FKBP5 variant moderates effects of childhood adversity in schizophrenia

**FK506 binding protein 5 (FKBP5) genotypes moderate the effects of childhood maltreatment on cognition in schizophrenia and healthy controls.**

Revision 3/6/15 for

*Journal of Psychiatric Research*

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**Word Counts:** Abstract 277; Main text 3383

**Key words:** FKBP5, childhood trauma, psychosis, GxE, cognitive impairment
**Abstract**

Common variants of the FK506 binding protein 5 (*FKBP5*) gene are implicated in psychotic and other disorders, via their role in regulating glucocorticoid receptor (GR) receptor sensitivity and effects on the broader function of the HPA system in response to stress. In this study, the effects of four *FKBP5* polymorphisms (rs1360780, rs9470080, rs4713902, rs9394309) on IQ and eight other cognitive domains were examined in the context of exposure to childhood maltreatment in 444 cases with schizophrenia and 292 healthy controls (from a total sample of 617 cases and 659 controls obtained from the Australian Schizophrenia Research Bank; ASRB). Participants subjected to any kind of maltreatment (including physical, emotional, or sexual abuse or physical or emotional neglect) in childhood were classified as ‘exposed’; cognitive functioning was measured with Repeatable Battery for the Assessment of Neuropsychological Status, the Controlled Oral Word Association Test, and IQ was estimated with the Weschler Test of Adult Reading. Hierarchical regressions were used to test the main effects of genotype and childhood maltreatment, and their additive interactive effects, on cognitive function. For rs1360870, there were significant main effects of genotype and childhood maltreatment, and a significant interaction of genotype with childhood trauma affecting attention in both schizophrenia and healthy participants (C-homozygotes in both groups showed worse attention in the context of maltreatment); in SZ, this SNP also affected global neuropsychological function regardless of exposure to childhood trauma, with T-homozygotes showing worse cognition than other genotypes. The mechanisms of trauma-dependent effects of *FKBP5* following early life trauma deserve further exploration in healthy and psychotic samples, and may be particularly informative in subgroups exposed to various other forms of early life adversity (i.e., birth complications, immigration).
Introduction

Exposure to traumatic experiences in the early stages of life, including maltreatment (i.e., physical, sexual or emotional abuse, and various forms of neglect), parental loss or divorce, parental substance abuse, and poverty (Rosenberg et al., 2007) are known to influence the development of severe mental disorders, including schizophrenia (Kessler et al., 2010, Varese et al., 2012). These early life experiences may set about a cascade of biological effects that result in dysregulation of the hypothalamic–pituitary–adrenal (HPA) axis, a key system in stress response (van Winkel et al., 2008a, van Winkel et al., 2008b). Variation in genetic regions known to regulate the stress response system (e.g., the FK506 binding protein 5 [FKBP5] gene) have been implicated as risk factors for bipolar disorder (Willour et al., 2009), and psychotic symptoms in the general population (Collip et al., 2013). The FKBP5 gene codes for the FKBP5 protein that is involved in the regulation of glucocorticoid function in response to stress; notably, elevated levels of FKBP5 confer reduced sensitivity of the glucocorticoid receptor to circulating cortisol, leading to decreased negative feedback regulation of the HPA axis, and thus an abnormally prolonged stress response as the system takes longer to reduce cortisol secretion (Binder, 2009). The relevance of these genetic variations for stress-related psychopathology is thus well established.

For example, a number of studies demonstrate significant interactions between childhood adversity and single nucleotide polymorphisms (SNPs) of the FKBP5 gene (most commonly rs1360870) affecting a range of psychopathologies, including post-traumatic stress disorder (Binder et al., 2008, Xie et al., 2010), depression (Appel et al., 2011, Zimmermann et al., 2011), suicide risk (Roy et al., 2010), aggression (Bevilacqua et al., 2012) and psychosis (Collip et al., 2013). This SNP has also been associated with biased attention toward threat and associated hippocampal function and structure (Fani et al., 2013), and neurophysiology of the cingulum (Fani et al., 2013). Taking one step further to study the
interaction between several \textit{FKBP5} SNPs and childhood maltreatment affecting threat-related amygdala reactivity, White et al. (2012) recently reported significant effects of two common variants (rs9470080 [in high Linkage Disequilibrium with rs1360780] and rs9394309) affecting amygdala function in healthy participants in the context of exposure to childhood emotional neglect (White et al., 2012).

Only two recent studies provide divergent evidence for the interaction of \textit{FKBP5} genotypes and childhood trauma in psychotic samples: one study reports that subclinical psychotic symptoms in a general population sample (N=401) are higher for certain \textit{FKBP5} genotypes (rs9296158, rs992105, rs1360780 [genotyped as rs3800373]) in the context of childhood trauma exposure (Collip et al., 2013); interestingly, only one of these SNPs (rs9296158) interacted with trauma to affect psychotic symptoms in a clinical group (N=195), and two (rs4713916, rs1043805) were associated with psychotic-like symptoms in the unaffected relatives (N=200) of psychosis patients. This study additionally examined genetic variation in association with cortisol levels, showing significant effects of two SNPs (rs9296158, rs4713916) in the context of childhood trauma, in which lower cortisol levels were found in A-homozygotes exposed to trauma in the general population sample. Notably, the analysis for rs1360780 (rs3800373) just failed to reach significance, but suggests that T-homozygotes in the general population (i.e., those reporting greater psychotic-like phenomena) also had lower cortisol levels (Collip et al., 2013). In another study by the same group, these four polymorphisms were examined in relation to their interactive effects with childhood trauma on cognitive performance and hippocampal volume in a relatively smaller sample of psychotic patients (N=89) and their unaffected siblings (N=95) (Hernaus et al., 2014); this study reported null results, and may have been underpowered considering that Collip et al.’s (2013) individual group sample sizes were significantly greater than those of Hernaus et al. (2014).
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Childhood maltreatment has been shown to affect cognitive performance in later life in previous studies of ostensibly healthy adults (Koenen et al., 2003, Pears et al., 2008, Perez and Widom, 1994), people with schizophrenia (Green et al., 2014, Lysaker et al., 2001, Shannon et al., 2009) and people diagnosed with borderline personality disorder (Afifi et al., 2011, Minzenberg et al., 2008). This study thus sought to further investigate potential interplay between four previously implicated genetic variants of FKBP5 (rs4713902, rs3800373, rs1360780, and rs9470080) and exposure to childhood maltreatment in relation to cognitive function (and symptom expression in patients) in a large sample of schizophrenia patients and healthy controls. It was hypothesized that these polymorphisms would moderate the influence of childhood maltreatment on cognitive performance in schizophrenia and healthy controls, and symptom expression in the schizophrenia cases.

Methods

All participants provided written informed consent according to study procedures approved by the Human Research Ethics Committee of the University of New South Wales (originally UNSW Protocol No. 07167; renewed in 2012 as HREC12384).

Participants

Research data obtained from the Australian Schizophrenia Research Bank (ASRB) included full sets of clinical and cognitive data for 617 clinical cases with an ICD-10 diagnosis of schizophrenia (n=526) or schizoaffective disorder (n=91), to be referred to collectively as ‘SZ’, and 659 healthy controls (HC). The ASRB represents a national biobank facility that is open for access by any team of scientists wanting to test hypotheses afforded by these data; formal access protocols ensure appropriate use of the data for relevant scientific purposes. The ASRB research data were collected over the years of 2006-2010 by scientific collaborators across five Australian states and territories (Loughland et al., 2011).
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Exclusion criteria comprised an inability to converse fluently in English, organic brain disorder, brain injury with greater than 24 hours post-traumatic amnesia, mental retardation (IQ < 70), movement disorders, current substance dependence, and/or electroconvulsive therapy received in the last 6 months. In addition, the control participants had no personal history of DSM-IV Axis 1 disorder and no history of psychotic disorder in their first-degree biological relatives. The majority of clinical cases in the ASRB sample were medicated at the time of testing, with 398 participants taking anti-psychotic medication, 69 taking a mood stabiliser, and 145 taking anti-depressants. Overall 612 out of 617 (99.19%) were receiving some type of medication (See Table 1).

Materials

Clinical assessments

Clinical and diagnostic information was obtained using the Diagnostic Interview for Psychosis (DIP), conducted by trained research staff (Castle et al., 2006). The severity of positive symptom was estimated by computing the total score of lifetime hallucination and delusion scores from the DIP (DIP items 49 to 53 and 58 to 64, respectively), and similarly for negative symptom severity was assessed with the Scale for the Assessment of Negative Symptoms (Andreasen, 1983).

Neuropsychological assessment

Premorbid IQ was assessed using the Wechsler Test of Adult Reading (Wechsler, 2001). Measures of neuropsychological function in seven cognitive domains was assessed using the Repeatable Battery for Assessment of Neuropsychological Status (RBANS) (Randolph, 1998) which provides an index five domains: immediate memory, delayed memory, attention, language, construction; in addition, the Letter-Number Sequencing test of the Weschler Adult Intelligent Scales (WAIS) was used as an index of working memory, and
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the Controlled Oral Word Association Test (COWAT) of verbal fluency was used as an index of executive functioning (Spreen and Benton, 1969).

**Childhood Maltreatment**

The Childhood Adversity Questionnaire (CAQ) (Rosenman and Rodgers, 2004) was used to measure both childhood maltreatment and adverse living circumstances (e.g., living in financial hardship, or living with a depressed parent). The CAQ comprises 20 items scored as yes or no that assess experiences of physical abuse, emotional abuse, sexual abuse, emotional abuse, emotional neglect, and family dysfunction. The sub-cohort of clinical cases with available CAQ data for analysis in this study comprised 454 clinical cases (with schizophrenia or schizoaffective disorder) and 502 healthy controls. Only items pertaining to deliberate maltreatment (not adverse living circumstances) were used to indicate exposure to maltreatment (see Analysis section, below).

**Genotyping**

Four common variants on the FKBP5 gene were extracted from an existing array conducted with Infinium Human 610 K BeadChips (Illumina); these were: rs1360780, rs9470080, rs4713902, rs9394309. There were six other available FKBP5 SNPs: rs7757037, rs2294807, rs992105, rs4798346, rs7748266, and rs1475774; however, there was no prior evidence of their association with childhood maltreatment or psychosis, or any relevant cognitive or brain dysfunction, to warrant their investigation in this hypothesis-driven study.

Briefly, genotyping was carried out using 200ug of gDNA was amplified, fragmented and denatured before hybridization. After washing, extension, and staining steps, the BeadChips were dried and scanned on the Illumina BeadArray Reader. A gene call threshold of 0.15 and standard cluster file, provided by Illumina using a multi-ethnic HapMap population, were used to calculate SNP and sample statistics. Population stratification artefact
was considered via Principal Components Analysis of these data on a genome-wide scale (Price et al., 2006), which revealed that less than 2% of cases analysed in the current study were likely to be of non-caucasian descent. Genotype information was available for approximately 93% of the total sample (See Table 2); rs9470080 was in high (but not perfect) LD with rs1360780 ($r^2 = 0.82$) and rs93493909 ($r^2 = 0.87$); no other genotypes were in LD above 0.75 (see Figure 1).

**Statistical Analysis**
All analyses were performed with SPSS version 22; significance threshold was set at $p<0.01$. Exposure to childhood maltreatment was defined by endorsement of any CAQ item representing physical abuse, emotional abuse, sexual abuse, emotional abuse, emotional neglect (Rosenman and Rodgers, 2004); this meant that 69.4% of the SZ cases and 44.2% of the HC group were treated as exposed to childhood maltreatment (Table 1). The definition of maltreatment thus did not include family dysfunction (i.e., perceived household tension household, parental drug/alcohol use, and parental depression) endorsed by approximately 90.7% of the SZ sample, and 71.5% of the HC group. Exposure to childhood maltreatment was coded ‘1’ and non-exposure ‘0’ for focal regression analyses; all SNPs were coded 0/1/2 according to the number of major alleles carried by the individual. A series of hierarchical regressions, conducted for SZ and HC groups separately, tested the main effects of childhood maltreatment and FKBP5 genotype/s, as well as their interactions, on cognitive variables (for SZ and HC) and positive and negative symptoms (for SZ only). Main-effects and interaction terms (G x E) were entered simultaneously into the regression models. Post-hoc ANOVAs were used to determine differences in cognitive performance between cases of the same genotype, within SZ and HC groups separately, in the context of exposure to maltreatment.
Results
Sample characteristics
Descriptive characteristics, and performance on cognitive domains, for the entire schizophrenia (N=617) and healthy control (N=659) groups are presented in Table 1; The SZ cases were more likely to be less educated and unemployed, and were significantly impaired in performance across all cognitive domains compared to healthy controls. Of these individuals, 454 SZ cases and 501 HC provide data on the CAQ. The SZ group reported greater total scores on the CAQ, relative to HC, and the SZ group were more likely to be exposed to all types of childhood adversity relative to HCs (see Table 1).

Genotype frequencies and differences among genotypes
Genotype data were available for the majority of participants (93-94%; min. N=439; max N=444) for each SNP considered. Table 2 presents frequencies of the FKBP5 genotypes for cases and controls; all FKBP5 genotypes were in Hardy-Weinberg equilibrium as calculated using PLINK version 1.07 (Purcell et al., 2007).

Effects of FKBP5 genotypes and childhood maltreatment on cognition
Tables 3 and 4 present the results of two series of regression analyses (one for each participant group, SZ and HC) to examine the main effects of four FKBP5 SNPs, the main effect of childhood maltreatment, and the interaction of these factors, on cognitive function in eight domains (and on positive and negative symptoms for SZ).

The most consistent associations were found in significant main effects of genotype (rs1360780), childhood adversity, and the interaction of these factors, on the cognitive domain of attention for both SZ cases and controls (See Tables 3 and 4; Figure 2). In addition, for SZ cases only, main effects for this SNP, for childhood maltreatment, and a significant interaction contributed to variation in RBANS total scores (See Table 3; Figure 2).
Post-hoc tests for rs1360780 suggested that participants homozygous for the major allele (CC genotypes) who had experienced maltreatment were significantly more impaired on the domain of attention, relative to CC counterparts not exposed to attention, although this finding did not reach significance in either group (SZ: F_{1,104} = 3.68, p<0.06; HC: F_{1,151} = 1.20, p<0.20).

In SZ cases only, interaction between rs9394309 and childhood maltreatment significantly affected language ability in schizophrenia, in the context of significant main effects of childhood maltreatment, and a near significant effect of genotype. Post hoc tests conducted for SZ cases only, suggested that this may have been driven by lower language ability in heterozygotes (AG) exposed to maltreatment; however the difference (relative to unexposed AG genotypes in SZ) was not significant (F_{1,159} = 0.33, p<0.86). There were no significant main effects or interactions for this SNP in the HC group.

For rs4713902, there was a significant interaction between genotype and childhood maltreatment affecting for IQ estimates derived from the Wechsler Adult Reading Test in the HC group only (see Table 4; Figure 2). Post hoc tests showed that this finding in the HC group reflected significantly lower IQ in major homozygotes (AA) who were exposed to maltreatment, compared to those genotypes who were not (HC: F_{1,151} = 6.19, p<0.01).

There were no significant models involving rs9470080 in either SZ or HC groups, although the model for attention showed a trend toward an interactive effect between genotype and childhood maltreatment (p<0.03), in line with the findings for rs1360780 (for which this SNP is in high LD).

**INSERT TABLE 2 ABOUT HERE**
Effects of \textit{FKBP5} genotypes and childhood maltreatment on symptoms

There were no significant main effects or interactions between exposure to childhood maltreatment and any \textit{FKBP5} genotypes in models testing effects on positive and negative symptoms in SZ.

Discussion

This study demonstrates that common variation in one \textit{FKBP5} genotype (rs1360780) moderates the effects of exposure to childhood trauma on the cognitive domain of attention, and in both schizophrenia and healthy individuals. An additional finding, for SZ cases only, was that this SNP (rs1360780) contributed to variation in general neuropsychological function (total RBANS scores). Effects for other genotypes were less consistent: rs9394309, affected language ability in schizophrenia, and its effects were moderated by childhood maltreatment. For rs4713902, there was a significant interaction between genotype and childhood maltreatment affecting IQ in the HC group, and significant interaction affecting attention in the SZ group (though the overall model did not reach significance). There were no significant models involving rs9470080 for either group.

The consistent effects of rs1360780 on attention in both SZ and HC participants were seen in somewhat worse attention deficits for CC homozygotes in the context of trauma exposure (compared to non-exposed CC homozygotes within each group; see Figures 1A and 1B). In SZ only, the main effect of genotype for rs1360780 in SZ participants appeared to reflect worse performance on attention in T-homozygotes regardless of childhood trauma history (Figure 1A), in line with the T-allele as that previously implicated in risk for psychosis (Collip et al., 2013). Variation in this SNP also contributed to broader neuropsychological dysfunction only in schizophrenia, with the T-homozygotes showing worse overall performance in a U-shaped model in which neuropsychological function was
optimal in the heterozygous (T/C) genotype (Figure 2A). This pattern was also seen in the healthy control group, where IQ estimates were highest for heterozygous (AC) genotypes of the rs4713902 SNP (Figure 2B), in the context of worst performance in the AA homozygotes.

These findings are consistent with a growing number of studies demonstrating the effects of this particular genotype on a variety of psychopathologies, including post-traumatic stress disorder (Binder et al., 2008), depression (Appel et al., 2011, Binder, 2009), suicide risk (Roy et al., 2010), aggression (Bevilacqua et al., 2012) and psychotic symptoms and cortisol levels in the general population (Collip et al., 2013). In the study by Collip et al. (2013), the T-allele of rs1360780 was associated with greater sub-clinical psychotic symptoms and decreased cortisol levels in the healthy population, but not psychotic patients; while this may seem counter-intuitive, it is consistent with the present lack of evidence for the effects of rs1360780 on psychotic symptoms in schizophrenia. However, our findings for the association between rs1368070 and attention (as well as other significant results for other SNPs and cognitive domains) are in contrast with the findings of Hernaus et al. (2014) who reported no interactive effects of this SNP on cognitive performance (verbal memory and working memory tasks) in psychotic patients and their unaffected siblings (Hernaus et al., 2014). It is possible that the study by Hernaus et al. lacked sufficient power (group Ns<100), or that these cognitive domains are specifically not affected by variation in FKB5 interacting with trauma; notably no significant results for these domains were revealed in the present study. Future studies might thus attempt to replicate the present effects of rs1360780 on attention in the context of childhood trauma, which was revealed consistently for both healthy control and schizophrenia participants in this study.

That trauma-dependent neurobiological effects of FKB5 variation may not be limited to psychopathology, but may also affect cognitive functions in healthy individuals, is perhaps not surprising with consideration of the complexity of these systems in previous
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studies of patients with post-traumatic stress disorder (PTSD) symptoms. These studies demonstrate different functional association between high protein expression of *FKBP5* (associated with certain genotypes including rs1360780 and 9470080; see Binder, 2009) and glucocorticoid-receptor (GR) resistance (sensitivity to cortisol secretion) in clinical and healthy populations; that is, the alleles associated with high protein expression confer increased GR resistance in those without clinical symptoms of trauma, while the alleles associated with lower protein expression confer reduced GR resistance in individuals with PTSD. These studies suggest that environmental effects on the expression *FKBP5* may reflect epigenetic processes (such as DNA methylation) of the *FKBP5* gene that have not been considered here. Future study of the epigenetic regulation of *FKBP5*, and effects on downstream molecular function, may provide new insights for the development of pharmacological agents that target systems affected by the neurobiology of stress and trauma exposure. These may form the basis of new treatments for cognition; indeed, one previous study shows positive effects of manipulation of glucocorticoid receptor (GR) function on cognitive performance in bipolar disorder (Young et al., 2004); however, interestingly, a similar study of GR manipulation in schizophrenia which was *not* effective in improving cognition (Gallagher et al., 2005).

Limitations of this study include those inherent to self-report instruments used to measure childhood adversity; in this case, the CAQ measured only adverse experiences perpetrated by parents, and may thus have precluded reporting of other potential traumatic events, such as abuse by non-immediate family members; this may account for the low rate of sexual abuse reported. Similarly, the effects of other types of significant life stresses (e.g., death of a close family member) were not captured by the CAQ. Neither did we attempt to study here the effects of specific types of childhood abuse or neglect, given the high rates of endorsement of multiple types of trauma (and low rates of sexual abuse) reported by the
participants. Finally, the potential confounding effects of medication cannot be ruled out, since we were unable to estimate the effects of medication dosage on cognitive function. This information was not collected owing to the limited reliability of collecting these details from outpatient participants in a collaborative national consortium of this size. Finally, there was high LD among some of the SNPs under investigation (especially between rs9470080 and two other SNPs; see Figure 1); in line with this, the effects of this SNP on attention in healthy controls approached significance in the same direction as those of rs1360780.

In summary, this study demonstrates significant interactive effects of *FKBP5* genotypes and childhood maltreatment on cognition in both the healthy population and people with schizophrenia. Future study of stress-related effects on the developing brain will be useful to determine the mechanisms by which these genetic variations contribute to the development of psychotic disorders and cognitive functioning, in the context of adverse environmental exposures. Trauma-dependent neurobiological effects of *FKBP5* variation may also be evident in people exposed to various other forms of early life adversity (i.e., birth complications, migration, sexual abuse), such that study of these groups is warranted.
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