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Association of *GAD1* gene polymorphism rs3749034 with the information processing and cognitive event-related potentials in schizophrenia patients and healthy subjects

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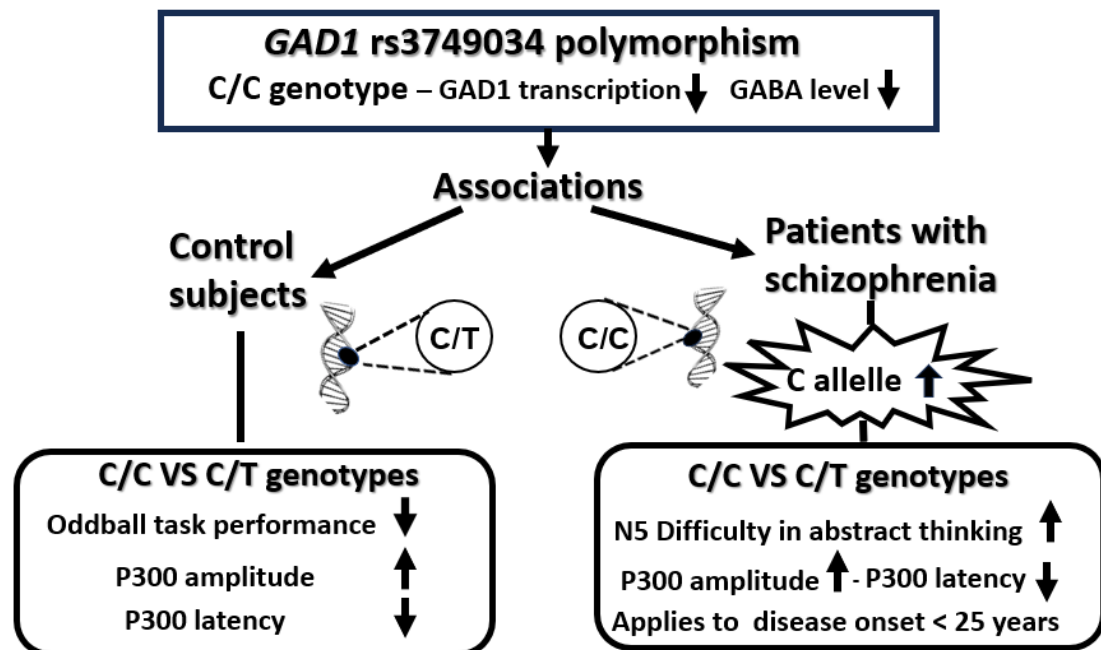
Running title: *GAD1* gene polymorphism effects in schizophrenic patients

Keywords: *GAD1* rs3749034 polymorphism, oddball paradigm, event-related potentials P300, schizophrenia.

Highlights

- An increased frequency of the cytosine-cytosine allele in *GAD1* rs3749034 polymorphism was found in schizophrenic patients.
- A decreased patient oddball task performance was found only in the cohort of cytosine-thymine carriers between study groups.
- A higher P300 amplitude and shorter latency in cytosine-cytosine versus cytosine-thymine carriers was observed for both groups.

Graphical Abstract



Abstract

Objective: Glutamic acid decarboxylase, an enzyme in GABA biosynthesis, is encoded by the *GAD1* gene, the transcriptional activity of which is affected by the rs3749034 polymorphism. The aim was to investigate the effects of rs3749034 on cognitive event-related potentials (P300) in healthy subjects and schizophrenic patients.

Methods: Determination of rs3749034 polymorphism was performed in 89 healthy volunteers and 109 schizophrenic patients (males). Two-stimulus oddball task performance and P300 auditory evoked potentials were analyzed and patient symptomatology was assessed using the Positive and Negative Syndrome Scale (PANSS).

Results: An increased frequency of C allele carriers was disclosed in patients. In controls, superior task performance was observed in cytosine-thymine carriers, while a greater P300 amplitude and shorter latency were found in C/C carriers. Analogous effects were found in patients with a disease onset before 25 years of age. Higher N5 and lower P3 and G5 PANSS scales were revealed in C/C homozygotes.

Conclusions: The findings substantiate an involvement of GABA-ergic mechanisms in maintaining an optimal excitatory-inhibitory balance and an association of rs3749034 with early-onset disorder and negative symptoms of schizophrenia.

Significance: These results are important for understanding underlying mechanisms and the development of evidence-based methods for assessing the risk of schizophrenia.

1. Introduction

Populations of inhibitory neurons play a crucial role in brain development and synaptogenesis, contributing extensively to the organization of activity in various brain circuits, synchronization of neural oscillations and cognitive function (Bucher et al., 2021). γ -Aminobutyric acid (GABA) is a major inhibitory central neurotransmitter, and prefrontal cortical GABA hypofunction is not only associated with impaired cognitive activity including attention and memory (Enomoto, Tse, and Floresco, 2011; Paine et al., 2011; Rolls et al., 2008) but also with a high level of schizotypy (Kozhuharova et al., 2021). Alterations in several markers of GABA neurotransmission in various cortical regions have been revealed in schizophrenic patients (Daskalakis et al., 2002; Mattay et al., 1997; Costa et al., 2004; Lewis et al., 2005).

A specific cognitive deficit is one of the most prominent symptoms in schizophrenia and involves a range of advanced cognitive processes, designated as executive functions. Executive functions rely heavily on frontal lobe structures and include directed attention, task planning, monitoring, working memory, and inhibition of irrelevant responses (Eisenberg and Berman, 2010). This is in line with the suggestion that frontal lobe dysfunction is crucially important in schizophrenic psychopathology. In this context, the activity of parvalbumin-positive GABA interneurons in the prefrontal cortex (PFC) is important for inhibiting inappropriate neural activation increasing signal-to-noise ratio, that in turn, is crucial for mechanisms of selective attention (Paine et al., 2011; Rolls et al., 2008).

The glutamic acid decarboxylase isoform, GAD67, is a critical enzyme in GABA biosynthesis, and a reduction of its mRNA expression and enzyme protein in postmortem brains of schizophrenic patients has been reported (Akbarian et al., 1995; Hashimoto et al. 2003; Volk et al., 2000). Accordingly, downregulation of GAD67 in fast-spiking parvalbumin-positive

interneurons of the dorsolateral PFC has also been found in postmortem studies of patients (Torrey et al., 2005; Volk et al., 2016).

The decrease in GAD67 brain expression in schizophrenic patients suggests that there is a role of the transcriptional activity of *GAD1* (the gene encoding this enzyme) in the pathogenesis of the disease. A polymorphism, rs3749034 (cytosine/thymine [C/T] substitution in the promoter zone), has been shown to affect the transcriptional activity of the *GAD1* gene (Straub et al. 2007; Tao et al. 2018). Homozygosity for cytosine is associated with a low level of *GAD1* transcription, decreased thickness of cortical grey matter, and a risk of childhood-onset schizophrenia (Brauns et al., 2013; Addington et al., 2005).

The influence of rs3749034 on processes of brain development and maturation have also been investigated, and it is evident that the C/C genotype is associated with an increase in a truncated transcript of the K^+ - Cl^- co-transporter (KCC2) in the prefrontal cortex (PFC). Moreover, a high level of this shortened transcript is present in patients with schizophrenia, and it is regarded as a sign of abnormal GABA-ergic transmission (Tao et al., 2012).

In a subsequent study by Lett et al., (2016), a decrease in PFC white matter fractional anisotropy in carriers of the T/T genotype of rs3749034 was detected. This finding concurs with data on the participation of GABA-ergic mechanisms in remodeling of myelin in the adult brain (Reyes-Haro et al., 2021). Thus, the C/C genotype displays an association with changes in gray matter volume and activity of local inhibitory circuits, whereas the T/T allele appears to be associated with changes in cortical connectivity.

The auditory event-related potential (ERP), P300, obtained in the active oddball paradigm, is widely applied to assess cognitive functioning and activity of distributed brain networks associated with attention and information processing (Luck et al., 2011; Polich, 2007; Huang et al., 2015). In the standard two-stimulus oddball task, a sequence of repetitive standard stimuli (tones) is presented with high probability (80-90%) and are interrupted by infrequently (10-20%) deviant target stimuli. The P300 component elicited in response to target stimuli reflects

voluntary detection of task-relevant salient stimuli and has been associated with both a regional neural inhibition involved in processing of expected stimuli and the updating of activity in corticolimbic circuits. (Polich, 2007; Luck et al., 2011; Bramon et al., 2005). A body of evidence indicates that P300 reflects contributions from widespread cortical and subcortical regions, including prefrontal, temporal, parietal, and occipital areas (Luck et al., 2011; Hariri et al, 2003; Turetsky et al., 2007; Turetsky et al., 2015). In addition, intracranial electrophysiological monitoring and functional magnetic resonance imaging (fMRI) studies have distinguished several sources of P300-like ERP activity including the hippocampus and thalamus (Turetsky et al., 2007). It should also be noted that the PFC is a component of a large attentional regulatory system, and it exerts a diffuse modulatory influence as well as functionally specific effects on P300 (Luck et al., 2011; Soltani and Knight, 2000).

P300 measures reveal variations in neural activity with cognition (Luck et al., 2011; Huang et al., 2015). Moreover, a reduced amplitude of the auditory oddball P300 response, has been widely observed in schizophrenia patients, and a high heritability has been demonstrated (Turetsky et al., 2007; Turetsky et al., 2015). Thus, the ERP may be useful for investigating genotype–phenotype associations, and to more directly capture the effects of genetic variation on information processing (Bramon et al., 2005; Hariri and Weinberger, 2003; Turetsky et al., 2007).

There have been investigations concerning the influence of GABA-ergic function on auditory P300 measures, and in pharmacological studies, modulation of P300 amplitude and latency by GABA-enhancing drugs has been identified (Urasaki et al., 1994; Watson et al., 2009). The interaction of GABA-ergic and cholinergic circuits has also been observed in relation to the effects of the *GAD1* polymorphism, rs7557793, on auditory evoked potentials in the oddball paradigm (Hadjis et al., 2018). However, the neurophysiological phenotypes of carriers of

different rs3749034 variants are poorly understood, so examining their impact on P300 characteristics should aid the comprehension of GABA-ergic activity on cognitive function.

There are epidemiological findings which support the hypothesis that the influence of gender on schizophrenia, is a significant transcultural phenomenon related to neurobiological differences between the sexes in the general population, which can elucidate differences in the nature and expression of schizophrenia (Hafner, 2003). The influence of sex on the level of gene expression and transmitter functioning, has also been reported. Sex is clearly a broad and important characterization of the individual context in which any given gene is expressed, much like a diagnosis. There are many examples of sexual dimorphism in the phenotypic consequences of genes. The systematic differences between male and female brains mean that any single functional genetic difference is likely to have different consequences for behavior in each sex (DeYoung and Clark, 2012). In the case of rs3749034, a genotype $\times$ gender interaction has also been found (Weber et al., 2012). To better understand the effects of genes, it seems fruitful to conduct studies in male and female samples separately.

Consequently, the aim of the present study was to evaluate oddball task behavioral and electrophysiological concomitant parameters in carriers of different variants of rs3749034 from male cohorts of healthy participants and schizophrenic patients.

2. Materials and methods

2.1 Subjects

A total of 198 right-handed male subjects (aged 19 - 55 years), who were white Caucasians, participated in the study. The control group included 89 healthy volunteers, none of whom had a previous personal or family history of psychiatric disease, developmental and neurological abnormalities or psychoactive drug addictions. The schizophrenic patient group included 109 subjects who were admitted to the National Federal Medical Research Centre of Psychiatry and Narcology to undergo forensic psychiatric examination and who also received an

ICD-10 diagnosis of a schizophrenic disorder (F20). Patient symptomatology was assessed by the Positive and Negative Symptom Scale (PANSS) and all patients were withdrawn from medication for a period exceeding one month before the examination.

After presentation of a detailed description of the study, written informed consent for the investigation was obtained from all subjects in accordance with the local ethics committee of the National Federal Medical Research Centre of Psychiatry and Narcology, and the study was performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

A subset of 124 subjects completed the ERP testing session and 107 subjects (48 controls and 59 patients) with artifact-free data were included in the analysis.

2.2 Genotyping

Extraction of DNA from saliva samples was accomplished using MagMAX™ DNA Multi-Sample Ultra Kits (Applied Biosystems Cat No A25597). Determination of *GAD1* rs3749034 polymorphism was performed employing real-time polymerase chain reaction using TaqMan® SNP Genotyping Assays C__2177452_1 for rs3749034 (Applied Biosystems, USA) with a LightCycler® Nano Instrument (Roche Molecular Systems Inc, USA).

2.3 Auditory oddball paradigm

A two-stimulus active oddball paradigm was employed and experimental stimuli were generated by the GENTASK software program (STIM2, Neuroscan Compumedics, USA).

Auditory tones (75 dB, 50 ms) were randomly presented binaurally with randomly varying inter-trial intervals of 1800 - 2200 ms. A 1000 Hz “standard” tone was presented in 80% of the trials and a 2000 Hz “target” was presented for the remaining 20% of trials. Overall, 60 target stimuli were presented.

The subjects were seated with their eyes closed while wearing headphones for the presentation of auditory stimuli. The task involved pressing a button on a button-box in response

to the target stimuli. All participants were briefly trained with respect to the target discrimination and motor response prior to data acquisition.

2.4 Behavioral measures

The number of correct or erroneous reactions to the target (T) stimuli was determined during the oddball task. Reaction times (RT) were assessed, and responses with RT < 600 ms were considered as correct. Apart from evaluating the total number of errors, two error types were analyzed, namely – missing responses and delayed responses (with RT > 600 ms).

2.5. ERP recording and analysis

Electrophysiological data were obtained with a 64 Channel Synamp2 system (Neuroscan Compumedics, USA). In accordance with the 10–20 system, 19 sites were recorded with impedances less than 10 k Ω and all scalp locations were referred to linked earlobes, and the ground was attached 3 cm above the nasion. EEG data were collected at a sampling rate of 1000 Hz with a band-pass filter of 1-70 Hz and zero phase digital filters. The half power filter with a slope of 24 dB/octave was applied, and the notch filter was 50 Hz. Following manual artifact rejection, continuous EEG was segmented into artifact-free epochs (–200 to +800 ms relative to stimulus onset, with a 200 ms pre-stimulus baseline), low-pass filtered (8.5 Hz) and corrected for baseline values. Only trials that received a correct response were included in the average. Between 25-30 trials were averaged to obtain individual evoked potentials.

The P300 component was defined automatically as the peak response between 250 and 500 ms. Subsequently, peaks were verified through visual inspection and the amplitude and latency of each P300 was evaluated relative to baseline. Mean ERP waveforms in response to target stimuli are presented in **Fig. 1**.

2.6 Statistical analysis

Statistical analysis of results was performed using SPSS 17.0 software. The investigators were blinded towards the rs3749034 polymorphism when conducting the study and the data analysis.

Demographic data, PANSS scores and behavioral measures of task performance were compared using the non-parametric Mann-Whitney test with False Discovery Rate correction for multiple comparisons.

The Hardy–Weinberg equilibrium calculation, as well as statistical assessment of the reliability of between-group difference in the frequency distribution of polymorphic alleles for GAD1 rs3749034 polymorphism, was calculated using χ^2 -test (Rodriguez et al., 2009). The Effect Size was estimated using its correlation-like measure ϕ and the level of Odds Ratio (OR).

ERP peak amplitude and latency were analyzed using ANOVA GLM with repeated measures. The analysis was provided for 15 sites (Fz, F3, F4, Cz, C3, C4, Pz, P3, P4, F7, F8, T3, T4, T5, T6). ANOVA analysis was carried out for 9 sites and included between-subject factors *Diagnosis* ($n = 2$: control subjects vs. schizophrenic patients) and *Genotype* ($n = 2$: C/C *GAD1* genotype carriers vs. C/T carriers). The within-subject factors were *Electrode* ($n = 3$: frontal (F3, Fz, F4) vs. central (C3, Cz, C4) vs. parietal (P3, Pz, P4) sites) and *Laterality* ($n = 3$: left vs. sagittal vs. right sites). For all main and interaction effects including within-subject factors with more than two levels, violations of the sphericity assumption were controlled by df adjustment. Hence, Greenhouse–Geisser corrected p values are reported. For post-hoc analysis of contrasts of group means, the univariate ANOVA was used with Tukey's Honest Significant Difference (HSD) test for multiple comparisons. Participant age was included in the analysis as a covariate in each ANOVA evaluation and effect size (d) was calculated.

Spearman's correlations between PANSS scales and ERP measures were analyzed separately for C/C and C/T *GAD1* genotype carriers in the schizophrenic patient group. Only significant ($p < 0.05$) correlations were examined. To compensate for multiple hypothesis tests

(total number of analyzed correlations for each group amounted to 495) we used Wilkinson's binomial expansion (Zaykin et al., 2002).

3. Results

3.1 Distribution of *GAD1* rs3749034 polymorphism in schizophrenic patients and healthy subjects

A frequency analysis of the distribution of *GAD1* rs3749034 polymorphism was carried out for the whole cohort of participants: 89 healthy subjects and 109 schizophrenic patients (**Table 1**). In both groups, the distribution of the *GAD1* genotype was within the Hardy-Weinberg equilibrium. The frequency of the C allele in the patient group, was significantly higher than it was in the healthy control subjects (74.3% compared to 62.9%, $p = 0.009$, $\phi=0.167$, $OR=4.91$). The frequency of the C homozygotes was also greater in the schizophrenia group compared to the healthy controls (56.9% versus 41.6%, $p = 0.045$, $\phi=0.104$, $OR=1.63$). These results are evidence of a relatively modest but significant association of rs3749034 with the risk of schizophrenia, and they corroborate our previous data obtained for a smaller sample (Kirenskaya et al., 2018).

Table 1. Genotype frequencies of rs3749034 (*GAD1*) in control subjects and schizophrenic patients.

Group	Frequencies % (n)							p (χ^2)
	Genotypes				Alleles			
	Total	C/C	C/T	T/T	Total	C	T	
Control subjects	100% (n = 89)	41.6% (37/89)	42.7% (38/89)	15.7% (14/89)	100% (n = 178)	62.9% (112/178)	37.1% (66/178)	p = 0.629 ($\chi^2= 0.642$)
Schizophrenic patients	100% (n = 109)	56.9%* (62/109)	34.9% (38/109)	8.2% (9/109)	100% (n =218)	74.3%** (162/218)	25.7% (56/218)	p = 0.824 ($\chi^2= 0.743$)

p(χ^2) - deviation from the Hardy–Weinberg equilibrium genotype distribution

*p = 0.045; **p = 0.009 relative to control group

3.2 Demographic characteristics in control and schizophrenic patient groups

Demographic characteristics of participants that completed the ERP testing session in relation to their *GAD1* genotype are presented in **Table 2**. The sample of T/T genotype carriers was only small ($n = 4$) in the schizophrenia group, consequently, between-group comparisons were provided for two *GAD1* genotypes – C/C and C/T, and there were genotype differences in their demographic data. The mean age in the control group was 25.8 ± 0.9 years and the effect of genotype was not significant (**Table 2**). However, the mean age of the schizophrenic patient group was 32.8 ± 1.3 years, and the effect of genotype was significant because the age of C/C genotype carriers was higher than that of C/T heterozygotes (**Table 2**).

ANOVA revealed significant effects of diagnosis ($F(1,102) = 27.447$, $p < 0.01$) and a diagnosis x genotype interaction ($F(1,87) = 7.883$, $p = 0.006$). The comparison of mean age for *GAD1* genotype variants in control and patient groups unveiled significant differences for C/C samples ($p < 0.001$) but no differences in terms of age were found for C/T samples. Essentially, the between-group differences in age were related to increased patient age with the C/C genotype. Due to the fact that age differed significantly between groups, ANOVA with age as the covariate, was used in further analysis.

Table 2. Demographic characteristics of healthy control subjects and schizophrenic patients that completed the ERP testing session in relation to their *GAD1* genotype

Parameters	Genotype Group	Genotypes				Significance of Differences* C/C vs. C/T
		Total	C/C	C/T	T/T	
Age (years) mean $\pm$ SEM	Control subjects	n = 46 25.8 ± 0.9	n = 22 24.9 ± 0.7	n = 16 28.4 ± 2.2	n = 8 23.2 ± 0.9	Genotype: $F(1,35) = 3.26$, $p=0.079$
	Schizophrenic patients	n = 59 32.8 ± 1.3	n=34 35.4 ± 1.5	n=19 28.3 ± 2.2	n=4 38.2 ± 6.4	Genotype: $F(1,51) = 7.38$, $p=0.009$

* Between-group differences by age: $F(1,102) = 27.447$, $p < 0.01$.

3.3 PANSS scores for *GAD1* genotypes in schizophrenic patients

The clinical characteristics of the patients are presented in **Table 3**. The C/C genotype carriers had a substantially elevated **N5** score (difficulty in abstract thinking) and an apparent **G8** (uncooperativeness) upturn which did not attain statistical significance compared with C/T carriers. On the other hand, C/T carriers had a statistically higher **P3** (hallucinatory behavior) score and **G5** (mannerism and posturing) score. The **G9** (unusual thought content) scores appeared to be greater than C/C carriers, but they did not achieve significance statistically.

Table 3. Symptom ratings (PANSS, mean $\pm$ SEM) in schizophrenic patients in relation to their total sample and *GAD1* genotype (C/C and CT) scores.

Groups PANSS scales	Total Sample n = 54	<i>GAD1</i> genotypes			Significance Mann-Whitney Test C/C vs. C/T
		C/C n = 33	C/T n = 17	T/T n = 4	
PANSS total score	84.60 $\pm$ 2.33	84.55 $\pm$ 3.13	84.71 $\pm$ 3.29	89.00 $\pm$ 5.64	–
PANSS positive score	18.74 $\pm$ 0.84	18.70 $\pm$ 1.25	19.18 $\pm$ 1.14	21.00 $\pm$ 1.58	–
PANSS negative score	23.29 $\pm$ 0.82	24.06 $\pm$ 1.07	22.00 $\pm$ 1.54	26.00 $\pm$ 3.11	–
PANSS general score	41.88 $\pm$ 1.17	41.79 $\pm$ 1.54	43.53 $\pm$ 2.14	44.50 $\pm$ 2.78	–
P3 – Hallucinatory behavior	2.52 $\pm$ 0.19	2.12 $\pm$ 0.25	3.29 $\pm$ 0.31	2.25 $\pm$ 0.75	Z = 2.620, p = 0.009
N5 – Difficulty in abstract thinking	3.00 $\pm$ 0.18	3.36 $\pm$ 0.23	2.35 $\pm$ 0.30	3.50 $\pm$ 0.50	Z = 2.561, p = 0.010
G5 – Mannerism and posturing	2.87 $\pm$ 0.13	2.64 $\pm$ 0.16	3.24 $\pm$ 0.23	3.11 $\pm$ 0.25	Z = 2.136, p = 0.033
G8 – Uncooperativeness	1.76 $\pm$ 0.14	1.94 $\pm$ 0.19	1.47 $\pm$ 0.26	1.44 $\pm$ 0.25	Z = 1.921, p = 0.055
G9 – Unusual thought content	3.81 $\pm$ 0.21	3.52 $\pm$ 0.30	4.29 $\pm$ 0.31	4.50 $\pm$ 0.29	Z = 1.835, p = 0.067

3.4 Oddball task performance

Analysis of pooled samples demonstrated an inferior oddball test performance in schizophrenic patients compared to controls: the total percentages of errors and delayed responses (with RT > 600 ms) were significantly increased (**Table 4**). Thus, patients exhibited an increased RT in response to target stimuli ($p < 0.05$) and this finding matches those of our previous study (Kirenskaya et al., 2021).

The *GAD1* genotype affected the measures of oddball task performance in the control subjects (**Table 4**), the best outcome being documented for C/T genotype carriers. In the samples of homozygotes (C/C and T/T) the percentage of delayed responses was significantly higher and

the reaction time of correct responses was protracted in comparison with the C/T genotype sample.

No significant effects of *GAD1* genotype on oddball task performance were found in schizophrenic patients.

Table 4. Oddball paradigm performance measures (Mean $\pm$ SEM) in control subjects and schizophrenic patients in total samples and *GAD1* genotypes.

Measures		Groups	Total samples	GAD1 genotypes			Differences between genotypes Mann-Whitney Test	
				C/C	C/T	T/T	C/C vs C/T	C/T vs T/T
Errors (%)	Missing responses	Control	n = 45 7.70 ± 1.34	n = 22 7.40 ± 1.67	n = 15 5.86 ± 1.49	n = 8 11.02 ± 5.09	ns	ns
		Patients	n = 59 8.27 ± 1.65	n = 35 8.02 ± 2.49	n = 20 7.37 ± 1.87	n = 4 3.75 ± 1.57	ns	ns
	Delayed responses	Control	2.84 ± 0.84	4.20 ± 1.45	0.80 ± .41	4.11 ± 2.00	Z = 2.110 p = 0.035 <i>d = 0.659</i>	Z = 2.270 p = 0.028 <i>d = 1.01</i>
		Patients	14.79 ± 3.00***	14.00 ± 3.42**	18.00 ± 6.77***	9.58 ± 7.90	ns	ns
	All responses	Control	10.56 ± 1.60	11.74 ± 2.15	6.67 ± 1.48	15.25 ± 5.62	ns	ns
		Patients	23.05 ± 3.40*	22.01 ± 4.38	25.35 ± 6.92*	13.35 ± 7.48	ns	ns
Reaction Time of correct responses (RT in ms)		Control	377.7 ± 9.0	385.0 ± 13.2	351.1 ±12.1	410.7 ± 17.3	Z = 1.792 p = 0.070 <i>d = 0.629</i>	Z = 2.643 p = 0.007 <i>d = 1.23</i>
		Patients	411.1 ± 9.1*	407.5 ± 11.0	420.6 ± 12.5**	395.5 ± 26.04	ns	ns

* - $p < .05$, ** - $p < .01$, *** - $p < .001$ when comparing patients and controls (Mann-Whitney Test)

3.5 P300

Due to the small number of T/T homozygote carriers in the experimental samples, ANOVA statistical analysis of P300 amplitude and latency was only feasible with the C/C and C/T genotypes. ERP waveforms in response to target stimuli for two genotypes in control and schizophrenic patient groups are presented at **Fig. 1**. Comparison of pooled samples of control subjects and patients divulged a high significance of *Diagnosis* for P300 amplitude ($F(1,78) = 26.451$, $p < 0.001$; $d = 1.164$), in addition to P300 latency ($F(1,78) = 6.745$, $p = 0.011$; $d = 0.590$). In accordance with the results of numerous studies, the P300 amplitude was lower, and the latency was longer in patients compared to controls at all recording electrodes (see **Fig. 2**).

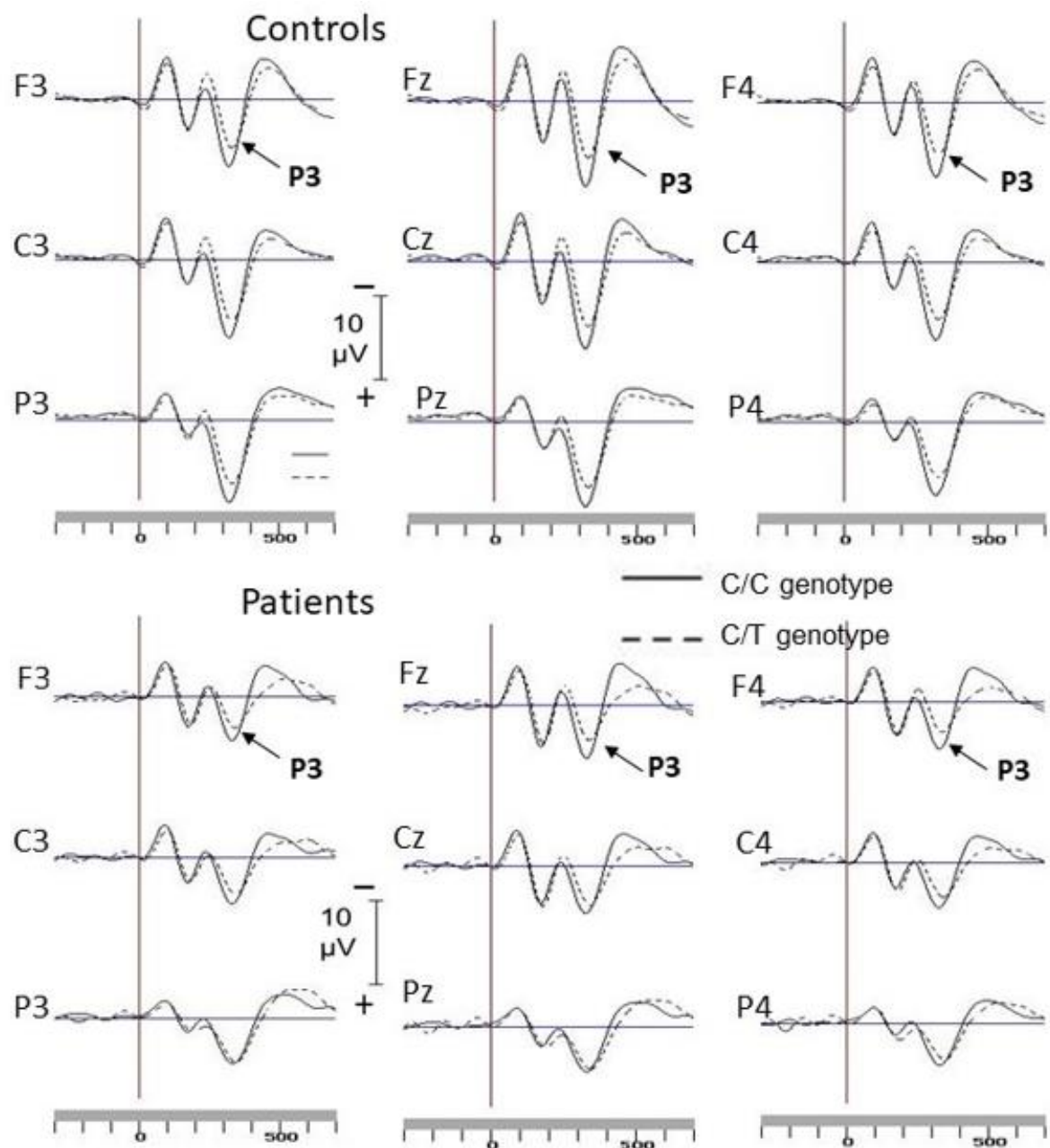


Figure 1. Overall average ERP waveforms for C/C and C/T genotypes in healthy subjects and schizophrenic patients (with a disease onset before the age of 25 years).

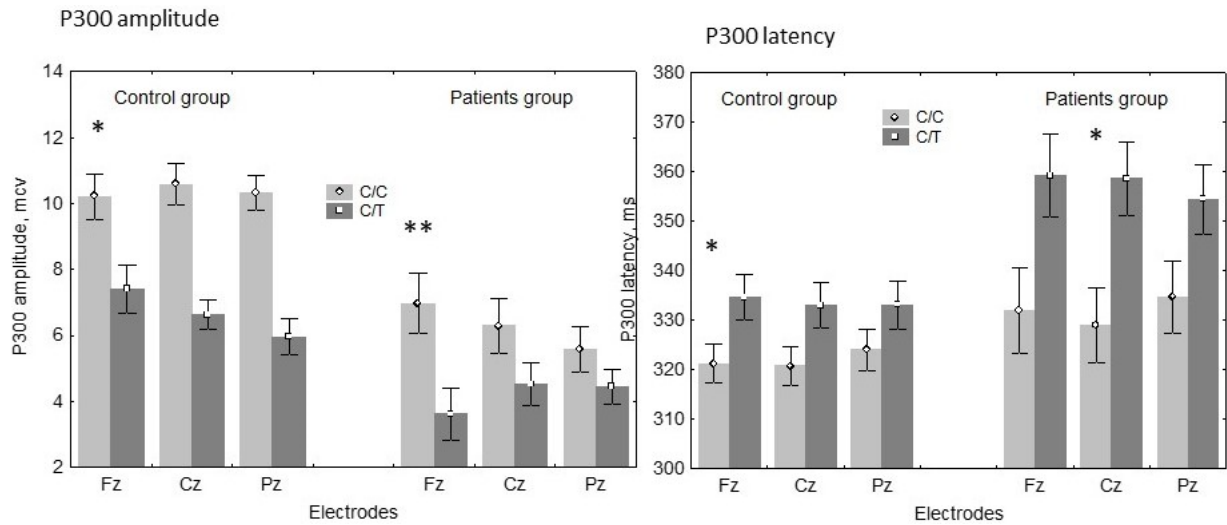


Figure 2. P300 amplitudes (A) and latencies (B) at midline sites for carriers of C/C and C/T *GAD1* genotypes in control and schizophrenic patients
Level of significance: * $p < 0.05$; ** $p < 0.01$ (after the Tukey's HSD correction).

There was also a significant main effect of *GAD1* genotype for P300 amplitude ($F(1,78) = 8.361, p = 0.005; d = 0.655$) in the total sample of participants due to the generalized increase of amplitude over the all scalp areas in C/C genotype carriers in comparison with C/T carriers. No significant *GAD1* genotype and diagnosis interaction was observed.

3.6 Control group

There was a significant effect of *GAD1* genotype ($F(1,33) = 12.861, p = 0.001; d = 1.247$) relating to the P300 amplitude. Post hoc comparisons in due course, revealed that the mean values of P300 amplitude were significantly greater in C/C homozygote carriers compared to their C/T genotype counterparts at frontal (Fz, $p = 0.012, d = 0.875$; F4, $p = 0.044, d = 0.784$) and right parietal (P4, $p = 0.020, d = 1.003$) sites (**Fig. 2**). Regarding P300 latency, a significant effect of *GAD1* genotype ($F(1,33) = 5.369, p = 0.027; d = 0.807$) was also disclosed by distinctly prolonged P300 latencies in subjects with the C/T genotype compared to C/C homozygote carriers. Post hoc comparisons demonstrated the differences between the two genotypes at frontal (Fz, $p = 0.041, d$

= 0.724; F_3 , $p = 0.036$, $d = 0.709$; F_4 , $p = 0.027$, $d = 0.748$) and left central (C3, $p = 0.048$, $d = 0.716$) sites (**Fig. 2**).

3.7 Schizophrenic patients

No significant effects of the *GAD1* genotype were found on P300 measures in the total patient group. In an ensuing analysis, we conducted comparisons between patients with an onset of schizophrenia before the age of 25 years. Youth-onset schizophrenia (17-25 years old) displays some specific congenital features, including genetic factors which play a leading role in the pathogenesis of schizophrenia, while after 40 years the contribution of sociocultural factors increases (Jones, 2013, Chen et al., 2018, Kessler et al., 2007, Morozova et al., 2019).

Out of a total of 33 participants, 28 completed the ERP testing session with artifact-free data (16 s in the C/C homozygote carriers and 12 s with the C/T genotype patients).

Statistical analysis (ANOVA) of the P300 amplitude revealed a significant effect of *Genotype* ($F(1,25) = 6.708$, $p = 0.006$; $d = 1.037$) and a marginal significance for the *Genotype* $\times$ *Electrode* interaction ($F(1,2,50) = 3.212$, $p = 0.049$ [after *Greenhouse–Geisser correction* $p = 0.075$; $d = 0.717$]), due to more pronounced differences between the two genotypes in the fronto-central region (**Fig. 2**). Post hoc comparisons exposed significant increases in P300 amplitude at the frontal (Fz, $p = 0.003$, $d = 0.783$; F_3 , $p = 0.015$, $d = 0.742$) and central (C3, $p = 0.022$, $d = 0.710$; C4, $p = 0.039$, $d = 1.230$) electrode locations in schizophrenic C/C homozygote carriers compared to the C/T heterozygotic patients.

A significant effect of *Genotype* was also found for P300 latency ($F(1,25) = 4.760$, $p = 0.039$; $d = 0.873$) (**Fig. 1**) due to a prolonged latency in C/T genotype carriers compared with the C/C homozygotic schizophrenics (**Fig. 2**). Post hoc comparison of means revealed that P300 latency was prolonged at frontal (F_4 , $p = 0.047$, $d = 0.640$) and central (Cz, $p = 0.046$, $d = 0.813$; C4, $p = 0.042$, $d = 0.855$) electrodes in C/T genotype carriers versus individuals with C/C homozygotic polymorphism.

3.8 Correlations between P300 measures and PANSS scores

3.8.1. P300 amplitude

A reduced P300 amplitude was primarily (94%) associated with increased PANSS scores for C/C genotype carriers. The largest number of significant correlations was obtained for negative scales (10/18 - 56%) - **N5** (Difficulty in abstract thinking) and total negative scale. Significant correlations were also obtained for central, parietal and temporal sites, among which 12 (67%) correlations were related to the right hemispheric sites and only 1 was associated with a site in the left hemisphere. (**Fig. 3**). Significant correlations for frontal sites were not found.

The incidence of correlations between P300 amplitude and negative scales (12/39 - 31%) for C/T carriers was much lower than the C/C carriers, but the percentage of correlations with positive and general scale scores was higher (**Fig. 3**). In contrast to the C/C group, an increase in positive scales was associated with elevated P300 amplitude. Positive correlations were also found for **G3** (Guilt feelings), **G4** (Tension), and **G9** (Unusual thought content) scales, whereas correlation of negative PANSS scores with P300 amplitude proved to be inversely related. Notwithstanding these outcomes, no significant correlations were found with respect to the **N5** scale. Analysis of cortical zones associated with clinical symptoms in the C/T group unveiled an involvement of frontal zones and a prevalence of contribution by left hemispheric sites compared to those in the right hemisphere (**Fig. 3**).

3.8.2. P300 latency

A prolongation of P300 latency was associated with increased PANSS scores in the case of C/C carriers and once again, most of the correlations (13/20 – 65%) occurred with negative scales (**Fig. 3**). Arising from 20 correlations, all (i.e. 100%) were associated with right hemispheric sites, and 15 (75%) were derived from temporal electrodes (T4, T6).

In the C/T genotype group, 15 correlations (36% - 15/42) for positive scales were obtained and increased **P1** (Delusions), **P3** (Hallucinations), and **P4** (Excitement) scales were associated with shortened P300 latency, while increased **P6** (Suspiciousness) and **P7** (Hostility) scales were linked to latency prolongation. There were 11 correlations with negative scales (27%) (**Fig. 3**), their directionality being identical to C/C carriers. In addition, correlations were observed with all recording sites among which, 22 (52%) occurred with left hemisphere electrodes and 14 (33%) were with right hemispheric sites (**Fig. 3**).

Thus, the patterns of correlation between P300 measures and PANSS scores differed by scales and cortical zones in the samples of C/C and C/T *GAD1* genotype carriers, while an inverse directionality of associations was found for positive syndromes.

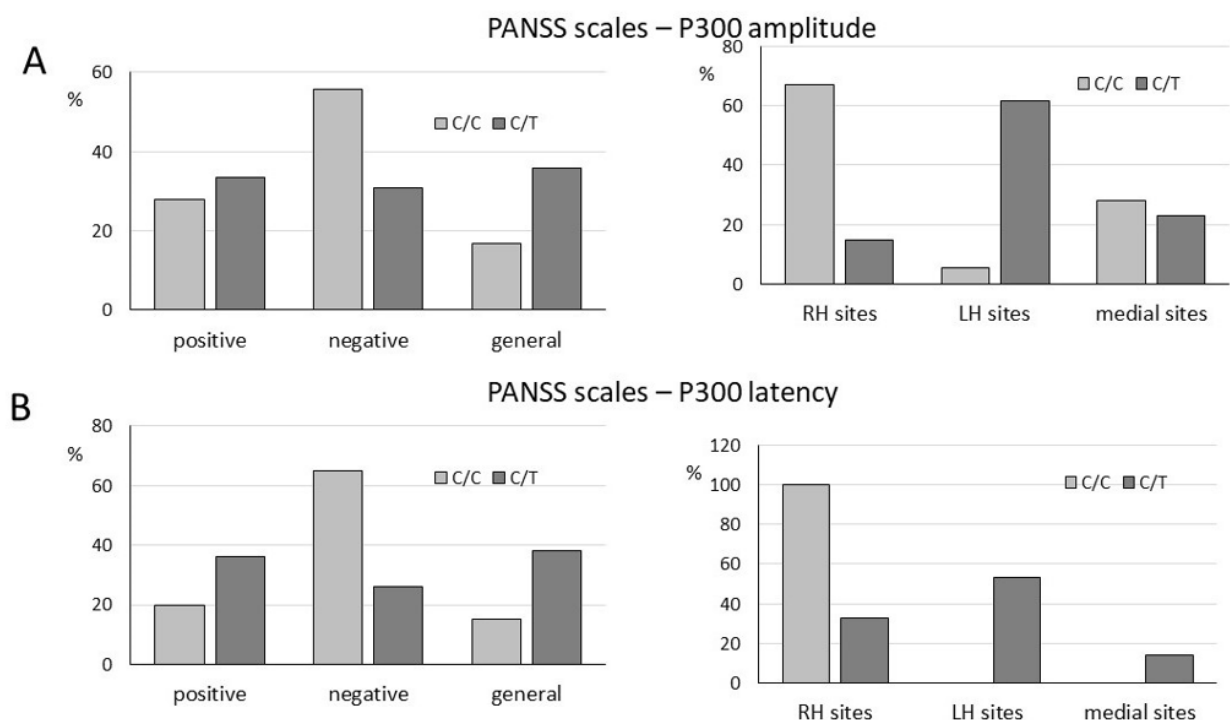


Figure 3. The incidence (%) of significant correlations between PANSS scales and P300 amplitude (A) and latency (B) with positive, negative and general scales (left hand panel) and with the electrodes located at the right hemisphere (RH), left hemisphere (LH) and at the midline (right hand panel).

4. Discussion

The key findings of this study of *GAD1* polymorphism outcomes in schizophrenic patients and healthy subjects involved firstly, an increased frequency of C/C allele carriers in *GAD1* rs3749034 polymorphism in patients compared to controls. Secondly, there were significant effects of *GAD1* genotypes on oddball task performance and P300 indices in the control subjects. A superior task performance was observed in C/T genotype carriers, while there was a higher P300 amplitude but shorter latency in C/C homozygotic individuals. Thirdly, analogous effects of *GAD1* genotypes on P300 measures were found for pooled samples of control subjects and schizophrenic patients, as well as for patients with a disease onset before the age of 25 years old. Lastly, different patterns of correlations between PANSS scores and P300 measures were ascertained in patients with C/C and C/T variants of the *GAD1* genotype.

The higher frequency of C/C carriers in the patient group, corresponded with the assumption concerning the importance of *GAD1* in the pathogenesis of schizophrenia. An association between the C/C allele and a schizophrenia diagnosis has also been described previously in several studies (Addington et al., 2005; Kirenskaya et al., 2018; Straub et al., 2007; Kogure et al., 2021).

Against this background, an influence of the *GAD1* genotype on oddball task performance was clear-cut in our control subjects, there being delayed responses as well as a prolonged reaction time for homozygote carriers. At the same time, C/T carriers were characterized by a reduced P300 amplitude and prolonged latency compared to C/C individuals. Notwithstanding this, enhanced cognitive processes reflecting sustained attention, target identification, content updating and decision making were detected in carriers of the heterozygote variant of the *GAD1* rs3749034 polymorphism as opposed to the C/C variants.

Several neurotransmitters including cholinergic, noradrenergic, dopaminergic, serotonergic and GABA-ergic systems are implicated in the generation and modulation of the P300 wave. GABA agonists have been shown to reduce wave amplitude and prolong its latency (Hansenne, 2000). Subsequently, GABA-ergic mechanisms responsible for attenuating the P300 wave have been attributed to general cognitive-behavioral retardation (Watson et al., 2009). What is more, studies have indicated that homozygosity for cytosine in the rs3749034 polymorphism is linked to a lowering of GABA levels (Brauns et al., 2013; Addington et al., 2005). In this regard, the enhanced P300 amplitude in C/C genotype carriers most likely relates to attenuation of inhibitory processes. Therefore, alterations in the fine-tuning of cortical networks may be responsible for inefficacy of cortical activation and poorer oddball paradigm performance in C/C genotype carriers. Our results showed that there was an increased error rate associated with elevated amplitude of the P300 wave in the C/T genotype sample. This finding is compatible with data supporting a strong relationship between higher GABA concentration in the auditory cortex and improved inhibitory control during the performance of an auditory-driven Go/No-go task (Cheng et al., 2017). It should also be noted that similar effects on P300 indices (reduced amplitude and prolonged latency) have been described for the GABA_A receptor agonist thiopental (Watson et al., 2009).

The differences in P300 measures between rs3749034 genotype variants were most pronounced in the frontal-central region. Regarding these outcomes, studies have identified an association between alternative transcription of *GAD1* and the level of methylation within the promoter of this gene in the prefrontal cortex (Hyde et al., 2011; Tao et al., 2018). The increase in P300 latency in C/T genotype carriers is also consistent with a report by Lett et al. (2016) who found lower fractional anisotropy in the prefrontal cortex region of rs3749034 T/T allele carriers than in C/C homozygotic patients. However, they also documented a worse working memory task performance in T allele carriers, but in our study the best oddball task performance was observed in the C/T genotype sample. This latent discrepancy of results may have been derived from

differences in the experimental groups. Consequently, in our study, male subjects with the C/T genotype were compared with C/C and T/T homozygote carriers, however, in the study by Lett and coworkers, both male and female subjects with the C/C genotype were examined in comparison with pooled samples of C/T and T/T genotypic carriers.

Our observed impairment of P300 measures in schizophrenic patients (a decline in P300 amplitude and latency prolongation) are in accord with numerous earlier descriptions (for reviews see Bramon et al., 2005; Turetsky et al., 2007; Turetsky et al., 2015).

The main upshot of *GAD1* gene polymorphism was recognized on P300 amplitude in the pooled sample of control subjects and patients. To be more precise, amplitude was greater in the C/C homozygotic individuals than in C/T carriers for both groups and this may be suggestive of an overall causal effect. Nevertheless, there were no meaningful effects of the *GAD1* genotype within the whole group of schizophrenic patients. Keeping in mind the possible role of *GAD1* in the processes of brain development and maturation (Addington et al., 2005; Brauns et al., 2013; Hyde et al., 2011; Tao et al., 2012; Tao et al., 2018), the sample of patients with a disease onset before the age of 25 years was analyzed separately. Traditionally (Kessler et al., 2007), the classification of schizophrenia according to age of onset included three periods: early-onset (prior to 40 years of age), late-onset (with manifestation between 40 and 60) and very-late-onset (after 60 years old). However, later data obtained from studying the associations of genetic and sociocultural factors with the risk of the disease, indicated the need to improve the classification by identifying new periods of disease onset as a specific feature within the already designated ones. In particular, youth-onset schizophrenia with an onset at 16-25 years of age has been identified. (Qiu et al. 2021; Kaleda, 2015; Jones, 2013).

In the youth-onset patient subgroup, the effects of the *GAD1* genotype with respect to P300 parameters, were parallel to those in the healthy controls suggesting a similarity of contributory mechanisms concerning P300 generation. The significant results obtained for

patients with the earlier disease onset may have been also attributable to a more homogeneous sample in this case.

In our study, an impact of the *GAD1* rs3749034 polymorphism on clinical symptomatology was encountered. Correspondingly, C/C genotype carriers displayed markedly higher **N5** assessments (Difficulty in abstract thinking) and C/T carriers had greater **P3** (Hallucinatory behavior) and **G5** (Mannerism and posturing) scale evaluations. Additionally, different patterns of correlations between P300 measures and PANSS scores were obtained for two variants of the *GAD1* genotype. In respect of this, for the C/C genotype, most correlations emerged with negative scores (total score of negative scales and difficulty in abstract thinking) and right hemispheric temporal sites. Alternatively, for the C/T genotype, the extent of correlations with negative scales was approximately two-fold lower, and there was a prevalence of left hemispherical associations.

Some investigators regard the GABA-ergic synaptic dysfunction present in C/C genotype carriers, as a contributory mechanism underlying cognitive impairment and the negative symptoms of schizophrenia (Gonzalez-Burgos et al., 2011). Our findings are also consistent with the model of schizophrenic syndromes based on hemispheric imbalances (Gruzelier, 2003). The model considers two quite different syndromes. One termed, an ‘activated’ syndrome found in patients with a left > right asymmetry in cortical activity and characterized by the positive symptoms of behavioral over-activity, pressure of speech, manic, grandiose and paranoid ideas associated with a late onset disorder. The other, designated as a ‘withdrawn’ syndrome, occurs in patients with a right > left pattern of asymmetry. It is characterized by the classical profile of social and emotional withdrawal, negative symptoms and motor retardation, tending to be associated with the early onset disorder. The profile of correlation in our sample of patients carrying the C/C genotype corresponds with the “withdrawn” syndrome, whereas patients possessing the C/T genotype tend to match the “activated” syndrome. An association of volume loss in the right temporal gyrus with clinical symptom severity, including thought disturbances,

has also been demonstrated by Lui and coworkers (2009). However, differences between the hetero- and homozygotic genotypes regarding hemispheric balance, have not been established for P300 indices and this warrants future investigation.

The current findings provide insights concerning a putative link between GABA neurotransmission, as well as attentional and executive processes related to the P300 wave in healthy controls and schizophrenic patients. However, these outcomes require further study with a larger sample.

Limitations.

Two conceivable limitations of this study relating to the participants should be documented. Firstly, only males took part in our investigation, because gender-specific effects to *GAD1* (Weber et al., 2012), as well as P300 (Melynyte et al., 2018) have been previously reported. Consequently, in future prospective studies, the inclusion, not only of females, but also other tasks involving selective attention and executive function would be expedient.

Secondly, our groups of healthy controls and patients with schizophrenia incorporated a proportion ($\leq 30\%$) of tobacco smokers. Smoking has been shown to be associated with a decrease in amplitude (but not latency) of auditory P300 (Hedges and Bennett, 2014; Houlihan et al., 1996). In regard to this, all our study subjects abstained from smoking two-hours prior to testing, and an abstinence of this period is inadequate in precipitating nicotine psychological withdrawal in nicotine dependent males (Xue et al., 2020). Moreover, more recently, evidence from ERP data, suggests that two-hour nicotine withdrawal improves inhibitory control dysfunction in nicotine dependent patients (Hou et al., 2024). Accordingly, our abstinence interval, may at least partially mitigate a smoking induced decrease in P300 amplitude. In any event, the interaction between nicotine, rs3749034 genotype, and disease warrants further investigation.

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Conflict of Interest Statement

None of the authors have potential conflicts of interest to be disclosed.

Author Contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Anna V. Kirenskaya, Zinaida I. Storozheva and Marina A. Gruden. Diagnostics and clinical examination of patients (including assessment of the PANSS scale) was performed by Denis V. Samylkin. The first draft of the manuscript was written by Anna V. Kirenskaya, Zinaida I. Storozheva, Marina A. Gruden and Robert D. E. Sewell and all authors commented on this version of the manuscript. All authors read and approved the final manuscript.

Ethics approval

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the local Ethics Committee of the Federal Medical Research Centre of Psychiatry and Narcology (Moscow, Russian Federation).

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