The emerging role of the FKBP5 gene polymorphisms in vulnerability-stress model of schizophrenia: further evidence from a Serbian population

Author list

Marina Mihaljevic, Clinic for Psychiatry, Clinical Centre of Serbia, Pasterova 2, 11000, Belgrade, Serbia;

Katarina Zeljic, Genetics& Evolution Department, Faculty of Biology, University of Belgarde, Studentskitrg 3,11000, Belgrade, Serbia;

Ivan Soldatovic, Institute for Biostatistics, Medical Informatics and Researches in Medicine, Faculty of Medicine, University of Belgrade, Dr Subotica 15 11000, Belgrade, Serbia;

Sanja Andric, Clinic for Psychiatry, Clinical Centre of Serbia, Pasterova 2, 11000, Belgrade, Serbia;

TijanaMirjanic, Special Hospital for Psychiatric Disorders Kovin, Cara Lazara 253, 26220, Kovin, Serbia;

Alexander Richards, MRC Centre for Neuropsychiatric Genetics and Genomics, Institute of Psychological Medicine and Clinical Neurosciences, Cardiff University School of Medicine, Cardiff CF24 4HQ, Wales, United Kingdom;

Kiran Mantripragada, MRC Centre for Neuropsychiatric Genetics and Genomics, Institute of Psychological Medicine and Clinical Neurosciences, Cardiff University School of Medicine, Cardiff CF24 4HQ, Wales, United Kingdom;

Tatjana Pekmezovic, Institute of Epidemiology, Faculty of Medicine, University of Belgrade, Visegradska 26, 11000, Belgrade, Serbia;

Ivana Novakovic, Institute of Human Genetics, Faculty of Medicine, University of Belgrade, Visegradska 26, 11000, Belgrade, Serbia;

Nadja P. Maric, Faculty of Medicine, University of Belgrade, Dr Subotica 8, 11000, Belgrade, Serbia;Clinic for Psychiatry, Clinical Centre of Serbia, Pasterova 2, 11000, Belgrade, Serbia;

Corresponding author: Marina Mihaljevic; e-mail: mihaljevicm@yahoo.com; telephone: +38163-89-201-44

Abstract word count: 247

Abstract

Increased reactivity to stress is observed in patients with schizophrenia spectrum disorders and their healthy siblings in comparison to the general population.

Additionally, higher levels of neuroticism, as a proposed psychological measure of stress sensitivity, increasethe risk for schizophrenia. HPA axis dysregulation is one of the possible mechanisms related to the vulnerability-stress model of schizophrenia and recent studies revealed a possible role of the functional genetic variants of FK506-binding protein 51 (FKBP5) gene which modulate activity of HPA axis.

The purpose of the present study was to investigate impact of FKBP5 on schizophrenia in Serbian patients, and to explore relationship between genetic variants and neuroticism by using the case-sibling-control design. In 158 subjects we measured psychotic experiences, childhood trauma and neuroticism. Nine single nucleotide polymorphisms (rs9295158, rs3800373, rs9740080, rs737054, rs6926133, rs9380529, rs9394314, rs2766533, rs12200498) were genotyped. The genetic influence was modeled using logistic regression, and the relationship between genetic variants and neuroticism was assessed by linear mixed model. Our results revealed genetic main effect of FKBP5 risk alleles (A allele of rs9296158 and T allele of rs3800373) and AGTC "risk" haplotype combination (rs9296158, rs3800373, rs9470080, rs737054, respectively) on schizophrenia, particularly when childhood trauma was set as a confounding factor. We confirmed strong relationship between neuroticism and psychotic experiences in patients and siblings and further showed relationship between higher levels of

neuroticism and FKBP5 risk variants suggesting potential link between biological and psychosocial risk factors. Our data support previous findings that trauma exposure shapes FKBP5 impact on schizophrenia.

Keywords: schizophrenia, healthy siblings, FKBP5 genetic variants, haplotypes, neuroticism

Introduction

The vulnerability-stress model has long been proposed as an important framework for the study of etiology and clinical courseof all major psychiatric disorders including schizophrenia and related psychotic disorders [1-3]. The model offered substantial evidence that schizophrenia isa stress-sensitive disorder and highlighted increased interpersonal sensitivity to environmental stressors in patients [4]. Regarding this hypothesis, several lines of evidence indicated that major neurobiological mechanism for vulnerability-stress model present the hypothalamic-pituitary-adrenal (HPA)axisdysregulation found across the illness course[5]. Blunted cortisol awaking response is consistently reported in drug-naive patients [6, 7], ultra-highrisk group for psychosis [8], andalso in converters to psychosis[9]. Elevated basal cortisol levelswerepresent in the different phases of schizophrenia, associated with symptoms severity and cognitive functioning [10, 11], while theresults derived from the post-mortem tissue of patients with schizophrenia revealed decreased mRNA expression of the glucocorticoid receptor (GR), the primary receptor responsive to cortisol and thus stress response[12]. Regarding hyperdopaminergic activity in the mezolimbic area observed in schizophrenia, it is suggested that HPA axis triggers a cascade of events resulting GR impairment and potential to increase activity of dopamine pathway involved in schizophrenia and related psychotic disorders [13].

The pivotal role in the regulation of GR activity hasa co-chaperone FK-506 binding protein 51 (FKBP51) which modulates GR sensitivity to cortisol [14]. Higher expression of FKBP51 leads to an altered GR responsiveness to cortisol and thus impaired regulation of the HPA axis negative feedback loop [15]. FKBP5 gene, located on the chromosome 6p21, encodes this important protein. According to the consistent evidence, FKBP5 comprises several functional single nucleotide polymorphisms (SNPs), such as rs1360780, rs9296158, rs3800373 and rs9470080,which genotypes containing minor allele (T, A, G, T, respectively) are related to the increased FKBP5 expression[16] and thus impaired HPA axis negative feedback loop[17] as well as to allele-specific FKBP5 epigenetic changes[18]after stress exposure . Particularly, these genetic variations determining expression of FKBP51 may explain higher sensitivity to trauma. Genotypes of these functional SNPs with minor (risk) allele have been consistently associated with broad range of stress-related psychiatric disorders such as PTSD, depression, bipolar disorder, borderline personality disorder [16-21] and pathological conditions (suicide, aggressive behavior) following trauma exposure [22, 23].The first evidence for the role of FKBP5 ina non-affective psychosis was reported by Collip and colleagues [24], revealing significant effect of the interaction between risk alleles and childhood trauma on psychotic symptoms. Alongside with the clinical population, given interaction was found at the subclinical level of psychotic symptoms, by evaluation of patients' healthy siblings and controls. After this publication, several studies confirmed the association between FKBP5risk alleles and psychosis in individuals that reported stressful environmental exposure [25, 26], moderating cognitive impairment [27] as well as their different response to clozapine [28]. In addition, FKBP5 mRNA levels were shown to be increased in the postmortem tissues of patients with schizophrenia [29, 30]. Regarding these evidences, recent review proposed the role of FKBP5 gene for vulnerability-stress model of schizophrenia related to gene-stress interaction [31].

The vulnerability-stress model also emphasizes important role of psychosocial risk factors in the etiology of schizophrenia. It is well established that personality trait neuroticism could be considered as a psychosocial risk factor as it is characterized by chronic negative affect and susceptibility to stress (distress proneness). Studies confirmed that young individuals with higher neuroticism have increased risk for later schizophrenia [32-34] and that this trait has been associated with

psychotic symptoms severity [35] as well as patients' functional outcomes [36].Interestingly, Boyette and colleagues revealed stronger association between neuroticism and psychotic symptoms in patients with psychosis and their healthy siblings compared to controls [37]. These findings are related to an increased stress sensitivity observed in patients and their healthy siblings, which suggest that altered stress-sensitivity should be considered as a familial risk for psychosis [38, 39]. Particularly, larger increase of negative affect in relation to stress has been found in patients and their siblings compared to controls [40]. Higher levels of neuroticism lead to more negative affect experiences and more sensitive response to environmental stressors which is exactly what vulnerability-stress model postulate. It is also suggested that the systems involved in adaptation, such as HPA axis, could play an important role in the neurobiological pathway of neuroticism [41]. In addition, recent study revealed strong correlation between negative affect and higher expression of FKBP51protein level suggesting potential biological explanation of interplay between neuroticism, as a measure of proneness to negative affectivity, and increased stress sensitivity [42].

The purpose of the present study was to analyze biological (FKBP5 genetic variants) and psychosocial (neuroticism) risk factors and their interrelationship as a pathway of altered stress reactivity proposed by vulnerability-stress model of schizophrenia. Regarding consistent evidence ofFKBP5 role in the stress-related disorders, including schizophrenia, we hypothesized thatin Serbian sample FKBP5risk variants would have an impact on patients' status following trauma exposure and, due to increased stress-sensitivity reported in healthy siblings, different distribution of FKBP5 risk variants between siblings and controls could be found. In conjunction with the findings that increased FKBP51 expression strongly correlates with negative affectivity, we hypothesized that neuroticism could be associated with risk ("high induction") allele carriers ofFKBP5 functional SNPs. Accordingly, the first objective of the present study was to investigate differences in genotype, allele and haplotype distribution of FKBP5 genetic variants among patients, their healthy siblings and controls. In addition, we examined the genetic main effects on patients' status taking into account childhood trauma as a confounding factor. The second objective of the research was to replicate the findings of Boyette and colleagues [37], showing stronger association between neuroticism and psychotic experience in patients and siblings compared to controls, and to further investigate relationship between neuroticism and potential biological risk factors such as specific FKBP5variants.

Materials & Methods

Participants

The data collection was conducted in Serbia, as a part of the European Network of National Networks studying Gene-Environment Interactions in Schizophrenia (EUGEI) [43]. In this cross-sectional study, the sample comprised of 52 patients within schizophrenia spectrum disorders, 55 of their healthy siblings (first-degree relatives), and 51 healthy controls. Patients were selected by clinicians from two regional mental health institutions from Belgrade and surrounding (University Psychiatric Clinic in Belgrade and Special Psychiatric Hospital in Kovin). Healthy siblings were sampled through participation of the patients, and controls were included through a local advertisement. Inclusion criteria for the patients were (I) age range 18-40, (II) IQ >70, (III) meeting DSM-IV [44] criteria for schizophrenia spectrum disorders, not caused by neurological disorder or substance abuse assessed by Mini International Neuropsychiatric Interview(MINI 5.0.0)[45], (IV) maximum duration of illness <10 years, (V) remission status (GAF scores >40) [46]. Siblings and controls had no evidence of current/past history of psychiatric disorder and no recent history of alcohol or drug abuse as verified by MINI.For the controls, the occurrence of psychotic disorder in any first-degree family member, assessed using the Family Interview for Genetic Studies (FIGS) [47], was constituted as an exclusion criterion. The control participants were matched by age and gender with sibling participant.

After full explanation of the study, all participants agreed to participate and written informed consent was obtained. The research was performed in compliance with the International Code of Medical Ethics of the World Medical Association (Declaration of Helsinki) and was approved by the appropriate hospitals' ethics committee and Ethics Committee of Faculty of Medicine, University of Belgrade.

Phenotype and confounding measures

Psychotic experiences

We used the Community Assessment of Psychic Experiences to rate self-reports of psychotic experiences. The CAPE measures the life time prevalence of the subclinical positive, negative and depressive symptoms as well as a distress related to the symptoms [48]. It provides a total score per these three dimensions by adding up the scores on the frequency items. Items are scored on a 4-point scale. Studies using CAPE in the general population have shown good psychometric properties in terms of reliability and validity [49]. It is used to assess subclinical psychotic symptoms on the psychosis continuum, as a clinical and extended psychosis phenotype [36, 50]. For the purposes of the present study, we included standardized sum scores of the subscale (score range 1-4) for positive symptoms (CAPE-P) and depressive symptoms (CAPE-D) in the further analyses.

Neuroticism

All participants were asked to fulfill a 30-item scale extracted from the Eysenck Personality Questionnaire (EPQ-103) which was measuring personality trait neuroticism [51]. Neuroticism is a very well established measure of distress proneness, increased sensitivity to stress, and tendency to experience more overall psychopathology. Higher levels of neuroticism reflect increased self-referential evaluation of negatively valence stimuli. It is confirmed that neuroticism is influenced by substantial genetic background [41]. Studies in schizophrenia patients reported stability of personality traits over time, but recent study suggested that depressive symptoms could have a strong influence on personality traits stability after three years of follow-up [52]. Thus, we included in our phenotype analyses CAPE-D as a covariate. Participants completed EPQ neuroticism (EPQ-N) on the same day when testing was performed.

Childhood trauma

Childhood adversities were measured by the Childhood Trauma Questionnaire (CTQ) [53]. It is a retrospective self-report questionnaire, consisting of 25 items rated on a 5-point Likert scale (1=never true, 5=very often true). CTQ is one of the most frequently used scales assessing childhood advertises related to different psychopathology as well as psychotic disorders [24, 36], with well confirmed validity and reliability in the general population[53]. It is measuring five types of childhood trauma: emotional abuse, physical abuse, emotional neglect, physical neglect and sexual abuse. Each domain of trauma severity is ranging from 1 to 5. Consistent with previous literature, we summed the mean score for all 25 items (CTQ-total) to obtain a continuous measure of CTQ symptoms severity (score range 1-5).

Genetic data

DNA extraction, SNP Genotyping

All participants included in this study were Caucasians, of the same Serbian ethnicity, with former Yugoslavian origins confirmed using FIGS. DNA was extracted from EDTA-treated whole blood using the salting-out method [54]. Genotyping for rs9296158 was performed using TaqManallelic discrimination

assay [55] on the ABI 7900HT (Applied Biosystems, Foster City, CA, USA). Genotyping for rs3800373, rs9740080, rs737054, rs6926133, rs9380529, rs9394314, rs2766533, rs12200498 were performed on the Illumina's Infinium platform using IPMCN chip (Institute of Psychological Medicine and Clinical Neurology, Cardiff University) custom-made for EUGEI sample [43]. Genotype data passed quality control, with call rate > 98% for all polymorphisms. A total of nine SNPs were included in genetic analyses for the genotype distribution, allele and haplotype frequencies. After these evaluations, for the association analyses we selected three SNPs (rs3800373, rs9470080, rs9296158) on the basis of their functional significance confirmed in the literature that we explained in the introduction.

Hapotype analysis

Hardy–Weinberg equilibrium (HWE), pairwise linkage disequilibrium (LD) (D′ and r2 values) and minor allele/haplotype frequencies of SNPs were computed using the Haploview software, version 4.2 [56]. Chromosomal position, allelic distribution, HWE and role of all genotyped FKBP5 SNPs are presented in the Table S1 (see Supplementary Materials). All of the analyzed SNPs were in Hardy–Weinberg equilibrium concordance, first examined for all participants taken together and then for each group separately. "Solid spine block" definition was used for haplotype block identification, following "solid-spine" criteria D' ≥ 0.80. Permutation testing to calculate corrected p values for multiple testing of 1,000 simulations was performed using Haploview software. Linkage disequilibrium plots, with D' among analyzed SNPs presented within groups and haplotypes frequencies (patients vs. controls/ siblings vs. controls) are presented in the Figure 1. Haploview computed two haploblocks, one consisted of four SNPs including three functional SNPs (rs3800373, rs9296158, rs9470080) and rs737054, and the other one consisted of five SNPs (rs6926133, rs9380529, rs9394314, rs2766533 and rs12200498). Based on the previous research [57], haplotype combination comprised of minor "risk" alleles (AGTC) and presented with one or two copies were grouped together and assigned as group '1', thus individuals with zero copies of this haplotype were assigned as group '0'. This dichotomous variable was utilized in subsequent analyses to examine main effect on patients' status and phenotype.

Statistical analysis

Data were analyzed using the SPSS software, version 20.00 (SPSS, Chicago, IL, USA). Data are presented as counts (percents), mean ± standard deviations or median (range), depending on data type and distribution. Between group differences in demographic characteristics (age and gender), phenotype measures (CAPE-P, EPQ-N) and confounding factors (CTQ-total, CAPE-D) were assessed using chi-square and Kruskal-Wallis test. Bonferroni adjustment was applied for multiple comparison.

Genetic analyses

First objective of the study was to compare genotype distribution and allele frequencies among the groups, for which contingency tables and the chi-square test were used. The two tailed Pearson X^2 was performed for 2x2 contingency tables to compare genotypes frequencies between the groups (patients vs. controls/ siblings vs. controls). Fisher's Exact Test was used for 2x2 contingency tables to compare allele and AGTC haplotype frequencies among the groups (patients vs. controls/siblings vs. controls). Bonferroni correction for multiple comparison was applied setting statistical significance threshold (α) at 0.025. Further, we performed a binary logistic regression analyses to assess the genotype and allele association with patients' status. We used general (AA as a reference genotype: AA vs. Aa/AA vs. aa), dominant (Aa+aa vs. AA) and recessive (AA+Aa vs. aa) genetic model. Additionally, we performed the logistic regression to asses

AGTC haplotype association with patients' status. All genetic main effects were analyzed in three models using OR (odds ratio) with 95% confidence interval (95%CI) as a measure of effect. First we preformed unadjusted analyses (Model 1), then we made adjustments for age, gender and depressive symptoms (Model 2) as previously suggested due to FKBP5 effects on depression phenotype [24], and in the third model we added childhood trauma as a confounding factors (Model 3). We preformed GxE interactions in the last set of the analyses, accounting genetic data as a dichotomous variable (risk allele carries vs. no-risk allele carriers). We did not correct our results for multiple testing as our SNPs are in high LD and regarding previous substantial evidence reported in the literature of their biological significance and influence on different psychopathology.

Phenotype analyses

According to the second objective of the present study, Spearman's Rho correlations and linear regression were used to analyze relationship between EPQ-N and CAPE-P. Further on, as we were particularly focused on neuroticism as a psychosocial risk factor, we utilized rigorous statistical method to assess the effect of SNPs/AGTC haplotype on neuroticism, performing in all participants and healthy individuals separately. For that purpose we used the linear mixed model. EPQ-N was transformed with square root transformation and set as an outcome variable. SNPs/AGTC haplotype were separately included in the model as a categorical variable ('0' - non risk allele carriers, '1' - risk allele carriers / '0' - zero copies of AGTC haplotype, '1' - 1 or 2 copies of AGTC haplotype, respectively). Analyses were controlled for several potential confounders: age, gender, group status, and CAPE-D. Intra-family correlation (Family ID) was set as a random factor as previously described [37]. Phenotype analyses were first performed for all participants and then only for healthy participants (sibling and controls). All reported p values were two-sided and considered as significant if less than 0.05.

Results

Description of the sample, phenotype and confounding measures

Main characteristics of all participants are presented in the Table 1. No differences between the groups in terms of demographic characteristics were observed. Patients reported significantly higher levels of phenotypic characteristics (CAPE-P, CAPE-D, and EPQ-N) compared to siblings ($p \leq 0.001$) and controls ($p < 0.001$), respectively. Patients experienced more trauma in childhood (CTQ-total) compared to controls (p=0.003), while no differences in trauma levels were evident between patients and siblings (p=0.264), and between siblings and controls (p=0.305).

Table 1

Association between neuroticism and psychotic experiences among the groups

Spearman's Rho correlations revealed that CAPE-P correlated strongly with EPQ-N in patients (r=0.583, p<0.001) and siblings (r=0.339, p=0.013), whereas no significant correlation was observed in the control group (r=0.204, p=0.231). The liner regression analysis (Table 2) confirmed the association between neuroticism and psychotic experiences in patients (b=0.301, p<0.001) and siblings (b=0.252, p=0.003) and, as expected, no significant association was found in the control group (b=0.035, p=0.224). Also, strong correlation between CAPE-D and EPQ-N in patients (r=0.732, p<0.001), siblings (r=0.434, p=0.001) and controls (r=0.595, p<0.001) was detected, thus CAPE-D was set as a confounding factor in the genetic and phenotype analyses.

Table 2

Genotype distribution and allele frequencies

Genotype distribution and allele frequencies of the studied functional SNPs (rs9296158, rs3800373, and rs9470080) are presented in the Table S2 (see Supplementary Materials). Among all studied SNPs there was a trend in genotype distribution between patients and controls only for rs380373 (p=0.041). Regarding the allele frequencies, differences between patients and controls were evident for minor "risk" allele of rs3800373 (G allele:37.5% vs. 22.5%, respectively; p=0.020), while trend was found for risk allele of rs9296158 (A allele: 43.1% vs. 30.0%, respectively; p=0.053). There were no differences in the aforementioned functional SNPs comparing sibling vs. controls. Differences between groups in genotype distribution and allele frequencies among other SNPs (rs737054, rs6926133, rs9380529, rs9394314, rs2766533, rs12200498) were not observed.

Haplotype frequencies

Haploview generated two haploblocks (Figure 1).First block contained three functional SNPs (rs9296158, rs3800373, rs9470080) and rs737054. Around 90% of participants in the first block were presented with GTCC, AGTC, or GTCT haplotype. AGTC haplotype comprised of three "risk" alleles (A, G, T, respectively) presented "risk" haplotype. Our results showed that AGTC haplotype was significantly more frequent in patients compared to controls (35.5% vs. 21.3%, p=0.026). GTCC haplotype, with no "risk" alleles, and thus observed as "non-risk" haplotype tended to be more frequent in controls compared to patients (42.8% vs. 29.7%, p=0.053) and siblings (42.8% vs. 29.1%, p=0.038), respectively. Further, there was a tendency that GTCT haplotype (with no "risk" alleles), also observed as "non-risk" haplotype, was more present in siblings compared to controls (35.4% vs. 22.4%, p=0.054). In the second block GTCTC, GTTCC, TTCCC haplotypes tended to be more frequent in siblings compared to controls (p=0.076, p=0.074, p=0.064, respectively), but taken together these blocks were presented in around 15% of participants. Differences in the second block between patients vs. controls were not found. After calculation of corrected p values obtained after 1,000 permutations, observed differences in the distribution were not significant. In addition to haplotype analyses and following haplotype assignment, individuals with one or two copies of AGTC "risk" haplotype were grouped together (group '1') and compared to individuals with zero copies of this haplotype(group '0'). Group '1' was more frequent in patients compared to controls (64.7% vs. 35.4%, respectively; p=0.005) while siblings were in intermediate position regarding AGTC haplotype (Table S3, Supplementary Materials).

Figure 1

Association analyses between functional SNPs/AGTC haplotype and schizophrenia

Major findings of case-control association analyses between functional SNPs/AGTC haplotype and schizophrenia are presented in the Table 3.The logistic regression revealed strong genetic main effect under the dominant genetic model for rs3800373 (TG+GG vs. TT), showing strong impact of "risk" G allele carriers on patients' status under unadjusted model (OR=2.742; p=0.014), with the pattern of "increasing risk" after including childhood trauma into the model (OR=4.261, p=0.004).The general genetic model for rs3800373 is presented in Table S3 (see Supplementary Materials). Results of the unadjusted logistic regression showed that heterozygous TG of rs3800373, with "risk" G allele, was associated with increased risk for schizophrenia spectrum disorders compared to homozygous TT (OR = 2.644; p=0.032). After adjusting, analyses yielded the same pattern in the second and particularly in the third model, where childhood trauma was included as a confounding factor(OR = 3.662; p=0.008). Additionally, after accounting childhood trauma, homozygous for "risk" G allele (GG) was strongly associated with increased risk for schizophrenia compared to homozygous TT (OR=8.561; p=0.027).

The similar pattern of the association was observed for rs9296158. Homozygous for "risk" A allele increased the risk for schizophrenia compared to carriers of GG genotype after taken into account childhood trauma (OR=6.738; p=0.030) (Table S3, Supplementary Materials). Following "risk" A allele contribution, the dominant genetic model (GA+AA vs. GG) showed borderline significance on patients' status particularly after adjusting for childhood trauma (OR=2.687; p=0.052) (Table 3). The general genetic model of rs9470080 revealed strong effect of homozygous for "risk" T allele compared to homozygous CC only after adjusting for childhood trauma (Table S3, Supplementary Materials) (OR=5.006; p=0.044). Regarding "risk" AGTC haplotype, the logistic regression presented in the Table 3 revealed that AGTC haplotype carriers have increased risk of developing schizophrenia compared to individuals with no copies of this haplotype in unadjusted analysis (OR = 3.343, p=0.004), whereas the risk for developing schizophrenia increased after adjustment for childhood trauma (OR = 5.191, p=0.002). GxE interactions were not detected (Table S4, Supplementary Materials).

Table 3

Association between neuroticism and the functional SNPs/AGTC haplotype

In the whole sample analysis, a strong association between risk allele carriers of the functional SNPs/risk AGTC haplotype with higher levels of neuroticism was found (Table 4). Analysis of the whole sample, revealed that risk allele of rs9296158 (b=0.338, p=0.003), rs3800373 (b=0.320, p=0.006), rs9470080 (b=0.317, p=0.006) and AGTC haplotype combination (b=0.342, p=0.004) were strong predictors of the higher levels of neuroticism, controlling for age, gender, group status, intra-family relationship and CAPE-D. After focusing on the non-clinical population only (patients were excluded), results were conclusive with previous, confirming that risk allele of rs9296158 (b=0.293, p=0.029), rs3800373 (b=0.306, p=0.025), rs9470080 (b=0.295, p=0.030) and AGTC haplotype combination (b=0.310, p=0.027) were strong predictors of higher neuroticism.

Table 4

Discussion

The results of the present study supported vulnerability-stress model for schizophrenia and hypothesis of increased stress-sensitivity reported in patients and their healthy siblings. We systematically presented three lines of evidence: 1) we confirmed genetic main effect of FKBP5 risk alleles on presence of schizophrenia particularly after including childhood trauma as a confounding factor, highlighting the importance of accounting exposures to environmental stressors in genetic studies; 2) we revealed for the first time higher frequencies of "risk" haplotype in patients and higher frequencies of "non-risk" haplotype in healthy siblings and controls; and 3) we confirmed a strong relationship between neuroticism and psychotic experiences in patients and siblings as similar toBoyette et al. study [37] and we further found significant association between risk allele carriers/risk haplotype and higher neuroticism, suggesting a potential psychosocial inherited risk phenotype for schizophrenia related to vulnerability-stress hypothesis.

Firstly, our results revealed an increased risk for schizophrenia under the dominant genetic model for rs3800373 and rs9296158, showing strong genetic influence of their risk alleles (G and A, respectively) on the disease particularly after accounting for childhood trauma. Previous findings from the literature suggested that FKBP5 risk variants contribute to psychosis risk only in the presence of specific environmental risk factors. For example, Collip et al. [24] presented evidence of GxE interaction impact on the psychotic symptoms across psychosis continuum, whereas environmental risk factor was childhood trauma

and genetic risk variants were risk A allele carriers of rs9296158 and rs471396. Similarly, we observed increased genetic impact of risk A allele of rs9296158 on schizophrenia when we included childhood trauma as the confounding factor. We did not observe GxE interaction probably because our childhood trauma scores in patients were lower than reported in their study (in our sample CTQ-total mean score was1.4, while Dutch sample had CTQ-total mean score 1.7).On the other hand, Ajnakina et al. highlighted genetic main effect on the presence of psychotic disorder but only after including environmental exposures as confounding factors [25]. In their study different environmental risk factors were set as confounding factors under the dominant genetic model for rs1360780, showing strong genetic effect of the risk T allele after accounting for parental separation and cannabis use. Our results under the dominant genetic model for rs3800373 are in line with the results of Ajnakina and colleagues. Specifically, our unadjusted genetic main effect and confidence interval of risk G allele carriers of rs3800373 (OR=2.74, 95%CI: 1.22 to 6.14, p=0.014) were very similar to their adjusted genetic main effect of risk T allele carriers of rs1360780 (OR=2.81, 95%CI: 1.23 to 6.43, p=0.002).Consistently, we observed that the risk for schizophrenia increased when childhood trauma was included as a confounding factor into the model (OR=4.26, p=0.004). In addition, we controlled association analyses for depressive symptoms to avoid the limitation reported in their study, and it did not alter the observed effect. Similar results between the research groups are expected as our analyzed SNPs are consistently reported in the strong LD with rs1360780 [16-18].Beside these findings in psychosis, several other research lines have shown genetic influence of risk G allele for rs3800373 and risk A allele for rs9296158, following childhood trauma exposure, on the development of PTSD and depression [16, 17, 19].

To the best of our knowledge this is the first study that preformed FKBP5 haplotype analysis in schizophrenia patients and their healthy siblings compared to controls. However due to the small sample size it should be considered as a pilot study. We observed that AGTC haplotype with three risk alleles (AGT), and assigned as a risk haplotype also in the literature [57, 58],was more frequent in patients compared to controls. GTCT haplotype (with no-risk alleles) was more frequent in controls either when we compared to patients or to siblings. Interestingly, GTCC haplotype,also comprised of no-risk alleles, was more frequent in siblings compared to controls. After performing permutation analysis, as a more conservative method, haplotype differences did not remain significant. This could be due to the sample size rather than false positive results, because our results are consistent with the previous findings. Studies showed that FKBP5 haplotype comprised of risk alleles for functional SNPs (rs9296158, rs3800373, rs1360780, rs9470080) had strong influence in interaction with trauma on stress-related psychiatric disorders [20, 57, 58]. Indeed, we found strong impact of FKBP5 risk haplotype on schizophrenia, with increased influence when we accounted childhood trauma.Same studies suggested that individuals with no-risk alleles in the haplotype had protective effect related to stress conditions. This could be one of the explanations of higher non-risk haplotype frequencies in the controls and siblings in our sample as a potential protective or resilient biological mechanism.

Our phenotype analyses revealed similar results to the observation of Boyette et al. [37], confirming strong association between neuroticism and psychotic experiences in patients and their siblings. Although we did not observe this association in the control group, possibly due to the limited sample size, we have shown the same pattern of "increasing association" between neuroticism and psychotic experiences in the control-sibling-patient direction. Furthermore, we found strong association between risk allele carriers of all three functional SNPs /risk AGTC haplotype and neuroticism as a phenotype of distress-proneness. Both findings could be linked to the reported evidence of increased stress-sensitivity in the first degree relatives of patients with psychosis, suggesting an inherited pathway and genetic influence on higher sensitivity to stress [59]. Additionally, recent studies revealed that the functional consequence of the interaction between risk haplotype comprised of the risk alleles of functional SNPs (rs9296158, rs3800373, rs9470080 and rs1360780) and trauma are limbic

irritability and an increased amygdale activity [57, 58]. Since the aforementioned regions are crucial for emotional functioning, their impairment could lead to anxiety, distress, and negative emotional processing that actually describes neuroticism as a phenotype and support our findings.

We would like to point out several limitations of the study. We did not have genotype data for rs1360780, as one of the promising risk markers for FKBP5related psychopathology, which could give us more information about the overall genetic impact. We did not measure some other biological data such as cortisol levels, mRNA expression or epigenetic changes of FKBP5, which could provide us deeper insight into regulatory nature of FKBP5 neurobiological pathway. Although the observed genetic associations with schizophrenia could be considered weak due to the small sample size, our results are exactly in line with the previous findings regarding FKBP5 association with psychosis and stress related psychiatric disorders. Further, we would like to emphasize that an interpretation of the strong genetic main effect, even under unadjusted analyses, in the Serbian patients' sample could be due to the overall stressful environment over the decades in this region. The "three-hit" hypothesis of vulnerability model suggests cumulative stress impact on psychiatric disorders [60]. War, sanctions and low economy over a decade in our country could present chronic cumulative stress exposure and risk factors particularly for individuals with the genetic predisposition for systemic changes in the HPA axis activity. Importantly, this stressful period was in time of the childhood and puberty of our participants which is suggested as a critical period for HPA axis disturbance sinceFKBP5 mRNA expression increases particularly during this time of the development [61]. The recent study did not find association between anxiety in adolescence and FKBP5 risk variants, but the reason actually could be lack of traumatic life events in their analyses [62]. Based on the concept of allostasis, stressful environment may disrupt functioning during sensitive period and contribute to the development of allostatic overload. Thus, prolonged stress results in dysregulation of HPA axis, leading to inefficient homeostasis and stress-related disorders [63]. Genetic vulnerability (such as risk FKBP5 variants) prior to the trauma exposure could influence stress sensitivity by modulating homeostatic elements in the stress cascade which is actually one of the neurobiological hypothesis for vulnerability-stress model of schizophrenia [5].

Conclusion

Altered sensitivity to the stressful environment makes adaptation a difficult process, leading to a higher susceptibility to stress and proneness to psychopathology. Our findings confirmed the role of FKBP5 gene in patients with psychosis exposed to trauma, serving as a basis to further evaluate etiologically similar subgroup of "schizophrenias" applicable for vulnerability-stress model. Additionally, our study results support previous findings that environmental stressors are warrant for shaping FKBP5 effect in psychotic disorders. Regarding overall FKBP5 research, its impact is observed in different stress-related psychiatric disorders which suggest importance of consideration for a similar stress-related pathway across diagnostic boundaries. The ability to discriminate high risk and resilience group under the stress-related biological pathway and to find an intermediate phenotype could be important framework for developing strategies of psychosis prevention and early intervention trough the personalized approach.

Conflict of interest

The authors declare no competing financial interests.

References:

1. Rosenthal D (1963)TheGenain Quadruplets: A Case Study and Theoretical Analysis of Heredity and Environment in Schizophrenia. Basic Books, New York

2. Zubin J, Spring B (1977) Vulnerability — A new view of schizophrenia. Journal of Abnormal Psychology 86:103−126

3. Nuechterlein KH, Dawson ME (1984) A heuristic vulnerability/stress model of schizophrenic episodes. Schizophr Bull 10:300−312

4. Walker EF, Diforio D (1997) Schizophrenia: A Neural Diathesis-Stress Model. Psychol Rev 104:667−685

5. Walker E, Mittal V, Tessner K (2008) Stress and the hypothalamic pituitary adrenal axis in the developmental course of schizophrenia. Annu Rev ClinPsychol 4:189−216

6. Ryan MC, Sharifi N, Condren R, Thakore JH (2004) Evidence of basal pituitary-adrenal overactivity in first episode, drug naïve patients with schizophrenia. Psychoneuroendocrinology 29:1065-1070

7. van Venrooij JA, Fluitman SB, Lijmer JG, Kavelaars A, Heijnen CJ, Westenberg HG, Kahn RS, Gispen-de Wied CC (2012) Impaired neuroendocrine and immune response to acute stress in medication-naive patients with a first episode of psychosis. Schizophr Bull 38:272-279

8. Day FL, Valmaggia LR, Mondelli V, Papadopoulos A, Papadopoulos I, Pariante CM, McGuire P (2014) Blunted cortisol awakening response in people at ultra high risk of developing psychosis. Schizophr Res 158:25-31. doi: 10.1016/j.schres.2014.06.041

9. Labad J, Stojanovic-Pérez A, Montalvo I, Solé M, Cabezas Á, Ortega L, Moreno I, Vilella E, Martorell L, Reynolds RM, Gutiérrez-Zotes A (2015) Stress biomarkers as predictors of transition to psychosis in at-risk mental states: roles for cortisol, prolactin and albumin. J Psychiatr Res 60: 163-169

10. Aas M, Dazzan P, Mondelli V, Toulopoulou T, Reichenberg A, Di Forti M, Fisher HL, Handley R, Hepgul N, Marques T, Miorelli A, Taylor H, Russo M, Wiffen B, Papadopoulos A, Aitchison KJ, Morgan C, Murray RM, Pariante CM (2011) Abnormal cortisol awakening response predicts worse cognitive function in patients with first-episode psychosis. Psychol Med 41:463-476

11. Walder DJ, Walker EF, Lewine RJ (2000) Cognitive functioning, cortisol release, and symptom severity in patients with schizophrenia. Biol Psychiatry 48:1121-1132

12. Webster MJ, Knable M., O'Grady J, Orthmann, J, Weickert CS (2002) Regional specificity of brain glucocorticoid receptor mRNA alterations in subjects with schizophrenia and mood disorders. Mol Psychiatry 7:985−994

13. vanWinkelR, Stefanis NC, Myin-Germeys I (2008) Psychosocial stress and psychosis. A review of the neurobiological mechanisms and the evidence for gene-stress interaction. Schizophr Bull 34:1095−1105

14. Binder EB (2009) The role of FKBP5, a co-chaperone of the glucocorticoid receptor in the pathogenesis and therapy of affective and anxiety disorders.Psychoneuroendocrinology 34:S186−S195

15. Scammell JG, Denny WB, Valentine DL, Smith DF (2001) Overexpression of the FK506-binding immunophilin FKBP51 is the common cause of glucocorticoid resistance in three New World primates. General and Comparative Endocrinology 124:152−165

16. Binder EB, Salyakina D, Lichtner P et al (2004) Polymorphisms in FKBP5 are associated with increased recurrence of depressive episodes and rapid response to antidepressant treatment. Nat Genet 36:1319–1325

17. Binder EB, Bradley RG, Liu W, Epstein MP, Deveau TC, Mercer KB, Tang Y, Gillespie CF, Heim CM, Nemeroff CB, Schwartz AC, Cubells JF, Ressler KJ(2008) Association of FKBP5 polymorphisms and childhood abuse with risk of posttraumatic stress disorder symptoms in adults. J Am Med Assoc 299:1291–1305. doi: 10.1001/jama.299.11.1291

18. Klengel T, Mehta D, Anacker C, Rex-Haffner M, Pruessner JC, Pariante CM, Pace TW, Mercer KB, Mayberg HS, Bradley B, Nemeroff CB, Holsboer F, Heim CM, Ressler KJ, Rein T, Binder EB (2013) Allele-specific FKBP5 DNA demethylation mediates gene-childhood trauma interactions. Nat Neurosci 16:33-41. doi: 10.1038/nn.3275

19. Zimmermann P, Brückl T, Nocon A, Pfister H, Binder EB, Uhr M, Lieb R, Moffitt TE, Caspi A, Holsboer F, Ising M (2011) Interaction of FKBP5 gene variants and adverse life events in predicting depression onset: results from a 10-year prospective community study. Am J Psychiatry 168:1107–1116.doi: 10.1176/appi.ajp.2011.10111577

20. Willour VL, Chen H, Toolan J, Belmonte P, Cutler DJ, Goes FS, Zandi PP, Lee RS, MacKinnon DF, Mondimore FM, Schweizer B; Bipolar Disorder Phenome Group; NIMH Genetics Initiative Bipolar Disorder Consortium, DePaulo JR Jr, Gershon ES, McMahon FJ, Potash JB (2009) Family-based association of FKBP5 in bipolar disorder. Mol Psychiatry 14:261-268. doi: 10.1038/sj.mp.4002141

21. Martín-Blanco A, Ferrer M, Soler J, Arranz MJ, Vega D, Calvo N, Elices M, Sanchez-Mora C, García-Martinez I, Salazar J, Carmona C, Bauzà J, Prat M, Pérez V, Pascual JC (2015) The role of hypothalamus-pituitary-adrenal genes and childhood trauma in borderline personality disorder. Eur Arch Psychiatry ClinNeurosci(in press)

22. Supriyanto I, Sasada T, Fukutake M, Asano M, Ueno Y, Nagasaki Y, Shirakawa O, Hishimoto A (2011) Association of FKBP5 gene haplotypes with completed suicide in the Japanese population. ProgNeuropsychopharmacolBiol Psychiatry 35:252-256. doi: 10.1016/j.pnpbp.2010.11.019

23. Bevilacqua L, Carli V, Sarchiapone M, George DK, Goldman D, Roy A, Enoch MA (2012) Interaction between FKBP5 and childhood trauma and risk of aggressive behavior. Arch Gen Psychiatry 69:62-70.doi: 10.1001/archgenpsychiatry.2011.152

24. Collip D, Myin-Germeys I, Wichers M, Jacobs N, Derom C, Thiery E, Lataster T, Simons C, Delespaul P, Marcelis M, van Os J, van Winkel R (2013)FKBP5 as a possible moderator of the psychosis-inducing effects of childhood trauma. Br J Psychiatry 202:261-8.doi: 10.1192/bjp.bp.112.115972

25. Ajnakina O, Borges S, Di Forti M, Patel Y, Xu X, Green P, Stilo SA, Kolliakou A, Sood P, Marques TR, David AS, Prata D, Dazzan P, Powell J, Pariante C, Mondelli V, Morgan C, Murray RM, Fisher HL, Iyegbe C (2014). Role of Environmental Confounding in the Association between FKBP5 and First-Episode Psychosis. Front Psychiatry 5:84.doi: 10.3389/fpsyt.2014.00084

26. Alemany S, Moya J, Ibáñez MI, Villa H, Mezquita L, Ortet G, Gastó C, Fañanás L, Arias B (2016) Research Letter: Childhood trauma and the rs1360780 SNP of FKBP5 gene in psychosis: a replication in two general population samples.Psychol Med 46:221-223.doi: 10.1017/S0033291715001695

27. Green MJ, Raudino A, Cairns MJ, Wu J, Tooney PA, Scott RJ, Carr VJ; Australian Schizophrenia Research Bank (2015) Do common genotypes of FK506 binding protein 5 (FKBP5) moderate the effects of childhood maltreatment on cognition in schizophrenia and healthy controls?JPsychiatr Res 70:9-17. doi: 10.1016/j.jpsychires.2015.07.019

28. Mitjans M, Catalán R, Vázquez M, González-Rodríguez A, Penadés R, Pons A, Massana G, Munro J, Arranz MJ, Arias B (2015) Hypothalamic-pituitary-adrenal system, neurotrophic factors and clozapine response: association with FKBP5 and NTRK2 genes. Pharmacogenet Genomics25:274-7

29. Sinclair D, Fillman SG, Webster MJ, Weickert CS (2013) Dysregulation of glucocorticoid receptor co-factors FKBP5, BAG1 and PTGES3 in prefrontal cortex in psychotic illness. Sci Rep 3:3539. doi: 10.1038/srep03539

30. Schmidt U, Buell DR, Ionescu IA, Gassen NC, Holsboer F, Cox MB, Novak B, Huber C, Hartmann J, Schmidt MV, Touma C, Rein T, Herrmann L (2015) A role for synapsin in FKBP51 modulation of stress responsiveness: Convergent evidence from animal and human studies. Psychoneuroendocrinology52:43-58

31. Daskalakis NP, Binder EB (2015)Schizophrenia in the spectrum of gene-stress interactions: the FKBP5 example.Schizophr Bull 41:323-9.doi: 10.1093/schbul/sbu189

32. Van Os J, Jones PB (2001) Neuroticism as a risk factor for schizophrenia. Psychol Med 31:1129-1134

33. Goodwin RD, Fergusson DM, Horwood LJ (2003) Neuroticism in adolescence and psychotic symptoms in adulthood. Psychol Med 33:1089-1097

34. Dinzeo TJ, Docherty NM (2007)Normal personality characteristics in schizophrenia: a review of the literature involving the FFM. NervMent Dis 195:421-429

35. Lysaker PH, Bryson GJ, Marks K, Greig TC, Bell MD (2004)Coping style in schizophrenia: associations with neurocognitive deficits and personality.Schizophr Bull 30(1):113-121

36. Boyette LL, van Dam D, Meijer C, Velthorst E, Cahn W, de Haan L; GROUP, Kahn R, de Haan L, van Os J, Wiersma D, Bruggeman R, Cahn W, Meijer C, Myin-Germeys I (2014) Personality compensates for impaired quality of life and social functioning in patients with psychotic disorders who experienced traumatic events.Schizophr Bull 40:1356-65. doi: 10.1093/schbul/sbu057

37. Boyette LL, Korver-Nieberg N, Verweij K, Meijer C, Dingemans P, Cahn W, de Haan L; GROUP (2013) Associations between the Five-Factor Model personality traits and psychotic experiences in patients with psychotic disorders, their siblings and controls. Psychiatry Res 210:491-497.doi: 10.1016/j.psychres.2013.06.040

38. Myin-Germeys I, van Os J (2007) Stress-reactivity in psychosis: evidence for an affective pathway to psychosis. ClinPsychol Rev 27:409-424

39. Aiello G, Horowitz M, Hepgul N, Pariante CM, Mondelli V (2012) Stress abnormalities in individuals at risk for psychosis: a review of studies in subjects with familial risk or with "at risk" mental state.Psychoneuroendocrinology 37:1600-1613.doi: 10.1016/j.psyneuen.2012.05.003

40. Myin-Germeys I, van Os J, Schwartz JE, Stone AA, Delespaul PA (2001) Emotional reactivity to daily life stress in psychosis. Arch Gen Psychiatry 58:1137-1144

41. Ormel J, Bastiaansen A, Riese H, Bos EH, Servaas M, Ellenbogen M, Rosmalen JG, Aleman A (2013) The biological and psychological basis of neuroticism: current status and future directions. NeurosciBiobehav Rev 37:59-72.doi: 10.1016/j.neubiorev.2012.09.004

42. Jovicic M, MaricNP, Soldatovic I, Lukic I, Andric S, Mihaljevic M, Pavlovic Z, Mitic M, Adzic M (2015) The role of glucocorticoid receptor phosphorylation in the model of negative affective states. World J Biol Psychiatry 16:301-311. doi: 10.3109/15622975.2014.1000375

43. European Network of National Networks studying Gene-Environment Interactions in Schizophrenia (EU-GEI) (2014) Identifying Gene-Environment Interactions in Schizophrenia: Contemporary Challenges for Integrated, large-scale investigations. Schizophr Bull 40:729-36.doi: 10.1093/schbul/sbu069

44. American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders. 4th text revision (2000)American Psychiatric Association, Washington

45. Sheehan DV, Lecrubier Y, Sheehan KH, et al (1998) The Mini-International Neuropsychiatric Interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. J Clin Psychiatry 59: 22-33

46. Hall RC (1995) Global assessment of functioning. A modified scale. Psychosomatics 36: 267-275

47. NIMH Genetics Initiative (1992) Family Interview for Genetic Studies (FIGS). National Institute of Mental Health, Rockville

48. Stefanis NC, Hanssen M, Smirnis NK, Avramopoulos DA, Evdokimidis IK, Stefanis CN, Verdoux H, Van Os J (2002) Evidence that three dimensions of psychosis have a distribution in the general population. Psychol Med 32:347-358

49. Konings M, Bak M, Hanssen M, van Os J, Krabbendam L (2006) Validity and reliability of the CAPE: a self-report instrument for the measurement of psychotic experiences in the general population. ActaPsychiatrScand 114:55–61

50. LatasterT, Verweij K, Viechtbauer W et al (2014) Effect of illness expression and liability on familial associations of clinical and subclinical psychosis phenotypes.ActaPsychiatrScand 129:44-53.doi: 10.1111/acps.12102

51. Eysenck HJ, Eysenck SBJ (1975)Manual of the Eysenck Personality Questionnaire. Hodder and Stoughton, London

52. Boyette LL, Nederlof J, Meijer C, de Boer F, de Haan L; GROUP (2015)Three year stability of Five-Factor Model personality traits in relation to changes in symptom levels in patients with schizophrenia or related disorders. Psychiatry Res 229:539-544. doi: 10.1016/j.psychres.2015.05.057

53. Bernstein D, Finkelhor L (1998) Childhood Trauma Questionnaire: A Retrospective Self-report. The Psychological Corporation, San Antonio

54. Miller SA, Dykes DD, Polesky HF (1988) A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 16:12-15

55. Livak KJ (1999) Allelic discrimination using fluorogenic probes and the 5′ nuclease assay. Genet Anal 14:143–149

56. Barett JC, Fry B, Maller J, Daly MJ (2005)Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics 21:263–265

57. Dackis MN, Rogosch FA, Oshri A, Cicchetti D (2012) The role of limbic system irritability in linking history of childhood maltreatment and psychiatric outcomes in low-income, high-risk women: moderation by FK506 binding protein 5 haplotype.DevPsychopathol 24:1237-1252.doi: 10.1017/S0954579412000673

58. White MG, Bogdan R, Fisher PM, Muñoz KE, Williamson DE, Hariri AR (2012) FKBP5 and emotional neglect interact to predict individual differences in amygdala reactivity. Genes BrainBehav11:869-78. doi: 10.1111/j.1601-183X.2012.00837

59. Lataster T, Collip D, Lardinois M, van Os J, Myin-Germeys I (2010) Evidence for a familial correlation between increased reactivity to stress and positive psychotic symptoms.ActaPsychiatrScand 122:395-404. doi: 10.1111/j.1600-0447.2010.01566

60. Daskalakis NP, Bagot RC, Parker KJ, Vinkers CH, de Kloet ER (2013) The three-hit concept of vulnerability and resilience: toward understanding adaptation to early-life adversity outcome. Psychoneuroendocrinology38:1858-1873.doi: 10.1016/j.psyneuen.2013.06.008

61. - Shannon Weickert C, Webster MJ, Boerrigter D, Sinclair D(2015) FKBP5 Messenger RNA Increases After Adolescence in Human Dorsolateral Prefrontal Cortex. Bio Psychiatrypii: S0006-3223(15)00957-9.doi: 10.1016/j.biopsych.2015.11.005

62. Bortoluzzi A, Blaya C, da Rosa ED, Paim M, Rosa V, Leistner-Segal S, Manfro GG (2015) What can HPA axis-linked genes tell us about anxiety disorders in adolescents? Trends Psychiatry Psychother. 37:232-7. doi: 10.1590/2237-6089-2015-0035

63. McEwen BS, Wingfield JC (2003) The concept of allostasis in biology and biomedicine. HormBehav43:2–15

a)
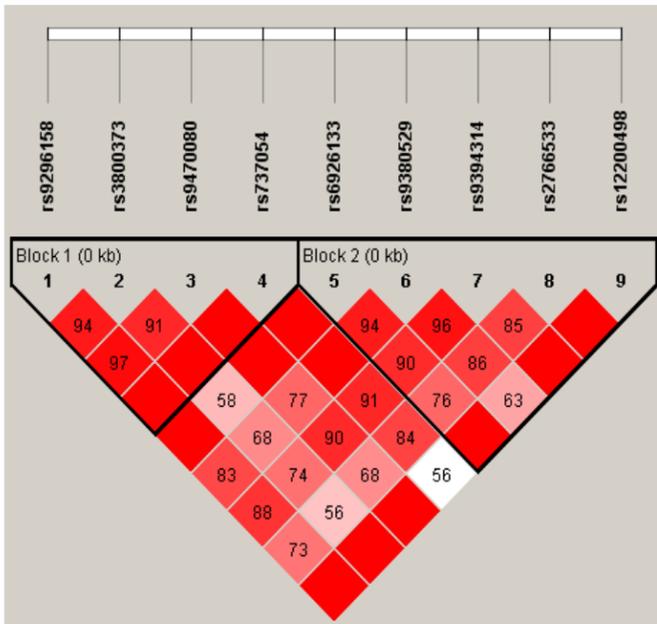
	Haplotypes	Total %	Patients %	Controls %	P value	Permutation P value
Block 1	GTCC	36.0	29.7	42.8	*0.0531*	0.338
	AGTC	28.6	35.5	21.3	**0.0264**	0.123
	GTCT	24.3	26.0	22.4	0.5605	1.000
	ATTC	7.5	5.9	9.3	0.3606	1.000
	GTTC	2.0	1.0	3.1	0.286	0.928
	GGCC	1.1	1.1	1.1	0.9807	1.000
Block 2	GCTCC	40.5	39.4	41.7	0.7357	1.000
	TTCTC	23.2	25.9	20.4	0.3499	1.000
	GTTTT	16.3	15.4	17.2	0.7203	1.000
	GTCTC	9.4	12.5	6.2	0.1227	0.554
	GTTCC	4.0	2.9	5.2	0.4051	1.000
	GCTTT	2.1	1.0	3.2	0.2701	0.900
	TTCCC	2.5	1.9	3.1	0.6028	1.000

b)
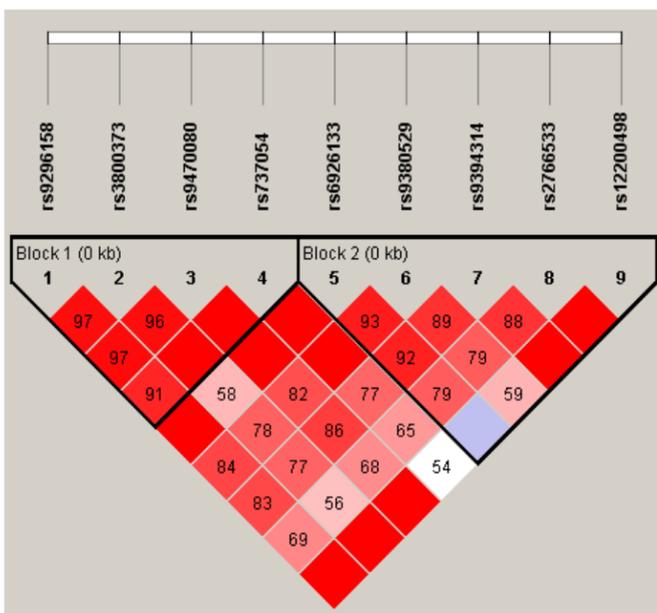
	Haplotypes	Total %	Siblings %	Controls %	Pvalue	Permutation P value
Block 1	GTCC	35.5	29.1	42.8	**0.0384**	0.2250
	AGTC	25.4	29.1	21.4	0.2045	0.9350
	GTCT	28.8	34.5	22.4	*0.0543*	0.2860
	ATTC	6.8	4.6	9.2	0.1849	0.9030
	GTTC	2.4	1.8	3.1	0.5555	1.000
Block 2	GCTCC	43.6	45.3	41.7	0.6008	1.000
	TTCTC	20.6	20.8	20.3	0.9281	1.000
	GTTTT	15.3	13.6	17.2	0.4668	1.000
	GTCTC	10.1	13.6	6.2	*0.0765*	0.4720
	GTTCC	2.9	1.0	5.1	*0.0745*	0.4420
	GCTTT	2.5	1.9	3.2	0.5428	1.000
	GCCTC	1.5	1.9	1.0	0.6033	1.000
	TTCCC	1.5	0	3.1	*0.0644*	0.3600

Figure 1. Linkage disequilibrium (LD) plots and distribution of FKBP5 haplotypes in the analyzed sample.The values within each square represent pair-wise linkage disequilibrium (D'). Box colors correspond as follows: Red- LOD>2, D'=1; Shades of pink- LOD>2, D'<1; Blue-LOD<2, D'=1, White- LOD<2, D'<1. a) LD plots for patient and control group taken together (on the left) anddistribution of identified haplotypes (on the right) b) LD plots for sibling and control group taken together (on the left)anddistribution of identified haplotypes (on the right).Presented p values are uncorrected and with performing permutation analyses;P<0.05 are presented in bold, P<0.1 are presented in italic

Table 1. Participants demographic, clinical and scale characteristics

Characteristics		Patients	Siblings	Controls	Patients vs. Controls	Patients vs. Siblings	Siblings vs. Controls
Age (years, M±SD)		29.3±5.9	28.5±6.8	29.8±6.3	P=0.695	P=0.554	P=0.335
Gender (male, %)		59.6	41.8	45.1	P=0.169	P=0.083	P=0.845
DSM diagnosis (N, %)			NA	NA			
Schizophrenia		27 (52%)					
Schizophreniform disorder		12 (23%)					
Schizoaffective disorder		7 (13%)					
Psychotic disorder NOS		6 (12%)					
Age of onset (years, M±SD)		24.4±5.1	NA	NA			
Duration of illness (months, M±SD)		62.7±56.7	NA	NA			
Scale	Score						
CAPE-P	Mean	1.54	1.19	1.23			
	SD	0.40	0.18	0.21	**P<0.001**	**P<0.001**	P=0.753
	Median	1.45	1.15	1.20			
	Range	1 - 2.85	1 - 1.80	1 - 2.30			
CAPE-D	Mean	1.85	1.49	1.54			
	SD	0.49	0.28	0.32	**P=0.001**	**P<0.001**	P=1.000
	Median	1.75	1.50	1.50			
	Range	1-3.25	1-2.75	1-2.88			
Neuroticism	Mean	13.13	7.89	7.72			
	SD	6.78	4.30	4.58	**P<0.001**	**P<0.001**	P = 1.000
	Median	13.00	7.00	6.50			
	Range	1 - 30	1 – 20	1 - 19			
CTQ-total	Mean	1.43	1.29	1.20			
	SD	0.41	0.27	0.25	**P=0.003**	P=0.264	P=0.305
	Median	1.28	1.18	1.12			
	Range	1 - 2.72	1 - 2.20	1 - 2.24			

Note: NA Non applicable, P<0.05 are presented in bold, Bonferroni adjustment was applied for all analysis

Table 2. Linear regression analyses: neuroticism as a predictor of subclinical psychotic symptoms

	Group status	R^2	F	Coefficient (b) (95% CI)	P value
	Patients	0.301	6.322	0.551 (0.121 - 0.329)	**<0.001**
Neuroticism	**Siblings**	0.252	5.494	0.406 (0.033 - 0.157)	**0.003**
	Controls	0.035	0.531	0.183 (-0.031 - 0.129)	0.224

Note: All analyses were adjusted for age and gender, P<0.05 are presented in bold