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ORIGINAL ARTICLE

Identification of a biological signature for schizophrenia in serum

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Biomarkers are now used in many areas of medicine but are still lacking for psychiatric conditions such as schizophrenia (SCZ). We have used a multiplex molecular profiling approach to measure serum concentrations of 181 proteins and small molecules in 250 first and recent onset SCZ, 35 major depressive disorder (MDD), 32 euthymic bipolar disorder (BPD), 45 Asperger syndrome and 280 control subjects. Preliminary analysis resulted in identification of a signature comprised of 34 analytes in a cohort of closely matched SCZ ($n=71$) and control ($n=59$) subjects. Partial least squares discriminant analysis using this signature gave a separation of 60–75% of SCZ subjects from controls across five independent cohorts. The same analysis also gave a separation of ~50% of MDD patients and 10–20% of BPD and Asperger syndrome subjects from controls. These results demonstrate for the first time that a biological signature for SCZ can be identified in blood serum. This study lays the groundwork for development of a diagnostic test that can be used as an aid for distinguishing SCZ subjects from healthy controls and from those affected by related psychiatric illnesses with overlapping symptoms.
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Introduction

Schizophrenia (SCZ) is a psychiatric disorder affecting up to 1% of the world population.[1] It negatively impacts on quality of life for patients and their families, and costs hundreds of billions of US dollars in healthcare provision alone.[2] Despite years of intensive investigation, the biological mechanisms underlying the pathophysiology of the disorder are still not completely understood. Advances have been made over recent decades in the diagnosis of SCZ using standardized systems and structured interviews such as DSM-IV and SCID,[3] although improvement in this area requires increased understanding of the molecular mechanisms involved in the onset and progression of the disease. This is because the accuracy of existing diagnostic methods can be confounded as subjects often show significant overlap of symptoms with those displayed by individuals affected by other psychiatric conditions such as bipolar disorder (BPD), major depressive disorder (MDD) and autism spectrum conditions (eg, Asperger syndrome). In addition, diagnosis can be hindered by frequently occurring confounding factors including substance abuse or manifestation of other medical conditions.[4] Such complications can lead to delays and inaccuracies of diagnosis which can, in turn, lead to uncertainties and further delays in designing a therapeutic strategy, resulting in a poorer outcome for the affected individuals.[5]

Recently, we described the identification of a serum-based analyte signature, capable of distinguishing SCZ from control subjects with high sensitivity and specificity.[6] Here, we have investigated the biological reproducibility of the component analytes across the same cohorts. Such biological signatures have proven crucial for increasing our understanding,

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aiding rapid clinical diagnosis and effective treatment of many disorders, but are currently lacking for psychiatric illnesses.[7] One reason for this is that it is not known whether disorders of the central nervous system can be identified by alterations in peripheral markers. However, peripheral pathways such as immune and metabolic systems can be regulated through crosstalk with the central nervous system via neuroanatomical networks, hormonal routes and molecular signaling mechanisms.[8] As there is mounting evidence for abnormalities in these pathways in SCZ,[9,10] it is likely that a distinguishing pattern of molecules in the peripheral circulation can be detected.

Here, we have used the HumanMAP Multi-Analyte Profiling platform (Supplementary Table 1) in collaboration with Rules Based Medicine (Austin, TX, USA) to analyze serum samples from 642 individuals comprised of 250 first or recent onset SCZ, 67 affective disorder (MDD and BPD), 45 Asperger syndrome and 280 control subjects, recruited from five psychiatric centres in Germany, Holland and the United Kingdom. This technology has been shown to be reproducible and robust and has already been applied successfully in numerous clinical studies and biomarker discovery projects of diseases such as epithelial ovarian cancer,[11] coronary artery disease,[12] myocardial infarction[13] and autoimmune disorders.[14]

The main objective of this study was to determine whether a robust signature for SCZ can be identified in blood serum in the early stages of the disorder. It was also of interest to determine the specificity of this signature for SCZ relative to other psychiatric conditions.

Methods

Clinical samples

The institutional ethical committees (see below) approved the protocols of the study, informed written consent was given by all participants and studies were conducted according to the Declaration of Helsinki. All diagnoses were carried out using DSM-IV and clinical tests were conducted by psychiatrists according to 'Good Clinical Practice Guidelines' to minimize variability. The demographic details are shown in Table 1. Subjects in cohorts 1–5 were independent and diagnosed as having the paranoid subtype of SCZ (295.30). The exclusive focus on the clinically well-defined paranoid SCZ, which embodies the most prevalent subtype of the illness, was intended to minimize variability arising from potential disease heterogeneity. SCZ patients featured high average scores on positive, as well as negative items of the positive and negative syndrome scale (PANSS, Table 1).[15] All SCZ patients from cohort 1, 2, 4 and 5, and 33 out of 45 patients from cohort 3 were antipsychotic-naïve at the time of sample collection. The patients in cohort 6 were acutely ill with MDD and were either antipsychotic-naïve ($n=22$) or drug free ($n=13$) for at least 6 weeks before sample collection. All patients were treated as inpatients for several weeks. The collaborating clinicians had access to all detailed clinical files including the medical history by proxy and referral letters from the general practitioners in order to verify the first episode state and drug status. Written informed consent was obtained for most patients before the collection of samples in individuals with full insight into the study objectives and obtained later in patients who were not able to give informed consent at first presentation because of impairments in their mental states. Medication was not withheld but administered after completion of diagnosis wherever possible. Patient blood was collected at time of first presentation. Samples were collected in expert centres from prodromal and early onset SCZ patients. This includes liaison with general practitioners to

Table 1 Demographics

	1 SCZ1	*2 SCZ2*	*3 SCZ3*	*4 SCZ4*	*5 SCZ5*	*6 MDD*	*7 BPD*	*8 Asperger*
Patients n	71	46	46	47	40	35	32	45
Controls n	59	46	45	40	40	40	59	50
Patients (M/F)	42/29	35/11	30/16	36/11	27/12	13/22	13/19	22/23
Controls (M/F)	31/28	35/11	27/18	33/7	26/14	26/14	31/28	26/24
Patients Age	31 ± 10	27 ± 9	35 ± 12	26 ± 8	35 ± 10	40 ± 14	34 ± 10	32 ± 9
Controls Age	30 ± 8	27 ± 9	34 ± 12	27 ± 4	36 ± 11	36 ± 11	30 ± 8	32 ± 7
Patients BMI	24 ± 5	22 ± 2	26 ± 5	na	25 ± 5	25 ± 7	25 ± 4	na
Controls BMI	23 ± 4	na	24 ± 4	na	24 ± 3	24 ± 3	23 ± 4	na
Patients Smoking (Y/N/na)	25/23/23	16/26/4	25/21/0	33/14/0	22/18/0	17/18/0	7/14/11	11/34/0
Controls Smoking (Y/N/na)	25/34/0	na	11/34/0	na	18/22/0	18/22/0	25/34/0	9/41/0
Patients Cannabis (Y/N/na)	33/22/16	15/27/4	8/38/0	23/24/0	7/33/0	0/40/0	14/7/11	2/20/23
Controls Cannabis (Y/N/na)	31/25/3	na	0/45/0	na	3/37/0	3/37/0	31/25/3	2/39/9
Medication free patients	all	all	33/45	all	all	all	4/32	36/45
PANSS positive item score	23 ± 6	18 ± 7	21 ± 5	na	23 ± 7	na	na	na
PANSS negative item score	23 ± 8	18 ± 7	22 ± 7	na	19 ± 8	na	na	na

Abbreviations: BMI, body mass index; BPD, bipolar disorder; MDD, major depressive disorder; M/F, male/female; na, not available; PANSS, positive and negative syndrome scale; SCZ, schizophrenia; Y/N, yes/no.
Values are shown as mean ± s.d. Control groups of cohorts 1 and 7, and those of cohorts 5 and 6 were identical.

avoid treatment with antipsychotics or antidepressants where clinically acceptable before referral to the specialized team, which also operated out of hours.

The subjects in cohort 7 were diagnosed as euthymic BPD type I (296.4) and type II (296.89). The individuals in cohort 8 were diagnosed as having Asperger syndrome. All subjects were matched for the indicated parameters and the medication status of each patient group is also given. Control subjects of cohorts 1 and 7, and those of cohorts 5 and 6 were identical. All controls were recruited from the geographical areas or institutes matching the respective patient populations as indicated for age, gender and social demographics. This careful matching was intended to circumvent problems arising from potential confounding factors. Controls with a family history of mental disease or with other medical conditions such as type II diabetes, hypertension, cardiovascular or autoimmune diseases were excluded from the study. SCZ, BPD, MDD and AS subjects with any of these other features were also excluded.

The cohorts used in this study were obtained from multiple centres. Cohorts 1 and 7 were from the University of Cologne, Germany (Ethical Committee of the Medical Faculty of the University of Cologne), cohort 2 from the University of Münster, Germany (Ethics Commission of the Physician Chamber Westphalia Lippe and the Medical Faculty of the Westfälische-Wilhelms University Münster), cohorts 3, 5 and 6 from the University of Magdeburg, Germany (Ethics committee of the Medical Faculty of the University of Magdeburg), cohort 4 from Erasmus University, Netherlands (Research Ethics Committee of the Erasmus Medical Centre) and cohort 8 from the Department of Psychiatry, University of Cambridge, UK (Cambridge Research Ethics Committee). Cohorts 1–5 were the same as those reported recently.[6]

Blood samples were collected from all subjects into S-Monovette 7.5 ml serum tubes (Sarstedt; Numbrecht, Germany). Serum was prepared by placing samples at room temperature for 2 h for blood coagulation, followed by centrifugation at $4000 \times g$ for 5 min. The resulting supernatants were stored at $-80\,^{\circ}C$ in low binding Eppendorf tubes (Hamburg, Germany) before analysis.

Multiplexed immunoassay

The HumanMAP multiplexed antigen immunoassay platform comprising 181 analytes was used to measure the concentrations of serum proteins and small molecules (Supplementary Table 1) in a clinical laboratory improvement amendments (CLIA)-certified laboratory at Rules Based Medicine (http://www.rulesbasedmedicine.com) as described previously.[6] Samples were randomized and blinded by code numbers until all biochemical assays were completed. Assays were calibrated using standards, raw intensity measurements converted to absolute protein concentrations by comparison with the standards, and performance was verified using quality control samples. Data analyses were carried out using the statistical software package R (http://www.r-project.org). The protocol for the study participants, clinical samples and test methods was carried out in compliance with the Standards for Reporting of Diagnostic Accuracy (STARD) initiative.[16]

Data analyses

Kolmogorov–smirnov analysis showed that the majority of the investigated analytes were non-normally distributed. Therefore, non-parametric, two-tailed Wilcoxon rank-sum tests were carried out to identify significant expression differences between patients and controls. In addition, correlation analyses indicated that several analytes were significantly associated with gender, age, BMI, smoking or cannabis consumption. Analysis of covariance (ANCOVA) was, therefore, carried out on log-transformed data to assess the effect of these demographic variables on the significance of identified marker candidates. *P*-values of less than 0.05 were considered to indicate statistical significance. The false discovery rate was controlled according to Benjamini and Hochberg.[17] Multivariate analysis was carried out using SIMCA P+ 10.5 (Umetrics; Umea, Sweden) for partial least squares discriminant analysis (PLS-DA) to visualize any separation between patient and control subjects as indicated. This technique was used to visually display the relationship between the combined set of analytes of interest and the disease state. The PLS model consists of PLS components that are linear combinations of the analytes used as input. As these linear combinations are chosen to contain the highest information content that is correlated to the disease state, the dimensionality of the multi-dimensional input space can be reduced to fewer dimensions. The PLS scores plots display each investigated subject in the space of reduced, two-dimensional space of PLS components. PLS weights indicate the contribution of each analyte to the score in the direction of a given PLS component.

Results

Identification of preliminary SCZ biomarker signature

The first stage of the study was aimed at identification of differentially expressed serum analytes in a single cohort of first onset anti-psychotic naive SCZ subjects and well-matched controls using the HumanMAP analysis. Cohort 1 was chosen for this analysis as this group was comprised of SCZ ($n = 71$) and controls ($n = 59$) who were matched for age, gender, body mass index, smoking, cannabis consumption and date of sample collection (Table 1). This analysis resulted in identification of 34 analytes, which were altered significantly in SCZ compared with control subjects using unpaired two-tailed Wilcoxon rank-Sum tests and remained significant after adjusting for covariates using ANCOVA (Table 2, Supplementary Table 2). The majority of these analytes were also significant after controlling the false discovery rate. Analytes

Table 2 Identification of differentially expressed serum analytes in cohort 1 (SCZ 1) subjects using HumanMAP analysis

Analyte	P–*value*	q–*value*	*FC*	*Unit*	*SCZ (mean ± s.d.)*	*NC (mean ± s.d.)*	*Weight*
α 1 Antitrypsin (a1AT)	0.005*	0.032	1.08	mg ml^{-1}	2.35 ± 0.42	2.17 ± 0.58	0.11
α 2 Macroglobulin (A2M)	< 0.001	0.001	1.21	mg ml^{-1}	1.4 ± 0.34	1.16 ± 0.32	0.21
Angiopoietin 2 (ANG2)	< 0.001	0.008	1.33	ng ml^{-1}	2.18 ± 0.97	1.67 ± 0.77	0.18
BDNF	0.004*	0.027	0.87	ng ml^{-1}	24.77 ± 8.84	28.54 ± 7.27	–0.14
Betacellulin	< 0.001	0.012	1.93	pg ml^{-1}	66.15 ± 38.27	55.15 ± 28.3	0.21
BMP6	< 0.001	0.007	2.02	ng ml^{-1}	1.7 ± 1.27	1.37 ± 0.87	0.17
CEA	0.001	0.002	1.75	ng ml^{-1}	1.78 ± 1.39	1.02 ± 0.62	0.21
CD40L	0.027	0.012	0.64	ng ml^{-1}	1.41 ± 1.24	2.16 ± 1.49	–0.17
Cortisol	0.003	0.036	1.14	ng ml^{-1}	118.24 ± 32.77	103.88 ± 35.94	0.13
CTGF	0.003	0.046	1.17	ng ml^{-1}	3.97 ± 1.15	3.4 ± 1	0.16
EGF	< 0.001	< 0.001	0.49	pg ml^{-1}	97.01 ± 93.91	176.97 ± 122.32	–0.25
Eotaxin 3	0.002	0.029	2.12	pg ml^{-1}	77.39 ± 71.8	40.63 ± 30.26	0.16
Factor VII	0039*	0.144	0.87	ng ml^{-1}	498.34 ± 131.34	573.08 ± 190.63	–0.14
FSH	0.001	0.062	2.41	ng ml^{-1}	0.71 ± 1.23	0.31 ± 0.2	0.14
GM-CSF	0.002	0.109	0.91	pg ml^{-1}	10.13 ± 14.06	7.69 ± 3.16	–0.02
GST	< 0.001*	< 0.001	1.30	ng ml^{-1}	1.06 ± 0.25	0.81 ± 0.21	0.30
Haptoglobin (HPT)	< 0.001*	0.002	1.68	mg ml^{-1}	1.44 ± 0.91	0.84 ± 0.58	0.22
ICAM 1	0.001	0.149	0.94	ng ml^{-1}	121.7 ± 41.92	128.8 ± 17.79	–0.07
IGFBP 2	0.045*	0.149	1.22	ng ml^{-1}	52.9 ± 27	43.29 ± 19.51	0.12
IL10	< 0.001	< 0.001	1.21	pg ml^{-1}	15.6 ± 5.91	12.87 ± 2.14	0.18
IL17	< 0.001	< 0.001	1.62	pg ml^{-1}	14.48 ± 7.85	9.42 ± 3.36	0.25
IL5	0.039	0.010	0.72	pg ml^{-1}	4.48 ± 2.82	5.59 ± 2.19	–0.14
LH	< 0.001	0.015	1.66	ng ml^{-1}	0.15 ± 0.14	0.09 ± 0.05	0.17
MIF	0.024	0.149	1.72	ng ml^{-1}	0.18 ± 0.28	0.1 ± 0.08	0.11
NrCAM	0.001	0.149	0.83	ng ml^{-1}	0.5 ± 0.33	0.58 ± 0.32	–0.09
PP	< 0.001	0.008	1.64	pg ml^{-1}	140.15 ± 145.45	85.34 ± 132.41	0.12
PAP	0.001	0.036	0.82	ng ml^{-1}	0.28 ± 0.1	0.34 ± 0.12	–0.16
RANTES (C-C motif chemokine 5)	0.005	0.121	1.17	ng ml^{-1}	22.14 ± 8.6	18.95 ± 8.88	0.11
Resistin	0.007	0.027	0.80	ng ml^{-1}	0.58 ± 0.3	0.72 ± 0.31	–0.14
SGOT	0.008	0.005	1.25	ug ml^{-1}	10.64 ± 3.86	8.51 ± 2.62	0.19
Sortilin	< 0.001	< 0.001	0.76	ng ml^{-1}	6.49 ± 2.15	8.59 ± 3.03	–0.24
SCF	0.033	0.149	0.93	pg ml^{-1}	389.14 ± 126.53	419.86 ± 100.94	–0.08
Thrombopoietin (TPO)	0.004	0.005	0.84	ng ml^{-1}	3.74 ± 1.1	4.44 ± 1.14	–0.19
Thrombospondin 1 (TSP1)	0.014	0.002	0.82	ng ml^{-1}	43264.8 ± 13882.9	52976.3 ± 11720.8	–0.22

Abbreviations: BDNF, brain-derived neurotrophic factor; BMP6, bone morphogenic protein 6; CEA, carcinoembryonic antigen; CD40L, CD40 ligand; CTGF, connective tissue growth factor; EGF, epidermal growth factor; FC, fold change; FSH, follicle stimulating hormone; GM-CSF, granulocyte macrophage colony stimulating factor; GST, glutathione S transferase; IGFBP2, insulin-like growth factor binding protein 2; IL, Interleukin; LH, luteinizing hormone; MIF, macrophage migration inhibitory factor; NC, normal control; PP, pancreatic polypeptide; PAP, prostatic acid phosphatise; SCF, stem cell factor; SGOT, serum glutamic oxaloacetic transaminase.
P-values were calculated using ANCOVA based on log-transformed data from SCZ patients and controls in cohort 1 (gender, age, BMI, smoking and cannabis consumption used as covariates), *Diagnosis-covariate interaction was significant and *P*-value was determined using non-parametric Wilcoxon rank-sum test instead.
q values represent FDR adjusted *P*-values derived from Wilcoxon rank-sum tests comparing SCZ patients and controls in cohort 1.
The weight represents the PLS weight for the first latent variable of a PLS model comparing SCZ and controls in cohort 1. FC denotes average intensity of analyte in SCZ divided by the average intensity in controls of cohort 1.

showing the highest magnitude of fold changes were betacellulin, bone morphogenic protein 6 (BMP6), eotaxin 3, follicle stimulating hormone (FSH) and epidermal growth factor (EGF), which were all altered by approximately twofold in SCZ compared with control subjects. To account for the possibility that the findings were related to stress, we used cortisol as a covariate for ANCOVA. Six analytes were nonsignificant after this adjustment (Eortaxin 3, $P = 0.051$; Factor VII, $P = 0.067$; ICAM 1, $P = 0.070$; IGFBP2, $P = 0.052$; IL5, $P = 0.070$ and SCF, $P = 0.105$), although all of these analytes showed a trend towards significance. For added confirmation of the results, the same serum samples were re-assayed ~3 months after the first analysis. This repeat analysis showed good reproducibility of the results with an average correlation across all analytes of 0.81 (Pearson's correlation coefficient) and 50% of the analytes had a correlation greater than 0.90 (data not shown).

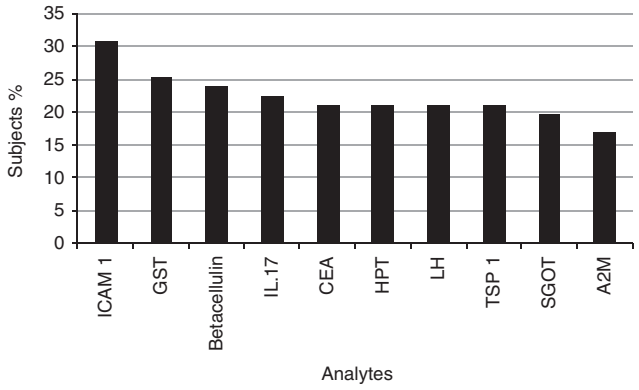

Figure 1 Biomarkers changed in more than 15% of individual patients. The y axis indicates the percentage of subjects, in which these biomarkers were altered in cohort 1. Differential expression was determined by identifying biomarkers that showed measurements varying by more than 2 s.d. in individual schizophrenia (SCZ) patients compared with the mean control value in the same cohort. The abbreviations are as indicated in the legend for Table 2.

We also determined the proportion of subjects in whom these biomarkers were altered across in cohort 1. In this case differential expression of a biomarker in a subject was indicated, if the measurement varied by more than 2 s.d. compared with that of the mean control measurement in the same cohort. Using these criteria, ICAM 1, glutathione S transferase (GST), Betacellulin and interleukin 17 (IL17) were altered in the highest proportion of subjects (Figure 1).

Validation of SCZ signature in other cohorts

One factor that renders diagnosis of SCZ imprecise is the heterogeneous nature of the disease and the overlap of SCZ symptoms with those of other psychiatric conditions.[18] In the next phase of the study, the 34 differentially expressed analytes identified in cohort 1 were tested as a combined panel using samples from SCZ and control subjects in the first three cohorts (SCZ1, SCZ2 and SCZ3) and in cohorts 7 (BPD) and 8 (Asperger syndrome), as these were profiled using HumanMAP analysis at the same time. PLS-DA was used to visualize any separation between patient and control subjects. This showed the 34-analyte panel resulted in a separation of SCZ patients from controls by 40–85% across cohorts 1–3 (Figure 2a). For comparison, euthymic BPD patients were tested as such patients can experience disruptions in cognitive behaviors as seen in SCZ.[19] Asperger syndrome subjects (cohort 8) were analyzed as they can also show overlap with SCZ in display of such symptoms as emotional lability, anxiety and poor social functioning.[20] In contrast to SCZ, the signature resulted in little or no separation of BPD patients (Figure 2b) or Asperger syndrome subjects (Figure 2c) from the respective controls. The analytes most important for the separation achieved in cohort 1 were GST, sortilin, IL17, CEA, EGF, TSP1, HPT, A2M, Betacellulin, SGOT, TPO and ANG2 in descending order (Figure 2d). It should be noted that the separation achieved in SCZ1 reflects the training performance of the multivariate model and is positively biased, as data from this cohort were used to establish the initial 34-analyte signature.

In addition, the 34-analyte panel was trained on cohort 4 (SCZ4) and tested on cohorts 5 (SCZ5) and 6 (MDD), as these samples were subjected to HumanMAP analysis at the same time. Classification of subjects using the panel showed a separation of 60–75% of SCZ patients from control subjects in cohorts 4 and 5 (Figure 3a). As before, the separation displayed for cohort 4 (SCZ4) represents the training performance of the multivariate model. MDD subjects were chosen for the comparative disease analysis because of the overlap of negative symptoms between this disorder and SCZ.[21] Approximately 50% of MDD patients also showed a separation from controls (Figure 3b). This suggested that the panel may not be entirely specific for SCZ. The most significant analytes for the separation achieved in cohort 4 were cortisol, resistin, PP, NrCAM, MIF, A1AT, GST, HPT and ICAM 1 in descending order (Figure 3c).

An example of the biological profile of HPT is given that shows significant alterations in all of the SCZ cohorts and no change in the any of the non-SCZ conditions (Figure 4). These findings show that some components of the 34-analyte biomarker panel were relatively specific for SCZ.

As a final step, we investigated the ability of the 34-analyte panel to discriminate between SCZ and the differential diagnosis groups collected at the respective clinical sites directly. We found a sensitivity and specificity of 86 and 78% when comparing SCZ (cohort 1) against BPD (cohort 7), sensitivity and specificity of 87 and 94% when comparing against MDD (cohort 2 vs cohort 6) and a sensitivity and specificity of 96 and 96% when comparing against Asperger's syndrome (cohort 1 vs cohort 8), respectively.

Discussion

These results demonstrate that a reproducible biological signature for SCZ can be identified in blood serum. One strong point of the current investigation is that samples were obtained from first onset antipsychotic naïve subjects who were well matched with controls for factors such as age, gender, substance abuse and lifestyle. This was an important consideration to maximize the capability of identifying molecular biomarkers associated with the disease and minimizes the chances of identifying those associated with potential confounding factors. Most previous SCZ studies have investigated chronic patients who have been treated with antipsychotic medications and who often have multiple co-morbidities, which can confound the results of biomarker investigations. Domenici *et al*.[22] recently described the identification of SCZ and MDD biomarkers, although the majority of the samples used for this study came from treated

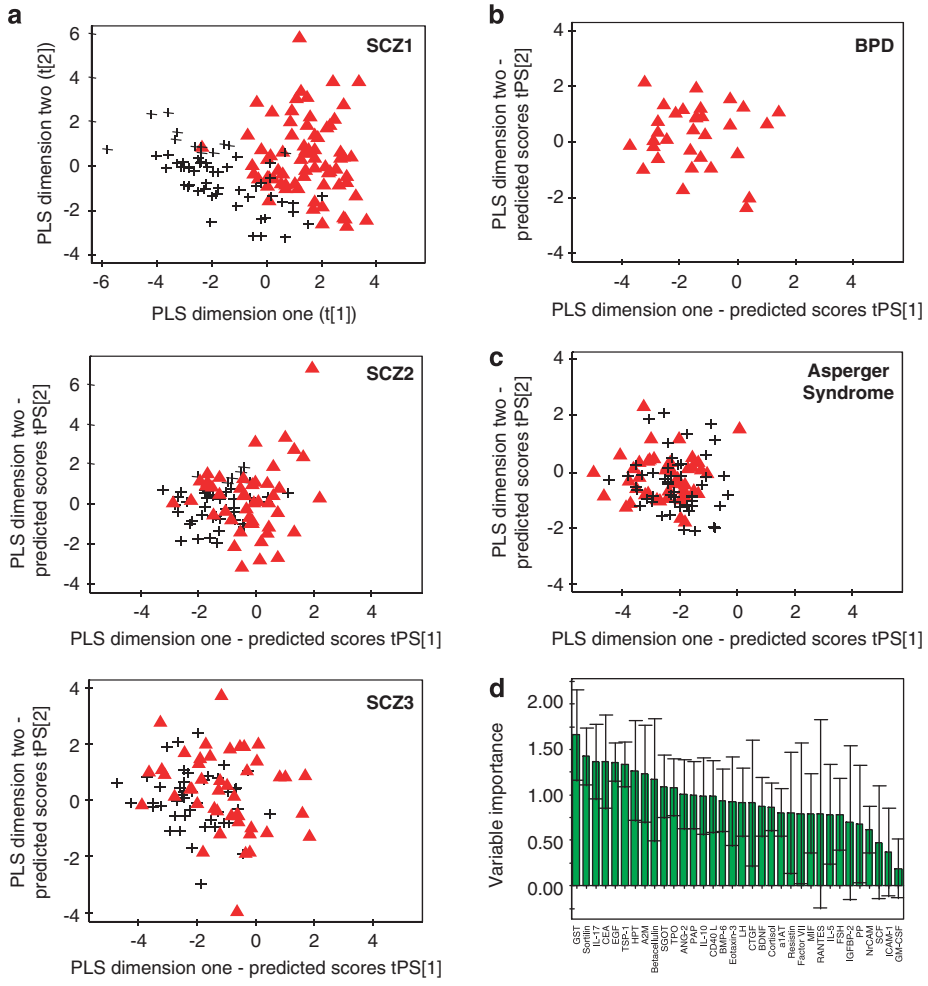

Figure 2 Partial Least Squares-Discriminant Analysis (PLS-DA) of schizophrenia (SCZ), bipolar disorder (BPD) and Asperger syndrome subjects. PLS-DA using the 34 serum analytes identified as differentially expressed in cohort 1 (SCZ1; see Table 2). Serum samples for SCZ1, SCZ2, SCZ3, BPD and Asperger syndrome were analyzed at the same time using the HumanMAP platform. In all, 34 analytes were identified as differentially expressed in SCZ and these were combined as a single SCZ panel. (**a**) The 34-analyte panel was trained on cohort 1 (SCZ1) and then tested blindly on cohorts 2 and 3 (SCZ2 and SCZ3) using PLS-DA. The red triangles indicate true SCZ patients and the plus symbols indicate the true controls. The gray enclosure approximates the position of the majority of the control population in SCZ 1 and this was maintained for all other cohorts as a reference. The 34-analyte panel was also tested on (**b**) euthymic BPD patients (red triangles; cohort 7) and (**c**) Asperger syndrome subjects (red triangles; controls = plus symbols; cohort 8). (**d**) The histogram shows the relative contribution of each analyte to the separation achieved in SCZ1. The values are the variable importance in the projection (VIP, determined by SIMCA-P + software) and the corresponding confidence interval based on a jack-knife procedure. The abbreviations of analytes are as listed in Table 2.

subjects. Studies involving first onset antipsychotic naïve patients are scarce because of the fact that even large psychiatric centres can only enlist around 20–30 of these subjects each year, and few centres follow strict standard operating procedures for collection of samples. We have overcome this problem by obtaining samples from first onset antipsychotic naïve and minimally treated SCZ patients from multiple clinical centres over a 10-year time span. All of the patients and controls underwent extensive clinical characterization, and sera were collected and stored according to strict standard operating procedures. In addition, all protocols involving clinical subjects, samples and test measurements were carried out in compliance with the Standards for Reporting of Diagnostic Accuracy (STARD) initiative[16] to maximize reliability and accuracy of the results.

Many of the proteins and small molecules identified as SCZ biomarkers have been implicated previously in acute or chronic inflammatory conditions, endothelial cell dysfunction, cardiovascular disease, type 2 diabetes mellitus and metabolic disorder.[23–25] This may be important as recent studies have explored the possibility of using either anti-inflammatory agents such as cyclo-oxygenase-2 (COX-2) inhibitors[26] or anti-diabetic compounds such as insulin-sensitizing agents[27] for improving cognitive deficits or other symptoms of SCZ. In this regard, a

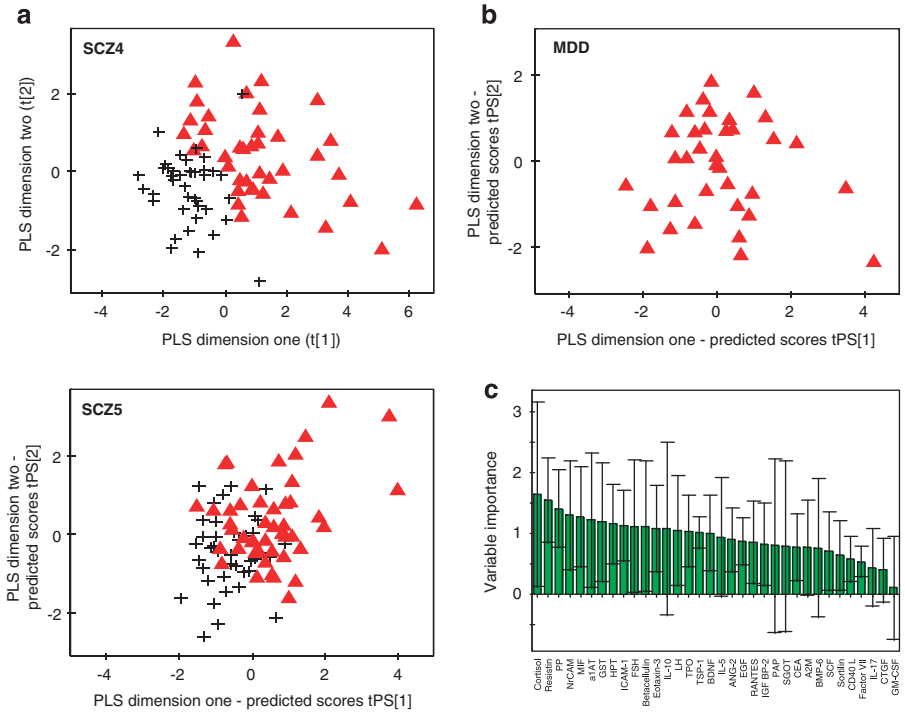

Figure 3 Partial Least Squares-Discriminant Analysis (PLS-DA) of schizophrenia (SCZ) and major depressive disorder (MDD) subjects. PLS-DA using the 34 serum analytes identified as differentially expressed in cohort 1 (SCZ1; see Table 2). Serum samples from SCZ4, SCZ5 and MDD subjects were analyzed at the same time using the HumanMAP platform. (**a**) The 34-analyte panel was trained on cohort 4 (SCZ4) and then tested blindly on cohort 5 (SCZ5) using PLS-DA. The red triangles indicate true SCZ patients and the plus symbols indicate the true controls. The gray enclosure approximates the position of the majority of the control population identified for cohort 4 (SCZ4). The 34-analyte panel was also tested on (**b**) MDD patients (red triangles; cohort 6). (**c**) The histogram shows the relative contribution of each analyte to the separation achieved in SCZ4 (VIP plot, see legend of Figure 2). The abbreviations of analytes are as listed in Table 2.

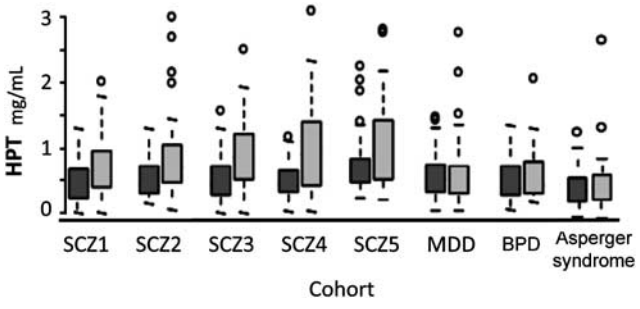

Figure 4 Altered expression of haptoglobin (HPT) across schizophrenia (SCZ) cohorts. Expression profile changes of HPT in patient and control populations across the SCZ and non-SCZ cohorts. The expression levels are given as box plots for patients (orange) and controls (blue).

recent study has demonstrated that administration of the insulin-sensitizing agent pioglitazone resulted in increased localized cerebral blood flow in Alzheimer's disease patients and significant improvement in cognitive testing.[28] Such compounds have only been tested as an adjunct to anti-psychotic treatment with the aim of minimizing the frequently occurring metabolic side effects.[29,30] Thus far, they have not been tested for any benefits on cognition or psychopathology as a monotherapy.

Altered inflammatory response or dysregulation of the adrenal cortex hormone cortisol has been associated with a number of psychiatric disorders.[31] We found a significant increase in cortisol levels across all SCZ cohorts and a nonsignificant trend for increase across all other non-SCZ cohorts ($P = 0.188$–0.268). Hypercortisolemia and hypothalamic–pituitary–adrenal hyperactivity may also be linked to the increased levels of MIF in the SCZ cohorts. MIF has been shown to have a central role in the progression of immunological disturbances associated with atherosclerotic plaque development,[32,33] oxidative stress and endothelial cell dysfunction.[34] Inflammatory disorders and SCZ also share an increased prevalence of insulin resistance, metabolic syndrome and type 2 diabetes.[35–38] This has been supported by our recent studies showing hyperinsulinaemia and insulin resistance in a subset of first onset antiosychotic naïve SCZ subjects,[27,39] and by our findings of abnormalities in glucose regulation and brain vasculature in *postmortem* SCZ brain tissue.[9,27,40]

Recent studies have demonstrated ultrastructural abnormalities of capillaries and the pericapillary environment, supporting the concept that blood–brain barrier dysfunction might contribute to the pathogenesis of SCZ.[41] The effects on brain endothelial cells are known to be mediated by alterations in

prostaglandin signaling. It is interesting in this regard that we identified alterations in endothelial cell biomarkers including endothelin 1 and CTGF as part of the SCZ signature. In addition, it has been known for decades that niacin can induce a visible skin flush response caused by prostaglandin-mediated vasodilatation.[42,43] Recent studies have demonstrated that the attenuated skin flushing in response to niacin administration in SCZ subjects may be secondary to the increased oxidative stress, alterations of nonspecific immune-response or inflammation-like processes.[44] Taken together, these findings suggest that conditions such as inflammation and metabolic disorder may be converging pathophysiological processes in SCZ and potentially other psychiatric and non-psychiatric disorders.[45]

In summary, the present biomarker signature has provided potential additional insights into the biological pathways underlying the onset or development of SCZ. In addition, the signature also shows potential in the development of a test for distinguishing SCZ patients from controls and from subjects with other psychiatric disorders. However, some components of the signature were also present in non-SCZ conditions including MDD and BPD, indicating that further work is required to complete and improve the performance of the panel for specific identification of SCZ. Incorporation of additional analytes targeting inflammatory, hormonal, metabolic and neurotrophic pathways in larger population studies, may lead to further improvements for identification of SCZ and provide improved classification of this disorder, which is known to be comprised of overlapping subtypes.[18] Furthermore, incorporation of analytes that are altered in other psychiatric conditions and that are not altered in SCZ, may improve the differential diagnostic capabilities of the panel.[6] Also, it will be important to investigate whether the discovered biomarker candidates reflect the state or the trait of the disorder. This time-dependent change of the biomarker signature will be assessed in future, longitudinal studies.

These results highlight the importance of evaluating biomarkers in larger studies with explicit assessment of the ability to classify subjects. The future success of biomarker strategies may depend on the discovery of new molecules to complement the most robust existing biomarkers, perhaps with the help of state-of-the-art targeted and non-targeted approaches. In addition, it should be noted that tests for disorders with a low incidence such as SCZ require exceptionally high specificities, if used in the general population. For this reason, the most effective use of such tests would be as a confirmatory diagnostic aid by a psychiatric specialist in conjunction with a clinical assessment. In this way, the test would be used in populations already enriched for SCZ, with the purpose of establishing and confirming a diagnosis more rapidly as compared with the requirement for 6-months duration of continuous symptoms using the current DSM IV-based diagnosis. Such an application of a biomarker test would help to initiate treatment of patients more rapidly and, therefore, reduce the duration of untreated psychosis and, in turn, improve patient outcomes.[46] This would be an important breakthrough by helping clinical psychiatrists to identify vulnerable patients early in the disease process, allowing for earlier or even preventative therapeutic intervention.

Conflict of interest

The authors declare no conflict of interest.

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