

# Sex differences in TGF $\beta$ signaling with respect to age of onset and cognitive functioning in schizophrenia

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**Abstract:** There are studies showing that gene polymorphisms within the transforming growth factor- $\beta$  (TGF- $\beta$ ) signaling constitute schizophrenia risk variants. However, the association between *TGFBI* gene polymorphisms (+869T/C and +915G/C), TGF- $\beta$  level with schizophrenia course, and its symptomatology together with cognitive functioning has not been investigated so far. We included 151 patients with schizophrenia and 279 healthy controls. Cognitive functioning was assessed using Rey Auditory Verbal Learning Test, Trail Making Test (TMT)-A and TMT-B, Verbal Fluency task, Stroop test, as well as selected subtests from the Wechsler Adults Intelligence Scale – Revised, Polish adaptation (WAIS-R-Pl): Digit Symbol Coding, Digit Span Forward and Backward, and Similarities. Additionally, serum TGF- $\beta$  levels were measured in 88 schizophrenia patients and 88 healthy controls. Serum TGF- $\beta$  level was significantly higher among patients with schizophrenia in comparison with healthy controls; however, the studied polymorphisms were not associated with TGF- $\beta$  level in schizophrenia patients. Subjects carrying the +869T allele performed significantly worse in comparison with +869CC homozygotes on Stroop task, Verbal Fluency task and Digit Symbol Coding task. There was a significant difference in age of psychosis onset in female schizophrenia patients with respect to the *TGFBI* +869T/C polymorphism. Additionally, adjustment for possible confounders revealed that there was a significant difference in cognitive performance on Digit Symbol Coding task with respect to the *TGFBI* +869T/C polymorphism among female schizophrenia patients. Our results suggest that TGF- $\beta$  signaling might be a valid link contributing to observed differences in age of onset and the level of cognitive decline between male and female schizophrenia patients.

**Keywords:** cognition, digit symbol coding task, gender differences, gene polymorphism, TGF, transforming growth factor-beta

## Introduction

Cytokine alterations are increasingly recognized as part of schizophrenia pathophysiology.<sup>1-3</sup> Some cytokines including interleukin (IL)-12, interferon- $\gamma$  (IFN- $\gamma$ ), and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) serve as trait markers of schizophrenia as they are increased in patients with first-episode psychosis and acute relapse, and during symptomatic stability. Other cytokines, including IL-1 $\beta$ , IL-6, and transforming growth factor- $\beta$  (TGF- $\beta$ ) appear to be state markers, which are increased during acute relapses in comparison with periods of symptomatic stability.<sup>4</sup>

TGF- $\beta$  is a regulatory cytokine that influences proliferation, differentiation, and survival of lymphocytes, natural killer cells, dendritic cells, macrophages, mast cells, and granulocytes.<sup>5</sup> Interestingly, TGF- $\beta$  has been found to regulate differentiation and survival of midbrain dopaminergic neurons.<sup>6-8</sup> The recent genome-wide association studies data analysis revealed that TGF- $\beta$  signaling is one of the top ranked pathways

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associated with schizophrenia.<sup>9</sup> In addition, overexpression of the *ZNF804A* gene, which is a susceptibility gene for schizophrenia supported by genome-wide association studies data, has been found to upregulate genes implicated in the TGF- $\beta$  signaling.<sup>10</sup> There is also one postmortem study showing that the TGF- $\beta$  signaling pathway genes are upregulated within hippocampal cells in schizophrenia patients.<sup>11</sup>

The TGF- $\beta$  gene (*TGFBI*) has biallelic single nucleotide polymorphisms (SNPs) at codon 10 (position +869) and at codon 25 (position +915), both causing amino acid substitutions and both known to influence alterations in TGF- $\beta$  synthesis.<sup>12,13</sup> Our investigation of these SNPs showed that the *TGFBI* +869T/C polymorphism is associated with schizophrenia susceptibility.<sup>14</sup> The risk of schizophrenia was more than twofold higher in carriers of the T allele (TT and TC genotypes) in comparison with patients with the CC genotype. Interestingly, when we analysed sex differences, this association was significant in females, while in males it showed a trend towards statistical significance, suggesting the influence of sex differences on the TGF- $\beta$  signaling.

Emerging evidence indicates that genetic factors influencing the TGF- $\beta$  signaling may be associated with cognitive deficits. Loeys et al<sup>15</sup> identified heterozygous mutations in genes encoding TGF- $\beta$  receptors (*TGFBR1* and *TGFBR2*) that underlie the development of the syndrome of cardiovascular, craniofacial, neurocognitive, and skeletal disturbances. Furthermore, transgenic mice models overexpressing TGF- $\beta$  are characterized by cognitive deficits mimicking those specific for Alzheimer's disease.<sup>16,17</sup> Finally, there is one study showing the association between the *TGFBI* +869T/C polymorphism and the risk of Alzheimer's disease.<sup>18</sup> However, there are no studies addressing the influence of the TGF- $\beta$  signaling on cognitive functioning in schizophrenia.

The aim of this study was to investigate whether polymorphisms in the *TGFBI* gene (+869T/C and +915G/C) or serum TGF- $\beta$  level are associated with clinical manifestation, course of the disorder, and cognitive performance in schizophrenia.

## Material and methods

### Subjects

We recruited 279 controls (61 females and 196 males of mean age 38.7 $\pm$ 8.8 years), and 158 patients with schizophrenia (89 females and 69 males of mean age 38.0 $\pm$ 11.9 years) including individuals recovered from acute relapse and stable outpatients.<sup>14</sup> All participants were Caucasians and came from the same geographic area – Lower Silesia. The study was approved by the Ethics Committee at Wrocław

Medical University and all subjects gave informed consent. The study was performed in accordance with the 1964 Declaration of Helsinki and its later amendments. Participants were excluded on the basis of the following criteria: history of traumatic brain injury, neurologic disorders, severe physical health impairments, and comorbid substance use disorders with the exception of nicotine. Additionally, they had no current infections, allergies, nor present and past history of autoimmune disorders. Present, past, and family history of psychiatric illness was negative in all control subjects.

Genotyping of the *TGFBI* polymorphisms (+869T/C and +915G/C) was performed in all participants according to the methodology described in our previous article,<sup>14</sup> while serum TGF- $\beta$  level was assessed in the randomly selected group of 88 schizophrenia subjects and 88 healthy controls matched for sex, age and genotype distribution.

### Assessment of psychopathology and cognitive functions

A diagnosis of schizophrenia was based on *Diagnostic and Statistical Manual of Mental Disorders IV* criteria and established by the same two senior board psychiatrists. Lifetime psychopathology and course of schizophrenia were assessed using the Operational Criteria for Psychotic Illness (OPCRIT) checklist.<sup>19</sup> In turn, psychopathology on the day of assessment was evaluated using the Positive and Negative Syndrome Scale (PANSS),<sup>20</sup> the Scale for Assessment of Positive Symptoms (SAPS),<sup>21</sup> and the Scale for Assessment of Negative Symptoms (SANS).<sup>22</sup> Assessment of cognitive performance was performed in schizophrenia patients using the following tests: Rey Auditory Verbal Learning Test,<sup>23</sup> Trail Making Test (TMT-A, TMT-B),<sup>24</sup> verbal fluency tests (F, A, and S letters,<sup>25</sup> and supermarket),<sup>26</sup> Stroop test,<sup>27</sup> as well as selected Wechsler Adults Intelligence Scale – Revised, Polish adaptation (WAIS-R-Pl) subtests (Digit Symbol Coding Test, Digit Span Forward and Backward, and Similarities).<sup>28</sup>

### Genotyping

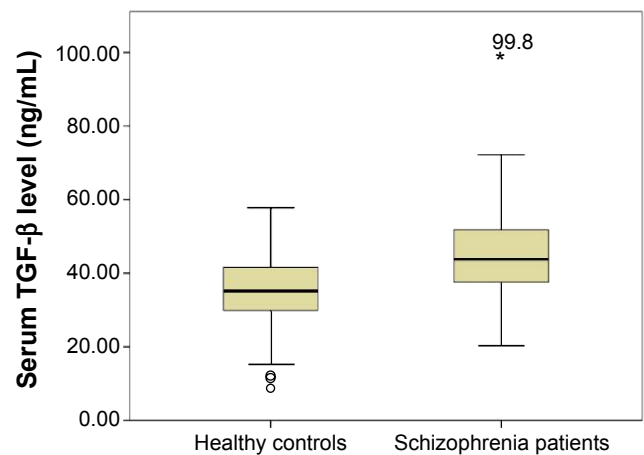
Genomic DNA was obtained from peripheral blood leukocytes (from whole frozen blood) using the QIAamp DNA Blood Mini Kit (Qiagen NV, Venlo, the Netherlands). The *TGFBI* +869T/C (rs1800470, c.29C>T) and the *TGFBI* +915G/C (rs1800471, c.74G>C) polymorphisms were examined by the polymerase chain reaction (PCR) with sequence specific primers technique (single specific primer-PCR) using the PCYTGEN kit (One Lambda, Canoga Park, CA, USA). The PCR products were visualized on 2% agarose gel.

## TGF- $\beta$ measurement

Serum samples were stored in aliquots at  $-80^{\circ}\text{C}$ . Serum concentrations were measured blinded for case and control status. Serum hs-CRP was measured with use of the C-reactive protein extended range method on the Dimension<sup>®</sup> clinical chemistry system (Siemens Healthcare Diagnostics Inc., Newark, NJ, USA). Serum levels of TGF- $\beta$  were measured using a commercially available Human TGF- $\beta$  Immunoassay (Quantikine<sup>®</sup> ELISA; R&D Systems, Inc., Minneapolis, MN, USA), according to the manufacturer's instructions.

## Statistics

The differences in serum levels of TGF- $\beta$  between subjects with different *TGFB1* +869T/C and +915G/C genotypes, as well as different allele carriers in both schizophrenia patients and healthy controls, were compared using Kruskal–Wallis test and Mann–Whitney *U*-test. The analysis of covariance was performed to control for age and sex. Demographic and clinical data with respect to the *TGFB1* +869T/C polymorphism among patients with schizophrenia were compared using Kruskal–Wallis test (body mass index [BMI], age, pack-year smoking index, chlorpromazine equivalent, years of education, disease duration, number of previous episodes) and  $\chi^2$  test (sex, education, family history of schizophrenia, course of the disorder). Kaplan–Meier survival analysis was used to assess the difference in the age of psychosis onset between subjects with different *TGFB1* +869T/C and +915G/C genotypes. Differences between two survival curves were tested with log-rank test. Cognitive performance and psychopathological manifestation with respect to the *TGFB1* +869T/C and +915G/C were compared using Kruskal–Wallis test and Mann–Whitney *U*-test. Correlations between clinical variables and cognitive functioning with serum TGF- $\beta$  level were assessed using Spearman's rank correlations (Spearman's rho). The association between the *TGFB1* polymorphisms and TGF- $\beta$  serum level with cognitive functioning was performed using linear regression analysis adjusting for age, education level, illness duration,



**Figure 1** Serum TGF- $\beta$  levels in healthy control subjects and patients with schizophrenia ( $P < 0.0001$ ).

total PANSS score, BMI, smoking, and chlorpromazine equivalent. Differences were considered as statistically significant if the two-tailed *P*-value was less than 0.05. All analyses were performed using the Statistical Package for Social Sciences version 20.

## Results

Serum TGF- $\beta$  levels were significantly higher in schizophrenia patients in comparison with healthy controls (median  $\pm$  interquartile range:  $43.56 \pm 13.36$  ng/mL and  $35.15 \pm 11.83$  ng/mL, respectively,  $P < 0.0001$ ) (Figure 1). This difference was still statistically significant after covarying for age and sex of the participants ( $F = 25.28$ ,  $P < 0.0001$ ).

All results for patients and controls were in Hardy–Weinberg equilibrium ( $P > 0.05$ ). The association of the *TGFB1* polymorphisms with serum TGF- $\beta$  level are presented in Tables 1 and 2. With respect to the *TGFB1* +869T/C polymorphism, healthy controls with CT and CC genotypes were characterized by higher levels of serum TGF- $\beta$  in comparison with subjects with TT genotype ( $P = 0.005$ ) (Table 1). There was no significant association between the *TGFB1* +869T/C polymorphism and TGF- $\beta$  level in schizophrenia patients. With respect to

**Table 1** The comparison of TGF- $\beta$  levels between schizophrenia patients and healthy controls with respect to distinct genotypes and alleles' carriers of the *TGFB1* +869T/C polymorphism

Study group	<i>TGFB1</i> +869T/C genotype			P-value <sup>a</sup>	<i>TGFB1</i> +869 T/C allele carriers		P-value <sup>b</sup>
	CC	CT	TT		CC	CT and TT	
SCH	43.26 (12.26) (n=21)	42.62 (15.00) (n=36)	44.47 (13.94) (n=31)	0.84	43.26 (12.26) (n=21)	44.09 (14.54) (n=67)	0.79
HC	38.84 (13.22) (n=14)	37.22 (13.06) (n=45)	30.31 (13.88) (n=29)	<b>0.005</b>	38.84 (13.22) (n=14)	34.51 (13.07) (n=74)	0.09

**Notes:** <sup>a</sup>Kruskal–Wallis test; <sup>b</sup>Mann–Whitney *U*-test. Median values and interquartile range of TGF- $\beta$  levels are presented (ng/mL). Significant association has been marked in bold characters ( $P$ -value  $< 0.05$ , two-tailed).

**Abbreviations:** HC, healthy control subjects; SCH, schizophrenia patients.

**Table 2** The comparison of TGF- $\beta$  levels between schizophrenia patients and healthy controls with respect to distinct genotypes and alleles' carriers of the *TGFBI* +915G/C polymorphism

Study group	<i>TGFBI</i> +915G/C genotype			P-value <sup>a</sup>	<i>TGFBI</i> +915G/C allele carriers		P-value <sup>b</sup>
	CC	CG	GG		CC and CG	GG	
SCH	NA (n=1)	38.25 (14.13) (n=14)	44.15 (13.71) (n=72)	0.31	38.36 (12.42) (n=15)	44.15 (13.71) (n=70)	0.12
HC	NA (n=0)	32.60 (10.47) (n=12)	35.87 (13.23) (n=76)	0.18	32.60 (10.47) (n=12)	35.87 (13.23) (n=76)	0.18

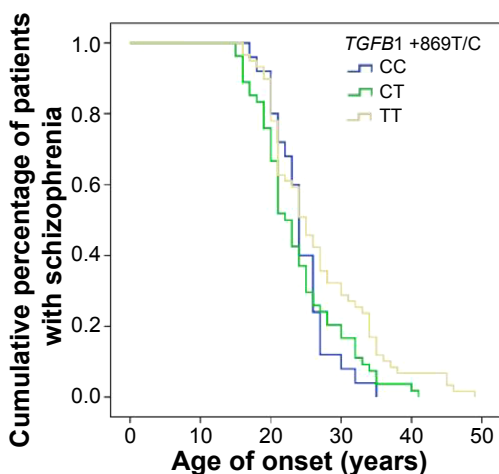
**Notes:** <sup>a</sup>Kruskal–Wallis test; <sup>b</sup>Mann–Whitney *U*-test. Median values and interquartile range of TGF- $\beta$  levels are presented (ng/mL).

**Abbreviations:** HC, healthy control subjects; NA, not applicable; SCH, schizophrenia patients.

the *TGFBI* +915G/C polymorphism, there was no significant association with serum TGF- $\beta$  either in schizophrenia patients or in healthy controls ( $P>0.05$ ) (Table 2).

There was no association between the *TGFBI* +869T/C nor +915G/C polymorphisms with domains evaluated by the OPCRIT checklist such as: disturbances related to speech and form of thought; affect and associated features; appearance and behavior; as well as abnormal beliefs and abnormal perceptions (data not shown). However, there was a significant difference in age of psychosis onset between schizophrenia patients with distinct *TGFBI* +869T/C polymorphism genotypes (log-rank test  $\chi^2=6.54$ ,  $P=0.038$ ) (Figure 2). When the patients were divided with respect to sex, there was still a significant association in females (log-rank test  $\chi^2=9.27$ ,  $df=2$ ,  $P=0.01$ ) but not in males (log-rank test  $\chi^2=0.57$ ,  $df=2$ ,  $P=0.75$ ). With respect to the *TGFBI* +915G/C polymorphism, there were no significant associations with age of psychosis onset either in female or in male subjects (data not shown).

General characteristics with respect to the *TGFBI* +869T/C polymorphism are provided in Table 3. There was no significant association between the *TGFBI* +869T/C



**Figure 2** Kaplan–Meier survival plot of genotype-specific age of onset distribution curves with respect to the *TGFBI* +869T/C polymorphism.

**Notes:** Long-rank test  $\chi^2=6.54$ ,  $df=2$ ,  $P=0.038$ .

polymorphism and demographic variables, basic clinical characteristics (number of previous episodes, illness duration, family history of schizophrenia, and chronic course of the disorder), or possible confounding factors (chlorpromazine equivalent, BMI, and pack-year index for cigarette smoking). Similarly, the *TGFBI* +915G/C polymorphism was not associated with any of these variables (data not shown). There was no statistically significant difference between demographic and clinical variables among male and female patients with respect to the *TGFBI* +869T/C and +915G/C polymorphisms (data not shown).

All of the patients had been medicated on the day of the assessment. The mean duration of treatment was  $12.24\pm 12.24$  years. The majority of patients were treated with second-generation antipsychotic drugs (14.58% olanzapine, 27.08% risperidone, 12.50% quetiapine, 21.87% clozapine, 11.45% ziprasidone, 2.08% aripiprazole, 2.08% sertindole), while the rest of the patients were treated with first-generation antipsychotics (2.08% chlorpromazine, 1.04% perazine, 1.04% zuclopenthixol, 4.16% haloperidol). The mean value of daily chlorpromazine equivalent dose was  $571.84\pm 401.98$  mg/day.<sup>29</sup>

Correlations between TGF- $\beta$  level and clinical variables are shown in Table 4. TGF- $\beta$  serum level was not associated with illness duration, number of previous episodes, number of years of completed education, age, pack-year smoking index, BMI, or chlorpromazine equivalent dosage. Serum TGF- $\beta$  was not associated either with schizophrenia psychopathology or cognitive performance (Table 5). There was no difference between male and female patients with respect to age ( $P=0.09$ ), number of previous episodes ( $P=0.56$ ), BMI ( $P=0.25$ ), pack-year index of cigarette smoking ( $P=0.18$ ), chlorpromazine equivalent dosage ( $P=0.72$ ), education level ( $P=0.17$ ), number of years of completed education ( $P=0.11$ ), PANSS total score ( $P=0.10$ ), or any of the cognitive tests ( $P>0.05$ ). However, there was a difference in the age of onset (females  $26.6\pm 7.8$  years, males  $23.12\pm 4.44$  years,  $P=0.016$ ),

**Table 3** General demographic and clinical characteristics of schizophrenia patients with respect to the *TGFBI* +869T/C polymorphism

Clinical and demographic variables	<i>TGFBI</i> +869T/C genotype			P-value <sup>a</sup>
	CC	CT	TT	
Age (years)	38.50±19.55	33.10±9.67	41.38±11.29	0.59
Female (%)	71.43	56.90	59.68	0.42 <sup>b</sup>
Education (%)				0.91 <sup>b</sup>
Primary	0	7.69	9.52	
Vocational	20.00	23.08	16.67	
Secondary	60.00	48.72	50.00	
Higher	20.00	20.51	21.43	
Education (years)	14.17±3.37	12.80±26.16	13.85±3.00	0.69
Age of onset (years)	29.50±9.40	22.80±3.26	26.54±8.81	0.12
Disease duration (years)	9.00±10.95	10.30±8.96	14.85±8.68	0.71
Number of previous episodes	4.50±3.27	4.50±3.41	5.46±4.18	0.78
Chlorpromazine equivalent	391.67±251.16	618.50±436.18	617.95±498.44	0.29
Body mass index (kg/m <sup>2</sup> )	24.89±2.99	25.14±4.37	28.72±9.13	0.58
Pack-year smoking index	7.28±14.82	6.34±12.13	14.35±18.79	0.19

Notes: <sup>a</sup>Kruskal–Wallis test; <sup>b</sup> $\chi^2$  test. Mean and standard deviation values are presented. Pack-year index is the number of cigarettes packs per month  $\times$  number of years smoking.

which is one of the most commonly described aspects of sex dimorphism in schizophrenia. There was no significant difference in TGF- $\beta$  level between female and male patients (median  $\pm$  interquartile range: 44.15 $\pm$ 13.70 ng/mL, 42.85 $\pm$ 14.10 ng/mL, respectively,  $P=0.47$ ). Additionally, the *TGFBI* +869T/C polymorphism did not influence TGF- $\beta$  level either in female ( $P=0.78$ ) or in male patients ( $P=0.19$ ). Similarly, the *TGFBI* +915G/C polymorphism did not influence TGF- $\beta$  level either in female ( $P=0.65$ ) or in male patients ( $P=0.14$ ).

There was no significant difference in psychopathological manifestation assessed using PANSS, SAPS, and SANS with respect to the *TGFBI* +869T/C and +915G/C polymorphisms ( $P>0.05$ ). There was no significant association between *TGFBI* +915G/C polymorphism and cognitive functioning in schizophrenia (data not shown); however, subjects carrying the +869T allele performed worse in comparison

with +869CC homozygotes on Stroop test ( $P=0.02$ ), Verbal Fluency task (F words) ( $P=0.03$ ), and on Digit Symbol Coding task ( $P=0.06$ ).

After adjustment for possible confounding factors, such as age, education level, illness duration, total PANSS score, BMI, smoking, chlorpromazine equivalent, and TGF- $\beta$  serum level, there was a statistically significant difference in cognitive performance on Digit Symbol Coding task with respect to the *TGFBI* +869T/C polymorphism among female schizophrenia patients ( $\beta=-0.57$ ,  $t=-2.78$ ,  $P=0.02$ ).

## Discussion

There are numerous studies showing the influence of the immune system deregulation on the risk of schizophrenia and its clinical manifestation.<sup>30,31</sup> However, the association between the *TGFBI* gene polymorphisms and TGF- $\beta$  level with schizophrenia course and symptomatology, together with cognitive functioning, has not been investigated so far.

In our study, we showed that TGF- $\beta$  level was significantly higher in patients with schizophrenia than in healthy control subjects, even after covarying for age and sex. The majority of previous studies on medicated and drug-naïve first-episode patients have also shown higher TGF- $\beta$  levels in schizophrenia subjects in comparison to healthy controls,<sup>32–34</sup> with the exception of one study that failed to show this association.<sup>35</sup>

Studies, which have attempted to establish the relationship between the *TGFBI* gene polymorphisms and TGF- $\beta$  level, have yielded ambiguous results.<sup>36,37</sup> In the present study, we

**Table 4** Correlations between TGF- $\beta$  level and clinical variables in patients with schizophrenia

Clinical variables	TGF- $\beta$ level	
	Rho	P-value <sup>a</sup>
Disease duration (years)	-0.42	0.72
Age (years)	-0.13	0.28
Number of previous episodes	-0.03	0.82
Number of years of completed education (years)	0.55	0.75
Pack year smoking index	-0.08	0.55
Body mass index (kg/m <sup>2</sup> )	0.20	0.16
Chlorpromazine equivalent	-0.03	0.84

Notes: <sup>a</sup>Spearman's rank correlations. Pack-year index is the number of cigarettes packs per month  $\times$  number of years smoking.

**Table 5** Psychopathological manifestation and cognitive performance with respect to the *TGFBI* +869T/C polymorphism

Psychopathological symptoms and cognitive performance	<i>TGFBI</i> +869T/C carriers			TGF- $\beta$ serum level	
	CC	CT and TT	P-value <sup>a</sup>	Rho	P-value <sup>b</sup>
<b>Psychopathology</b>					
PANSS – positive symptoms	19, 17.71 $\pm$ 5.63	20, 20.11 $\pm$ 7.26	0.41	<0.01	0.92
PANSS – negative symptoms	20.50, 21.50 $\pm$ 8.05	23, 23.27 $\pm$ 8.24	0.52	<0.01	0.96
PANSS – general psychopathology	39.50, 39.43 $\pm$ 8.16	39, 40.76 $\pm$ 11.10	0.95	0.07	0.57
PANSS – total score	84, 78.64 $\pm$ 17.56	81, 84.14 $\pm$ 23.88	0.69	0.01	0.92
PANSS – depression item score	1, 1.86 $\pm$ 1.23	1, 1.80 $\pm$ 1.08	0.91	0.11	0.35
SAPS	29.50, 29.86 $\pm$ 18.58	30, 31.24 $\pm$ 20.56	0.78	0.07	0.55
SANS	44, 38.14 $\pm$ 19.28	37, 40.82 $\pm$ 21.77	0.70	<0.01	0.99
<b>Cognitive tasks</b>					
Trail making test, part A	36, 38 $\pm$ 15.24	42, 46.57 $\pm$ 20.40	0.16	-0.13	0.30
Trail making test, part B	100.50, 103.8 $\pm$ 55.7	110, 143.4 $\pm$ 104.4	0.16	-0.19	0.13
Stroop, congruent	<b>32, 37.47<math>\pm</math>11.13</b>	<b>41, 45.39<math>\pm</math>19.56</b>	<b>0.02</b>	0.01	0.95
Stroop, incongruent	70, 78.47 $\pm$ 47.20	76, 82.33 $\pm$ 33.74	0.29	0.16	0.18
RAVLT, immediate recall 1st–5th	33.50, 35.72 $\pm$ 11.76	36, 35.86 $\pm$ 10.90	0.92	<0.01	0.93
RAVLT, interference	7.50, 6.88 $\pm$ 3.26	7, 7.39 $\pm$ 2.82	0.74	-0.01	0.91
RAVLT, delayed recall	6, 6.00 $\pm$ 3.33	7, 6.50 $\pm$ 2.96	0.89	-0.06	0.61
RAVLT, recognition	11, 9.69 $\pm$ 4.04	10, 9.41 $\pm$ 3.94	0.72	0.08	0.52
Verbal fluency, F words	<b>8, 8.47<math>\pm</math>3.24</b>	<b>6, 6.49<math>\pm</math>3.76</b>	<b>0.03</b>	0.07	0.56
Verbal fluency, A words	9, 7.67 $\pm$ 10.09	5, 6.25 $\pm$ 3.62	0.13	0.07	0.59
Verbal fluency, S words	9, 9.53 $\pm$ 3.64	8, 8.00 $\pm$ 3.74	0.44	-0.09	0.46
Verbal fluency, supermarket	16, 17.20 $\pm$ 6.53	15, 16.06 $\pm$ 6.83	0.80	0.09	0.47
Forward Digit Span	6, 5.82 $\pm$ 1.63	6, 6.11 $\pm$ 1.89	0.67	-0.01	0.91
Backward Digit Span	5, 1.33 $\pm$ 1.33	5, 5.04 $\pm$ 2.10	0.78	<0.01	0.95
Digit Symbol Coding	<b>39, 42.18<math>\pm</math>13.11</b>	<b>35, 35.05<math>\pm</math>14.00</b>	<b>0.06*</b>	-0.01	0.98
Similarities	17, 16.67 $\pm$ 4.24	15.5, 15.88 $\pm$ 5.34	0.92	0.23	0.06

**Notes:** <sup>a</sup>Mann–Whitney *U*-test; <sup>b</sup>Spearman's rank correlations. Median, mean and standard deviation values are presented. Significant associations have been marked in bold characters (*P*-value <0.05, two-tailed) and trend level association is indicated by bold characters marked with\* (*P*-value <0.1, two-tailed).

**Abbreviations:** PANSS, Positive and Negative Syndrome Scale; RAVLT, Rey Auditory Verbal Learning Test; SAPS, Scale for Assessment of Positive Symptoms; SANS, Scale for Assessment of Negative Symptoms.

found that the *TGFBI* +869T/C and +915G/C polymorphisms are not associated with TGF- $\beta$  level in schizophrenia patients. However, we revealed that the *TGFBI* +869T/C but not the *TGFBI* +915G/C polymorphism is associated with TGF- $\beta$  level in healthy controls. The explanation of a differential contribution of the *TGFBI* +869T/C polymorphism in schizophrenia patients and healthy controls would originate from a complex regulation of gene expression within the TGF- $\beta$  signaling pathway. In the recent study by Umeda-Yano et al<sup>10</sup> it was found that overexpression of the *ZNF804A* gene may lead to altered expression of genes encoding proteins acting on the TGF- $\beta$  signaling pathway. Notably, large data sets indicate that the SNP (rs1344706) in the *ZNF804A* gene is one of the top genetic variants associated with schizophrenia.<sup>38</sup>

In our study, we showed no association between TGF- $\beta$  levels and clinical variables, including current (PANSS, SANS, SAPS) or lifetime (OPCRIT) psychopathological manifestation of schizophrenia. Other researchers have also found no correlations with current clinical status as assessed by the Brief

Psychiatric Rating Scale<sup>30</sup> or PANSS.<sup>39</sup> However, the analysis of counter-regulatory cytokines ratio, such as IFN- $\gamma$ /TGF- $\beta$  ratio, has shown a weak negative correlation with negative symptoms and general psychopathology subscales of PANSS,<sup>31</sup> as well as the total PANSS score.<sup>31,32</sup> Additionally, a weak negative correlation has been found between the IL-17/TGF- $\beta$  ratio and the negative and general psychopathology PANSS subscales.<sup>32</sup>

We found no significant association between the *TGFBI* gene polymorphisms and schizophrenia current and life-time symptomatology, or variables associated with course of the disorder. However, we found that the *TGFBI* +869T/C polymorphism may predict age of psychosis onset in female patients. Indeed, females with the +869TT and +869CT genotypes had significantly later age of psychosis onset in comparison with females with the +869CC genotype. These findings are in agreement with the results that were previously published by our group.<sup>14</sup> We found that the risk of schizophrenia is more than twofold higher in the +869T allele carriers in comparison with those with the +869CC genotype. When we

conducted a separate analysis of male and female subjects, we found that this association was significant in females, while in males it reached a trend toward significant difference. Several lines of evidence support strong heritability of age at onset in schizophrenia.<sup>40–42</sup> Moreover, there is a plethora of studies linking SNPs to age of psychosis onset. Although the majority of these studies show that the risk allele for schizophrenia is simultaneously associated with earlier age of psychosis onset, there is a considerable number of studies linking the risk polymorphic variant to later age of psychosis onset.<sup>43–45</sup>

Cognitive impairment serves as the core component of schizophrenia symptomatology that accounts for poor functional outcome.<sup>46,47</sup> Although the efficacy of current pharmacological strategies with respect to psychotic symptoms seems to be sufficient, their impact on cognition leaves much to be desired.<sup>48</sup> Therefore, our understanding of biological mechanisms underlying cognitive dysfunction in schizophrenia patients appears to be a major challenge for current psychiatric research. However, immune alterations are sparsely addressed in studies focused on cognitive impairment in schizophrenia patients. There is evidence that C-reactive protein (CRP) level negatively correlates with cognitive functioning.<sup>49,50</sup> It has also been found that this relationship is augmented by Herpes Simplex Virus-1<sup>51,52</sup> and cytomegalovirus seropositivity.<sup>52</sup> Additionally, the leukemia inhibitory factor gene polymorphism has been associated with deterioration in working memory function in patients with schizophrenia.<sup>53</sup> With respect to cytokine serum levels in schizophrenia, cognitive impairment has been associated with higher IL-6<sup>50</sup> and IL-18<sup>54</sup> as well as lower IL-2<sup>55</sup> levels.

Our study is the first to address cognitive correlates of TGF- $\beta$  level and the *TGFB1* gene polymorphisms in schizophrenia. We found no association between TGF- $\beta$  level and cognition; however, our findings suggest that the *TGFB1* +869T/C polymorphism is associated with cognitive functioning in schizophrenia. In our study, the +869T allele carriers performed worse on tests measuring processing speed (Digit Symbol Coding task, Stroop congruent task) and verbal fluency (F words). Interestingly, after adjustment for potential confounders, such as age, education level, illness duration, total PANSS score, BMI, smoking, chlorpromazine equivalent, and TGF- $\beta$  serum level, the +869T allele carriers scored significantly lower only on Digit Symbol Coding task in comparison with the +869CC homozygotes, among female patients. The meta-analysis of neuropsychological measures in schizophrenia revealed that Digit Symbol Coding task shows significantly larger effect in differentiating schizophrenia and healthy subjects in comparison with other

cognitive measures including verbal memory, executive functioning, or working memory tasks.<sup>56</sup> Digit Symbol Coding task is considered to be a cognitive endophenotype – a measurable biomarker that is correlated with an illness due to shared underlying genetic influences. It has been shown to have high heritability and stable trait-like qualities, and is associated with prognosis, as well as functional outcome among patients with schizophrenia.<sup>56</sup>

Interestingly, studies investigating the polymorphism in the *ZNF804A* gene (rs1344706), known to upregulate genes implicated in the TGF- $\beta$  signaling,<sup>10</sup> have shown its correlation with several domains of cognitive performance including episodic and working memory, executive functions, verbal learning, and recall.<sup>57–61</sup> Most interestingly, the *ZNF804A* gene polymorphism (rs1344706) has been found to predict poorer executive control of attention in schizophrenia, overlapping partially with the results of our study.<sup>60</sup> Additionally, there is one study that established a susceptibility link between the *TGFB1* +869T/C polymorphism and Alzheimer's disease,<sup>18</sup> suggesting that cognitive decline both in schizophrenia and dementia may be partially influenced by the same set of genes associated with the immune system.<sup>62,63</sup>

Our results, showing the association of the *TGFB1* +869T/C polymorphism with age of onset and cognitive performance in female patients, point to the list of sex differences in schizophrenia (for review see<sup>64,65</sup>). Indeed, female patients with schizophrenia tend to have later age of psychosis onset;<sup>66</sup> more affective<sup>67</sup> and less negative symptoms;<sup>68</sup> as well as less severe course of the disorder.<sup>69</sup> The presence of sex differences in schizophrenia has been attributed to neuroprotective and antidopaminergic properties of estradiol.<sup>65,69,70</sup> To support hormonal underpinnings, it has been found that there is a fluctuation in the severity of psychopathological manifestation across the menstruation cycle, with deterioration of symptomatology during low-estrogen phases.<sup>71</sup> Furthermore, chronic psychosis is prone to improvement during pregnancy.<sup>72</sup> Finally, low-estrogen lifespan phases including perimenstrual phase of the menstrual cycle, postmenopause, and the period after abortion are characterized by higher susceptibility to psychosis.<sup>71</sup> Interestingly, it has been found that females with first-episode psychosis are characterized by significantly higher TGF- $\beta$  levels.<sup>33</sup> There is also a crosstalk between TGF- $\beta$  and estradiol. It has been found that estradiol increases the production of TGF- $\beta$  in cortical and hypothalamic astrocytes.<sup>73,74</sup> On the other hand, TGF- $\beta$  may stimulate the production of estradiol via promotion of the basal secretion of follicle-stimulating hormone.<sup>75</sup> However, these interactions are yet to be investigated in schizophrenia patients.

Our results should be interpreted with caution due to limitations that require further discussion. Firstly, it should be noted that our sample size was limited; however, it is one of the largest studies analyzing serum cytokine levels with respect to cognitive functioning in schizophrenia.<sup>50,54,55,76</sup> Secondly, all our patients were medicated, while the association of TGF- $\beta$  level with cognitive functioning in unmedicated first-episode schizophrenia patients would be much more reliable. However, it should be noted that we found no association between TGF- $\beta$  level and daily chlorpromazine equivalent dose, and previous studies have shown higher TGF- $\beta$  levels in both drug-naïve and medicated schizophrenia patients in comparison to controls.<sup>32</sup> Additionally, our study assessed cognitive functions only at one point while, in the future, longitudinal design might bring insight into how changes in antipsychotic treatment are translated into changes in TGF- $\beta$  level and cognitive performance with time, especially in first-episode patients. Moreover, in our study, we did not assess menstrual cycle stage in female participants. This should be included in the future studies' protocols, as this information may shed more light on the reciprocal interaction between estrogen and TGF- $\beta$  levels in the brain, possibly playing a role in the sex dimorphism of schizophrenia. Finally, future studies should look into the *TGFB1* +869T/C polymorphism together with TGF- $\beta$  level on all indices associated with metabolic syndrome, including hypertension and glucose intolerance, especially due to their high prevalence in schizophrenia and their influence on cognition in this group of patients.

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## Disclosure

The authors report no conflicts of interest in this work.

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