



Identification of a Biological Signature for Schizophrenia in Serum

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► To cite this version:

Sabine Bahn, Emanuel Schwarz, Paul Guest, Hassan Rahmoune, Laura Harris, et al.. Identification of a Biological Signature for Schizophrenia in Serum. *Molecular Psychiatry*, 2011, 10.1038/mp.2011.42 . hal-00631258

HAL Id: hal-00631258

<https://hal.science/hal-00631258>

Submitted on 12 Oct 2011

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1 Identification of a Biological Signature for 2 Schizophrenia in Serum

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43 **Abstract**

44 Biomarkers are now used in many areas of medicine but are still lacking for psychiatric conditions
45 such as schizophrenia (SCZ). We have used a multiplex molecular profiling approach to measure
46 serum concentrations of 181 proteins and small molecules in 250 first and recent onset SCZ, 35
47 major depressive disorder (MDD), 32 euthymic bipolar disorder (BPD), 45 Asperger syndrome and
48 280 control subjects. Preliminary analysis resulted in identification of a signature comprised of 34
49 analytes in a cohort of closely-matched SCZ (n=71) and control (n=59) subjects. Partial least squares
50 discriminant analysis using this signature gave a separation of 60-75% of SCZ subjects from controls
51 across 5 independent cohorts. The same analysis also gave a separation of approximately 50% of
52 MDD patients and 10-20% of BPD and Asperger syndrome subjects from controls. These results
53 demonstrate for the first time that a biological signature for SCZ can be identified in blood serum. This
54 study lays the groundwork for development of a diagnostic test that can be used as an aid for
55 distinguishing SCZ subjects from healthy controls and from those affected by related psychiatric
56 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 U.S. 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 (e.g. 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, 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 signalling 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 (**Supp. 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 5 psychiatric centres in Germany, Holland and the UK. This technology has been shown to be reproducible and robust and has already been applied successfully in numerous clinical studies or 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 performed 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 schizophrenia, which embodies the most prevalent subtype of the illness, was intended to minimize variability arising from potential disease heterogeneity. Schizophrenia 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 prior to 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 prior to 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 due to 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 schizophrenia patients. This includes liaison with general practitioners to avoid treatment with antipsychotics or antidepressants where clinically acceptable prior to referral to the specialised 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. Schizophrenia, 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 7.5mL serum tubes (Sarstedt; Numbrecht, Germany). Serum was prepared by placing samples at room temperature for 2 hours for blood coagulation, followed by centrifugation at 4,000 X g for 5 minutes. The resulting supernatants were stored at -80°C in Low Binding Eppendorf tubes (Hamburg, Germany) prior to analysis.

MULTIPLEXED IMMUNOASSAY

The HumanMAP[®] multiplexed antigen immunoassay platform comprising 181 analytes was used to measure the concentrations of serum proteins and small molecules (**Supp. Table 1**) in a Clinical Laboratory Improvement Amendments (CLIA)-certified laboratory at Rules Based Medicine (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 to the standards, and performance was verified using quality control samples. Data analyses were performed 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. Since 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 since 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 to control subjects using unpaired two-tailed Wilcoxon Rank Sum tests and remained significant after adjusting for covariates using ANCOVA (**Table 2**, **Supp. Table 2**). The majority of these analytes were also significant after controlling the false discovery rate. Analytes showing the highest magnitude 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 2-fold in SCZ compared to 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 non-significant after this adjustment (Eotaxin 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 approximately 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).

We also determined the proportion of subjects in which 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 two standard deviations compared to that of the mean control measurement in the same cohort. Using these criteria, ICAM 1, GST, Betacellulin, GST and IL 17 were altered in the highest proportion of subjects (**Figure 1**).

Validation of SCZ signature in other cohorts

One factor which 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 3 cohorts (SCZ1, SCZ2 and SCZ3) and in cohorts 7 (BPD) and 8 (Asperger syndrome) since 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 behaviours as seen in SCZ (19). Asperger syndrome subjects (cohort 8) were analyzed since 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) since 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 due to the overlap of negative symptoms between this disorder and schizophrenia (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 which 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 was relatively specific for SCZ.

As a final step, we investigated the ability of the 34 analyte panel to discriminate between schizophrenia and the differential diagnosis groups collected at the respective clinical sites directly. We found a sensitivity and specificity of 86% and 78% when comparing schizophrenia (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 minimize 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 recently described the identification of SCZ and MDD biomarkers, although the majority of the samples used for this study came from treated subjects (22). Studies involving first onset antipsychotic naïve patients are scarce due to 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 II 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 cyclooxygenase-2 (COX-2) inhibitors (26) or anti-diabetic compounds such as insulin-sensitizing agents (27) for improving cognitive deficits or other symptoms of schizophrenia. In this regard, a 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 non-significant 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 play 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 schizophrenia also share an increased prevalence of insulin resistance, metabolic syndrome and type II diabetes (35-38). This has been supported by our recent studies showing hyperinsulinaemia and insulin resistance in a subset of first onset antipsychotic naïve SCZ subjects (27, 39), and by our findings of abnormalities in glucose regulation and brain vasculature in *post-mortem* 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 signalling. 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 which are altered in other psychiatric conditions and which 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-dependant 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 schizophrenia with the purpose of establishing and confirming a diagnosis more rapidly, as compared to 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.

REFERENCES

1. Saha S, Chant D, Welham J, McGrath J. A systematic review of the prevalence of schizophrenia. *PLoS Med* 2005 May; **2**(5): e141.
2. National Institute of Mental Health. (<http://www.nimh.nih.gov/>), last updated 15 February 2008.
3. First MB, Spitzer RL, Gibbon M, Janet BW. *Structured Clinical Interview for DSM-IV Axis I Disorders, Clinician Version (SCID-CV)*. American Psychiatric Press, Inc: Washington, D.C, 1996.
4. Csernansky JG, Schuchart EK. Relapse and rehospitalisation rates in patients with schizophrenia: effects of second generation antipsychotics. *CNS Drugs* 2002; **16**(7): 473-484.
5. Moller HJ. Course and long-term treatment of schizophrenic psychoses. *Pharmacopsychiatry* 2004 Nov; **37 Suppl 2**: 126-135.
6. Schwarz E, Izmailov R, Spain M, Barnes A, Mapes JP, Guest PC *et al*. Validation of a blood-based laboratory test to aid in the confirmation of a diagnosis of schizophrenia. *Biomark Insights*; **5**: 39-47.
7. Amur S, Frueh FW, Lesko LJ, Huang S-M. Integration and use of biomarkers in drug development, regulation and clinical practice: a US regulatory perspective. *Biomarkers Med* 2008; **2**(3): 305-311.
8. Eskandari F, Webster JI, Sternberg EM. Neural immune pathways and their connection to inflammatory diseases. *Arthritis Res Ther* 2003; **5**(6): 251-265.
9. Harris LW, Wayland M, Lan M, Ryan M, Giger T, Lockstone H *et al*. The cerebral microvasculature in schizophrenia: a laser capture microdissection study. *PLoS One* 2008; **3**(12): e3964.
10. Muller N, Schwarz MJ. Neuroimmune-endocrine crosstalk in schizophrenia and mood disorders. *Expert Rev Neurother* 2006 Jul; **6**(7): 1017-1038.
11. Bertenshaw GP, Yip P, Sessaiah P, Zhao J, Chen TH, Wiggins WS *et al*. Multianalyte profiling of serum antigens and autoimmune and infectious disease molecules to identify biomarkers dysregulated in epithelial ovarian cancer. *Cancer Epidemiol Biomarkers Prev* 2008 Oct; **17**(10): 2872-2881.
12. Gurbel PA, Kreutz RP, Bliden KP, DiChiara J, Tantry US. Biomarker analysis by fluorokine multianalyte profiling distinguishes patients requiring intervention from patients with long-term quiescent coronary artery disease: a potential approach to identify atherosclerotic disease progression. *Am Heart J* 2008 Jan; **155**(1): 56-61.

- 458
- 459 13. Escobar GP, Lindsey ML. Multi-Analyte Profiling of Post-Myocardial Infarction Plasma
460 Samples. *FASEB J* 2007; **21**(746.11).
- 461
- 462 14. Delaleu N, Immervoll H, Cornelius J, Jonsson R. Biomarker profiles in serum and saliva of
463 experimental Sjogren's syndrome: associations with specific autoimmune manifestations.
464 *Arthritis Res Ther* 2008; **10**(1): R22.
- 465
- 466 15. Kay SR, Fiszbein A, Opler LA. The positive and negative syndrome scale (PANSS) for
467 schizophrenia. *Schizophr Bull* 1987; **13**(2): 261-276.
- 468
- 469 16. Bossuyt PM, Reitsma JB, Bruns DE, Gatsonis CA, Glasziou PP, Irwig LM *et al.* Towards
470 complete and accurate reporting of studies of diagnostic accuracy: the STARD initiative.
471 Standards for Reporting of Diagnostic Accuracy. *Clin Chem* 2003 Jan; **49**(1): 1-6.
- 472
- 473 17. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful
474 approach to multiple testing. *J Roy Statist Soc Ser B* 1995; **57**: 289-300.
- 475
- 476 18. Seaton BE, Goldstein G, Allen DN. Sources of heterogeneity in schizophrenia: the role of
477 neuropsychological functioning. *Neuropsychol Rev* 2001 Mar; **11**(1): 45-67.
- 478
- 479 19. Ferrier IN, Stanton BR, Kelly TP, Scott J. Neuropsychological function in euthymic patients
480 with bipolar disorder. *Br J Psychiatry* 1999 Sep; **175**: 246-251.
- 481
- 482 20. Raja M, Azzoni A. Asperger's disorder in the emergency psychiatric setting. *Gen Hosp*
483 *Psychiatry* 2001 Sep-Oct; **23**(5): 285-293.
- 484
- 485 21. Fleischhacker W. Negative symptoms in patients with schizophrenia with special reference
486 to the primary versus secondary distinction. *Encephale* 2000 Oct; **26 Spec No 1**: 12-14.
- 487
- 488 22. Domenici E, Wille DR, Tozzi F, Prokopenko I, Miller S, McKeown A *et al.* Plasma protein
489 biomarkers for depression and schizophrenia by multi analyte profiling of case-control
490 collections. *PLoS One*; **5**(2): e9166.
- 491
- 492 23. Fessel WJ, Solomon GF. Psychosis and systemic lupus erythematosus: a review of the
493 literature and case reports. *Calif Med* 1960 Apr; **92**: 266-270.
- 494
- 495 24. Goldberg RB. Cytokine and cytokine-like inflammation markers, endothelial dysfunction, and
496 imbalanced coagulation in development of diabetes and its complications. *J Clin Endocrinol*
497 *Metab* 2009 Sep; **94**(9): 3171-3182.
- 498
- 499 25. Volp AC, Alfenas Rde C, Costa NM, Minim VP, Stringueta PC, Bressan J. [Inflammation
500 biomarkers capacity in predicting the metabolic syndrome]. *Arq Bras Endocrinol Metabol*
501 2008 Apr; **52**(3): 537-549.
- 502
- 503 26. Muller N, Riedel M, Schwarz MJ, Engel RR. Clinical effects of COX-2 inhibitors on cognition in
504 schizophrenia. *Eur Arch Psychiatry Clin Neurosci* 2005 Apr; **255**(2): 149-151.
- 505
- 506 27. Guest P, Wang L, Harris L, Burling K, Levin Y, Ernst A *et al.* Increased levels of circulating
507 insulin-related peptides in first onset, antipsychotic naïve schizophrenia patients. *Molecular*
508 *psychiatry (in press)*.

- 509
- 510 28. Sato T, Hanyu H, Hirao K, Kanetaka H, Sakurai H, Iwamoto T. Efficacy of PPAR-gamma agonist
511 pioglitazone in mild Alzheimer disease. *Neurobiol Aging* 2009 Nov 16.
- 512
- 513 29. Baptista T, Rangel N, Fernandez V, Carrizo E, El Fakih Y, Uzcategui E *et al.* Metformin as an
514 adjunctive treatment to control body weight and metabolic dysfunction during olanzapine
515 administration: a multicentric, double-blind, placebo-controlled trial. *Schizophr Res* 2007 Jul;
516 **93**(1-3): 99-108.
- 517
- 518 30. Henderson DC, Fan X, Sharma B, Copeland PM, Borba CP, Boxill R *et al.* A double-blind,
519 placebo-controlled trial of rosiglitazone for clozapine-induced glucose metabolism
520 impairment in patients with schizophrenia. *Acta Psychiatr Scand* 2009 Jun; **119**(6): 457-465.
- 521
- 522 31. Rybakowski JK, Wykretowicz A, Heymann-Szlachcinska A, Wysocki H. Impairment of
523 endothelial function in unipolar and bipolar depression. *Biol Psychiatry* 2006 Oct 15; **60**(8):
524 889-891.
- 525
- 526 32. Santos LL, Morand EF. Macrophage migration inhibitory factor: a key cytokine in RA, SLE and
527 atherosclerosis. *Clin Chim Acta* 2009 Jan; **399**(1-2): 1-7.
- 528
- 529 33. Burger-Kentischer A, Goebel H, Seiler R, Fraedrich G, Schaefer HE, Dimmeler S *et al.*
530 Expression of macrophage migration inhibitory factor in different stages of human
531 atherosclerosis. *Circulation* 2002 Apr 2; **105**(13): 1561-1566.
- 532
- 533 34. Bruchfeld A, Carrero JJ, Qureshi AR, Lindholm B, Barany P, Heimbürger O *et al.* Elevated
534 serum macrophage migration inhibitory factor (MIF) concentrations in chronic kidney
535 disease (CKD) are associated with markers of oxidative stress and endothelial activation. *Mol*
536 *Med* 2009 Mar-Apr; **15**(3-4): 70-75.
- 537
- 538 35. Chung CP, Avalos I, Oeser A, Gebretsadik T, Shintani A, Raggi P *et al.* High prevalence of the
539 metabolic syndrome in patients with systemic lupus erythematosus: association with disease
540 characteristics and cardiovascular risk factors. *Ann Rheum Dis* 2007 Feb; **66**(2): 208-214.
- 541
- 542 36. Wajed J, Ahmad Y, Durrington PN, Bruce IN. Prevention of cardiovascular disease in systemic
543 lupus erythematosus--proposed guidelines for risk factor management. *Rheumatology*
544 *(Oxford)* 2004 Jan; **43**(1): 7-12.
- 545
- 546 37. De Hert M, van Winkel R, Van Eyck D, Hanssens L, Wampers M, Scheen A *et al.* Prevalence of
547 diabetes, metabolic syndrome and metabolic abnormalities in schizophrenia over the course
548 of the illness: a cross-sectional study. *Clin Pract Epidemiol Ment Health* 2006; **2**: 14.
- 549
- 550 38. Shoelson SE, Lee J, Goldfine AB. Inflammation and insulin resistance. *J Clin Invest* 2006 Jul;
551 **116**(7): 1793-1801.
- 552
- 553 39. Steiner J, Walter M, Guest P, Myint AM, Schiltz K, Panteli B *et al.* Elevated S100B levels in
554 schizophrenia are associated with insulin resistance. *Mol Psychiatry* Jan; **15**(1): 3-4.
- 555
- 556 40. Prabakaran S, Swatton JE, Ryan MM, Huffaker SJ, Huang JT, Griffin JL *et al.* Mitochondrial
557 dysfunction in schizophrenia: evidence for compromised brain metabolism and oxidative
558 stress. *Mol Psychiatry* 2004 Jul; **9**(7): 684-697, 643.
- 559

41. Uranova NA, Zimina IS, Vikhreva OV, Krukov NO, Rachmanova VI, Orlovskaya DD. Ultrastructural damage of capillaries in the neocortex in schizophrenia. *World J Biol Psychiatry* Apr; **11**(3): 567-578.
42. Rybakowski J, Weterle R. Niacin test in schizophrenia and affective illness. *Biol Psychiatry* 1991 Apr 15; **29**(8): 834-836.
43. Morrow JD, Awad JA, Oates JA, Roberts LJ, 2nd. Identification of skin as a major site of prostaglandin D2 release following oral administration of niacin in humans. *J Invest Dermatol* 1992 May; **98**(5): 812-815.
44. Smesny S, Klemm S, Stockebrand M, Grunwald S, Gerhard UJ, Rosburg T *et al.* Endophenotype properties of niacin sensitivity as marker of impaired prostaglandin signalling in schizophrenia. *Prostaglandins Leukot Essent Fatty Acids* 2007 Aug; **77**(2): 79-85.
45. Steiner J, Walter M, Wunderlich MT, Bernstein HG, Panteli B, Brauner M *et al.* A new pathophysiological aspect of S100B in schizophrenia: potential regulation of S100B by its scavenger soluble RAGE. *Biol Psychiatry* 2009 Jun 15; **65**(12): 1107-1110.
46. Riecher-Rossler A, Gschwandtner U, Borgwardt S, Aston J, Pfluger M, Rossler W. Early detection and treatment of schizophrenia: how early? *Acta Psychiatr Scand Suppl* 2006; (429): 73-80.

ACKNOWLEDGEMENTS

This study was supported by the Stanley Medical Research Institute (SMRI), Psynova Neurotech Inc and the European Union FP7 SchizDX research programme (grant reference 223427). We want to thank Anke Dudeck, Jeanette Schadow, Dr. Wolfgang Jordan, Dr. Bernd Hahndorf, Dr. Florian Kästner, Dr. Anya Pedersen, Dr. Ansgar Siegmund, Dr. Katja Kölkebeck, Torsten Schoenborn, Dr. Christoph W. Gerth, Dr. Christian Mauss, Dr. Brit M. Nolden, Dr. M. A. Neatby, Dr. Liliana Ruta and Dr. Erin Ingudomnukul for their participation in sample characterization and collection. Thanks to all members of the Bahn Laboratory for discussions, help, and encouragement. Most of all, thanks to all patients and healthy volunteers for their selfless donation of samples used in this study.

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601 **FIGURE LEGENDS**

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603 **Figure 1. Biomarkers changed in more than 15% of individual patients.** The y axis indicates the
604 percentage of subjects in which these biomarkers were altered cohort 1. Differential expression was
605 determined by identifying biomarkers that showed measurements varying by more than two standard
606 deviations in individual SCZ patients compared to the mean control value in the same cohort. The
607 abbreviations are as indicated in the legend for Table 2.

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

622 **Figure 3. Partial Least Squares-Discriminant Analysis (PLS-DA) of SCZ and MDD subjects.**
623 PLS-DA using the 34 serum analytes identified as differentially expressed in cohort 1 (SCZ1; see
624 **Table 2**). Serum samples from SCZ4, SCZ5 and MDD subjects were analyzed at the same time using
625 the HumanMAP platform. **A)** The 34 analyte panel was trained on cohort 4 (SCZ4) and then tested
626 blindly on cohort 5 (SCZ5) using PLS-DA. The red triangles indicate true SCZ patients and the plus
627 symbols indicate the true controls. The grey enclosure approximates the position of the majority of the
628 control population identified for cohort 4 (SCZ4). The 34 analyte panel was also tested on **B)** MDD
629 patients (red triangles; cohort 6). **C)** The histogram shows the relative contribution of each analyte to
630 the separation achieved in SCZ4 (VIP plot, see legend of Fig. 2). The abbreviations of analytes are as
631 listed in **Table 2**.

Figure 4. Altered Expression of Haptoglobin (HPT) across 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).

TABLES

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

M/F = male/female, BMI = body mass index, Y/N = yes/no, na = not available. Values are shown as mean ± sd. Control groups of cohorts 1 and 7, and those of cohorts 5 and 6 were identical.

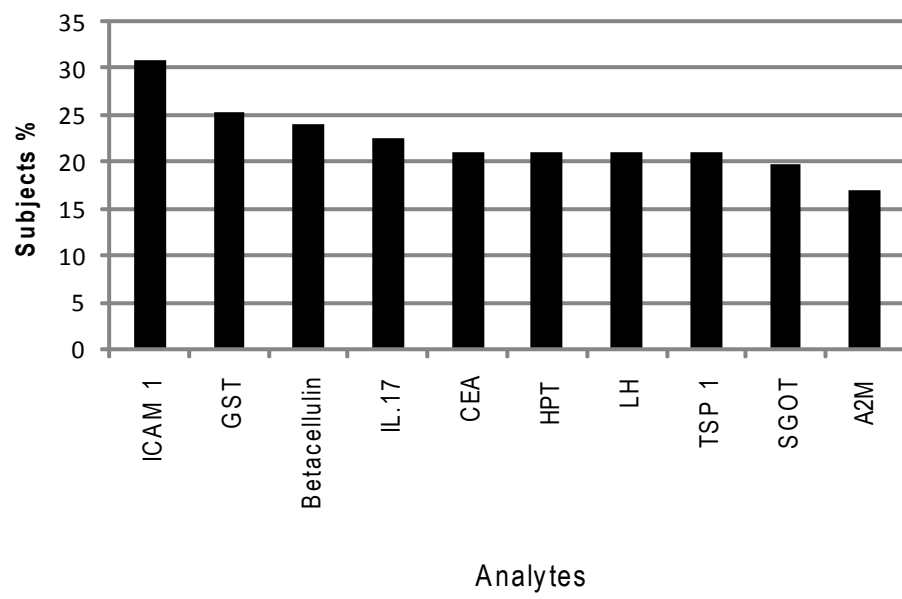
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660 **Table 2.** Identification of differentially expressed serum analytes in cohort 1 (SCZ 1) subjects using
661 HumanMAP® analysis

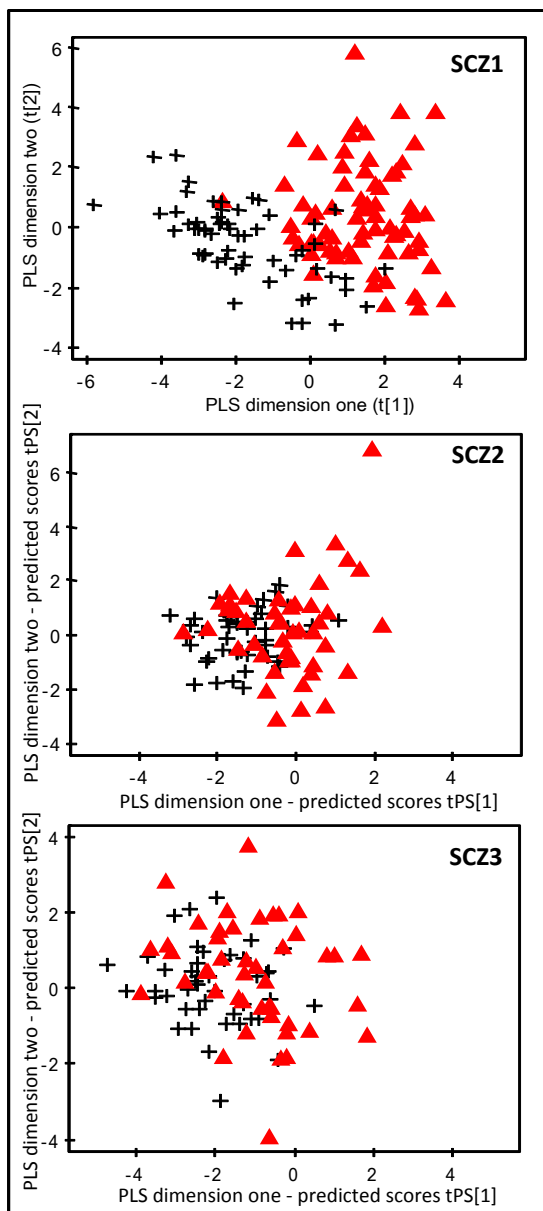
Analyte	p-value	q-value	FC	Unit	SCZ (mean ± sd)	NC (mean ± sd)	weight
α 1 Antitrypsin (a1AT)	0.005*	0.032	1.08	mg/mL	2.35 ± 0.42	2.17 ± 0.58	0.11
α 2 Macroglobulin (A2M)	<0.001	0.001	1.21	mg/mL	1.4 ± 0.34	1.16 ± 0.32	0.21
Angiopoietin 2 (ANG2)	<0.001	0.008	1.33	ng/mL	2.18 ± 0.97	1.67 ± 0.77	0.18
Brain derived neurotrophic factor (BDNF)	0.004*	0.027	0.87	ng/mL	24.77 ± 8.84	28.54 ± 7.27	-0.14
Betacellulin	<0.001	0.012	1.93	pg/mL	66.15 ± 38.27	55.15 ± 28.3	0.21
Bone morphogenic protein 6 (BMP6)	<0.001	0.007	2.02	ng/mL	1.7 ± 1.27	1.37 ± 0.87	0.17
Carcinoembryonic Antigen (CEA)	0.001	0.002	1.75	ng/mL	1.78 ± 1.39	1.02 ± 0.62	0.21
CD40 Ligand (CD40L)	0.027	0.012	0.64	ng/mL	1.41 ± 1.24	2.16 ± 1.49	-0.17
Cortisol	0.003	0.036	1.14	ng/ml	118.24 ± 32.77	103.88 ± 35.94	0.13
Connective tissue growth factor (CTGF)	0.003	0.046	1.17	ng/mL	3.97 ± 1.15	3.4 ± 1	0.16
Epidermal growth factor (EGF)	<0.001	<0.001	0.49	pg/mL	97.01 ± 93.91	176.97 ± 122.32	-0.25
Eotaxin 3	0.002	0.029	2.12	pg/mL	77.39 ± 71.8	40.63 ± 30.26	0.16
Factor VII	0039*	0.144	0.87	ng/mL	498.34 ± 131.34	573.08 ± 190.63	-0.14
Follicle stimulating hormone (FSH)	0.001	0.062	2.41	ng/ml	0.71 ± 1.23	0.31 ± 0.2	0.14
GM-CSF	0.002	0.109	0.91	pg/mL	10.13 ± 14.06	7.69 ± 3.16	-0.02
Glutathione S transferase (GST)	<0.001*	<0.001	1.30	ng/mL	1.06 ± 0.25	0.81 ± 0.21	0.30
Haptoglobin (HPT)	<0.001*	0.002	1.68	mg/mL	1.44 ± 0.91	0.84 ± 0.58	0.22
ICAM 1	0.001	0.149	0.94	ng/mL	121.7 ± 41.92	128.8 ± 17.79	-0.07
IGFBP 2	0.045*	0.149	1.22	ng/mL	52.9 ± 27	43.29 ± 19.51	0.12
Interleukin 10 (IL 10)	<0.001	<0.001	1.21	pg/mL	15.6 ± 5.91	12.87 ± 2.14	0.18
IL 17	<0.001	<0.001	1.62	pg/mL	14.48 ± 7.85	9.42 ± 3.36	0.25
IL 5	0.039	0.010	0.72	pg/mL	4.48 ± 2.82	5.59 ± 2.19	-0.14
Luteinizing Hormone (LH)	<0.001	0.015	1.66	ng/ml	0.15 ± 0.14	0.09 ± 0.05	0.17
MIF	0.024	0.149	1.72	ng/mL	0.18 ± 0.28	0.1 ± 0.08	0.11
NrCAM	0.001	0.149	0.83	ng/mL	0.5 ± 0.33	0.58 ± 0.32	-0.09
Pancreatic Polypeptide (PP)	<0.001	0.008	1.64	pg/ml	140.15 ± 145.45	85.34 ± 132.41	0.12
Prostatic acid phosphatase (PAP)	0.001	0.036	0.82	ng/mL	0.28 ± 0.1	0.34 ± 0.12	-0.16
RANTES (C-C motif chemokine 5)	0.005	0.121	1.17	ng/mL	22.14 ± 8.6	18.95 ± 8.88	0.11
Resistin	0.007	0.027	0.80	ng/ml	0.58 ± 0.3	0.72 ± 0.31	-0.14
SGOT	0.008	0.005	1.25	ug/mL	10.64 ± 3.86	8.51 ± 2.62	0.19
Sortilin	<0.001	<0.001	0.76	ng/mL	6.49 ± 2.15	8.59 ± 3.03	-0.24
Stem Cell Factor (SCF)	0.033	0.149	0.93	pg/mL	389.14 ± 126.53	419.86 ± 100.94	-0.08
Thrombopoietin (TPO)	0.004	0.005	0.84	ng/mL	3.74 ± 1.1	4.44 ± 1.14	-0.19
Thrombospondin 1 (TSP1)	0.014	0.002	0.82	ng/mL	43264.8 ± 13882.9	52976.3 ± 11720.8	-0.22

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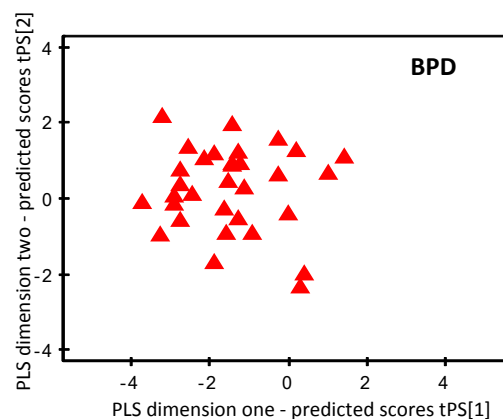
FC = fold change (average intensity of analyte in SCZ divided by the average intensity in controls of cohort 1).
p-values were calculated using ANCOVA based on log-transformed data from SCZ patients and controls in cohort 1 (gender, age, BMI, smoking and cannabis consumption used as covariates),
*Diagnosis-covariate 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
GM-CSF = granulocyte macrophage colony stimulating factor, IGFBP2 = insulin-like growth factor binding protein 2, MIF = macrophage migration inhibitory factor, SGOT = serum glutamic oxaloacetic transaminase.
The weight represents the PLS weight for the first latent variable of a PLS model comparing SCZ and controls in cohort 1.



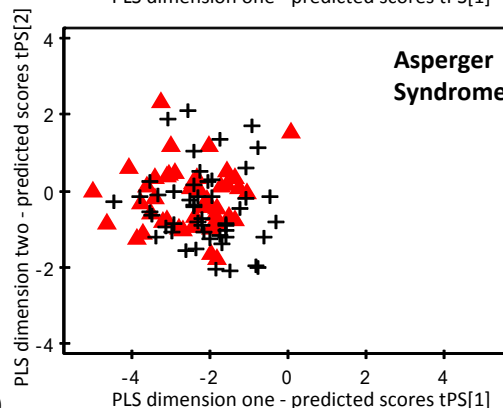
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C



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