balance between *FKBP4* and *FKBP5* expression, and the balance would be disrupted in MDD, MDD/Psychosis, or HIV/MDD. In Fig. 2, we plotted the *FKBP4* expression on the *y*-axis and *FKBP5* on the *x*-axis and analyzed the possible correlation in expression of the two genes, separating the groups. Pearson's test for correlation showed a correlation between *FKBP4* and *FKBP5* ($R=0.745$) expression in the control group (circles), which is absent in all other groups. In the MDD (filled squares) and MDD/Psych (empty squares) groups, no correlation was observed between *FKBP4* and *FKBP5* ($R=0.389$ and -0.287, respectively). Even though expression was elevated in both HIV (filled triangles) and HIV/MDD (empty triangles), the correlation

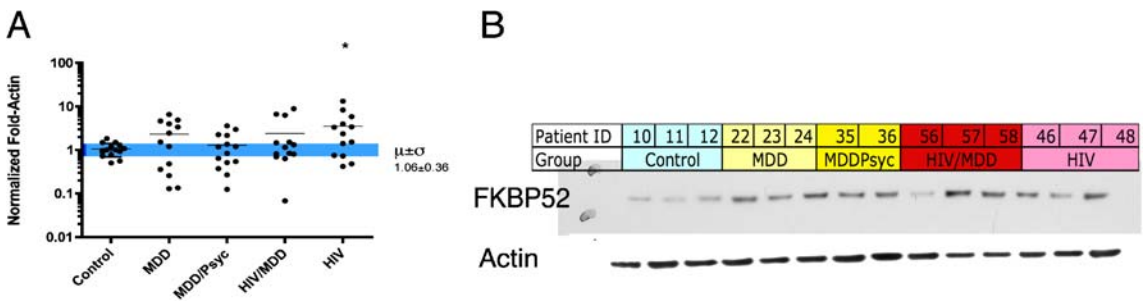

Fig. 4 FKBP52 protein levels are increased in the HIV group. Cases representing each study group were included in every gel. After transfer and immobilization on PVDF membrane, proteins were probed by Western blot for FKBP52; representative blot showed in **b**. Membranes were stripped of antibodies and reprobed for actin. Values are normalized to controls on their respective gels, Normalized Fold-Actin = $\text{Fold-Actin}_{\text{Patient}}/\text{Fold-Actin}_{\text{ControlMean}}$. The *area within the bar* delineates the standard deviation about the mean ($\mu \pm \sigma$). Kruskal–Wallace and Dunn's multiple comparison test were used to test for significance (*$p<0.05$; **a**)

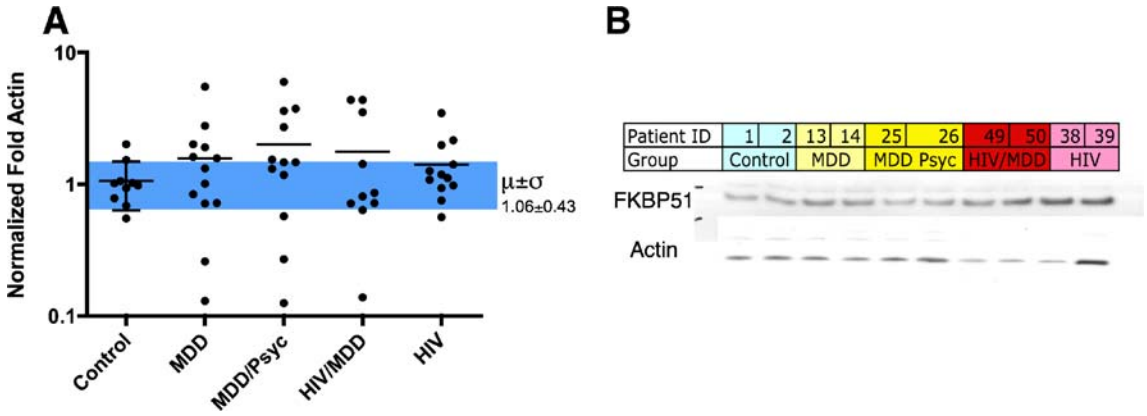

Fig. 5 FKBP51 protein levels are altered in MDD groups compared to control. Values were normalized to controls on their respective gels, Normalized Fold-Actin = $\text{Fold-Actin}_{\text{Patient}}/\text{Fold-Actin}_{\text{ControlMean}}$. The *area within the bar* delineates the standard deviation about the mean ($\mu \pm \sigma$). Kruskal–Wallace and Dunn's multiple comparison test were used to test for significance at $p<0.05$ (**a**). Representative blot is showed in **b**

of the two genes observed in the control group was no longer present when looking at the separate diagnostic groups ($R=-0.178$ and -0.203).

In the tissue acquired from the CNTN, we had available patient history of the MDD and MDD/Psych patients regarding their most recent MDD ,episode and it was either within the last month or 6 months before death. The *FKBP4* and *FKBP5* expression was higher in the MDD patients who had MDD episodes within 1 month of death, but did not reach statistical significance, $p=0.11$ and $p=0.15$, respectively (Fig. 3).

Protein analysis

The protein analysis of FKBP52 and 51 illustrated in Figs. 4 and 5, respectively, showed a wide variation in expression of both immunophilins in the control group, indicating that expression varies in the population at large and likely is dependent on many factors. FKBP52 was elevated in HIV Group compared to control patients, $p=0.0084$. Compared to HIV without MDD, the HIV/MDD group, although more variable than the control, had lower levels of FKBP52. Amongst the MDD and MDD/Psychosis Groups, expression of FKBP52 is more variable than control, with the MDD mean above the standard deviation of the mean control. FKBP51 showed higher mean protein expression in the MDD, MDD/Psych, and HIV/MDD groups, however, not statistically significant; there were patients both above and below the standard deviation of the control group.

Single nucleotide polymorphism analysis

Using the allelic discrimination assay, we tested two polymorphisms: rs38000373, an A to C transversion substitution in the 3′ untranslated region (3′UTR) of the *FKBP5* gene and rs1360780, which is a C to T transition substitution in the second intron of the *FKBP5* gene. The rs1360780 polymorphism is located in a region where transcription factors may bind, where hormone response elements (HREs) are located as shown in Fig. 6, which may alter transcription and have been implicated in mood disorders (Hubler and Scammell 2004; Billing et al. 2007). The rs3800373 SNP is located in a region which may alter stability and half-life of the mRNA molecule.

The SNP rs3800373 had associations with MDD and MDD with Psychosis. The heterozygous genotype, AC, and the minor allele homozygous CC were significantly more frequent than expected based on Hardy–Weinberg assumptions of the North American Caucasian population and previously published allelic and genotype frequencies (Table 2; Haga et al. 2002). The HIV^{+}MDD did not have the same deviation from expected as the HIV-negative MDD group. The MDD/Psych group had increased

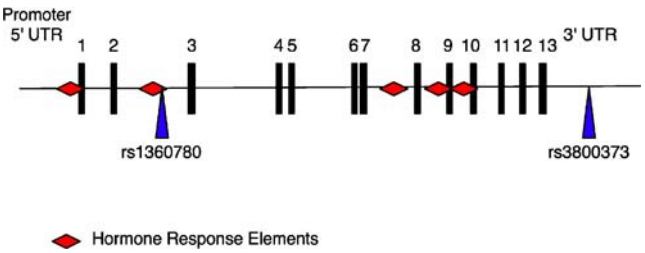

Fig. 6 Transcription factor binding sites and SNP locations on *FKBP5* gene. The genomic organization of the *FKBP5* gene is illustrated; exons are *numbered with black bars*; *distance between bars* represents relative lengths of the introns. The locations of the polymorphisms rs1360780 and rs3800373 are indicated by *triangles*, and the locations of hormone-responsive elements are indicated with *diamonds* (information from Hubler and Scammell 2004)

Table 2 Genotype frequencies for SNPs rs3800373 and rs1360780 of *FKBP5* gene

	rs3800373							rs1360780						
	Expected			Observed			χ^2	Expected			Observed			χ^2
	AA	AC	CC	AA	AC	CC	*p* value	AA	AC	CC	AA	AC	CC	*p* value
Control (*n*=13)	5.4	7	0.5	8	4	1	0.232	5.4	7	0.5	8	4	1	0.232
MDD (*n*=11)	5	6.5	0.5	7	2	2	0.015	5	6.5	0.5	7	2	2	0.015
MDD Psych (*n*=10)	4.6	6	0.5	2	6	2	0.037	4.6	6	0.5	2	6	2	0.037
HIV (*n*=9)	3.8	4.9	0.4	2	6	1	0.35	3.8	4.9	0.4	2	6	1	0.35
HIV MDD (*n*=8)	3.3	4.3	0.3	3	5	0	0.79	3.3	4.3	0.3	3	5	0	0.79

Genotypes were determined from genomic DNA by allelic discrimination assay described in "Materials and methods". Based on the published allelic frequencies for rs3800373: *f*(A)=0.688, *f*(C)=0.312 and for rs1360780: *f*(C)=0.758, *f*(T)=0.242, *Expected* genotype frequencies are calculated based on Hardy–Weinberg equilibrium and compared to *Observed* genotypes for the study groups. χ^2 analysis comparing *Expected* with *Observed* values determines significant deviation in the patient groups from the population; significance is considered at $p<0.05$

frequency of the C allele compared with the North American Caucasian population and the control group in this study (Table 3).

In the, genotype frequencies of the Intron II polymorphism were significantly different in the MDD/Psych group from North American Caucasian population. The AC and CC genotypes were increased in the MDD/Psych as well as significant increase in the minor, C, allele (Tables 2 and 3). A possible confounder to this analysis is that the control and the HIV populations differed from the Expected counts.

Discussion

The observation of unequal variance of transcriptional levels of both *FKBP4* and *FKBP5* among the groups may indicate a nonuniform distribution in the general population and suggests that multiple mechanisms can be involved. Nonetheless, the trends in variation in the MDD, MDD/Psychosis, HIV, and HIV/MDD groups suggest that the interplay between FKBP51 and FKBP52 in modulating the effects of cortisol at the cellular level may be of consequence.

Our observation of higher *FKBP5* gene transcription levels in the MDD group that did not reach statistical significance (Fig. 1), combined with previous reports that *FKBP5* is responsive to cortisol (Davies et al. 2002), likely indicates increased expression in response to hypercortisolemia in MDD. In HIV patients, immunophilin expression is clearly elevated, probably in response to chronic inflammation and microglial activation. Achim and Avramut showed higher expression of the immunophilin FKBP12 in the HIV-infected brain (Avramut and Achim 2003). If the immunophilins share similar promoter regions, containing SP1 binding elements and CCAAT boxes, it would make sense that immunophilins genes would be upregulated by overlapping mechanisms.

FKBP5 transcript was much more variable in HIV^+MDD than HIV^+, which could indicate two things. First, that transcription of *FKBP5* would be elevated in direct response to hypercortisolemia, and second, that there is a

Table 3 Allelic frequencies for SNPs rs3800373 and rs1360780 of *FKBP5* gene

	rs3800373					rs1360780				
	Expected		Observed		χ^2	Expected		Observed		χ^2
	A	C	A	C	*p* value	C	T	C	T	*p* value
Control (*n*=26)	18	8	20	6	0.371	20	6	17	9	0.215
MDD (*n*=22)	15	7	16	6	0.691	18	6	15	9	0.128
MDD Psych (*n*=20)	14	6	10	10	0.07	17	5	12	10	0.02
HIV (*n*=18)	12	6	10	8	0.225	14	4	9	9	0.011
HIV MDD (*n*=16)	11	5	11	5	0.997	12	4	11	5	0.51

Genotypes were determined from genomic DNA by allelic discrimination assay described in "Materials and methods". Based on the published allelic frequencies for rs3800373: *f*(A)=0.688, *f*(C)=0.312 and for rs1360780: *f*(C)=0.758, *f*(T)=0.242, *Expected* allele frequencies are calculated based on group size and compared to *Observed* genotypes for the study groups. χ^2 analysis comparing *Expected* with *Observed* values determines significant deviation in the patient groups from the population; significance is considered at $p<0.05$

subgroup of patients whose neurons did not increase expression of *FKBP5* concordantly to compensate resulting in abnormally high GR signaling rendering them susceptible to the effects of the glucocorticoid cascade.

The SNP, rs1360780, located in Intron II, which contains HREs, potential alternative promoter regions, and distal enhancer elements (Hubler and Scammell 2004), had genotype frequencies significantly different from expected in control, MDD/Psychosis, and HIV. After genotyping the *FKBP5* gene for two SNPs, we found that rs3800373 CC genotype is higher in the MDD and MDD/Psychosis group (Table 2); this SNP is located in the 3′UTR of the mRNA and could potentially affect protein translation. In Fig. 5, we show higher protein levels of the *FKBP5* gene product, FKBP51, in portions of the MDD and MDD/Psychosis groups as compared to control, while we did not find significant changes in transcription levels; it may be that this 3′UTR polymorphism affects translation efficiency. Our data analysis methods, based on comparing to controls, preclude determining whether higher protein amounts are found in the C allele, but it would be an interesting next step. Furthermore, new research on micro-RNAs show that mRNA stability, based on micro-RNA binding in 3′UTRs, affects the half-life of a particular transcript (Lai 2002); this would be another possibility of yet another level of modulation of cellular FKBP51 levels and thereby modulation of glucocorticoid signaling and HPA axis function.

It should be noted that our design study was correlative in analyzing gene and protein expressions in postmortem analyses; the results will be correlative in nature. We cannot determine whether FKBP51 and FKBP52 are dysregulated in the frontal cortex as a process *resulting from* MDD or as part of a process that *led to* MDD. Further, we do not know the causes of the increase observed to correlate to HIV infection; we hypothesize it to occur in neurons resulting from chronic inflammatory signals from infected monocyte-derived macrophages and microglia, from cytokine and chemokine secretion, since FKBP51 and FKBP52 are present in neurons of the frontal cortex, and neurons are not infected with HIV, but rather respond to signals from surrounding glia. However, it may be due to part of a cellular "stress response" from shedded viral particles.

We hypothesized that FKBP51, as modulator of the nuclear translocation of the GR, would be lower in MDD. Instead, elevated FKBP51 at both the transcript and protein levels correlates with MDD. However, FKBP52, the adapter protein that facilitates active GR trafficking, was variable in both directions, elevated, *and* decreased in MDD and MDD/Psych. In contrast to *FKBP5*, *FKBP4* fold change was not significantly increased in HIV, but in HIV^{+}MDD. Perhaps immunophilins as a class of chaperone and adapter proteins are upregulated during chronic inflammation by a common mechanism, and if the agonist, in this case *FKBP5*, is not significantly upregulated to compensate for the increased ability of GR to be functionally active, cortical neurons would be rendered more susceptible to the effects of glucocorticoids and the cascade leading to the subtle pathologies of depression.

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The San Diego HIV Neurobehavioral Research Center [HNRC] group is affiliated with the University of California, San Diego, the Naval Hospital, San Diego, and the Veterans Affairs San Diego Healthcare System and includes the following: Director—Igor Grant, M.D.; Co-Directors—J. Hampton Atkinson, M.D., Ronald J. Ellis, M. D., Ph.D., and J. Allen McCutchan, M.D.; Center Manager—Thomas D. Marcotte, Ph.D., Melanie Sherman; Naval Hospital San Diego—Braden R. Hale, M.D., M.P.H. (P.I.); Neuromedical Component—Ronald J. Ellis, M.D., Ph.D. (P.I.), J. Allen McCutchan, M.D., Scott Letendre, M.D., Edmund Capparelli, Pharm.D., Rachel Schrier, Ph.D., Jennifer Marquie-Beck, Terry Alexander, R.N., Janis Durelle; Neurobehavioral Component—Robert K. Heaton, Ph.D. (P.I.), Mariana Cherner, Ph.D., Steven Paul Woods, Psy.D., David J. Moore, Ph.D., Matthew Dawson; Neuroimaging Component—Terry Jernigan, Ph.D. (P.I.), Christine Fennema-Notestine, Ph.D., Sarah L. Archibald, M.A., John Hesselink, M.D., Jacopo Annese, Ph.D., Michael J. Taylor, Ph.D., Brian Schweinsburg, Ph.D.; Neurobiology Component—Eliezer Masliah, M.D. (P.I.), Ian Everall, FRCPsych., FRCPath., Ph.D., Cristian Achim, M.D., Ph.D.; Neurovirology Component—Douglas Richman, M.D., (P.I.), David M. Smith, M.D.; International Component—J. Allen McCutchan, M.D., (P.I.); Developmental Component—Ian Everall, FRCPsych., FRCPath., Ph.D. (P.I.), Stuart Lipton, M.D., Ph.D.; Clinical Trials Component—J. Allen McCutchan, M.D., J. Hampton Atkinson, M.D., Ronald J. Ellis, M.D., Ph.D., Scott Letendre, M.D.; Participant Accrual and Retention Unit—J. Hampton Atkinson, M.D. (P.I.), Rodney von Jaeger, M.P.H.; Data Management Unit—Anthony C. Gamst, Ph.D. (P.I.), Clint Cushman (Data Systems Manager), Daniel R. Masys, M.D. (Senior Consultant); Statistics Unit—Ian Abramson, Ph.D. (P.I.), Florin Vaida, Ph.D., Christopher Ake, Ph.D.

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