

## Study of Novel Autoantibodies in Schizophrenia

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**A recent genome-wide association (GWA) study confirmed 108 genetic loci that were strongly associated with schizophrenia. Fifteen schizophrenia-associated genes were selected for this study based on a number of selection criteria including their high expression in both brain tissues and B-lymphocyte cells. We aimed to investigate whether individuals with schizophrenia showed different levels of plasma IgG antibodies against protein-derived fragments encoded by these 15 genes. A total of 356 plasma samples were used to analyze circulating IgG antibodies against 18 target peptide antigens using an in-house enzyme-linked immunosorbent assay. Of 18 antigens tested, 6 (derived from DPYD, MAD1L1, ZNF804A, DRD2, TRANK1, and MMP16, respectively) showed increased IgG levels and 3 (derived from TSNARE1, TCF4, and VRK2, respectively) showed decreased IgG levels in patients with schizophrenia compared with control subjects. Receiver operating characteristic (ROC) curve analysis revealed that the anti-TRANK1 IgG assay had the area under the ROC curve of 0.68 (95% CI = 0.62–0.73), with the highest sensitivity of 20.7% against specificity of 95.2% among all 18 tests. There was no difference in positivity of anti-double strand DNA IgG between the patient group and the control group and no correlation between total IgG levels and each individual IgG level tested. Although risperidone treatment showed confounding effects on overall IgG levels in the circulation (combined  $P = .005$ ), anti-TRANK1 IgG levels did not appear to be significantly affected ( $t = 1.358$ ,  $P = .176$ ). In conclusion, this study suggests that circulating anti-TRANK1 IgG is likely to serve as a biomarker for identification of a subgroup of schizophrenia.**

**Key words:** autoantibodies/ELISA/TRANK1/schizophrenia

### Introduction

The World Health Organization identifies schizophrenia as one of the top 10 causes of disability for both men and women, affecting around 0.7% of the population worldwide.<sup>1</sup> Antipsychotic medication is the first line treatment of schizophrenia but 20%–30% of patients do not have a good response to antipsychotic treatment. Although clozapine can be effective in up to one-third of patients, side effects are severe.<sup>2</sup> A definitive etiology for the development of schizophrenia remains complex and unclear, suggesting that there may be a number of underlying mechanisms involved. The most well-documented biochemical mechanisms are the disturbances of dopamine and glutamate systems, although dysfunction of these neurotransmitter systems cannot account for all cases and psychotic symptoms.<sup>3</sup>

Schizophrenia is a highly heritable disease with complex modes of transmission. A recent genome-wide association (GWA) study analyzed more than 30 000 case-control samples and confirmed 108 genetic loci significantly associated with risk of the disease.<sup>4</sup> The expression levels of the candidate genes in most loci were found to be the highest in brain tissues and B-lymphocytes (CD20<sup>+</sup> and CD19<sup>+</sup> cells) over 50 human tissues and cell lines analyzed, supporting the hypothesis that an immunological component is likely to be involved in the development of schizophrenia.<sup>4</sup> Further support of an autoimmune role in schizophrenia is the genetic association for the human leukocyte antigen (HLA) region in the short arm of chromosome 6.<sup>4</sup>

Microglia are the resident immune cells in the brain, compromising around 15% of total cells in the central nervous system (CNS).<sup>5</sup> Microglia can be activated either in response to a systemic inflammatory challenge or

through the process of priming, whereby different stimuli sensitize the microglia so that a weak stimulus is able to produce an exaggerated immune response.<sup>6</sup> Priming is key in triggering immune and inflammatory responses within the CNS; eg, stress-induced immune activation can lead to some psychotic symptoms.<sup>5</sup> This compliments the theory that infection early in childhood, followed by either re-infection or exposure to new stimuli may induce a release of sensitized cytokines resulting in neurotransmission disturbances.<sup>6</sup>

Microglial activation and subsequent cytokine production have the ability to disrupt the blood–brain barrier, thus allowing movement of some inflammatory cytokines and other molecules into the brain.<sup>7</sup> Altered cytokine levels are found in both drug naïve, first-episode psychosis patients and chronically medicated patients, suggesting a problem independent of the effects of antipsychotic medications.<sup>7</sup> The concentrations of some inflammatory molecules may be clinically state-dependent. Inflammatory state-related markers include interleukin 1-beta (IL-1 $\beta$ ), IL-6, transforming growth factor beta (TGF- $\beta$ ); the concentrations of these molecules are increased during acute psychotic episodes, returning to normal levels during the period of clinical stability.<sup>8</sup> IL-12, interferon-gamma (IFN- $\gamma$ ), and tumor necrosis factor alpha (TNF- $\alpha$ ) are inflammatory markers that are elevated in peripheral blood in both stable patients and first-episode psychoses.<sup>8</sup> Degraded products of inflammatory molecules have been identified in the cerebrospinal fluid (CSF) and brain tissues in around 50% of patients with schizophrenia.<sup>9</sup> These observations suggest that there are significant changes in the inflammatory signaling process at least in a subgroup of schizophrenia patients. Gross inflammatory changes have not been found in neuroimaging or neuropathological studies of schizophrenia, but a decrease in brain volume has been observed as early as the first episode of psychosis occurs, followed by progressive loss in brain volume during further development of the disease.<sup>9</sup>

Infection during a critical period of brain development may increase risk of developing schizophrenia later in life.<sup>10</sup> Antibodies against different viruses were detected although these findings have been inconsistent across the studies.<sup>11</sup> A recent study reported that 20% of patients with first-episode psychosis carried serum antibodies against neuronal cell surface molecules.<sup>12</sup> Schizophrenia-associated changes in immunoglobulin levels in both plasma and CSF support an immunological role in the pathogenesis of the disease.<sup>11</sup> Benros et al<sup>13</sup> have recently reviewed evidence that pre-existing autoimmune disease might be a risk factor for schizophrenia, according to the link between schizophrenia and coeliac disease, rheumatoid arthritis, and other autoimmune conditions.

Goldsmith and Rogers<sup>14</sup> investigated the role of autoimmunity in schizophrenia and found contrasting reports of autoantibody levels, due to heterogeneity between studies and failure to account for the confounding effects

of antipsychotic medication. The current study aimed to identify potential autoantigens encoded by selected genes present in schizophrenia-associated loci confirmed in the 2014 GWA study and to explore the association of circulating autoantibodies with schizophrenia.

## Methods

### *Study Samples*

A total of 356 archived plasma samples collected from patients with schizophrenia ( $n = 169$ , 132 males and 37 females), aged  $42.0 \pm 13.3$  years, and control subjects ( $n = 187$ , 125 males and 62 females), aged  $44.1 \pm 12.8$  years, were used to examine circulating antibodies against the linear peptide antigens derived from the target proteins. These case–control samples were collected through the University of Aberdeen in the period between 2003 and 2008, and stored long term at  $-80^{\circ}\text{C}$  without thawing until they were aliquoted for antibody testing. Subjects were all classified as British Caucasian including English, Scottish, Welsh, and Irish individuals. All healthy controls had no previous history or current diagnosis of any mental health condition. All patients were diagnosed as having schizophrenia based on the DSM-IV criteria. Antipsychotic drugs prescribed to schizophrenia patients at the time of sampling are listed in supplementary table S1, with 128 patients taking a single antipsychotic drug, 14 taking more than one drug, and 27 without medication details. All control subjects were recruited during the same period from local communities and screened for psychiatric disorders as described previously.<sup>15</sup> All subjects gave written informed consent to donate blood samples for the study of the pathogenesis of schizophrenia. This study was approved by a local ethics committee and conformed to the provisions of the Declaration of Helsinki.

### *Detection of Antibodies Against Linear Peptide Antigens*

Target proteins encoded by the genes harboring or near to the index SNPs confirmed recently were identified from the NCBI protein database (<http://www.ncbi.nlm.nih.gov/protein>).<sup>4</sup> They were selected based on the following criteria: 1) the genes were highly expressed in both B-lymphocytes and brain tissues based on the BioGPS gene expression database (<http://biogps.org>), 2) odds ratio (OR) from combined samples should be  $>1.08$  or  $<0.93$  based on the GWA study, and 3) the number of genes present in a schizophrenia-associated locus of interest should be less than 3.<sup>4</sup> A total of 15 proteins were selected in this study, which are encoded by the following genes: NRG1, TCF4, MMP16, ZNF804A, VRK2, CACNA1C, DPYD, SLC39A8, DRD2, TSNARE1, MAD1L1, TRANK1, FANCL, ERCC4, and IGSF9B.

Supplementary table S2 summarizes their location in the human genome, index SNPs, and physiological function.

Linear peptide antigens were designed based on the computational prediction of the HLA-II epitopes and on the epitope information for human disease in the Immune Epitope Database (<http://www.iedb.org/>). All peptide antigens were synthesized by solid-phase chemistry with a purity of >95% (Severn Biotech Ltd). An in-house enzyme-linked immunosorbent assay (ELISA) was developed in-house with each antigen listed in [table 1](#) to coat maleimide activated 96-well plates (ThermoFisher Scientific) according to the manufacturer's guidelines. Each peptide antigen was dissolved in 67% acetic acid to a concentration of 5 mg/ml and stored at -20°C. The stock solution of each antigen was then diluted in coating buffer (0.1 M phosphate buffer containing 0.15 M NaCl and 10 mM EDTA, pH 7.2) to a concentration of 20 µg/ml as working solution. Each plate was coated with 100 µl of working solution and incubated overnight at 4°C. Plates were washed 3 times using 200 µl of wash buffer 1 (0.1 M phosphate buffer containing 0.15 M NaCl and 0.05% Tween-20, pH 7.2), and blocked using 10 µg/ml cysteine in coating buffer for 1 h at room temperature. Plates were washed twice with 200 µl of wash buffer 1 and dried at 40°C for 2 h. Plates, once dried, were sealed with sealing film and stored at 4°C until use. Sealing film was removed just before use and the plates were washed twice with 200 µl of wash buffer 2 (phosphate-buffered saline (PBS) containing 0.1% Tween-20), in each well to rehydrate. The plasma sample (including positive control, PC) was diluted 1:150 in assay buffer (PBS containing 0.5% bovine serum albumin) and 50 µl of the sample was loaded into each sample well; 50 µl of assay buffer was added to each negative control (NC) well. Following incubation at room temperature for 90 minutes, the plate was washed 3 times

with 200 µl of wash buffer 2 and 50 µl of peroxidase-conjugated goat anti-human IgG Fc (ab98624, Abcam) diluted 1:50 000 in assay buffer was then added and incubated for 60 min at room temperature. After incubation the plate was washed 3 times with 200 µl of wash buffer 2, 50 µl of 3,3',5,5'-tetramethylbenzidine (TMB, SB02, Life Technologies) was added and the plate was incubated in the dark for 20 min before 25 µl of the stop solution was added (SS04, Life Technologies). The optical density (OD) of each well was then measured within 10 min with a plate reader at 450 nm with a reference wavelength of 620 nm. All samples were tested in duplicate and the specific binding ratio (SBR) was calculated for each sample using the following formula:

$$\text{SBR} = \frac{\text{OD}_{\text{sample}} - \text{OD}_{\text{NC}}}{\text{OD}_{\text{PC}} - \text{OD}_{\text{NC}}}$$

#### Detection of Total IgG Levels and Double Stranded DNA Antibodies

To look at how specific each individual IgG test was in identification of schizophrenia subgroups, all plasma samples were tested for total IgG levels using the human IgG total Ready-SET-Go! ELISA kit (ThermoFisher Scientific) and also for IgG antibodies against double-stranded DNA using the anti-dsDNA-NcX ELISA (IgG) kit (EUROIMMUN UK Ltd).

#### Data Analysis

Shapiro-Wilk statistic was used to test a normal distribution of the resulting data; 15 of the 18 tests showed significantly skewed distributions in the patient group and 14 in the control group (supplementary table S3). Mann-Whitney *U*-test was thus used to examine the differences

**Table 1.** Sequences of Peptide Antigens Derived From Target Proteins Tested

Antigen Name	Protein Coding Gene	Sequence
DPYD1	DPYD	DIE SIL ALN PRT QTH ATL CST SAK KLD
CA1Ca	CACNA1C	CKLSWQAAIDAARQAKLMGSAGNATI
ARE1a	TSNARE1	RCW LQE LFQ EMS ANV FRI NSS VTS LER
SLC39a	SLC39A8	DFV ILL NAG MST RQA LLF NFL SAC SH
D1L1a	MAD1L1	LIE VHL RRQ DSI PAF LSS ELF SRC
F804A-1	ZNF804A	ECY YIV ISS THL SNG HFR NIK GVF RGP LID
NRGN-1	NRGN	CDIPLDDPGANAAAQIQASFRGHMARKKIK
TCF4a	TCF4	CLK SDK PQT KLL ILH QAV AVI LSL EQQ VRE
SB2-1	TCF4	ILH QAV AVI LSL EQQ VRE RNL NPK AAC
VRK2a	VRK2	CKT ISQ FTL SEE TNA DVY III PVL H
DRD2a	DRD2	DLC AIS IDR YTA VAM PML YNT RYS SKR
MP16-1	MMP16	LLTFSTGRRLDFVHHSGVFFLQTLWILC
MP16-2	MMP16	DVYTVFQFKRKGTPRHILYCKRSMQEWVD
SB2-2	TRANK1	CLQ WDP TYV KGV YRA GYS LLR LHQ RYE A
SB2-3	TRANK1	NIL ILS VRD ARD WLM KTE TRL KKE C
SB2-4	FANCL	CKS LIS IYS QFL AAI ESL KAF WDV MDH
ERC4a	ERCC4	CQE AFI LRL FRQ KNK RGF IKA FTD NAV D
F9B1	IGSF9B	VYF QND LKL RVR ILI DGT LTI FRC

in antibody levels between these 2 groups, and Spearman correlation analysis was applied to test the correlation in circulating levels between individual IgG antibodies tested and the correlation between total IgG levels and each individual IgG level; a  $P$ -value of  $<.003$  was considered to be statistically significant as 18 antigens were tested in this study. Linear regression was applied to determine the effects, if any, of the antipsychotic medication on the secretion of circulating antibodies with adjustment for age and sex. In such analysis, IgG levels were used as the dependent variable, and medication, age, and sex were used as the independent variables. Fisher's combining probability test was applied to determine the combined  $P$ -value for each medication on overall antibody levels; a  $P$ -value of  $<.006$  was considered to be statistically significant, due to the number of groups tested.<sup>16</sup> Receiver operating characteristic (ROC) curve analysis was carried out on each of the antigens to calculate the area under the ROC curve (AUC) with the in-house ELISA sensitivity against a specificity of  $\geq 95\%$  that was used to determine the cut-off for calculation of the positive predictive value (PPV) and the negative predictive value (NPV). The coefficient of variation (CV) was used to represent an inter-assay deviation estimated using pooled plasma samples, called quality control (QC) sample, which were randomly collected from  $>20$  healthy subjects and tested on every 96-well plate.

## Results

The reproducibility of the in-house ELISA test was excellent, with inter-assay deviation below 15% from every IgG assay (supplementary table S4). As shown in table 2, 9 of the 18 antigens tested in this study showed

significant differences in plasma IgG levels between the patient group and the control group ( $P < .003$ ), in which 6 showed an increase in plasma IgG levels including DPYD1 ( $Z = -3.22$ ,  $P = .001$ ), D1L1a ( $Z = -4.05$ ,  $P < .001$ ), F804A-1 ( $Z = -3.41$ ,  $P = .001$ ), DRD2a ( $Z = -3.31$ ,  $P = .001$ ), SB2-3 ( $Z = -5.71$ ,  $P < .001$ ), and MP16-2 ( $Z = -3.99$ ,  $P < .001$ ), and 3 showed a decrease including ARE1a ( $Z = -6.47$ ,  $P < .001$ ), TCF4a ( $Z = -8.70$ ,  $P < .001$ ) and VRK2a ( $Z = -3.25$ ,  $P = .001$ ).

ROC curve analysis revealed that of 18 IgG tests, the anti-TRANK1 (SB2-3) IgG assay was the most sensitive, with a sensitivity of 20.7% against the specificity of 95.2% (AUC = 0.68, 95% CI = 0.62–0.73); anti-TCF4a IgG assay showed the largest AUC (AUC = 0.78, 95% CI = 0.73–0.83) but its sensitivity was just 10.7%. The remaining of IgG tests all had a sensitivity of less than 15% (table 3).

Risperidone was the only antipsychotic drug that was likely to affect overall IgG levels (supplementary table S5) (combined  $P = .005$ ) with individual effects on circulating IgG against VRK2a ( $P = .018$ ), F804A-1 ( $P = .01$ ), ARE1a ( $P = .005$ ), and SB2-4 ( $P = .007$ ). All other antipsychotic drugs were not found to have significant effects on circulating IgG against these linear antigens tested. Kruskal–Wallis (supplementary table S6) analysis revealed that risperidone could up-regulate the secretion of plasma IgG against VRK2a ( $H = 4.92$ ,  $P = .027$ ), ARE1a ( $H = 6.08$ ,  $P = .014$ ) and SB2-4 ( $H = 5.88$ ,  $P = .015$ ); however, all these tests failed to survive the Bonferroni corrections. There was a significant correlation in circulating levels between all IgG antibodies tested ( $P < .003$ ) in the patient group and between most IgG antibodies in the control group; their coefficients and  $P$ -values are given in tables 4 and 5.

**Table 2.** Analysis of Circulating IgG Against Each of 18 Antigens Tested

Antigens	Patients, Mean $\pm$ SD ( $n$ )	Controls, Mean $\pm$ SD ( $n$ )	Z	$P^a$
DPYD1	0.56 $\pm$ 0.24 (169)	0.48 $\pm$ 0.22 (187)	-3.22	.001
CA1Ca	0.54 $\pm$ 0.34 (168)	0.51 $\pm$ 0.34 (187)	-1.44	.150
ARE1a	0.36 $\pm$ 0.18 (169)	0.57 $\pm$ 0.37 (187)	-6.47	<.001
SLC39a	0.46 $\pm$ 0.32 (169)	0.42 $\pm$ 0.28 (187)	-1.40	.161
D1L1a	1.00 $\pm$ 0.25 (169)	0.90 $\pm$ 0.22 (187)	-4.05	<.001
F804A-1	0.47 $\pm$ 0.17 (169)	0.42 $\pm$ 0.15 (187)	-3.41	.001
NRGN-1	0.59 $\pm$ 0.29 (169)	0.56 $\pm$ 0.28 (187)	-1.12	.240
TCF4a	0.21 $\pm$ 0.07 (169)	0.41 $\pm$ 0.21 (187)	-8.70	<.001
SB2-1	0.72 $\pm$ 0.27 (169)	0.74 $\pm$ 0.24 (187)	-1.18	.236
VRK2a	0.54 $\pm$ 0.18 (169)	0.61 $\pm$ 0.21 (187)	-3.25	.001
DRD2a	0.82 $\pm$ 0.26 (169)	0.74 $\pm$ 0.25 (187)	-3.31	.001
MP16-1	0.73 $\pm$ 0.28 (169)	0.69 $\pm$ 0.24 (187)	-1.193	.233
MP16-2	0.42 $\pm$ 0.15 (169)	0.36 $\pm$ 0.13 (187)	-3.99	<.001
SB2-2	0.84 $\pm$ 0.25 (169)	0.81 $\pm$ 0.21 (187)	-1.30	.193
SB2-3	1.06 $\pm$ 0.63 (169)	0.75 $\pm$ 0.40 (187)	-5.71	<.001
SB2-4	1.16 $\pm$ 0.39 (169)	1.14 $\pm$ 0.35 (187)	-0.18	.856
ERC4a	1.01 $\pm$ 0.88 (169)	0.77 $\pm$ 0.59 (187)	-2.90	.004
F9B1	0.85 $\pm$ 0.23 (169)	0.78 $\pm$ 0.21 (187)	-2.67	.008

<sup>a</sup> $P < .003$  was considered to be statistically significant.

**Table 3.** ROC Curve Analysis of Plasma IgG Antibodies Against Each Antigen in Schizophrenia

Antigens	Sensitivity (%) <sup>a</sup>	AUC	SE	Asymptotic 95% CI		PPV	NPV
				Lower Bound	Upper Bound		
DPYD1	12.5	0.60	0.030	0.54	0.66	0.72	0.52
CA1Ca	6.5	0.55	0.032	0.49	0.61	0.58	0.50
ARE1a	10.1	0.70	0.028	0.65	0.75	0.68	0.51
SLC39a	6.5	0.54	0.032	0.48	0.60	0.58	0.50
D1L1	11.2	0.62	0.030	0.57	0.68	0.70	0.52
F804A-1	11.8	0.61	0.030	0.55	0.67	0.71	0.52
NRGN-1	7.1	0.54	0.031	0.48	0.59	0.60	0.51
TCF4a	10.7	0.78	0.025	0.73	0.83	0.69	0.52
SB2-1	9.5	0.53	0.031	0.47	0.59	0.66	0.51
VRK2a	7.7	0.60	0.030	0.54	0.66	0.62	0.51
DRD2a	13.0	0.60	0.030	0.54	0.66	0.73	0.52
MP16-1	8.9	0.54	0.031	0.48	0.59	0.65	0.51
MP16-2	13.6	0.62	0.031	0.56	0.68	0.74	0.52
SB2-2	6.5	0.54	0.031	0.48	0.60	0.58	0.50
SB2-3	20.7	0.68	0.028	0.62	0.73	0.81	0.55
SB2-4	11.2	0.51	0.031	0.45	0.57	0.70	0.52
ERC4a	13.6	0.59	0.030	0.53	0.65	0.74	0.52
F9B1	5.9	0.58	0.030	0.52	0.64	0.55	0.50

Note: ROC, receiver operating characteristic; AUC, are under the ROC curve; PPV, positive predictive value; NPV, negative predictive value.

<sup>a</sup>Against a specificity of 95.2%.

Three individuals in the patient group and 3 in the control group were positive for double stranded DNA antibodies, and the chi-square test failed to show statistically significant ( $\chi^2 = 0.02$ ,  $P = .9$ ). There was no significant difference in total IgG levels ( $Z = 1.28$ ,  $P = .221$ ) between patients with schizophrenia ( $27.3 \pm 10.8$  mg/ml) and control subjects ( $26.7 \pm 12.6$  mg/ml); no significant correlation between total IgG levels and each individual IgG level was shown in either the patient group (supplementary table S7) or the control group (supplementary table S8).

## Discussion

The present study demonstrated that patients with schizophrenia had significant changes in circulating levels of IgG antibodies against peptide antigens encoded by schizophrenia-associated genes.<sup>4</sup> ROC curve analysis is a plot of the true positive against the false positive rate, the AUC provides a measure of how well each parameter is to distinguish between case and control groups. It is particularly important that the anti-TRANK1 IgG assay gave a sensitivity (true positive) of 20.7% against the specificity (true negative) of 95.2% (table 3) and failed to show a significant correlation with total IgG levels in plasma (supplementary tables S7 and S8), suggesting that anti-TRANK1 IgG test could serve as useful biomarker for a subgroup of schizophrenia. The TRANK1 gene is localized in the short arm of chromosome 3, 3p22.2, and encodes tetratricopeptide repeat and ankyrin repeat containing 1. Although its function remains largely unclear,

autoantibodies against TRANK1 protein (also called lupus brain antigen 1) have been reported to be associated with systemic lupus erythematosus (SLE).<sup>17</sup> It is worth noting that the proportion of individuals positive for plasma IgG against double stranded DNA was less than 2% in our samples, with no difference between the case and control groups, suggesting that SLE is unlikely to be a cause of schizophrenia. A recent study demonstrated that IFN- $\alpha$  could up-regulate expression of the TRANK1 gene in differentiated hepatic cells by activation of the STAT-JAK pathway.<sup>18</sup> The IFN pathway is involved in regulating the cellular innate immune system to eliminate invading pathogens, so an increase in anti-TRANK1 IgG levels may disrupt IFN-mediated immune function in schizophrenia. Further investigation is required to explore the precise mechanism by which TRANK1 plays a central role in regulating IFN-mediated immune response and protecting individuals from the development of schizophrenia.

All IgG antibodies tested in this study were significantly correlated to each other in the patients group (table 4) while most IgG antibodies (but not all tested) were significantly correlated to each other in the control group (table 5), suggesting that there may be something wrong with the humoral immunity in schizophrenia although there was no significant difference in total IgG levels between patients with schizophrenia and control subjects. Of 18 antigens studied, 6 showed an increase in circulating IgG levels in schizophrenia. The increased antibody levels may result from a breakdown of immune tolerance to the products of these schizophrenia-associated variants, causing an

**Table 4.** Spearman Correlation Analysis for the Correlation in Plasma Levels Between Individual IgG Antibodies Tested in Patients With Schizophrenia

	TCF4a	NRGN-1	MP16-1	MP16-2	VRK2a	F804A-1	CAICa	DPYD1	ARE1a	SLC39a	D1L1	DRD2a	SB2-1	SB2-2	SB2-3	SB2-4	ERC4a	
NRGN-1	<i>r</i> <sup>a</sup> .525																	
	<i>P</i> <sup>b</sup> <.001																	
MP16-1	<i>r</i> .453	.458																
	<i>P</i> <.001	<.001																
MP16-2	<i>r</i> .610	.437	.704															
	<i>P</i> <.001	<.001	<.001															
VRK2a	<i>r</i> .444	.327	.508	.390														
	<i>P</i> <.001	<.001	<.001	<.001														
F804A-1	<i>r</i> .508	.427	.581	.523	.877													
	<i>P</i> <.001	<.001	<.001	<.001	<.001													
CAICa	<i>r</i> .434	.335	.445	.494	.602	.588												
	<i>P</i> <.001	<.001	<.001	<.001	<.001	<.001												
DPYD1	<i>r</i> .511	.322	.490	.570	.517	.552	.770											
	<i>P</i> <.001	<.001	<.001	<.001	<.001	<.001	<.001											
ARE1a	<i>r</i> .446	.444	.694	.585	.574	.646	.523	.491										
	<i>P</i> <.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001										
SLC39a	<i>r</i> .283	.404	.598	.576	.395	.433	.581	.491	.699									
	<i>P</i> <.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001									
D1L1	<i>r</i> .371	.327	.615	.475	.575	.512	.389	.476	.452	.388								
	<i>P</i> <.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001								
DRD2a	<i>r</i> .548	.478	.600	.587	.576	.578	.495	.519	.551	.485	.749							
	<i>P</i> <.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001							
SB2-1	<i>r</i> .484	.380	.642	.568	.416	.434	.416	.438	.481	.501	.623	.564						
	<i>P</i> <.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001						
SB2-2	<i>r</i> .507	.403	.678	.575	.458	.516	.343	.401	.561	.468	.694	.609	.804					
	<i>P</i> <.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001					
SB2-3	<i>r</i> .410	.430	.614	.538	.507	.541	.414	.461	.510	.380	.606	.652	.533	.612				
	<i>P</i> <.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001				
SB2-4	<i>r</i> .412	.453	.657	.551	.651	.593	.503	.506	.615	.502	.712	.649	.619	.641	.746			
	<i>P</i> <.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001			
ERC4	<i>r</i> .473	.358	.478	.412	.405	.457	.233	.295	.467	.379	.390	.457	.363	.423	.432	.420		
	<i>P</i> <.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001		
F9B1	<i>r</i> .447	.295	.650	.496	.534	.540	.321	.377	.543	.441	.725	.563	.668	.745	.555	.657	.587	
	<i>P</i> <.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001

<sup>a</sup>Spearman correlation coefficient.

<sup>b</sup>*P* <.003 was considered to be significantly significant.

Table 5. Spearman Correlation Analysis for the Correlation in Plasma Levels Between Individual IgG Antibodies Tested in Control Subjects

	TCF4a	NRGN-1	MP16-1	MP16-2	VRK2a	F804A-1	CA1Ca	DPYDI	ARE1a	SLC39a	DIL1	DRD2a	SB2-1	SB2-2	SB2-3	SB2-4	ERC4a
NRGN-1	<i>r</i> <sup>a</sup> .466																
	<i>P</i> <sup>b</sup> <.001																
MP161	<i>r</i> .210	.444															
	<i>P</i> .004	<.001															
MP162	<i>r</i> .239	.392	.653														
	<i>P</i> .001	<.001	<.001														
VRK2a	<i>r</i> .488	.411	.462	.342													
	<i>P</i> <.001	<.001	<.001	<.001													
F804A-1	<i>r</i> .239	.443	.448	.415	.722												
	<i>P</i> .001	<.001	<.001	<.001	<.001												
CA1Ca	<i>r</i> .141	.307	.455	.439	.414	.439											
	<i>P</i> .057	<.001	<.001	<.001	<.001	<.001											
DPYDI	<i>r</i> .082	.276	.333	.384	.346	.329	.696										
	<i>P</i> .271	<.001	<.001	<.001	<.001	<.001	<.001										
ARE1a	<i>r</i> .740	.378	.391	.348	.598	.325	.306	.271									
	<i>P</i> <.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001									
SLC39a	<i>r</i> .150	.361	.468	.498	.209	.244	.483	.317	.396								
	<i>P</i> .042	<.001	<.001	<.001	.004	.001	<.001	<.001	<.001								
DIL1	<i>r</i> -.033	.360	.608	.444	.330	.439	.337	.291	.113	.291							
	<i>P</i> .650	<.001	<.001	<.001	<.001	<.001	<.001	<.001	.126	<.001							
DRD2a	<i>r</i> .155	.423	.550	.482	.348	.421	.398	.312	.259	.431	.765						
	<i>P</i> .035	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001						
SB2-1	<i>r</i> .370	.537	.638	.472	.465	.397	.308	.252	.454	.369	.608	.573					
	<i>P</i> <.001	<.001	<.001	<.001	<.001	<.001	<.001	.001	<.001	<.001	<.001	<.001					
SB2-2	<i>r</i> .251	.408	.612	.509	.409	.401	.323	.272	.331	.381	.633	.592	.845				
	<i>P</i> .001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001				
SB2-3	<i>r</i> -.028	.396	.427	.357	.256	.466	.481	.332	.097	.442	.456	.423	.505	.508			
	<i>P</i> .704	<.001	<.001	<.001	<.001	<.001	<.001	<.001	.188	<.001	<.001	<.001	<.001	<.001			
SB2-4	<i>r</i> .254	.534	.522	.321	.500	.509	.397	.333	.276	.352	.539	.505	.666	.631	.650		
	<i>P</i> <.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001		
ERC4	<i>r</i> .049	.317	.242	.376	.221	.297	.331	.315	.093	.298	.366	.360	.403	.423	.488	.399	
	<i>P</i> .501	<.001	.001	<.001	.002	<.001	<.001	<.001	.207	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001
F9B1	<i>r</i> .224	.481	.592	.476	.444	.454	.277	.275	.299	.374	.677	.599	.781	.807	.466	.653	.502
	<i>P</i> .002	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001

<sup>a</sup>Spearman correlation coefficient.<sup>b</sup>*P* <.003 was considered to be significantly significant.

autoimmune response.<sup>14</sup> Alternatively, this could result from a hyperactivity of the immune system as part of the etiology of schizophrenia, which would be concordant with current literature suggesting an increase in pro-inflammatory molecules with an increased immune response in those undergoing both acute psychosis and chronic schizophrenia.<sup>11</sup> Adjunctive trials of anti-inflammatory agents alongside anti-psychotic medication have demonstrated only modest clinical gains, potentially targeting effect rather than cause.<sup>19</sup> It could therefore be suggested that targeting of the immune system may gain more effective outcomes from clinical practices. Decreased antibody secretion was also observed in this study such as significant low levels of circulating IgG against peptide antigens derived from TCF4, TSNARE1, and VRK2. Possibly, these IgG antibodies have a protective effect on schizophrenia. Several lines of research have suggested that natural antibodies play an important role not only in eliminating pathogens invaded but also in maintaining homeostasis of the immune system through suppression of inflammatory reactions.<sup>20,21</sup> Since both autoantibodies and natural antibodies are produced by the B1 type of lymphocytes in the absence of external antigen stimulation or immunization, the balance between them may be very important for human health.<sup>22,23</sup>

Based on linear regression analysis (supplementary table S5), risperidone is the only antipsychotic drug affecting antibody levels in plasma, suggesting that its antipsychotic effect may partially depend on a regulatory process of the immune system. A systematic review demonstrated altered levels of some autoantibodies in drug-naïve patients with first-episode psychosis and also suggested a link between disease-associated SNPs identified and a number of autoantibodies.<sup>24</sup> Risperidone administration has previously been demonstrated to have an effect on inflammatory process and the immune system, consistent with our finding in this study.<sup>25,26</sup> The relationship between anti-VRK2 IgG levels and risperidone treatment is particularly interesting. Those patients prescribed with the drug showed an increase in circulating antibodies against VRK2 in comparison to controls, whereas those who did not take risperidone appeared to have a significant decrease in this antibody level (supplementary table S6). However, there is only a small number of the cases prescribed risperidone in comparison to the number of nonrisperidone prescriptions. This initial finding needs replication in a large sample size.

There are several limitations in this study. First, it was impossible to fully control the confounding effects of lifestyle factors such as alcohol consumption, diets, and use of any legal or illegal substances on the antibody levels measured, as samples were already collected and detailed information available was limited. Second, this study contains no subjects who were either antipsychotic-free or drug-naïve, so that the true effects of antipsychotic medication on the immune system may be masked or overestimated. Third, there was little clinical information of case-control subjects such as history of autoimmune disorders that may

affect antibody analysis although the prevalence of severe autoimmune conditions is less than 4% in the European populations. It will also be useful to test these autoantibodies in individuals with bipolar, major depressive disorders or other psychiatric diseases to determine if abnormalities of the humoral immunity are limited to schizophrenia. Obviously, the addition of further control groups would demonstrate the possibility of using the findings of GWA study to identify autoantigen targets.

### Supplementary Material

Supplementary data are available at *Schizophrenia Bulletin* online.

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