Neuroendocrinology Letters Volume 42 No. 5 2021
ISSN: 0172-780X; ISSN-L: 0172-780X; Electronic/Online ISSN: 2354-4716
Web of Knowledge / Web of Science: Neuroendocrinol Lett
Pub Med / Medline: Neuro Endocrinol Lett

ORIGINAL ARTICLE

Increased antibody reactivity against insulin receptor-A and insulin like growth factor 1 receptor and their ligands in cerebrospinal fluid and serum of patients with schizophrenia or related psychosis.

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Submitted: 2021-06-21 Accepted: 2021-07-28 Published online: 2021-07-28

Key words: **schizophrenia; antibodies; CDH5; HSP; IGF1; IGF1R; INS; INSR; NGF; PAGE2B;2;5; VEGFA**

Neuroendocrinol Lett 2021; **42**(5):339–358 **PMID:** 34506098 NEL420521A09 © 2021 Neuroendocrinology Letters • **www.nel.edu**

Abstract

OBJECTIVES: Evidence has accumulated that an autoimmune-mediated process in the central nervous system may underlie the development of schizophrenia. Various antibodies have also previously been detected in serum of patients with schizophrenia. Therefore, the aim of this study was to analyze antibody reactivity against proteins, selected based on potential schizophrenia disease relevance, in both cerebrospinal fluid and serum of patients with schizophrenia.
MATERIAL AND METHODS: Cerebrospinal fluid and serum from 17 patients with schizophrenia or related psychosis and 12 controls were analyzed regarding antibody reactivity, using bead-based antigen arrays of protein fragments or peptides of 21 selected proteins. Additionally, the patients were accessed for clinical symptoms with the Positive and Negative Syndrome Scale (PANSS) for schizophrenia.
RESULTS: Increased antibody reactivity was found in patients compared to controls against the insulin receptor (INSR), PAGE2B;2;5 and heat shock proteins (HSPs) in both cerebrospinal fluid and serum, and against the insulin like growth factor 1 receptor (IGF1R), insulin (INS), insulin like growth factor 1 (IGF1), cadherin 5 (CDH5), nerve growth factor (NGF) and vascular endothelial growth factor A (VEGFA) in serum alone. Moreover, patients' antibody reactivity in serum against PAGE2B;2;5, IGF1R or NGF correlated positively to their PANSS scores.
CONCLUSIONS: Taken together, these results point to that an autoimmune-mediated process underlies the development of a core group of schizophrenia cases and that the INSR and IGF1R, their ligands (INS and IGF1) and related inter- and intracellular proteins (CDH5, PAGE2B;2;5, HSPs, NGF and VEGFA) may constitute antigen targets.

INTRODUCTION

Schizophrenia is a psychotic disorder that affects approximately 0.5% of the population worldwide (Charlson *et al.* 2018; McGrath *et al.* 2008). In general, it is disabling with a chronic course, beginning in late adolescence or early adulthood and continuing throughout life (Freedman, 2003). The literature provides strong evidence for a role of genetic factors in its aetiology, and of the genetic risk loci identified to date, it is the gene region encompassing the major histocompatibility complex (MHC) on chromosome 6p playing an important role in the immune system, that is the most significant and consistent, followed by genes involved in calcium ion import into cells, cell membrane depolarization during action potential and synaptic transmission (Allen *et al.* 2008; Bray & O'Donovan, 2019; Chen *et al.* 2015; Giegling *et al.* 2017; Lam *et al.* 2019; Li *et al.* 2017; Loureiro *et al.* 2019; McGuffin & Power, 2013; Pardiňas *et al.* 2018; Ptacek *et al.* 2011; Ripke *et al.* 2014; Sekar *et al.* 2016; Yu *et al.* 2017). However, a substantial proportion of the heritability for schizophrenia is still unknown; only about 30-40% can be explained by the up – to – now identified genetic risk loci (Pardiňas *et al.* 2018). There are also indications that schizophrenia is a systemic disorder and not only a brain disease (Flyckt, 2001; Kirkpatrick *et al.* 2014; Moises *et al.* 2002) and that somatic comorbidity in schizophrenia is relatively common (Chen *et al.* 2012; Leucht *et al.* 2007; Melkersson & Wernroth, 2017; Meltzer, 1976; Mukherjee *et al.* 1996; Nasrallah, 2005; Sørensen *et al.* 2015).

Over the years, evidence has accumulated indicating that an inflammatory, probably autoimmune-mediated, process in the central nervous system (CNS), combined with an aberrant immune system, may underlie the development of schizophrenia, at least in a subgroup of patients (Al-Diwani *et al.* 2017; Bergink *et al.* 2014; Braun *et al.* 2017; Ermakov *et al.* 2017; Horváth & Mirnics, 2014; Jeppesen & Eriksen Benros, 2019; Johansson *et al.* 2012; Laskaris *et al.* 2016; Mané-Damas *et al.* 2019; Melkersson & Bensing, 2018; Miller *et al.* 2011; Mobarrez *et al.* 2013; Momtazmanesh *et al.* 2019; Müller *et al.* 2000; Schmitt *et al.* 2011; Schwarz *et al.* 2000; Trépanier *et al.* 2016; Upthegrove *et al.* 2014; Wang *et al.* 2018; Wetterberg *et al.* 2002). Earlier studies have also reported signs of impaired blood-brain barrier in patients with schizophrenia (Bechter *et al.* 2010; Kirch *et al.* 1985, 1992; Melkersson & Bensing, 2018; Müller & Ackenheil, 1995). The aberrance of the immune system in schizophrenia includes reduced capacity of both the cellular and humoral immune responses, as patients with schizophrenia exhibit both a decreased cutaneous cellular immune response to stimulation with a foreign protein or tuberculin, and a reduced antibody production after, for example, vaccination with a salmonella antigen (Glass *et al.* 2017; Melkersson, 2013; Melkersson & Bensing, 2018; Molholm, 1942; Müller *et al.* 1991, 2000; Steiner *et al.* 2010; Özek *et al.* 1971). Nevertheless, antibodies directed against brain tissue, blood cells, heat shock proteins (HSPs) and nerve growth factor (NGF) have previously been detected in serum of patients with schizophrenia (Abramson, 1967; Ebert *et al.* 2013; Kagami *et al.* 1987; Kilidireas *et al.* 1992; Kim *et al.* 2001; Klyushnik *et al.* 1999; Popova, 1977; Schwarz *et al.* 1998, 1999; Shcherbakova *et al.* 2004; Shinitzky *et al.* 1991; Spivak *et al.* 2009a, 2009b; Wang *et al.* 2003). Furthermore, patients with paraneoplastic ovarian teratoma-associated autoimmune encephalitis due to autoantibodies directed against the *N*-N-Methyl-D-Aspartate-aspartate receptor (NMDAR) have been presenting with schizophrenia-like psychosis (Dahm *et al.* 2014; Dalmau *et al.* 2007, 2016; Linnoila *et al.* 2014; Steiner *et al.* 2013, 2015; Vitaliani *et al.* 2005).

In this study, we therefore analysed immunoglobulin (Ig)G antibody reactivity against protein fragment- and peptide antigens of total 21 proteins of potential interest for schizophrenia in cerebrospinal fluid (CSF) and serum of patients with schizophrenia or related psychosis, and compared with that in CSF and serum of control subjects.

MATERIAL AND METHODS

Ethical approval

The study was approved by The Ethics Committee of Karolinska Institutet and The Regional Ethical Review Board, Stockholm, Sweden, and all patients and control subjects participated after giving informed consent.

Patients and control subjects

Consecutive outpatients at psychiatric polyclinics in the region of Stockholm, Sweden, diagnosed with schizophrenia or schizoaffective disorder according to the DSM-5 criteria (American Psychiatric Association, 2013), were invited to participate in this study. Any patients having a substance-related disorder, or a physical illness that could influence the evaluation were excluded. In total, 17 patients were included. In addition, 12 sex- and age-matched individuals diagnosed with non-inflammatory neurological diseases were included as control subjects.

Characteristics of the patients and control subjects are given in Table 1. All patients were Caucasians and had a diagnosis of schizophrenia, except one woman who was diagnosed with schizoaffective disorder. Twelve (70.6%) of the patients had heredity for schizophrenia or related psychosis, i.e. they had one or more first-, second-, third-, or fourth-degree relatives, siblings included, with such a disorder (Melkersson, 2009). None (0.0%) of the male patients compared to seven (70.0%) of the female patients were smokers (p=0.010), otherwise no sex differences in characteristics were found among the patients. Male and female patients were also treated with similar antipsychotics,

Tab. 1. Characteristics of the patients and control subjects

	Ethnicity, n	Age[a], y	Smoking, n (%)	Diagnosis, n	Heredity for schizophrenia or related psychosis[b], n (%)	Duration of psychotic disorder[a], y	Type of current antipsychotic, n	Treatment time with current antipsychotic[a], y
Patients (n=17: 7 men, 10 women)	Caucasian (n=17)	43 (9)	7 (41.2)	Schizophrenia[c] (n=16) Schizoaffective disorder[c] (n=1)	12 (70.6)	18.7 (9.5)	Haloperidol (n=1) Clozapine (n=3) Olanzapine (n=6) Perphenazine (n=1) Risperidone (n=5) Zuclopentixol (n=1)	5.4 (4.0)[d]
Control subjects (n=12: 4 men, 8 women)	Caucasian (n=11) Asian (n=1)	43 (9)	nda	Non-inflammatory neurological disease (n=12)	nda	na	na	na

Abbreviations: n=number, na=not applicable, nda=no data available, y=year; a) The data are given as mean (standard deviation); b) I.e. patients who had one or more first-, second-, third-, or fourth-degree relatives, siblings included, with schizophrenia or related psychosis (Melkersson, 2009); c) According to DSM-5 (American Psychiatric Association, 2013); d) 1 missing value

and the only concomitant medications used were benzodiazepine derivatives (n=3), lithium (n=1), orphenadrine (n=1), propiomazine (n=1), zopiclon (n=1) and zopiderm (n=1). The control subjects were all Caucasians except one woman who was Asian, and none had any diagnosis of psychotic disorder; neither did they use any drugs with anti-inflammatory effect that could influence their neurological disease, such as cortisone, interferon or cytostatics.

Clinical evaluation and laboratory analyses

The clinical evaluation of the patients was done by a psychiatrist on the day before the taking of CSF and serum specimens by using the Positive and Negative Syndrome Scale (PANSS) for schizophrenia (Kay *et al.* 1987; Von Knorring & Lindström, 1992). The PANSS consists of four subscales of symptom complexes (positive symptoms, negative symptoms, positive and negative symptoms combined, and general psychiatric symptoms), each of the items is rated on different point scales.

The lumbar punctures and collections of blood samples were carried out in the morning after the patients and control subjects had been fasting overnight, and the samples were frozen directly and stored in -80°C until analysis. Cerebrospinal fluid- and serum samples from the patients and control subjects were then analyzed for IgG antibody reactivity by bead-based antigen arrays of protein fragments or peptides, as previously described (Ayoglu *et al.* 2013, 2014, 2016b). For design of the antigen arrays, a set of target proteins was selected based on potential schizophrenia disease relevance according to previous literature (Table 2; Ayoglu *et al.* 2016a; Bernstein *et al.* 2009; Collste *et al.* 2017; Hallford *et al.* 2016; Melkersson, 2013; Melkersson & Persson, 2011, 2012; Melkersson *et al.* 2011, 2015; Mobarrez *et al.* 2013; Plavén-Sigray *et al.* 2018; Zandian *et al.* 2017), resulting in 50 protein fragment- and 64 peptide antigens of total 21 proteins. Detailed information about the protein fragments and peptides selected are given in Tables 3 and 4.

The protein fragments were designed and produced within the Human Protein Atlas framework, using a whole-genome bioinformatics approach based on the principle of lowest sequence similarity to other human proteins (www.proteinatlas.org; Uhlén *et al.* 2015). They were 20-148 amino-acid long, with a median of 76, and were all expressed in *Escherichia coli* with a six histidine and albumin binding protein (His_6ABP) tag. The peptides were designed to complete the first part of the study, where the protein fragments were used as antigens. They were 15 amino-acid long, and ordered with a biotin and amino hexanoic acid spacer conjugated to their N-terminals (PEPscreen, SigmaAldrich).

The protein fragments and four assay controls were immobilized on colour coded magnetic beads (MagPlex, Luminex Corporation, Austin, TX, USA) using COOH-NH_2 chemistry. The controls consisted of His_6ABP (control of binding to protein fragment tag), buffer (control of binding to bead particles), rabbit anti human IgG (sample loading control; 309-005-082, Jackson Immunoresearch), and Ebstein Barr Nuclear Antigen 1 (EBNA1, high reactivity frequency expected; ab138345, abcam). The coupling efficiency was tested

Tab. 2. Published studies regarding antibodies in schizophrenia in chronological order[a]

Antibody directed to	Results	References
Erythrocytes from chicken	Plasmas of schizophrenic subjects were demonstrated to have greater damaging effect on chicken erythrocytes than the plasmas of nonschizophrenic subjects, presumably due to an antibody	Ryan *et al.* 1966
Brains from schizophrenic and nonschizophrenic patients	A protein fraction called taraxein was isolated from sera of schizophrenic patients and shown to be an antibody against brain from schizophrenic, but not from nonschizophrenic, patients	Abramson, 1967
Thymocytes from mouse and rabbit	Sera from acutely/ subacutely ill, but not from chronic, schizophrenia patients contained considerably higher antibodies activity against thymic antigens, compared with that from healthy controls	Luria & Domashneva, 1974 Singal *et al.* 1975 Watanabe *et al.* 1982
Brain autoantigen	46.5% (93/200) of schizophrenia patients versus 3.4% (11/320) of healthy blood donors had brain autoantigen in serum	Popova, 1977
Lymphocytes	46.2% (12/26) of chronic schizophrenia patients versus 4.5% (1/22) of healthy controls were positive for a cytotoxic anti-lymphocyte IgM antibody in serum	Kagami *et al.* 1987
Septal region homogenate of rhesus monkey brain	96% (24/25) of unmedicated schizophrenic spectrum patients versus 0% (0/72) of nonschizophrenic controls had sera containing low quantity of an IgG antibody directed against the septal region homogenate	Heath *et al.* 1989
Cerebrum of rabbit	15.2% (7/46) of schizophrenia patients and 0% (0/42) of healthy controls had anti-cerebral antibodies in serum	Sugiura *et al.* 1989
Thrombocytes	Serum titers of anti-thrombocyte antibodies were significantly higher in schizophrenia patients than in healthy controls, with most pronounced increments in patients with clinical symptoms	Shinitzky *et al.* 1991 Spivak *et al.* 2009a, 2009b Ebert *et al.* 2013
60 kDa proteins (principally heat shock protein 60 (HSP60) and protein associated with MYC (PAM))	15-44% of schizophrenia patients versus 3-8% of controls had anti-60 kDa protein antibodies in serum	Kilidireas *et al.* 1992 Schwarz *et al.* 1998 Wang *et al.* 2003
Smith antigen (i.e. subcellular small nuclear RNAs complexed with protein)	15.4% (18/117) of schizophrenia patients versus 5.8% (11/189) of blood donors ($p<0.01$) tested positive for anti-Smith antibodies	Sirota *et al.* 1993
Nuclear antigen	21.2% (18/85) of chronic schizophrenia patients versus 5.4% (2/37) of healthy controls ($p<0.05$) had anti-nuclear antibodies in serum	Spivak *et al.* 1995
80-85 kDa protein from human neuroblastoma cells	30.8% (8/26) of schizophrenia patients versus 0% (0/22) of healthy controls had sera reacting with an unknown 80-85 kDa protein	Mazeh *et al.* 1998

using antibodies against His_6ABP. The CSF samples were diluted 1:5 in assay buffer (3% BSA in PBS supplemented with 0.05% Tween-20 and 160 µg/ml His_6ABP tag), and the serum samples were diluted 1:250 in assay buffer (3% BSA and 5% milk in PBS supplemented with 0.05% Tween-20 and 160 µg/ml His_6ABP tag). Commercial CSF (991-19-P, Lee Biosolutions) and plasma (HMPLEDTA2-F, Seralab) were diluted similarly and added to the dilution plates as technical controls. Before incubation with the protein fragment antigen bead array, all samples incubated for 1 hour at room temperature in order for the His_6ABP in the buffer to pre-block any potential antibodies against the albumin binding protein domain, since this is derived from gram-positive bacteria. Subsequently, the CSF samples incubated with the protein fragment antigen bead array overnight, and the serum samples for 2 hours. Goat fab fragments anti human IgG Fγ labeled with R-phycoerythrin (R-PE, H10104, Invitrogen) were thereafter added for 30 minutes to enable a read out.

The peptide antigen bead array was prepared in accordance with a previously described antigen coupling protocol (Ayoglu *et al.* 2014) with minor modifications. In brief, neutravidine (31000, Thermo Scientific) and three non-peptide controls were immobilized on colour coded magnetic beads (MagPlex, Luminex Corporation, Austin, TX, USA) using COOH-NH_2 chemistry. The peptides and one positive control peptide were subsequently coupled to the neutravidine-coated beads by binding with the N-terminally conjugated biotins. The controls consisted of buffer (control of binding to bead particles), rabbit anti human IgG (sample loading

Antibody directed to	Results	References
Nerve growth factor	Serum anti-nerve growth factor antibody titers were significantly higher in schizophrenia patients than in healthy controls, and correlated also to the clinical picture of schizophrenia	Klyushnik *et al.* 1999 Shcherbakova *et al.* 2004
70 kDa and 90 kDa heat shock proteins (HSPs)	23-31% of schizophrenia patients versus 1-3% of controls had anti-HSP70 antibodies in serum, and 20% of schizophrenia patients versus 5% of controls had anti-HSP90 antibodies in serum	Schwarz *et al.* 1999 Kim *et al.* 2001
N-methyl-D-aspartate receptor (NMDAR)	The first four cases of young women with psychosis and teratoma-associated autoimmune encephalitis were reported 2005, and two years later, the involved autoantibodies to the NMDAR were characterized. Parallel to this discovery, also other autoantibodies against neurotransmitter receptors and synaptic proteins were studied in psychosis and schizophrenia, but found only at low frequencies. In 2019, a study reported elevated serum anti-NMDAR antibody levels in patients with schizophrenia, compared to controls (p=0.002), and also correlations between anti-NMDAR antibody levels and PANSS scores	Vitaliani *et al.* 2005 Dalmau *et al.* 2007 Steiner *et al.* 2013 Dahm *et al.* 2014 Linnoila *et al.* 2014 Steiner *et al.* 2015 Dalmau, 2016 Tong *et al.* 2019
Glutamic acid decarboxylase (GAD)	Case report of a woman with a clinical syndrome, consisting of schizophrenia, elevated serum anti-GAD antibody titer and subcortical brain gliosis and infiltration, that improved with immunotherapy	Najjar *et al.* 2012 Grain *et al.* 2017
Voltage-gated potassium channel (VGKC) complexes, including cell surface protein complexed to Kv potassium channel subunits as leucine-rich glioma inactivated 1 (Lgi1) protein and contactin-associated protein-like 2 (Caspr2)	Case report of a 22-year old woman, who fulfilled DSM-IV criteria for schizophrenia and had VGKC antibodies in serum	Zandi *et al.* 2011 Deakin *et al.* 2014 Dalmau, 2016 Grain *et al.* 2017
Transglutaminase 6 (TG6) (primarily expressed in the brain)	Higher prevalence of TG6 IgA (but not IgG) antibodies in sera from schizophrenia patients than that in sera from controls	Cascella *et al.* 2013
Brains from rat and monkey	30-46.7% of schizophrenia patients versus 2.6-5.1% of controls had anti-brain antibodies in serum	Margari *et al.* 2013, 2015
24 neuroantigens (i.e. Glu receptors, Bdnf, ERBB4, KCNIP1-4, HTR4, Nrxn1, DRD1, DRD2 and DRD5)	No antibodies against the 24 neuroantigens were found in sera of 104 patients with schizophrenia or schizophreniform disorder	van Mierlo *et al.* 2015
Gamma-aminobutyric acid (B1)-receptor (GABA(B1)R)	Case report of a 21-year old woman, who was diagnosed with paranoid schizophrenia and found to have anti-GABA(B1)R antibodies in serum and cerebrospinal fluid	Dalmau, 2016 Haussleiter *et al.* 2017

Abbreviations: Bdnf=brain-derived neurotrophic factor; DR=dopamine receptor; ERBB4=receptor tyrosine-protein kinase erbB4; Glu=glutamate; HTR4=5-hydroxytryptamine receptor 4; KCNIP1-4=Kv channel-interacting protein 1-4; Nrxn=neurexin-1; PANSS=Positive and Negative Syndrome Scale; a) Studies in which patients were on antipsychotic treatment are excluded, as antipsychotics potentially may influence the antibody production (Ezeoke *et al.* 2013)

control; 309-005-082, Jackson Immunoresearch), full length EBNA1 (high reactivity frequency expected; ab138345, abcam), and a 20-mer peptide representing EBNA1 (high reactivity frequency expected and control of peptide coupling step; PEPscreen, SigmaAldrich). The coupling was confirmed using antibodies from the Human Protein Atlas, raised against sequences overlapping with the peptides. The CSF samples were diluted 1:5 and the serum samples 1:150 in assay buffer (5% BSA in PBS supplemented with 0.05% Tween-20 and 10 µg/ml neutravidine). Commercial CSF (991-19-P, Lee Biosolutions) and plasma (HMPLEDTA2-F, Seralab) were diluted similarly and added to the dilution plates as technical controls. Before incubation with the peptide antigen bead array, all diluted samples incubated for 1 hour at 4°C in order to preblock any potential antibodies against neutravidine. Subsequently, the CSF samples incubated with the peptide antigen bead array overnight, and the serum samples for 1 hour. Goat fab fragments anti human IgG Fγ labeled with R-phycoerythrin (R-PE, H10104, Invitrogen) were thereafter added for 30 minutes to enable a read out.

To read out, a FlexMap 3D instrument (Luminex Corporation, Austin, TX, USA) was used. The read out consisted of the median fluorescent intensity (MFI) and count of number of beads for each antigen in each

sample. Quality control was done using both MFI and bead count to exclude any antigens and samples not reaching technical criteria. No antigens or samples were excluded, however the two antigens heat shock protein family A (Hsp70) member 4 (HSPA4)_HPRR1920102 and PAGE family member 2B;2;5 (PAGE2B;2;5)_ HPRR3340273 were flagged as potentially unreliable due to low signals in the coupling efficiency test.

Statistical methods

The continuous data age, duration of psychotic disorder and treatment time with current antipsychotic are given as mean and standard deviation (SD), and PANSS scores as mean and SD as well as median and range. Comparison between patients and control subjects regarding age was performed using Student´s t-test. The categorical data smoking and heredity for schizophrenia or related psychosis are summarized using frequency counts and percentages. Fisher´s exact test was used to compare smoking habits between male and female patients. The MFI value for each protein fragment antigen was transformed per sample into number of median absolute deviations (MADs) from the sample median, in order to adjust for sample specific backgrounds, using the algorithm: $MADs_{sample}=MFI_{sample}-median_{sample(MFI)}/MAD_{sample(MFI)}$ (Neiman *et al.* 2019). Due to variable antigen backgrounds, the MFI values for the specific peptide antigens were first adjusted to the same median per peptide, and then the adjusted MFI value for each peptide antigen was transformed per sample into number of MADs from the sample median, in order to adjust for sample specific backgrounds, using the algorithm: $MADs_{sample}=MFI_{sample}-median_{sample(adjusted\ MFI)}/MAD_{sample(adjusted\ MFI)}$. Median absolute deviations of IgG antibody reactivity in CSF and serum against the 50 protein fragment- and 64 peptide antigens are presented as median and (min – max) range. Mann-Whitney U test was used to compare patients and control subjects regarding these variables. Moreover, Spearman rank correlation coefficient (r_s) was calculated to measure the association between pairs of variables. A *p*-value of less than 0.05 was considered statistically significant. All calculations were made with the statistical program Statistica for Windows 13.5 (TIBCO Software Inc., Palo Alto, CA, USA).

RESULTS

Median absolute deviations of antibody reactivity in CSF and serum of patients and control subjects against the 50 protein fragments and 64 peptides of the total 21 proteins investigated are given in Tables 5-6 and Figures 1-2. Significantly higher antibody reactivity was found in patients compared to control subjects against the protein fragments heat shock protein 90 alpha family class B member 1 (HSP90AB1)_HPRR4440047* in CSF, and cadherin 5 (CDH5)_ HPRR2050196, HSP90AB1_ HPRR4440047, insulin like growth factor 1 (IGF1)_HPRR3860093, insulin like growth factor 1 receptor (IGF1R)_HPRR650064, NGF_HPRR1320012;_ HPRR3730585 (=a part of HPRR1320012) and vascular endothelial growth factor A (VEGFA)_HPRR3730128 in serum. Regarding the peptides, significantly higher antibody reactivity was found in patients compared to control subjects against HSPA4_2*, insulin receptor (INSR)_3* and PAGE2B;2;5_1* in CSF, and HSPA4_1,4, IGF1R_2,3,6,8,12,13,15,18, insulin (INS)_1,6,7,9, INSR_2,3,4,7 and PAGE2B;2;5_6,7 in serum.

Mean (SD) and median (min-max) of PANSS scores in patients (n=16) are given in Table 7. Antibody reactivity in serum of patients, expressed as MADs, against the protein fragments NGF_HPRR1320012 or NGF_ HPRR3730585 correlated positively to the PANSS scores of positive symptoms (r_s=0.63, *p*=0.01 and r_s=0.63, *p*=0.01, respectively) (Figure 3a-b) and of positive and negative symptoms combined (r_s=0.54, *p*=0.03 and r_s=0.55, *p*=0.03, respectively). Regarding the peptides, antibody reactivity in serum of patients, expressed as MADs, against IGF1R_15 correlated positively to the PANSS scores of negative symptoms (r_s=0.58, *p*=0.02) (Figure 3c), and that against PAGE2B;2;5_7 correlated positively to the PANSS scores of positive and negative symptoms combined (r_s=0.51, *p*=0.04) (Figure 3d). In contrast, antibody reactivity in serum of patients against the peptide INS_2 was inversely correlated to the PANSS scores of negative symptoms (r_s=–0.57, *p*=0.02). Regarding this peptide, a tendency was also found towards lower antibody reactivity in serum in patients than in control subjects (*p*=0.067; Table 6). Between antibody reactivity in CSF of patients against the protein fragments or peptides and the PANSS scores of positive, negative or positive and negative symptoms combined, no significant correlations were found. Neither were any significant correlations found between antibody reactivity in CSF or serum of patients against the protein fragments or peptides and the PANSS scores of general psychiatric symptoms.

DISCUSSION

We show that patients with schizophrenia or related psychosis have increased antibody reactivity in CSF against the proteins INSR, PAGE2B;2;5, HSPA4 (also known as Hsp70) and HSP90AB1, compared to control subjects. To the best of our knowledge, this is the first study reporting increased antibody reactivity against these four proteins in CSF of patients with schizophrenia or related psychosis, pointing to an on-going autoimmune-mediated process in CNS in schizophrenia. Increased antibody reactivity against these proteins was observed in patients even if they were in partial remission, and not acutely psychotic. In addition, increased antibody reactivity in CSF of patients against these proteins was accompanied by increased antibody reactivity against the same proteins in serum, supporting the notion that schizophrenia is a systemic

Tab. 3. Data regarding the 50 protein fragments selected

Protein/Gene[a,b]	UniProt ID[a]	Protein fragment ID (amino-acid sequence)[a,c]
ANO2	Q9NQ90	HPRR3070034 (932-1003) HPRR3070036 (79-167) HPRR3070037 (9-78)
CDH5	P33151	HPRR2050196 (306-453) HPRR4240093 (63-134)
GLS	O94925	HPRR2920208 (617-669) HPRR3890163 (81-133)
GLS2	Q9UI32	HPRR3070446 (1-78) HPRR3890773 (567-602)
GLUL	P15104	HPRR1350059 (68-208) HPRR1350060 (227-365)
HSP90AA1	P07900	HPRR3690019 (75-135) HPRR4290083 (1-75)
HSP90AB1	P08238	HPRR3540067 (691-719) HPRR4440047 (279-339) HPRR4440048 (239-265)
HSPA4	P34932	HPRR1920102 (699-808) HPRR4220528 (526-577)
IDE	P14735	HPRR650042 (454-593) HPRR4180402 (620-708) HPRR4180403 (191-292)
IGF1	P05019	HPRR350040 (61-191) HPRR3720070 (96-153) HPRR3860093 (142-191)
IGF1R	P08069	HPRR650064 (631-774) HPRR650065 (177-323) HPRR2750058 (464-531) HPRR2750059 (1277-1366) HPRR2750060 (853-925) HPRR3350014 (644-716)
IGF2	P01344	HPRR380014 (40-165)
IGF2R	P11717	HPRR670260 (161-291) HPRR670338 (1369-1498)
INS	P01308	HPRR380034 (33-109)
INSR	P06213	HPRR1010005 (608-742) HPRR1010006 (764-902) HPRR2980052 (246-326)
IRS4	O14654	HPRR231035 (969-1090) HPRR4340060 (325-417)
NGF	P01138	HPRR1320012 (34-143) HPRR3730584 (134-190) HPRR3730585 (57-130)
PAGE2B;2;5[d]	Q5JRK9;Q7Z2X7;Q96GU1	HPRR3340273 (47-109) HPRR3340274 (8-46)
TGM6	O95932	HPRR3310212 (446-540)
TSPO[d]	B1AH88;P30536	HPRR3140230 (6-43) HPRR3870247 (66-85)
VEGFA	P15692	HPRR330133 (56-200) HPRR3730126 (22-79) HPRR3730128 (2-68)

Abbreviations: ANO2=anoctamin 2; CDH5=cadherin 5; GLS=glutaminase; GLS2=glutaminase 2; GLUL=glutamate-ammonia ligase; HSP90AA1=heat shock protein 90 alpha family class A member 1; HSP90AB1=heat shock protein 90 alpha family class B member 1; HSPA4=heat shock protein family A (Hsp70) member 4; IDE=insulin degrading enzyme; IGF1=insulin like growth factor 1; IGF1R=insulin like growth factor 1 receptor; IGF2=insulin like growth factor 2; IGF2R=insulin like growth factor 2 receptor; INS=insulin; INSR=insulin receptor; IRS4=insulin receptor substrate 4; NGF=nerve growth factor; PAGE2B;2;5=PAGE family member 2B,2,5; TGM6=transglutaminase 6; TSPO=translocator protein; VEGFA=vascular endothelial growth factor A; a) According to https://www.ncbi.nlm.nih.gov/protein; b) According to https://www.ncbi.nlm.nih.gov/gene; c) 1-6 protein fragments per UniProt ID were selected; d) The protein fragment sequences are not specific for one protein/gene, but for a family of proteins/genes

Tab. 4. Data regarding the 64 peptides selected

Protein/Gene[a,b]	Peptide position and amino-acid sequence[a]
HSPA4	1. 699–713 FEELGKQIQQYMKII 2. 714–728 SSFKNKEDQYDHLDA 3. 729–743 ADMTKVEKSTNEAME 4. 744–758 WMNNKLNLQNKQSLT 5. 759–773 MDPVVKSKEIEAKIK 6. 774–788 ELTSTCSPIISKPKP 7. 789–803 KVEPPKEEQKNAEQN 8. 794–808 KEEQKNAEQNGPVDG
IGF1	1. 142–156 KNTKSQRRKGWPKTH 2. 156–170 HPGGEQKEGTEASLQ 3. 159–173 GEQKEGTEASLQIRG 4. 162–176 KEGTEASLQIRGKKK 5. 177–191 EQRREIGSRNAECRG
IGF1R	1. 629–643 VKWNPPSLPNGNLSY 2. 631–645 WNPPSLPNGNLSYYI 3. 717–731 EYRKVFENFLHNSIF 4. 722–736 FENFLHNSIFVPRPE 5. 730–744 IFVPRPERKRRDVMQ 6. 745–759 VANTTMSSRSRNTTA 7. 760–774 ADTYNITDPEELETE 8. 464–478 EVTGTKGRQSKGDIN 9. 479–493 TRNNGERASCESDVL 10. 494–508 HFTSTTTSKNRIIIT 11. 509–523 WHRYRPPDYRDLISF 12. 517–531 YRDLISFTVYYKEAP 13. 853–867 PEPENPNGLILMYEI 14. 868–882 KYGSQVEDQRECVSR 15. 883–897 QEYRKYGGAKLNRLN 16. 898–912 PGNYTARIQATSLSG 17. 911–925 SGNGSWTDPVFFYVQ 18. 916–930 WTDPVFFYVQAKTGY 19. 915–927+ 2 a.a. SWTDPVFFYVQAKRY
INS	1. 33–47 SHLVEALYLVCGERG 2. 40–54 YLVCGERGFFYTPKT 3. 48–62 FFYTPKTRREAEDLQ 4. 55–69 RREAEDLQVGQVELG 5. 59–73 EDLQVGQVELGGGPG 6. 73–87 GAGSLQPLALEGSLQ 7. 79–93 PLALEGSLQKRGIVE 8. 88–102 KRGIVEQCCTSICSL 9. 95–109 CCTSICSLYQLENYC
INSR	1. 608–622 AKSDIIYVQTDATNP 2. 623–637 SVPLDPISVSNSSSQ 3. 638–652 IILKWKPPSDPNGNI 4. 653–667 THYLVFWERQAEDSE 5. 668–682 LFELDYCLKGLKLPS 6. 683–697 RTWSPPFESEDSQKH 7. 698–712 NQSEYEDSAGECCSC 8. 713–727 PKTDSQILKELEESS 9. 715–729 TDSQILKELEESSFR 10. 728–742 FRKTFEDYLHNVVFV 11. 743–757 PRKTSSGTGAEDPRP 12. 758–772 SRKRRSLGDVGNVTV
IRS4	1. 872–886 SPSKPSDHEPPKNKA 2. 872–886 SPSKPSDYEPPKNKA
PAGE2B;2;5[c]	1. 6–20 RTRSQSSERGNDQES 2. 7–21 TRSQSSERGNDQESS 3. 8–22 RSQSSERGNDQESSQ 4. 9–23 SQSSERGNDQESSQP 5. 14–28 RGNDQESSQPVGSVI 6. 15–29 GNDQESSQPVGSVIV 7. 16–30 NDQESSQPVGSVIVQ 8. 18–32 QESSQPVGSVIVQEP 9. 33–47 TEEKRQEEEPPTDNQ

Abbreviations: A=alanine; C=cysteine; D=aspartic acid; E=glutamic acid; F=phenylalanine; G=glycine; H=histidine; HSPA4=heat shock protein family A (Hsp70) member 4; I=isoleucine; IGF1=insulin like growth factor 1; IGF1R=insulin like growth factor 1 receptor; INS=insulin; INSR=insulin receptor; IRS4=insulin receptor substrate 4; K=lysine; L=leucine; M=methionine; N=asparagine; P=proline; PAGE2B;2;5=PAGE family member 2B,2,5; Q=glutamine; R=arginine; S=serine; T=threonine; V=valine; W=tryptophan; Y=tyrosine; a) According to https://www.ncbi.nlm.nih.gov/protein; b) According to https://www.ncbi.nlm.nih.gov/gene; c) The peptide sequences are not specific for one protein/gene, but for a family of proteins/genes

disorder and not only a brain disease (Flyckt, 2001; Kirkpatrick *et al.* 2014; Moises *et al.* 2002).

Regarding the INSR protein, increased antibody reactivity was found in both CSF and serum against peptide no 3 in patients, compared to control subjects. In serum alone, increased antibody reactivity was also found against the two adjacent peptides no's 2 and 4 and peptide no 7. The INSR is a heterotetrameric cell surface receptor, consisting of two extracellular α-subunits and two ß-subunits (containing an extracellular region, a transmembrane domain and a cytoplasmic extension), which is expressed as two alternatively spliced isoforms that differ by the absence (isoform A) or presence (isoform B) of a 12 amino-acid insert (amino-acids 745-756) at the carboxy terminus of the α-subunits (Ebina *et al.* 1985; Seino & Bell, 1989; Seino *et al.* 1989; Ullrich *et al.* 1985). Studies regarding the two isoforms of the INSR have revealed different relative expression in cells (i.e. INSR-A is predominantly expressed in the CNS, peripheral nervous system, blood cells and spleen, while INSR-B is predominantly expressed in the liver, and in other kinds of cells the two isoforms are expressed in various proportions), as well as different ligand affinities with an approximately two-fold higher affinity of INS for INSR-A, compared with INSR-B (Kotzke *et al.* 1995; McClain, 1991; Moller *et al.* 1989; Mosthaf *et al.* 1990; Roth *et al.* 1986; Sesti *et al.* 1994; Yamaguchi *et al.* 1991, 1993). The targeted peptides no´s 2,3,4 and 7 (corresponding to amino-acids 623-667 and 698-712) are all located in the proximal part of the extracellular α-subunit of the INSR, but just distal to the carboxy-terminal α-chain (αCT) segment (consisting of the amino-acids 731-744 and 757-758 without the insert of amino-acids 745-756) that

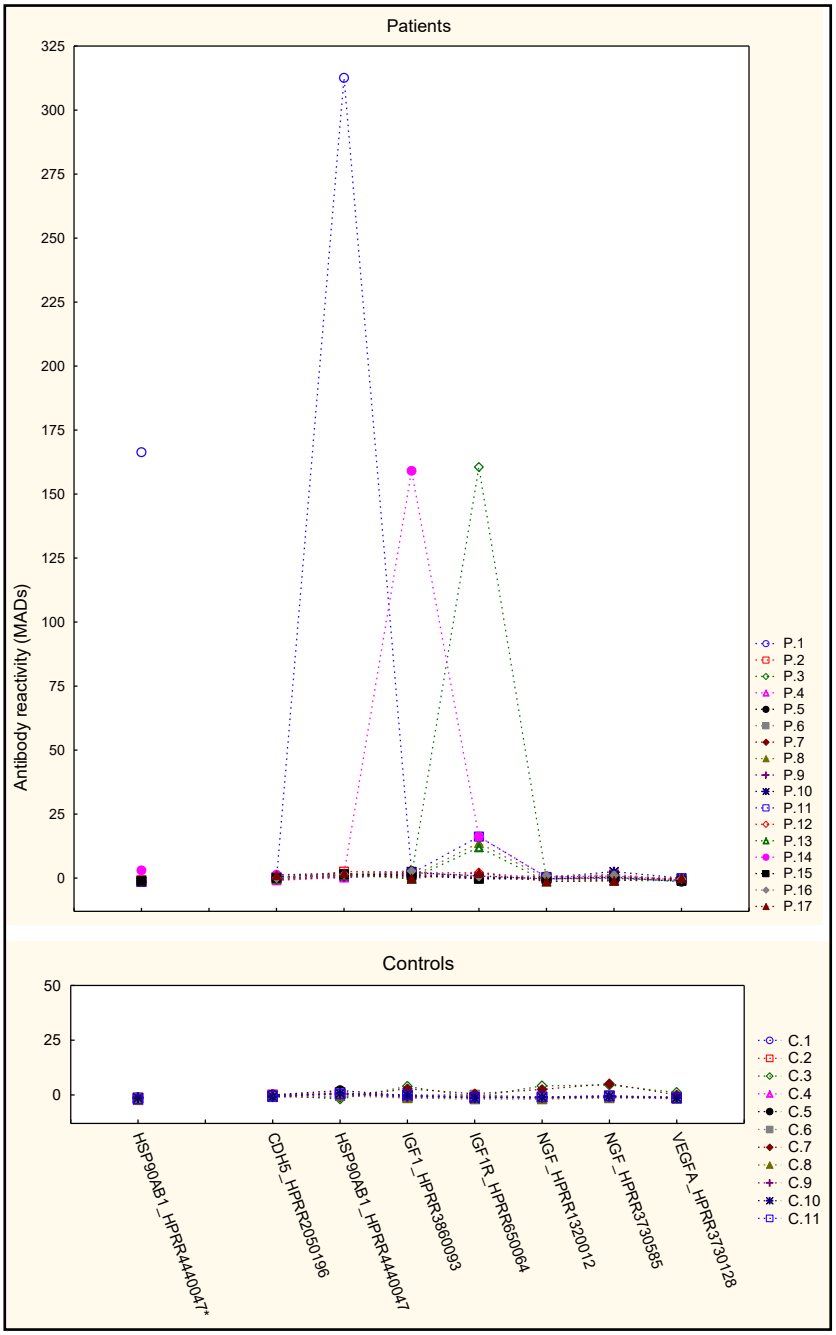

Fig. 1. Antibody reactivity, expressed as median absolute deviations (MADs), was significantly higher in patients (P) compared to controls (C) against the protein fragments heat shock protein 90 alpha family class B member 1 (HSP90AB1) _ HPRR4440047* in cerebrospinal fluid (P.1-P.4, P.8-P.12, P.14-P.16 versus C.1-C.11), and cadherin 5 (CDH5)_HPRR2050196, HSP90AB1_HPRR4440047, insulin like growth factor 1 (IGF1)_HPRR3860093, insulin like growth factor 1 receptor (IGF1R)_ HPRR650064, nerve growth factor (NGF)_HPRR1320012;_HPRR3730585 and vascular endothelial growth factor A (VEGFA)_HPRR3730128 in serum (P.1-P.17 versus C.1-C.11).

earlier has been demonstrated to be the primary binding site for INS, i.e. where two surfaces of the INS molecule are understood to interact with either α-subunit of the INSR (Menting *et al.* 2013). We did not find any increased antibody reactivity in patients against INSR_peptides no´s 10,11 and 12 that cover this αCT segment of the INSR (Menting *et al.* 2013). This proximal part of the α-subunit (except five amino-acids) represents also another part of the INSR than a more distal part consisting of the amino-acids 477-628, which in another earlier study has been shown to be an immunogenic region that antibodies which inhibit INS binding are directed against (Zhang & Roth,1991). Hence, it is most probable that the increased antibody reactivity directed against this proximal part of the α-subunit revealed in this study inhibits connecting (C)-peptide-, and not INS-, binding to the INSR, as available data show that the INSR is a bivalent molecule that is capable of binding two molecules of ligand with different affinities (Schäffer, 1994; Yip & Jack, 1992), providing a framework for a molecular explanation of a possible combined binding of INS and C-peptide to the INSR. In all probability, it is also the short isoform of the INSR (i.e. INSR-A that predominately is expressed in the CNS), and not the long isoform of the INSR (i.e. INSR-B), that constitutes the primary antigen target against which the autoimmune reactivity is directed in schizophrenia.

As concerns the PAGE2B;2;5 protein, increased antibody reactivity was found in CSF against peptide no 1 and in serum against peptides no's 6 and 7 in patients, compared to control subjects. These three peptides are all located in the N-terminal portion of the PAGE2B;2;5 protein against which increased antibody reactivity in serum earlier has been demonstrated in schizophrenia (Zandian *et al.* 2017). Our findings regarding PAGE2B;2;5 confirm this earlier finding (Zandian *et al.* 2017), and show in addition an increased antibody reactivity in CSF of patients against PAGE2B;2;5_ peptide no 1, and a positive correlation between antibody reactivity in serum against PAGE2B;2;5_peptide no 7 and PANSS scores of positive and negative symptoms combined in patients. The PAGE protein family is a group of proteins which have antiapoptotic properties (Gjerstorff & Ditzel, 2008). The expression of the PAGE2B;2;5 protein in peripheral organs is limited mainly to germ cells of testis and ovary and malignancies in these tissues, and in the brain, immunohistochemistry analysis with antisera to the N-terminal fragment of PAGE2B;2;5 has confirmed binding to extracellular

Tab. 5. Antibody reactivity in cerebrospinal fluid and serum, expressed as median absolute deviations (MADs), for the 50 protein fragments investigated in patients compared to controls

Protein/Gene[a,b]	Protein fragment[a]	Cerebrospinal fluid – MADs[c] Patients (n=12)	vs Controls (n=11)[d]	*P*-value[e]	Serum – MADs[c] Patients (n=17)	vs Controls (n=11)[d]	*P*-value[e]
ANO2	HPRR3070034	-0.02 (-0.79-42.29)	0.26 (-0.53-4.31)	0.829	-0.62 (-1.97-80.53)	2.40 (-1.00-22.67)	0.259
	HPRR3070036	-0.86 (-1.55-7.29)	0.05 (-1.00-27.33)	0.051	0.00 (-0.73-32.04)	2.53 (-1.63-238.18)	0.510
	HPRR3070037	1.31 (0.64-3.18)	1.59 (0.72-3.13)	0.878	0.23 (-0.36-30.00)	0.60 (-0.40-3.26)	0.204
CDH5	HPRR2050196	-0.35 (-0.76-3.00)	-0.60 (-1.23-0.13)	0.255	0.07 (-0.92-1.33)	-0.13 (-0.85-0.00)	**0.040**
	HPRR4240093	-0.23 (-0.64-5.00)	-0.17 (-0.33-2.00)	0.281	0.33 (-0.49-8.39)	0.20 (-0.38-2.50)	1.000
GLS	HPRR2920208	1.02 (0.42-49.29)	1.20 (0.23-3.47)	0.644	-0.03 (-1.00-134.86)	0.89 (-0.27-28.88)	0.132
	HPRR3890163	-1.45 (-2.00--1.00)	-1.42 (-2.33-0.00)	0.688	-1.91 (-3.25-6.45)	-1.87 (-3.00-1.00)	0.814
GLS2	HPRR3070446	-1.04 (-1.60--0.76)	-0.80 (-1.60-5.60)	0.175	-1.52 (-2.29--0.53)	-1.57 (-2.33-5.91)	0.760
	HPRR3890773	1.34 (-0.84-18.50)	-0.39 (-1.38-14.00)	0.091	2.39 (-2.08-17.65)	-0.50 (-1.48-3.00)	0.323
GLUL	HPRR1350059	2.31 (1.55-7.83)	2.06 (0.83-3.33)	0.712	0.92 (0.25-5.06)	1.22 (0.73-7.22)	0.158
	HPRR1350060	0.00 (-0.10-0.86)	0.28 (-0.27-1.60)	0.537	-0.12(-0.75-0.74)	-0.27 (-0.67-1.83)	0.621
HSP90AA1	HPRR3690019	1.43 (-0.71-7.04)	2.00 (-0.67-5.21)	0.782	0.06 (-0.86-6.21)	0.00 (-0.53-2.14)	0.510
	HPRR4290083	-1.34 (-1.58--0.91)	-1.47 (-2.00--1.20)	0.102	0.97 (0.38-2.25)	1.00 (-0.99-2.33)	0.906
HSP90AB1	HPRR3540067	1.20 (-0.21-7.12)	1.72 (-0.40-121.13)	0.255	-0.32 (-1.85-5.22)	0.80 (-1.19-381.93)	**0.004**
	HPRR4440047	-1.28 (-1.55-166.42)	-1.40 (-2.27--1.20)	**0.036**	1.32 (0.16-312.57)	0.44 (-1.88-2.00)	**0.010**
	HPRR4440048	-0.35 (-1.04-5.27)	-0.40 (-1.08-2.50)	0.975	-0.89 (-2.41-33.00)	-1.29 (-1.83-29.00)	0.925
HSPA4	HPRR1920102[f]	5.41 (-1.20-25.22)	2.67 (-2.00-42.00)	0.689	31.82 (-1.00-290.24)	20.57 (-0.51-127.84)	0.638
	HPRR4220528	0.84 (-1.29-45.58)	0.40 (-1.47-1.85)	0.325	1.68 (-1.40-88.47)	0.13 (-1.44-18.05)	0.371
IDE	HPRR650042	5.06 (0.43-22.79)	3.72 (-0.33-8.79)	0.518	0.40 (-1.14-9.61)	1.33 (-0.90-4.60)	0.672
	HPRR4180402	0.38 (-0.68-0.71)	0.00 (-0.85-1.20)	0.166	0.09 (-0.78-1.40)	0.68 (-0.88-2.40)	0.180
	HPRR4180403	1.42 (0.04-2.50)	1.20 (-0.23-3.67)	0.356	1.62 (0.27-3.15)	2.00 (-0.93-3.89)	0.925
IGF1	HPRR350040	0.57 (-1.29-3.75)	1.53 (-1.33-3.20)	0.926	0.22 (-1.18-74.07)	-0.37 (-1.00-3.38)	0.110
	HPRR3720070	-0.29 (-0.71-1.08)	0.05 (-1.00-2.53)	0.281	-0.86 (-1.43-0.65)	-1.00 (-1.50-3.39)	0.741
	HPRR3860093	-0.17 (-0.71-4.00)	0.60 (-1.00-2.53)	0.518	1.82 (-0.32-159.13)	-0.26 (-1.33-3.90)	**0.008**
IGF1R	HPRR650064	-0.92 (-1.33-21.29)	-1.06 (-1.62--0.13)	0.079	0.60 (-0.29-160.57)	-0.93 (-1.83-0.56)	**<0.001**
	HPRR650065	-0.78 (-2.25-481.00)	-0.54 (-3.00-37.13)	0.926	-2.12 (-2.95-996.13)	0.08 (-3.00-102.14)	0.397
	HPRR2750058	0.14 (-0.71-32.44)	0.00 (-0.47-0.20)	0.157	0.97 (-0.32-96.70)	0.40 (-0.74-1.74)	0.070
	HPRR2750059	-0.11 (-1.09-15.00)	16.00 (-0.83-119.77)	0.079	3.07 (-1.85-329.58)	22.65 (-0.33-884.93)	0.100
	HPRR2750060	-1.00 (-1.29-0.00)	-1.20 (-1.63--0.67)	0.072	0.00 (-0.93-6.39)	-0.50 (-1.17-1.30)	0.204
	HPRR3350014	1.82 (0.68-37.11)	3.31 (0.05-164.80)	0.735	5.63 (1.45-132.19)	9.11 (-0.03-245.86)	0.188
IGF2	HPRR380014	0.14 (-0.36-1.00)	0.00 (-0.68-4.67)	0.280	0.88 (-0.27-2.59)	0.20 (-0.50-2.93)	0.259
IGF2R	HPRR670260	0.41(-0.76-5.74)	0.58 (-0.40-1.84)	0.975	0.95 (-0.22-16.73)	1.20 (-1.03-2.40)	0.742
	HPRR670338	0.73 (-0.07-3.57)	0.40 (-0.07-1.80)	0.479	0.32 (-0.50-3.73)	0.05 (-0.50-0.80)	0.335
INS	HPRR380034	-0.68 (-1.24-0.14)	-0.80 (-1.21-0.92)	0.758	-0.88 (-1.62-1.82)	-0.80 (-1.28-11.50)	0.158
INSR	HPRR1010005	1.49 (0.14-5.35)	1.61 (-0.53-3.84)	0.829	-1.00 (-1.40-5.43)	-0.20 (-1.13-3.25)	**0.043**
	HPRR1010006	-0.90 (-1.26--0.35)	-0.68 (-1.47-12.20)	0.372	-1.18 (-1.78-2.12)	-0.98 (-2.11-17.11)	0.269
	HPRR2980052	-1.06 (-1.36--0.67)	-1.07 (-1.54-0.67)	0.734	-0.55 (-1.29-4.82)	-0.30 (-1.00-1.16)	0.981

Protein/Gene[a,b]	Protein fragment[a]	Cerebrospinal fluid – MADs[c] Patients (n=12)	vs	Controls (n=11)[d]	*P*-value[e]	Serum – MADs[c] Patients (n=17)	vs	Controls (n=11)[d]	*P*-value[e]
IRS4	HPRR231035	1.51 (0.80-15.22)		0.94 (-0.28-4.00)	0.406	-1.00 (-2.09-1.64)		2.50 (-2.03-6.86)	**0.043**
	HPRR4340060	2.34 (0.14-4.42)		2.60 (0.33-5.00)	0.805	0.48 (-0.36 -17.96)		0.40 (-0.25-1.67)	0.906
NGF	HPRR1320012	-1.20 (-1.50--0.60)		-1.00 (-1.63-3.93)	0.131	-0.24 (-1.32-1.00)		-1.20 (-1.83-4.13)	**0.021**
	HPRR3730584	1.96 (1.40-3.40)		2.00 (0.80-9.53)	0.580	3.08 (1.54-4.53)		4.08 (-0.19-6.27)	0.110
	HPRR3730585	-0.38 (-0.65-0.22)		-0.16 (-0.60-5.93)	0.310	0.29 (-1.10-2.52)		-0.60 (-1.33-5.10)	**0.036**
PAGE2B;2;5[g]	HPRR3340273[f]	-1.30 (-1.74-0.43)		-1.40 (-2.15-2.33)	0.460	-1.55 (-3.12-9.29)		-1.30 (-1.84-4.56)	0.510
	HPRR3340274	-0.68 (-1.64-8.87)		-0.90 (-1.21-104.46)	0.518	-1.42 (-2.57-18.00)		-1.20 (-2.43-319.83)	0.424
TGM6	HPRR3310212	-0.22 (-0.40-0.14)		0.00 (-0.47-2.73)	0.339	-0.31 (-1.13-0.10)		-0.16 (-1.00-1.81)	0.742
TSPO[g]	HPRR3140230	0.73 (0.03-1.11)		0.62 (0.15-4.80)	0.853	-0.47 (-1.06-0.00)		-0.25 (-0.83-5.49)	0.145
	HPRR3870247	0.14 (-0.86-3.35)		0.00 (-1.50-2.05)	1.000	-1.13 (-1.65-0.86)		-0.78 (-1.42-2.80)	**0.045**
VEGFA	HPRR330133	-0.87 (-1.57-0.44)		-0.80 (-1.67-0.30)	0.579	-1.00 (-1.62-0.00)		-1.20 (-1.84-0.88)	0.203
	HPRR3730126	-0.65 (-1.93-1.04)		-0.80 (-2.00--0.33)	0.355	-1.43 (-2.03-0.57)		-0.60 (-1.83-0.86)	**0.008**
	HPRR3730128	-1.70 (-1.91--1.32)		-1.85 (-3.00--0.80)	0.340	-0.63 (-1.43-0.02)		-1.20 (-1.67-1.18)	**0.041**

Abbreviations: ANO2=anoctamin 2; CDH5=cadherin 5; GLS=glutaminase; GLS2=glutaminase 2; GLUL=glutamate-ammonia ligase; HSP90AA1=heat shock protein 90 alpha family class A member 1; HSP90AB1=heat shock protein 90 alpha family class B member 1; HSPA4=heat shock protein family A (Hsp70) member 4; IDE=insulin degrading enzyme; IGF1=insulin like growth factor 1; IGF1R=insulin like growth factor 1 receptor; IGF2=insulin like growth factor 2; IGF2R= insulin like growth factor 2 receptor; INS=insulin; INSR=insulin receptor; IRS4=insulin receptor substrate 4; NGF=nerve growth factor; PAGE2B;2;5=PAGE family member 2B,2,5; TGM6=transglutaminase 6; TSPO=translocator protein; VEGFA=vascular endothelial growth factor A; a) According to https://www.ncbi.nlm.nih.gov/protein; b) According to https://www.ncbi.nlm.nih.gov/gene; c) Expressed as median (min-max); d) One of the 12 controls was found to have increased antibody reactivity in cerebrospinal fluid (CSF) and serum against the calcium-activated chloride-channel protein fragment ANO2_HPRR3070036 (MADs: CSF=74.20, serum=180.44), that is identified as an autoimmune target in multiple sclerosis (Ayoglu *et al.* 2016a; Tengvall *et al.* 2019), and was therefore excluded from the study; e) *P*-values <0.05 are written in bold text with significantly higher antibody reactivity in patients compared to controls underlined; f) Potentially unreliable due to low signals in the coupling efficiency test; g) The protein fragment sequences are not specific for one protein/gene, but for a family of proteins/genes

structures of the cerebral cortex, but not proven presence of the whole protein (Gjerstorff & Ditzel, 2008; Zandian *et al.* 2017). If not present, it is however possible that our patients' CSF autoantibodies in this respect are directed against an antigen of a different protein in the CNS, cross-reactive with the N-terminal fragment of PAGE2B;2;5. As regards the increased antibody reactivity against PAGE2B;2;5 in serum of our patients, it may be caused by an on-going autoimmune-mediated process directed mainly against germ cells of testis and ovary. This autoimmune-mediated process against germ cells may also at least in part explain the reduced fertility observed among patients with schizophrenia in general, and among male patients with schizophrenia in particular (Nimgaonkar, 1998). Of all protein fragments and peptides against which patients in this study had increased antibody reactivity, we found two overlaps between amino-acid sequences of PAGE2B;2;5 and the other proteins: 1. either glutamine–serine–serine (QSS) in peptide no 1 or SSQ in peptides no's 6 and 7 of PAGE2B;2;5 (corresponding to amino-acids 10-12 or 20-22) and SSQ in peptide no 2 of INSR (corresponding to amino-acids 635-637), and 2. glutamic acid–arginine–glycine (ERG) in peptide no 1 of PAGE2B;2;5 (corresponding to amino-acids 13-15) and ERG in peptide no 1 of INS-(ß-chain) (corresponding to amino-acids 45-47). Possibly, the two overlaps QSS + ERG (corresponding to amino-acids 10-15) in PAGE2B;2;5 constitute an antigen target in peripheral germ cells that is cross-reactive with QSS + ERG in an INSR-A–INS-(ß-chain) complex in the CNS.

For the HSPA4 protein, increased antibody reactivity was found in CSF against peptide no 2, and in serum against peptides no's 1 and 4; and for the HSP90AB1 protein, increased antibody reactivity was found in both CSF and serum against protein fragment HPRR4440047, in patients, compared to control subjects. Our findings show for the first time increased antibody reactivity in CSF against HSPA4 and HSP90AB1 in schizophrenia, and confirm also previous studies reporting elevated serum levels of anti-HSP70- and anti-HSP90 antibodies in patients with schizophrenia (Kim *et al.* 2001; Schwarz *et al.* 1999). The HSPA4 and HSP90AB1 proteins are present in the CNS and peripheral organs and play an

Tab. 6. Antibody reactivity in cerebrospinal fluid and serum, expressed as median absolute deviations (MADs), for the 64 peptides investigated in patients compared to controls

Protein/Gene[a,b]	Peptide[c]	Cerebrospinal fluid – MADs[d] Patients (n=12) vs	Controls (n=11)[e]	*P*-value[f]	Serum – MADs[d] Patients (n=17) vs	Controls (n=11)[e]	*P*-value[f]
HSPA4	1.	-0.71 (-1.39-306.42)	-0.76 (-2.93-4.66)	0.601	-0.89 (-3.65-149.05)	-2.87 (-5.07-8.01)	**0.008**
	2.	0.03 (-2.47-413.20)	-1.60 (-4.07-65.70)	**0.042**	-2.04 (-6.49-152.93)	-1.23 (-5.16-127.38)	0.672
	3.	0.05 (-0.52-2.68)	0.22 (-0.80-0.92)	0.975	0.82 (0.37-1.90)	0.41 (-1.12-2.81)	0.158
	4	-1.35 (-2.28--0.17)	-0.95 (-5.80-19.28)	0.622	-0.21 (-1.01-29.06)	-0.84 (-2.65-2.35)	**0.041**
	5.	0.34 (-0.60-1.41)	0.82 (-0.82-2.10)	0.853	-0.24 (-0.79-5.31)	-0.08 (-1.98-0.94)	0.851
	6.	-0.84 (-2.72-6.07)	-1.00 (-1.99-11.75)	0.926	-1.72 (-2.77-8.25)	0.22 (-0.04-1.58)	0.249
	7.	-0.25 (-2.04-2.47)	0.52 (-2.04-1.52)	0.176	-0.84 (-1.21-2.28)	1.59 (-0.01-3.28)	**<0.001**
	8.	-0.73 (-2.15-1.27)	-0.63 (-2.24-9.63)	0.926	0.40 (-1.39-1.66)	1.04 (-1.00-88.29)	0.452
IGF1	1.	-5.46 (-11.66-7.84)	7.82 (-8.89-60.49)	**0.007**	-6.50 (-14.64-123.44)	14.76 (-6.52-101.69)	**0.008**
	2.	-0.03 (-1.44-1.65)	0.49 (-1.79-1.81)	0.498	-0.30 (-1.00-1.98)	1.17 (0.00-1.41)	**0.001**
	3.	0.94 (-1.42-3.02)	1.66 (-0.31-2.45)	0.601	0.17 (-0.58-3.67)	0.50 (-0.70-1.00)	0.588
	4.	-2.74 (-6.27-4.40)	-2.01 (-3.60-16.57)	0.242	-0.71 (-2.37-79.61)	0.46 (-2.26-13.06)	**0.024**
	5.	-1.94 (-3.90-4.50)	-1.80 (-3.87-4.76)	0.735	-0.97 (-2.12-6.76)	-0.32 (-1.10-2.48)	**0.013**
IGF1R	1.	0.96 (-0.84-2.58)	0.94 (-1.22-2.13)	0.951	-0.82 (-2.91-5.16)	-0.36 (-1.94-12.63)	0.158
	2.	0.79 (-0.05-2.46)	0.20 (-2.38-4.80)	0.140	0.91 (-1.41-3.80)	-1.48 (-2.21-2.21)	**0.002**
	3.	-0.01 (-1.23-1.96)	-0.67 (-1.17-1.16)	0.103	1.29 (-1.16-10.45)	-0.58 (-2.25-5.77)	**0.005**
	4.	-0.28 (-1.79-9.85)	-0.63 (-1.71-7.02)	0.559	0.19 (-0.42-14.29)	0.15 (-1.74-16.83)	0.541
	5.	-2.00 (-4.43-58.52)	0.44 (-8.03-16.61)	0.689	1.89 (-0.24-173.08)	1.20 (-2.25-3.89)	0.121
	6.	-0.71 (-1.21-334.78)	-0.58 (-2.99-0.95)	0.601	-0.35 (-1.46-795.86)	-1.20 (-2.45--0.57)	**0.003**
	7.	0.11 (-1.40-10.19)	-0.07 (-1.41-1.05)	0.340	0.35 (-2.29-18.23)	1.21 (-3.29-2.63)	0.981
	8.	0.67 (-0.41-2.14)	-0.10 (-1.15-1.32)	0.140	1.16 (-0.49-2.21)	-0.34 (-1.00-3.91)	**0.014**
	9.	-1.85 (-3.07-4.21)	-1.24 (-3.60-2.17)	0.782	-0.79 (-1.42-5.33)	1.42 (0.29-2.85)	**<0.001**
	10.	-0.56 (-1.59-0.00)	1.02 (-1.46-14.19)	**0.018**	-1.99 (-4.26-5.25)	3.76 (-2.12-31.99)	**0.006**
	11.	-2.17 (-4.76-1.41)	-1.72 (-5.73-15.11)	0.926	-2.83 (-6.53-20.46)	9.95 (-8.94-72.34)	0.240
	12.	-0.63 (-1.70-1.46)	-0.18 (-1.90-1.00)	0.712	2.78 (-1.12-5.46)	1.00 (-1.51-2.55)	**0.004**
	13.	1.27 (-0.47-2.45)	0.17 (-0.75-2.02)	0.074	0.82 (-1.84-15.48)	-0.81 (-4.22-0.17)	**0.001**
	14.	-1.88 (-3.36-4.50)	-1.74 (-2.63-2.71)	0.423	-1.19 (-2.52-7.17)	1.89 (-0.61-3.03)	**0.001**
	15.	-1.74 (-3.57-36.22)	-1.27 (-4.60-11.24)	0.601	0.72 (-1.66-162.09)	-2.03 (-5.02-10.50)	**0.016**
	16.	0.60 (-1.66-2.51)	1.51 (-0.26-2.86)	0.372	0.24 (-0.08-0.95)	0.21 (-0.43-1.81)	0.689
	17.	0.29 (-1.49-4.50)	-0.54 (-2.54-25.28)	0.406	-0.18 (-3.71-1.13)	-1.22 (-3.72-47.78)	0.572
	18.	0.19 (-0.89-3.08)	0.28 (-1.74-2.52)	0.689	1.00 (-2.13-251.05)	-2.98 (-5.26-2.73)	**<0.001**
	19.	0.92 (-3.25-3.32)	-2.30 (-4.13-9.42)	0.281	-1.72 (-3.78-90.60)	-3.22 (-5.66-2.47)	0.188
INS	1.	-0.62 (-4.03-5.58)	-2.29 (-4.04-7.19)	0.644	1.86 (-1.66-7.17)	-0.70 (-2.88-0.89)	**<0.001**
	2.	-0.70 (-4.91-116.70)	-3.25 (-5.17-78.45)	0.310	-5.22 (-10.25-46.30)	-4.00 (-5.95-272.58)	0.067
	3.	0.06 (-2.07-1.16)	0.13 (-0.82-7.45)	0.498	1.24 (-0.52-2.80)	1.11 (0.00-5.88)	0.925
	4.	0.61 (-0.60-2.13)	0.45 (-0.22-1.03)	0.712	0.26 (-1.17-1.01)	0.88 (0.15-1.52)	**0.004**
	5.	0.10 (-2.52-4.91)	-0.53 (-1.36-1.82)	0.325	0.57 (-0.75-2.06)	0.46 (-0.99-9.24)	0.525
	6.	0.92 (-0.07-2.20)	0.26 (-0.65-2.24)	0.139	0.18 (-1.53-1.37)	-0.84 (-2.15-0.27)	**0.002**
	7.	0.95 (0.04-3.82)	0.43 (-1.60-1.61)	0.060	0.12 (-1.19-36.47)	-0.66 (-1.69--0.24)	**<0.001**
	8	-1.05 (-3.65-4.11)	-1.82 (-2.80-5.90)	0.926	-1.23 (-3.43-6.39)	-0.44 (-1.90-1.59)	0.115
	9.	-0.81 (-2.16-0.64)	-0.48 (-2.36-0.58)	0.406	2.99 (-0.74-6.37)	-0.87 (-2.89--0.17)	**<0.001**

Protein/Gene[a,b]	Peptide[c]	Cerebrospinal fluid – MADs[d] Patients (n=12)	vs	Controls (n=11)[e]	*P*-value[f]	Serum – MADs[d] Patients (n=17)	vs	Controls (n=11)[e]	*P*-value[f]
INSR	1.	-0.14 (-0.94-0.72)		-0.21 (-1.13-4.53)	1.000	0.12 (-1.29-5.33)		-0.41 (-1.84-2.64)	0.082
	2.	0.95 (-0.09-1.73)		0.52 (-2.70-1.92)	0.442	-0.14 (-3.07-0.97)		-1.17 (-1.72-0.27)	**0.004**
	3.	1.29 (0.48-4.53)		0.54 (-0.59-2.02)	**0.045**	1.50 (-1.46-2.97)		-0.56 (-1.45-1.02)	**<0.001**
	4.	0.23 (-1.64-10.23)		-0.30 (-2.18-7.02)	0.406	4.32 (-1.05-8.16)		0.82 (-4.57-3.34)	**0.003**
	5.	-0.97 (-3.65-18.02)		-2.17 (-4.09-3.91)	0.442	-1.49 (-3.67-35.21)		-1.00 (-2.98-2.21)	0.347
	6.	1.10 (-0.99-6.37)		0.90 (-1.29-1.88)	0.406	-0.13 (-1.06-3.43)		-0.28 (-1.84-0.29)	0.638
	7.	0.26 (-0.99-1.70)		-0.07 (-1.32-1.64)	0.389	2.10 (-0.77-10.52)		1.01 (0.27-2.02)	**0.006**
	8.	0.39 (-0.46-1.47)		0.06 (-1.20-2.17)	0.518	-0.33 (-1.64-1.44)		-0.72 (-1.65-0.00)	0.180
	9.	-0.59 (-1.52-0.74)		0.08 (-1.35-1.08)	0.157	0.75 (-0.55-3.20)		0.37 (-0.07-1.14)	0.180
	10.	-0.73 (-2.30-1.41)		-1.13 (-6.02-9.13)	0.255	0.38 (-1.95-2.85)		-3.04 (-6.60-23.67)	0.240
	11.	0.63 (-0.85-1.92)		0.72 (-0.21-3.54)	0.926	-0.34 (-1.43-2.68)		-0.17 (-1.52-0.60)	0.981
	12	-1.45 (-7.50-41.48)		3.86 (-8.42-86.60)	0.310	-3.80 (-8.44-15.89)		83.63 (-4.63-232.37)	**0.001**
IRS4	1.	1.50 (0.24-3.48)		1.54 (-0.70-3.09)	1.000	-1.13 (-2.56-4.34)		0.03 (-1.00-2.32)	**<0.001**
	2.	-0.65 (-1.92-2.05)		0.06 (-2.43-1.12)	0.442	-1.25 (-2.21-3.78)		-0.51 (-1.86-0.98)	0.100
PAGE2B;2;5[g]	1.	0.71 (-0.36-2.30)		0.11 (-1.53-0.46)	**0.025**	-0.40 (-1.05-2.95)		0.00 (-0.83-1.07)	0.165
	2.	-0.25 (-1.21-3.65)		-0.07 (-1.68-2.52)	0.951	-1.05 (-1.94-1.90)		-1.49 (-2.50-0.41)	0.115
	3.	0.24 (-0.55-1.86)		0.56 (-0.81-1.68)	0.622	-0.34 (-1.02-1.60)		0.48 (-0.30-1.16)	**0.011**
	4.	0.05 (-0.80-1.92)		0.42 (-1.22-2.16)	0.782	0.20 (-0.84-1.41)		1.12 (0.09-1.58)	**0.017**
	5.	0.62 (-0.71-3.57)		0.74 (-1.61-2.22)	0.735	0.00 (-0.80-1.58)		0.15 (-0.66-0.63)	0.588
	6.	0.50 (-0.50-2.02)		0.47 (-0.92-1.69)	0.878	0.16 (-0.99-4.02)		-0.20 (-1.16-0.47)	**0.022**
	7.	-0.37 (-1.63-1.30)		-0.45 (-1.70-0.95)	0.758	1.00 (-0.91-2.37)		0.04 (-0.64-0.50)	**0.002**
	8.	0.13 (-0.92-164.70)		0.08 (-1.13-1.43)	0.878	0.30 (-1.17-52.51)		0.88 (-0.62-3.20)	0.082
	9.	-0.65 (-2.42-0.08)		-0.17 (-2.14-1.53)	0.230	-0.64 (-1.78-0.37)		0.66 (-0.83-2.37)	**<0.001**

Abbreviations: HSPA4=heat shock protein family A (Hsp70) member 4; IGF1=insulin-like growth factor 1; IGF1R=insulin-like growth factor 1 receptor; INS=insulin; INSR=insulin receptor; IRS4=insulin receptor substrate 4; PAGE2B;2;5=PAGE family member 2B;2;5; a) According to https://www.ncbi.nlm.nih.gov/protein; b) According to https://www.ncbi.nlm.nih.gov/gene; c) Peptide numbering according to Table 4; d) Expressed as median (min-max); e) One of the 12 controls was found to have increased antibody reactivity in cerebrospinal fluid (CSF) and serum against the calcium-activated chloride-channel protein fragment ANO2_HPRR3070036 (MADs:CSF=74.20, serum=180.44), that is identified as an autoimmune target in multiple sclerosis (Ayoglu *et al.* 2016a; Tengvall *et al.* 2019), and was therefore excluded from the study; f) *P*-values <0.05 are written in bold text with significantly higher antibody reactivity in patients compared to controls underlined; g) The peptides are not specific for one protein/gene, but for a family of proteins/genes

important role in the repair, maintenance and removal of proteins in cells, both in the normal cell system and in disease states (Birnbaum, 1995). Increased antibody reactivity against HSPs is however not found exclusively in schizophrenia, as this is seen also in other disease states, for example neurodegenerative diseases like Alzheimer's disease and other autoimmune diseases like multiple sclerosis (Birnbaum, 1995).

Increased antibody reactivity, but in serum alone, was also found against the proteins IGF1R, INS, IGF1, VEGFA, CDH5 and NGF in patients, compared to control subjects. Of these results, those regarding IGF1R, INS, IGF1 and VEGFA are new, whereas those regarding CDH5 and NGF confirm previous findings (Klyushnik *et al.* 1999; Mobarrez *et al.* 2013; Shcherbakova *et al.* 2004; Smirnova *et al.* 2019). For CDH5 that is a calcium-dependent cell-cell adhesion molecule which is present in both CNS and peripheral organs and plays a role in endothelial adherence junction assembly and maintenance (Redies *et al.* 2012), earlier studies, although not regarding antibodies, have shown higher serum concentration of CDH5 in schizophrenia patients with positive symptoms than in schizophrenia patients with negative symptoms (Smirnova *et al.* 2019), and ten times higher number of endothelial-cell-derived CDH5 microparticles in CSF of patients with schizophrenia than in CSF of healthy controls (Mobarrez *et al.* 2013). For NGF that is involved in the regulation of growth and differentiation of nerve cells in the central and peripheral nervous systems, previous studies have shown, as in this study, higher serum

Tab. 7. Scores obtained with the Positive and Negative Syndrome Scale (PANSS) for schizophrenia in the patients studied[a]

	PANSS scores			
	Positive symptoms	**Negative symptoms**	**Positive and negative symptoms combined**	**General psychiatric symptoms**
Mean (SD)	10.4 (3.4)	9.3 (1.1)	1.1 (3.7)	20.4 (3.5)
Median (min-max)	10.0 (7.0-17.0)	9.0 (7.0-12.0)	1.0 (-5.0-9.0)	21.0 (16.0-26.0)
Reference range[b]	7-49	7-49	–42-42	16-112

a) n=16 due to missing PANSS data in 1 patient; b) According to Kay *et al.* 1987

NGF antibody titres in patients with schizophrenia than in controls, and a correlation between serum NGF antibody titres and the clinical picture of schizophrenia (Klyushnik *et al.* 1999; Shcherbakova *et al.* 2004).

Regarding the IGF1R, it is a heterotetrameric cell surface receptor, consisting of two extracellular α-subunits (amino-acids 31-736) and two transmembrane and intracellular ß-subunits (amino-acids 741-1367), which is expressed in both CNS and peripheral organs and binds IGF1 with high affinity, and INS with somewhat lower affinity (Abbott *et al.* 1992; McCowen & Smith, 2005; Sara *et al.* 1982). In structure, the IGF1R is relatively closely related to the INSR with 60% overall amino-acid homology (http://biogps.com). However, despite this similarity, the two receptors differ in tissue distribution and biological roles; whereas the INSR functions primarily in the regulation of body metabolism, the IGF1R mediates predominantly longer term actions on growth and development (Abbott *et al.* 1992). Two distinct forms of the IGF1R that differ in ß-subunit molecular weight and relative levels of expression in tissues have been demonstrated, and these two forms of IGF1R have also been identified in the CNS (Abbott *et al.* 1992; Gammeltoft *et al.* 1985; Yee *et al.* 1989). In this study, increased antibody reactivity was found in serum against IGF1R_protein fragment HPRR650064 and IGF1R_peptides no's 2,3,6,8,12,13,15 and 18 in patients, compared to control subjects. A positive correlation was in addition found between antibody reactivity in serum against IGF1R_peptide no 15 and the PANSS scores of negative symptoms in patients. The protein fragment and peptides against which increased antibody reactivity was found in serum are located in both the extracellular α-subunit and transmembrane/intracellular ß-subunit of the IGF1R. Together, these results may reflect an on-going autoimmune-mediated process directed against IGF1Rs on peripheral cells. Moreover, we compared the IGF1R´s and INSR´s protein fragment and peptides against which patients had increased antibody reactivity and found a high degree of overlap between the amino-acid sequences: proline–proline–serine–leucine–proline–asparagine–glycine–asparagine (PPSLPNGN) in the protein fragment HPRR650064 and peptide no 2 of IGF1R (corresponding to amino-acids 633-640) and proline–proline–serine–aspartic acid–proline–asparagine–glycine–asparagine (PPSDPNGN) in the peptide no 3 of INSR (corresponding to amino-acids 644-651), pointing to a possible cross-reactivity between these two receptors in schizophrenia.

As concerns the INS protein, its gene codes proinsulin in the pancreatic ß-cells, and after removal of the signal peptide (amino-acids 1-24), proinsulin is cleaved into three peptides: the ß-chain (amino-acids 25-54; cleavage site: amino-acids 55-56) and α-chain (cleavage site: amino-acids 88-89; amino-acids 90-110) peptides, which are covalently linked via two disulfide bonds to form INS, and C-peptide (amino-acids 57-87) (Rhodes, 2003; Steiner, 2004). Both INS and C-peptide are present in the CNS and peripheral organs (Adamo *et al.* 1989; Dorn *et al.* 1982, 1983; Kahn & Saltiel, 2005). In this study, increased antibody reactivity was found in serum against INS_peptides no's 1,6,7 and 9 in patients, compared to control subjects. Two of these four peptides are totally (no 6) or almost totally (no 7) located in C-peptide with cleavage site amino-acids 88-89, and include both the pentapeptide: glutamic acid–glycine–serine–leucine–glutamine (EGSLQ) that has been demonstrated to be essential for the capacity of C-peptide to bind to cell membranes and to elicit biological effects (Pramanik *et al.* 2001; Sima *et al.* 2009; Wahren *et al.* 2004). The other two peptides are located in INS; no 1 in the ß-chain and no 9 in the α-chain. Interestingly, T cells from pancreatic lymph nodes of subjects with type 1 diabetes mellitus (T1DM) have been demonstrated to recognize the epitope of amino-acids 96-110 in the α-chain of INS, but not other epitopes in the INS protein, indicating that this epitope may be an autoimmune target in T1DM (Kent *et al.* 2005); at the same time as – in this study – we have shown increased antibody reactivity in serum against INS_peptide no 9 (i.e. amino-acids 95-109) in schizophrenia. This reveals a link between these two autoimmune diseases, which also in part may explain an earlier finding of a register study that schizophrenia is associated with increased risk for developing subsequent T1DM (Melkersson, 2020). Antibody reactivity in serum against INS_peptide no 2 with location in the ß-chain of INS was, in addition, inversely correlated to the PANSS scores of negative symptoms in patients in this study. To compare, other T1DM associated autoantibodies have earlier shown no

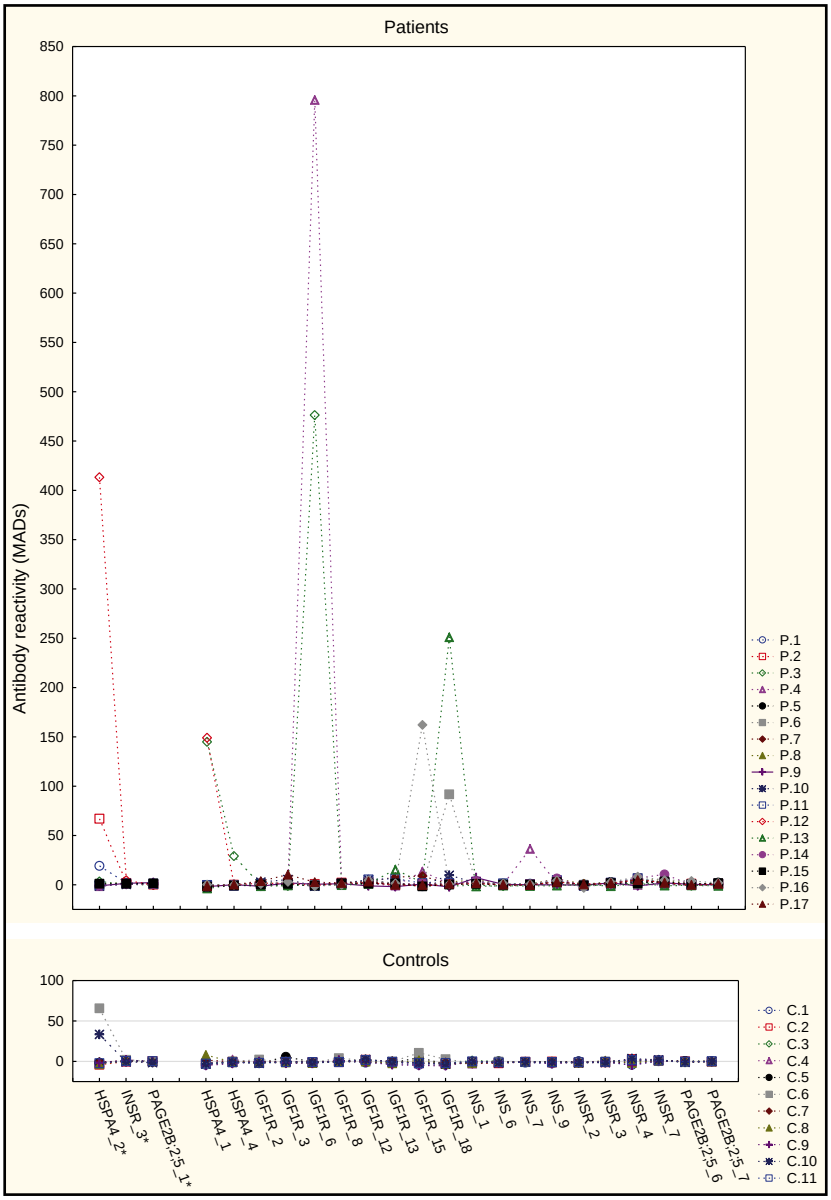

Fig. 2. Antibody reactivity, expressed as median absolute deviations (MADs), was significantly higher in patients (P) compared to controls (C) against the peptides heat shock protein family A member 4 (HSPA4)_2*, insulin receptor (INSR)_3* and PAGE family member 2B;2;5 (PAGE2B;2;5)_1* in cerebrospinal fluid (P.1-P.4, P.8-P.12, P.14-P.16 versus C.1-C.11), and HSPA4_1,4, insulin like growth factor 1 receptor (IGF1R)_2,3,6,8,12,13,15,18, insulin (INS)_1,6,7,9, INSR_2,3,4,7 and PAGE2B;2;5_6,7 in serum (P.1-P.17 versus C.1-C.11).

differences in plasma levels of IgG against insulinoma-associated antigen 2a (IA2a) and glutamic acid decarboxylase (GAD), or decreased plasma levels of IgG against insulinoma-associated antigen 2b (IA2b), in patients with schizophrenia, compared to control subjects (Hallford *et al.* 2016). In contrast however, having T1DM and being on therapy with INS, but not being substituted with C-peptide (i.e. lack ß-cells with INSR_peptides no's 2,3,4 and 7 and C-peptide with INS_ peptides no's 6 and 7 as antigen targets) has been shown in earlier register studies to be associated with a decreased risk of developing subsequent schizophrenia (Eaton *et al.* 2006; Finney, 1989; Juvonen *et al.* 2007; Melkersson & Wernroth, 2019).

Insulin like growth factor 1 is also a protein involved in mediating growth and development that is present in both CNS and peripheral organs (Adamo *et al.* 1989). Its gene codes two IGF1 variants, IGF1a and IGF1b, which differ in the length and sequence of their carboxyl-terminal extensions (Sara & Carlsson-Skwirut, 1988). The IGF1a variant that has a truncated amino-acid terminal region and preferentially binds to the IGF1R, has increased neurotrophic potency and has been identified in the CNS, whereas the IGF1b variant that has a non-truncated amino-acid terminal region, constitutes the circulating IGF1 in the body (Carlsson-Skwirut *et al.* 1986; Sara & Carlsson-Skwirut, 1988; Sara & Hall, 1990). In this study, increased antibody reactivity was found in serum of patients against protein fragment IGF1_HPRR3860093, i.e. against the terminal region of the non-truncated IGF1b protein which includes the pentapeptide: glutamic acid–alanine–serine–leucine–glutamine (EASLQ) that is very similar in structure and function to the pentapeptide EGSLQ in C-peptide (Pramanik *et al.* 2001). Hence, this increased antibody reactivity in serum of patients against IGF1_HPRR3860093 may be due to crossreactivity between the pentapeptide EASLQ in IGF1b and the pentapeptide EGSLQ in C-peptide. The fact that IGF1a (not including EASLQ), but not IGF1b (including EASLQ), is present in the CNS (Sara & Carlsson-Skwirut, 1988), may also explain why increased antibody reactivity was found only in serum and not in CSF of our patients.

For VEGFA, a growth factor that is expressed in both CNS and peripheral organs, and that induces proliferation and migration of vascular endothelial cells and is essential for angiogenesis (Hirashima, 2009), increased antibody reactivity against VEGFA_HPRR3730128 was found in serum of patients in this study, indicating that also peripheral cells producing VEGFA may be involved in the autoimmune-mediated process in schizophrenia.

The major strength of this study includes the use of a methodological and technical new antigen array for antibody profiling (Ayoglu *et al.* 2016b) that gives a possibility to select and investigate a larger number of protein fragment- and peptide antigens of potential interest for schizophrenia, thereby revealing new

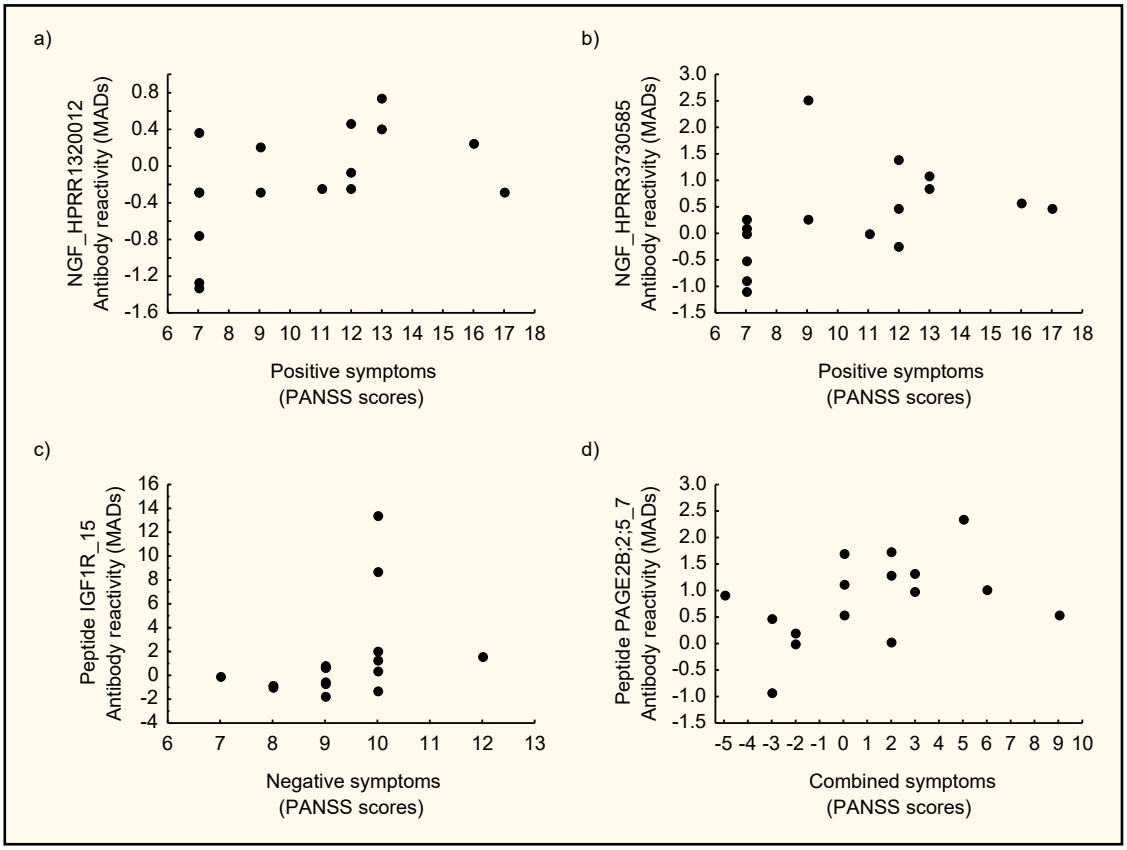

Fig. 3a-d. Relationships between antibody reactivity in serum against protein fragments or peptides, expressed as median absolute deviations (MADs), and PANSS scores of positive, negative or positive and negative symptoms combined in patients (n=16 due to missing PANSS data in 1 patient). Correlation coefficients for the relationships: a) r_s=0.63, p=0.01, b) r_s=0.63, p=0.01, c) r_s=0.58, p=0.02 and d) r_s=0.51, p=0.04.

antigens of importance and also verifying earlier findings with a new method. A further strength includes the narrow diagnostic selection of mainly only patients with a diagnosis of schizophrenia, allowing investigation of a diagnostically homogeneous patient group. The limitations, on the other hand, consist of the fact that the patients in this study were on antipsychotic drug treatment and not were acutely psychotic, which may have moderated the increments of the antibody reactivities observed (Ezeoke *et al.* 2013). The limitations also include that the control group in this study did not comprise healthy individuals. However, it seems unlikely that the control group selected, in which all control subjects were diagnosed solely with non-inflammatory neurological diseases and did not have any diagnosis of psychotic disorder, can have confounded our results.

In summary, we show increased antibody reactivity against INSR, PAGE2B;2;5, HSPA4 and HSP90AB1 in both CSF and serum, and against IGF1R, INS, IGF1, CDH5, NGF and VEGFA in serum alone, in patients with schizophrenia or related psychosis, compared to control subjects. We describe also possible cross-reactive amino-acid sequences between INSR and IGF1R, INSR-INS and PAGE2B;2;5, and C-peptide and IGF1. Together the results point to that an autoimmune-mediated process in CNS and to some extent in peripheral organs underlie the development of a core group of schizophrenia cases and that INSR-A and IGF1R, their ligands C-peptide, INS and IGF1, and the related inter- and intracellular proteins CDH5, PAGE2B;2;5, HSPA4, HSP90AB1, NGF and VEGFA constitute antigen targets, and to some extent also may cross-react. The results are in line with our previously described hypothesis that impaired cellular signalling via the INSR-A, and probably also via the IGF1R, may underlie known abnormalities in the CNS and peripheral organs in schizophrenia (Melkersson & Persson, 2011, 2012; Melkersson *et al.* 2011), and are also supported by several studies by others, reporting occurrence of microparticles in CSF, defective INSRs in postmortem brains and insulin-signalling abnormalities in patients with schizophrenia (Johansson *et al.* 2012; Kapogiannis *et al.* 2019; Mobarrez *et al.* 2013; Wetterberg *et al.* 2002; Zhao *et al.* 2006). In conclusion, our present findings of increased antibody reactivity in CSF and serum of patients with schizophrenia found in this study are of great importance to increase the understanding of the mechanisms underlying a core group of schizophrenia cases.

ACKNOWLEDGEMENTS

This study was supported by a grant from the Foundation Tornspiran (K.M.). We also thank Affinity Proteomics SciLifeLab, Royal Institute of Technology, Stockholm, Sweden for professionally conducted antibody assessments and Professor in Neurology Tomas Olsson at Karolinska Institutet, Stockholm, Sweden for the contribution of control subjects to the study.

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