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Polymorphisms in immune-inflammatory response genes and the risk of deficit schizophrenia

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ABSTRACT

Polymorphisms in immune-inflammatory response genes are believed to impact schizophrenia susceptibility. However, it remains unknown whether immunogenetic factors play a role in the etiology of deficit schizophrenia (D-SCZ). Therefore, we genotyped four polymorphisms in genes encoding two immune system regulatory proteins (*CTLA-4* rs231775 and *CD28* rs3116496), interleukin-6 (*IL6* rs1800795) and transforming growth factor-β (*TGFB1* rs1800470) in 513 schizophrenia patients and 374 controls. The *CD28* rs3116496-CC genotype and C-allele were significantly more frequent in the whole group of patients and D-SCZ patients compared to controls. Our results indicate that the *CD28* rs3116496 polymorphism might impact the risk of schizophrenia, especially D-SCZ.

1. Introduction

Schizophrenia is increasingly being recognized as a heterogeneous mental illness with imprecise diagnostic boundaries. This observation has been proposed as a potential explanation of mixed findings in the field of schizophrenia genetics and provided grounds for approaching schizophrenia etiology with more dimensional distinctions. One of such conceptualizations has been proposed by Carpenter et al. (1988), who developed the concept of deficit schizophrenia (D-SCZ) to capture a subgroup of patients with primary and enduring negative symptoms. Validity of the D-SCZ concept has been confirmed in several studies (for review see (Bucci and Galderisi, 2017)). Several clinical and socio-demographic characteristics of D-SCZ have been found, including i.e. poorer premorbid adjustment and more impairment in general cognitive abilities (Galderisi et al., 2002), worse clinical and functional outcome (Strauss et al., 2010; Tek et al., 2001) as well as more severe neuro-structural and neuro-functional abnormalities (Galderisi et al., 2008; Spalletta et al., 2015). Previous studies have also suggested that D-SCZ may share specific genetic background (Bakker et al., 2007; Minoretti et al., 2006; Wonodi et al., 2006).

Emerging evidence indicates that patients with D-SCZ might present higher levels of subthreshold inflammatory state in terms of elevated interleukin(IL)-6 and C-reactive protein (CRP) levels compared to patients with non-deficit schizophrenia subtype (ND-SCZ) (Garcia-Rizo et al., 2012). These findings are also in agreement with more recent studies showing elevated levels of IL-6 in patients with chronic schizophrenia accompanied by deterioration (Frydecka et al., 2015b) and the association between poor response to treatment and higher levels of IL-6 and interferon-γ in first-episode psychosis patients (Mondelli et al., 2015).

Importantly, it has been reported that genetic variation in immune-inflammatory response genes might impact schizophrenia risk. This hypothesis has been confirmed in the largest known genome-wide association study of schizophrenia cases and controls (Riepke and

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Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014). A recent meta-analysis revealed that polymorphisms in genes encoding such cytokines as interleukin(IL)-1β (*IL1B*), IL-6 (*IL6*) and soluble IL-6 receptor (*sIL6R*) might confer schizophrenia susceptibility (Hudson and Miller, 2016). There are also studies showing that variation in the transforming growth factor-β gene (*TGFB1*) as well as genes encoding two regulators of T-cell activity – CD28 and CTLA-4 might affect schizophrenia risk (Frydecka et al., 2013a; Frydecka et al., 2013b). However, the role of genetic factors related to immune functions in the etiology of D-SCZ has not been investigated so far. Therefore, in this study we tested the hypothesis whether polymorphisms in immune-inflammatory response genes, namely *IL6* (rs1800795), *TGFB1* (rs1800470), *CTLA-4* (rs231775) and *CD28* (rs3116496) might impact D-SCZ risk. All polymorphisms located in cytokine genes, together with the *CD28* rs3116496 polymorphism have been associated with blood levels of these molecules, while the *CTLA-4* rs231775 polymorphism has been found to impact T-cell activation (Awad et al., 1998; Fishman et al., 1998; Isitmangil et al., 2016; Maurer et al., 2002).

2. Material and methods

We recruited 513 patients with schizophrenia (267 males aged 36.3 ± 10.4 years and 246 females aged 42.5 ± 10.9 years), coming from two independent samples. The first sample represented 313 patients recruited in the Markers of Deficit Schizophrenia (MODIS) study, which was a multi-centric study performed in 46 Polish centres across Poland (Bienkowski et al., 2015). In this sample, the majority of patients were recruited in hospitals providing long-term care. The second sample was recruited at Department and Clinic of Psychiatry in Szczecin (Pomeranian Medical University, Poland) and included 200 patients (Pelka-Wysiecka et al., 2016; Pelka-Wysiecka et al., 2013). A diagnosis of schizophrenia was based on ICD-10 and DSM-IV criteria. Additionally, in the second sample representing 200 patients, a diagnosis of schizophrenia was validated using the Operational Criteria for Psychotic Illness (OPCRIT) checklist (McGuffin et al., 1991). A diagnosis of D-SCZ was based on the Schedule for Deficit Schizophrenia (SDS), which requires the presence of at least two primary and enduring negative symptoms during at least 12 preceding months (Kirkpatrick et al., 1989). Negative symptoms must be present during clinical stability and cannot occur as a consequence of depression, anxiety, mental retardation or adverse effects of medications. Additionally, psychopathological manifestation on the day of recruitment was assessed using the Positive and Negative Syndrome Scale (PANSS) in both samples (Kay et al., 1987). We used a 5-factor model of PANSS proposed by Wallwork et al. (2012) to assess positive symptoms (items: P1, P3, P5 and G9), negative symptoms (items: N1, N2, N3, N4, N6 and G7), disorganization (items: P2, N5 and G11), depressive symptoms (G2, G3 and G6) and excitement (items: P4, P7, G8 and G14). Healthy controls were enrolled at Department and Clinic of Psychiatry (Wroclaw Medical University, Poland) and represented participants with negative family history of psychotic and affective disorders, recruited in our previous studies (Frydecka et al., 2013a; Frydecka et al., 2015a; Frydecka et al., 2015b; Frydecka et al., 2015c). All psychiatrists responsible for recruitment of patients underwent training in assessment with SDS and PANSS before the study.

DNA was obtained from saliva samples or peripheral blood leukocytes. Selected polymorphisms: *CTLA-4* c.49A > G (rs231775), *CD28* c.17 + 3 T > C (rs3116496), *TGFB1* T29C (rs1800470), and *IL6*-572G > C (rs1800796) were genotyped using the allelic discrimination technique based on the validated TaqMan®SNP Genotyping Assays (C___2415786_20, C__25922478_10, C___1839697_20, and C__22272997_10, respectively).

Distribution of genotypes and alleles between schizophrenia patients and controls was compared using the χ^2 test. Similarly, assessment of the Hardy-Weinberg equilibrium (HWE) was performed by comparing observed and expected distributions using the χ^2 test. Adjustment for multiple testing was performed using the Bonferroni correction, taking into account the number of genotyped polymorphisms. Differences in continuous variables were tested using the Mann-Whitney *U* test and the Kruskal-Wallis test. Effect size estimates for Mann-Whitney *U* test were calculated according to the formula: $\eta^2 = Z^2/N - 1$. Results of statistical analysis were considered as statistically significant if the *p*-value was <0.05. Power calculations were performed using Quanto 1.2.4 Software (Gauderman, 2002). We examined dominant and recessive models for odds ratio (OR) ranging from 1.4 to 2.0 ($\alpha = 0.05$, two-tailed test).

3. Results

Patients with D-SCZ, compared to ND-SCZ patients, had significantly higher scores of positive symptoms (8.9 ± 4.0 vs. 7.5 ± 3.6, $\eta^2 = 0.025$, $p < 0.001$), negative symptoms (21.2 ± 5.5 vs. 12.6 ± 6.4, $\eta^2 = 0.257$, $p < 0.001$), depressive symptomatology (7.0 ± 3.0 vs. 5.0 ± 2.6, $\eta^2 = 0.094$, $p < 0.001$), disorganization (9.7 ± 3.0 vs. 6.5 ± 3.1, $\eta^2 = 0.155$, $p < 0.001$) and excitement (7.8 ± 3.4 vs. 6.1 ± 2.5, $\eta^2 = 0.063$, $p < 0.001$). Distribution of genotypes and alleles in the whole group of patients with schizophrenia and controls was presented in Table 1. Results of genotyping were in agreement with Hardy-Weinberg equilibrium in the group of patients and controls. The *CD28* rs3116496 C-allele was

Table 1
Distribution of genotypes and alleles in patients with schizophrenia and controls.

Polymorphism	SCZ patients, n (%)	Controls, n (%)	OR	95%CI	p
CTLA-4 rs231775					
AA	175 (34.3)	121 (33.2)	Referent	Referent	0.445
AG	248 (48.5)	168 (46.2)	1.02	0.75–1.38	
GG	88 (17.2) HWE: $p = 0.993$	75 (20.6) HWE: $p = 0.237$	0.81	0.55–1.19	
A allele (AA + AG)	598 (58.5)	410 (56.3)	Referent	Referent	0.558
G allele (AG + GG)	424 (41.5)	318 (43.7)	0.91	0.75–1.11	
CD28 rs3116496					
TT	355 (69.6)	277 (74.1)	Referent	Referent	0.050
TC	135 (26.5)	92 (24.6)	1.14	0.84–1.56	
CC	20 (3.9) HWE: $p = 0.696$	5 (1.3) HWE: $p = 0.391$	3.12	1.16–8.42	
T allele (CT + TT)	845 (82.8)	646 (86.4)	Referent	Referent	**0.044***
C allele (CC + CT)	175 (17.2)	102 (13.6)	1.31	1.01–1.71	
IL6 rs1800795					
CC	139 (27.4)	64 (23.4)	Referent	Referent	0.366
CG	236 (46.4)	128 (46.7)	0.85	0.59–1.22	
GG	133 (26.2) HWE: $p = 0.111$	82 (29.9) HWE: $p = 0.307$	0.75	0.50–1.12	
C allele (CC + CG)	514 (50.6)	256 (46.7)	Referent	Referent	0.144
G allele (CG + GG)	502 (49.4)	292 (53.3)	0.86	0.69–1.05	
TGFB1 rs1800470					
TT	172 (35.0)	90 (32.4)	Referent	Referent	0.253
CT	235 (47.9)	127 (45.7)	0.97	0.69–1.35	
CC	84 (17.1) HWE: $p = 0.807$	61 (21.9) HWE: $p = 0.203$	0.72	0.47–1.09	
T allele (TT + CT)	579 (59.0)	307 (55.2)	Referent	Referent	0.249
C allele (CT + CC)	403 (41.0)	249 (44.8)	0.86	0.70–1.06	

Unadjusted significant differences were marked in bold characters.
SCZ – schizophrenia, HWE – Hardy-Weinberg equilibrium.
* *p*-value non-significant after Bonferroni correction ($p > 0.0125$).

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