Psychiatr. Pol. 2016; 50(4): 761–770
PL ISSN 0033-2674 (PRINT), ISSN 2391-5854 (ONLINE)
www.psychiatriapolska.pl
DOI: http://dx.doi.org/10.12740/PP/61273

Family association study of Transforming Growth Factor Beta1 gene polymorphisms in schizophrenia

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Summary

Aim. Schizophrenia is a serious mental illness with chronic symptoms and significant impairment in psychosocial functioning. An etiopathological role for immunologic abnormalities in schizophrenia was hypothesized. Inflammatory markers are well-known etiological factors for psychiatric disorders, including schizophrenia. Several studies have investigated the possible effects of antipsychotics on inflammation and neurogenesis. Additionally, antiinflammatory adjuvant therapy has been under investigation as a treatment option for schizophrenia. Transforming Growth Factor Beta 1 (TGFB1) signaling is critical for many biological processes, including proliferation, development, differentiation and regeneration. Multiple members of the TGFB1 superfamily play a role in the developing nervous system and are regulated by neuronal activity. We conducted family-based study to assess whether *TGFB1* gene is associated with susceptibility to schizophrenia in Polish population.

Methods. Two functional polymorphisms: rs1800469 (C-509T) and rs1800470 (T869C) of *TGFB1* gene were analyzed within a group of 147 trios (patients diagnosed with schizophrenia and their healthy parents) using Transmission Disequilibrium Test (TDT).

Results. No association of these polymorphisms with schizophrenia was found in Polish population.

Conclusions. Further studies on larger groups along with correlation with circulating protein levels are needed.

Key words: schizophrenia, polymorphism, Transforming Growth Factor Beta 1

This study was supported by the grant of Polish Ministry of Science and High Education No. NN 402407339. The authors were not influenced at all in the course of research and development.

Introduction

The etiology of schizophrenia remains unclear, while there has been a growing amount of evidence for the immunogenetics, which are characterized by an increased serum concentration of several pro-inflammatory cytokines [1]. Cytokines can often act both as immunomodulators and as neuromodulators [2]. Cytokines are actively transported through the blood-brain barrier and are also produced by neuronal and glial cells in the central nervous system [3]. Furthermore, it has been demonstrated that cytokines are involved in the regulation of many neuronal functions such as neurotransmission, neuronal survival and synaptic plasticity [4]. The activation of the cytokine systems may be involved in the neuropathological changes occurring in the central nervous system of schizophrenic patients. Numerous studies report that treatment with antipsychotic drugs affects the cytokine network [5]. The influence of antipsychotics on the cytokine system may be at least partially responsible for their clinical effectiveness. One could expect that antipsychotics with proven effect on cytokines would bring an additional benefit to the patients by normalization of preexisting cytokine aberrations [5].

Transforming growth factor beta (TGFB) represents a family of cytokines with closely related isoforms, encoded by three different genes. *TGFB1, TGFB2* and *TGFB3* are expressed in several central nervous system (CNS) cell types, including neurons, astrocytes, and microglia [6, 7]. TGFBs are potent survival factors for midbrain dopaminergic neurons [8]. TGFB1 is a multifunctional cytokine and a key regulator of cell growth and differentiation, immune modulation, wound healing and embryogenesis [9]. TGFB1 plays an important role in neuronal survival and recovery of normal neuronal functions following central nervous system diseases [10] and might be a crucial regulator in central nervous system development [11]. TGFB1 also have trophic effects on dopaminergic neurons [12].

In a pathway analysis of GWAS (Genome Wide Association Study) data TGFB signaling pathway was consistently found to be top ranked and likely associated with schizophrenia. Results are supported by two studies conducted by Jia et al. with the use of advanced statistical methods (GSEA – Gene Set Enrichment Analysis; hypergeometric test; gamGWAS – generalized additive model for GWAS analysis) [13, 14]. This pathway is involved in many cellular processes including neuronal protection against both apoptosis and excitotoxicity [15]. TGFB signaling is involved in multiple aspects of neurodevelopment [16] and adult neurogenesis [17, 18]. It controls a set of cellular processes, including cell differentiation, apoptosis, proliferation, recognition, etc. [19, 20]. The canonical TGFB signaling pathway is critical for use-dependent modulation of $GABA_A$ synaptic transmission and dendritic homeostasis; furthermore, a disruption in the balance of the excitatory and inhibitory hippocampal network can result in psychiatric-like behavior [21]. It has been found that components of TGFB signaling pathway are altered in the hippocampus in human psychiatric conditions such as schizophrenia [22].

No significant differences in TGFB1 and TGFB2 levels were found in the cerebrospinal fluid samples of patients with schizophrenia comparing with healthy control subjects [23]. Serum levels of TGFB1 were significantly higher in a group of first episode psychosis [24] and schizophrenia in relapse patients than in controls [25]. In their study on Polish population, Frydecka et al. showed that TGFB serum level was significantly higher in patients with schizophrenia than in healthy control subjects [26]. Other studies detected no difference in TGFB serum levels between drug-free chronic schizophrenic patients in an acute phase of the disease and control subjects [27, 28]. Study conducted by Kim et al. showed increased plasma level of TGFB1 in schizophrenic patients and its normalization during treatment [29]. Meta-analysis by Miller et al. confirmed a significant decrease in TGFB blood level following antipsychotic treatment [24]. Also recent study on Polish population showed a significant reduction in the serum level of TGFB1 after 28 days of aripiprazole treatment in chronic schizophrenia patients [30]. TGFB appeared to be state marker, as it was increased in acutely relapsed inpatients with schizophrenia and first episode psychosis and normalized with antipsychotic treatment [24]. Studies reported of evidence for activation of the compensatory anti-inflammatory response syndrome – a counter-regulatory mechanism that inhibits the primary inflammatory response and involves an adaptive reprogramming of leukocytes in schizophrenia [31], including increased TGFB in acute exacerbations that decreased with antipsychotic treatment [24].

Data collected by Pietersen et al. revealed that a number of genes within the TGFB superfamily were differentially expressed in subjects with schizophrenia. They identified 1,331 mRNAs that were differentially expressed in schizophrenia, including genes that belong to the transforming growth factor beta (TGFB) signaling pathway and found that the TGFB signaling canonical pathway was highly dysregulated in schizophrenia with up-regulation of several genes immediately downstream from the TGFB1 receptor. Based on their findings, one might hypothesize that neurons derived from induced pluripotent stem cells from patients with schizophrenia may be particularly vulnerable to oxidative stress, which may then lead to dysregulated TGFB signaling [32].

TGFB1 gene is located on chromosome 19q13.1-3 [33]. TGFB1 protein levels are predominantly under genetic control [34]. For our study we have chosen two functional polymorphisms: rs1800469 (C-509T) and rs1800470 (T869C) which both affect serum/plasma levels of TGFB1 protein. Polymorphism rs1800469 is located in the proximal negative regulatory region of the *TGFB1* gene. Polymorphism rs1800470 causes leucine to proline substitution at codon 10 of the amino acid sequence, located within the signal peptide [9, 34–37].

In a recent report, Frydecka et al. have found rs1800470 to be associated with schizophrenia in Polish population. Risk of schizophrenia was more than two-fold higher in carriers of T allele (CT+TT genotype) comparing with individuals with CC genotype. This association was significant in females [38]. In their study, Lee and Kim showed that the C allele at position +869 (codon 10) was more frequent in the schizophrenia group than in controls but, the allelic difference did not reach statistical

significance after correction for multiple comparisons. They found that the C allele was possibly associated with better response to antipsychotics. The distribution of *TGFB1* gene polymorphism in Koreans is different from that in Caucasians so, because of these ethnic differences in the distribution of the genotypes, it is difficult to generalize the result of this genetic study [39].

Material

The study was performed on a group of 147 trio (patients diagnosed with schizophrenia and their healthy parents). There were 66 males (mean age 24.3; SD 6.7) and 81 females (mean age 26.9; SD 6.8) in patient's group. Mean age of onset of first psychotic episode was 22.9 years. In 21 patients family history of schizophrenia and in 42 patients family history of other psychiatric disorders have been confirmed. Mean age of parents at the recruitment time was: fathers 55.9 (SD 8.44), mothers 53.0 (SD 7.7) years. Both parents were psychiatrically screened and found to be mentally healthy. Consensus diagnosis of schizophrenia was made for each patient using Structured Clinical Interview for DSM-IV Axis I Disorders (SCID) [40]. All patients were evaluated for lifetime psychiatric symptomatology using the Operational Criteria for Psychotic Illness (OPCRIT) [41]. Participants were recruited from inpatients, treated at the Department of Adult Psychiatry, Poznan University of Medical Sciences. All subjects were of Caucasian origin and they were native Polish population from the Greater Poland region. The study was performed in accordance with the ethical standards established in the Declaration of Helsinki and was approved by the medical ethics committee of the Poznan University of Medical Sciences. All participants gave written informed consent before participating in the study.

Method

DNA was extracted using the salting out method [42]. The polymorphisms: rs1800470 and rs1800469 of *TGFB1* gene were genotyped with the use of TaqMan SNP Genotyping Assays (LifeTechologies™) in ABI PRISM® 7900HT Sequence Detection System (Applied Biosystems). Data acquisition and analysis was performed using the allelic discrimination analysis module in SDS v2.4 software (Applied Biosystems). The genotyping was performed without knowing the clinical status of the subjects.

Statistical methods

Transmission disequilibrium test (TDT) was performed using PLINK (http://pngu.mgh.harvard.edu/~purcell/plink/). Hardy-Weinberg equilibrium, LD plot and minor allele frequencies were obtained with the use of Haploview v4.2 (http://www.broadinstitute.org/). Power analysis was done using Quanto v.1.2.4 (http://hydra.usc.edu/gxe/).

Results

All polymorphisms did not deviate from Hardy-Weinberg equilibrium. No association of the polymorphisms: rs1800470 (p = 0.7216, OR = 0.9385; CI 0.6617–1.331) and rs1800469 (p = 0.7884, OR = 0.9531; CI 0.6712–1.354) from *TGFB1* gene with schizophrenia was found. Power to detect genetic association was 6.6% and 5.9% (respectively). Results of single polymorphism association are presented in Table 1.

Both polymorphisms are in linkage disequilibrium (D' = 1, $r^2 = 0.78$). No association of haplotypes (AG, GA, GG) with schizophrenia was found. Results of haplotype analysis are presented in Table 2.

Dividing group into male and female proband subgroups we also were not able to find any association of both studied polymorphism (Table 1 and Table 2).

Table 1. **Association of *TGFB1* SNPs with schizophrenia**

SNP	A1	A2	OVT	MAF	T	U	OR (CI)	p	Power	p males	p females
rs1800470	G	A	A	0.428	61	65	0.9385 (0.6617–1.331)	0.7216	6.6%	1.0000	0.4533
rs1800469	A	G	G	0.369	61	64	0.9531 (0.6712–1.354)	0.7884	5.9%	0.7995	0.9042

SNP – single nucleotide polymorphism; MAF – minor allele frequency; T – transmitted allele; U – undertransmitted allele; OVT – overtransmitted allele; OR – odds ratio, CI – confidence interval

Table 2. **Results of the haplotype analysis for *TGFB1* gene**

Block	Haplotype Frequency	Transmitted: Undertransmitted ratio	p	p males	p females
AG	0.572	66:58	0.4725	0.8084	0.6172
GA	0.369	60:65	0.6547	1	0.9042
GG	0.059	14:17	0.5900	0.6171	0.4656

Discussion

There are data suggesting that the –509T allele (rs1800469) is associated with increased TGFB1 plasma levels [34, 35]. Other studies reported decreased TGFB1 plasma levels in –509T allele carriers [43] or lack of association between extracellular TGFB1 concentration and the C-509T polymorphism [44]. The C allele of rs1800470 (T869C) polymorphism is associated with a higher level of TGFB1 mRNA and protein compared to the T allele [45, 46].

Although TGFB1 has been considered to be important in psychoneuroimmunology, there have been relatively few reports about the role of TGFB1 in mental disorders. In a recent report, Frydecka et al. have found rs1800470 to be associated with schizophrenia in Polish population. Risk of schizophrenia was more than two-fold higher in carriers of T allele (CT+TT genotype) comparing with individuals with CC genotype.

This association was significant in females [38]. In their recent study on Polish population, Frydecka et al. showed that the TGFB +869T/C and +915G/C polymorphisms were not associated with TGFB level in schizophrenia patients [26]. Association of rs1800470 with suicide behavior [47], the risk to develop late-onset Alzheimer's disease and depressive symptoms in Alzheimer's disease [48] have been found. Meta-analysis by Chang et al. did not provide an evidence of confirming association between the +869T/C and C-509T polymorphisms and Alzheimer's disease [49]. Other studies conducted in autism [11] and major depressive disorder with or without suicide behavior [50] did not found any association of TGFB1 polymorphisms with these diseases.

Our study did not confirm results published by Frydecka et al., where case-control study on 151 schizophrenia patients and 279 healthy control subjects was conducted also in Polish population. Our study has family-based design, which is robust to population stratification [51]. Family-based association (transmission disequilibrium test – TDT) is less prone to false-positive findings than the approach of selecting ethnically similar controls, but because of potentially reduced power, it may be more prone to false-negative results [52].

A concept of an endophenotype, also termed as an internal endophenotype, is used in genetic studies on psychiatric disorders. A marker can be considered an endophenotype if it meets the following criteria: association with a disease in a population, heritability, state-independence, familial association (the endophenotype is more prevalent in the affected individuals, their affected and non-affected family members in comparison to the normal population), co-segregation (the endophenotype is more prevalent among ill family members of ill probands compared with healthy relatives). Neurological soft signs are also considered candidates for endophenotypes of schizophrenia [53]. Using endophenotypes in genetic studies on psychiatric disorders gives opportunity of more homogeneous groups of examined patients.

Conclusions

Our results do not support theory that polymorphisms rs1800470 and rs1800469 of *TGFB1* gene are involved in the pathogenesis of schizophrenia. It seems advisable to carry out further examinations of the role of these polymorphisms in schizophrenia by means of TDT method and case-control association on more numerous groups of patients along with correlation with circulating protein levels.

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