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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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## 84 INTRODUCTION

85  
86 Schizophrenia (SCZ) is a psychiatric disorder affecting up to 1% of the world population (1). It  
87 negatively impacts on quality of life for patients and their families and costs hundreds of billions of  
88 U.S. dollars in healthcare provision alone (2). Despite years of intensive investigation, the biological  
89 mechanisms underlying the pathophysiology of the disorder are still not completely understood.  
90 Advances have been made over recent decades in the diagnosis of SCZ using standardized systems  
91 and structured interviews such as DSM-IV and SCID (3), although improvement in this area requires  
92 increased understanding of the molecular mechanisms involved in the onset and progression of the  
93 disease. This is because the accuracy of existing diagnostic methods can be confounded as subjects  
94 often show significant overlap of symptoms with those displayed by individuals affected by other  
95 psychiatric conditions such as bipolar disorder (BPD), major depressive disorder (MDD) and Autism  
96 Spectrum Conditions (e.g. Asperger syndrome). In addition, diagnosis can be hindered by frequently  
97 occurring confounding factors including substance abuse or manifestation of other medical conditions  
98 (4). Such complications can lead to delays and inaccuracies of diagnosis which can, in turn, lead to  
99 uncertainties and further delays in designing a therapeutic strategy, resulting in a poorer outcome for  
100 the affected individuals (5).

101 Recently, we described the identification of a serum-based analyte signature, capable of  
102 distinguishing SCZ from control subjects with high sensitivity and specificity (6). Here, we have  
103 investigated the biological reproducibility of the component analytes across the same cohorts. Such  
104 biological signatures have proven crucial for increasing our understanding, aiding rapid clinical  
105 diagnosis and effective treatment of many disorders but are currently lacking for psychiatric illnesses  
106 (7). One reason for this is that it is not known whether disorders of the central nervous system can be  
107 identified by alterations in peripheral markers. However, peripheral pathways such as immune and  
108 metabolic systems can be regulated through crosstalk with the central nervous system via  
109 neuroanatomical networks, hormonal routes and molecular signalling mechanisms (8). As there is  
110 mounting evidence for abnormalities in these pathways in SCZ (9, 10), it is likely that a distinguishing  
111 pattern of molecules in the peripheral circulation can be detected.

112 Here, we have used the HumanMAP<sup>®</sup> Multi-Analyte Profiling platform (**Supp. Table 1**) in  
113 collaboration with Rules Based Medicine (Austin, TX, USA) to analyze serum samples from 642  
114 individuals comprised of 250 first or recent onset SCZ, 67 affective disorder (MDD and BPD), 45  
115 Asperger syndrome and 280 control subjects, recruited from 5 psychiatric centres in Germany,  
116 Holland and the UK. This technology has been shown to be reproducible and robust and has already  
117 been applied successfully in numerous clinical studies or biomarker discovery projects of diseases  
118 such as epithelial ovarian cancer (11), coronary artery disease (12), myocardial infarction (13) and  
119 autoimmune disorders (14).

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

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## 124 **METHODS**

### 125 126 **CLINICAL SAMPLES**

127 The institutional ethical committees (see below) approved the protocols of the study, informed written  
128 consent was given by all participants and studies were conducted according to the Declaration of  
129 Helsinki. All diagnoses were carried out using DSM-IV and clinical tests were performed by  
130 psychiatrists according to “Good Clinical Practice Guidelines” to minimize variability. The demographic  
131 details are shown in **Table 1**. Subjects in cohorts 1-5 were independent and diagnosed as having the  
132 paranoid subtype of SCZ (295.30). The exclusive focus on the clinically well defined paranoid  
133 schizophrenia, which embodies the most prevalent subtype of the illness, was intended to minimize  
134 variability arising from potential disease heterogeneity. Schizophrenia patients featured high average  
135 scores on positive as well as negative items of the Positive and Negative Syndrome Scale (PANSS,  
136 **Table 1**) (15). All SCZ patients from cohort 1, 2, 4 and 5 and 33 out of 45 patients from cohort 3 were  
137 antipsychotic-naïve at the time of sample collection. The patients in cohort 6 were acutely ill with MDD  
138 and were either antipsychotic-naïve (n=22) or drug-free (n=13) for at least 6 weeks prior to sample  
139 collection. All patients were treated as inpatients for several weeks. The collaborating clinicians had  
140 access to all detailed clinical files including the medical history by proxy and referral letters from the  
141 general practitioners in order to verify the first episode state and drug-status. Written informed  
142 consent was obtained for most patients prior to the collection of samples in individuals with full insight  
143 into the study objectives and obtained later in patients who were not able to give informed consent at  
144 first presentation due to impairments in their mental states. Medication was not withheld but  
145 administered after completion of diagnosis wherever possible. Patient blood was collected at time of  
146 first presentation. Samples were collected in expert centres from prodromal and early onset  
147 schizophrenia patients. This includes liaison with general practitioners to avoid treatment with  
148 antipsychotics or antidepressants where clinically acceptable prior to referral to the specialised team,  
149 which also operated out of hours.

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

160 The cohorts used in this study were obtained from multiple centres. Cohorts 1 and 7 were  
161 from the University of Cologne, Germany (Ethical Committee of the Medical Faculty of the University  
162 of Cologne), cohort 2 from the University of Münster, Germany (Ethics Commission of the Physician

163 Chamber Westphalia Lippe and the Medical Faculty of the Westfälische-Wilhelms University  
164 Münster), cohorts 3, 5 and 6 from the University of Magdeburg, Germany (Ethics committee of the  
165 Medical Faculty of the University of Magdeburg), cohort 4 from Erasmus University, Netherlands  
166 (Research Ethics Committee of the Erasmus Medical Centre) and cohort 8 from the Department of  
167 Psychiatry, University of Cambridge, UK (Cambridge Research Ethics Committee). Cohorts 1-5 were  
168 the same as those reported recently (6).

169 Blood samples were collected from all subjects into S-Monovette 7.5mL serum tubes  
170 (Sarstedt; Numbrecht, Germany). Serum was prepared by placing samples at room temperature for 2  
171 hours for blood coagulation, followed by centrifugation at 4,000 X g for 5 minutes. The resulting  
172 supernatants were stored at -80°C in Low Binding Eppendorf tubes (Hamburg, Germany) prior to  
173 analysis.

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## 176 **MULTIPLEXED IMMUNOASSAY**

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

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## 188 **DATA ANALYSES**

189 Kolmogorov-smirnov analysis showed that the majority of the investigated analytes were non-normally  
190 distributed. Therefore, non-parametric, two-tailed Wilcoxon Rank sum tests were carried out to  
191 identify significant expression differences between patients and controls. In addition, correlation  
192 analyses indicated that several analytes were significantly associated with gender, age, BMI, smoking  
193 or cannabis consumption. Analysis of covariance (ANCOVA) was, therefore, carried out on log-  
194 transformed data to assess the effect of these demographic variables on the significance of identified  
195 marker candidates. P-values of less than 0.05 were considered to indicate statistical significance. The  
196 false discovery rate was controlled according to Benjamini and Hochberg (17). Multivariate analysis  
197 was carried out using SIMCA P+ 10.5 (Umetrics; Umea, Sweden) for partial least squares  
198 discriminant analysis (PLS-DA) to visualize any separation between patient and control subjects as  
199 indicated. This technique was used to visually display the relationship between the combined set of  
200 analytes of interest and the disease state. The PLS model consists of PLS components that are linear  
201 combinations of the analytes used as input. Since these linear combinations are chosen to contain the  
202 highest information content that is correlated to the disease state, the dimensionality of the multi-

203 dimensional input space can be reduced to fewer dimensions. The PLS scores plots display each  
204 investigated subject in the space of reduced, two-dimensional space of PLS components. PLS  
205 weights indicate the contribution of each analyte to the score in the direction of a given PLS  
206 component.

## 207 **RESULTS**

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### 209 **Identification of preliminary SCZ biomarker signature**

210 The first stage of the study was aimed at identification of differentially expressed serum analytes in a  
211 single cohort of first onset anti-psychotic naive SCZ subjects and well-matched controls using the  
212 HumanMAP analysis. Cohort 1 was chosen for this analysis since this group was comprised of SCZ  
213 (n=71) and controls (n=59) who were matched for age, gender, body mass index, smoking, cannabis  
214 consumption and date of sample collection (**Table 1**). This analysis resulted in identification of 34  
215 analytes which were altered significantly in SCZ compared to control subjects using unpaired two-  
216 tailed Wilcoxon Rank Sum tests and remained significant after adjusting for covariates using  
217 ANCOVA (**Table 2, Supp. Table 2**). The majority of these analytes were also significant after  
218 controlling the false discovery rate. Analytes showing the highest magnitude fold-changes were  
219 betacellulin, bone morphogenic protein 6 (BMP6), eotaxin 3, follicle stimulating hormone (FSH) and  
220 epidermal growth factor (EGF), which were all altered by approximately 2-fold in SCZ compared to  
221 control subjects. To account for the possibility that the findings were related to stress, we used  
222 Cortisol as a covariate for ANCOVA. Six analytes were non-significant after this adjustment (Eotaxin  
223 3, p=0.051; Factor VII, p=0.067; ICAM 1, p=0.070; IGFBP2, p=0.052; IL5, p=0.070 and SCF,  
224 p=0.105) although all of these analytes showed a trend towards significance. For added confirmation  
225 of the results, the same serum samples were re-assayed approximately 3 months after the first  
226 analysis. This repeat analysis showed good reproducibility of the results with an average correlation  
227 across all analytes of 0.81 (Pearson's correlation coefficient) and 50% of the analytes had a  
228 correlation greater than 0.90 (data not shown).

229 We also determined the proportion of subjects in which these biomarkers were altered across  
230 in cohort 1. In this case, differential expression of a biomarker in a subject was indicated if the  
231 measurement varied by more than two standard deviations compared to that of the mean control  
232 measurement in the same cohort. Using these criteria, ICAM 1, GST, Betacellulin, GST and IL 17  
233 were altered in the highest proportion of subjects (**Figure 1**).

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### 235 **Validation of SCZ signature in other cohorts**

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237 One factor which renders diagnosis of SCZ imprecise is the heterogeneous nature of the disease and  
238 the overlap of SCZ symptoms with those of other psychiatric conditions (18). In the next phase of the  
239 study, the 34 differentially expressed analytes identified in cohort 1 were tested as a combined panel  
240 using samples from SCZ and control subjects in the first 3 cohorts (SCZ1, SCZ2 and SCZ3) and in  
241 cohorts 7 (BPD) and 8 (Asperger syndrome) since these were profiled using HumanMAP analysis at

242 the same time. PLS-DA was used to visualize any separation between patient and control subjects.  
243 This showed the 34-analyte panel resulted in a separation of SCZ patients from controls by 40-85%  
244 across cohorts 1-3 (**Figure 2A**). For comparison, euthymic BPD patients were tested as such patients  
245 can experience disruptions in cognitive behaviours as seen in SCZ (19). Asperger syndrome subjects  
246 (cohort 8) were analyzed since they can also show overlap with SCZ in display of such symptoms as  
247 emotional lability, anxiety and poor social functioning (20). In contrast to SCZ, the signature resulted  
248 in little or no separation of BPD patients (**Figure 2B**) or Asperger syndrome subjects (**Figure 2C**) from  
249 the respective controls. The analytes most important for the separation achieved in cohort 1 were  
250 GST, sortilin, IL17, CEA, EGF, TSP1, HPT, A2M, Betacellulin, SGOT, TPO and ANG2 in descending  
251 order (**Figure 2D**). It should be noted that the separation achieved in SCZ1 reflects the training  
252 performance of the multivariate model and is positively-biased as data from this cohort were used to  
253 establish the initial 34 analyte signature.

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

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

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

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## 291 **DISCUSSION**

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293 These results demonstrate that a reproducible biological signature for SCZ can be identified in blood  
294 serum. One strong point of the current investigation is that samples were obtained from first onset  
295 antipsychotic naïve subjects who were well-matched with controls for factors such as age, gender,  
296 substance abuse and lifestyle. This was an important consideration to maximize the capability of  
297 identifying molecular biomarkers associated with the disease and minimize the chances of identifying  
298 those associated with potential confounding factors. Most previous SCZ studies have investigated  
299 chronic patients who have been treated with antipsychotic medications and who often have multiple  
300 co-morbidities, which can confound the results of biomarker investigations. Domenici et al recently  
301 described the identification of SCZ and MDD biomarkers, although the majority of the samples used  
302 for this study came from treated subjects (22). Studies involving first onset antipsychotic naïve  
303 patients are scarce due to the fact that even large psychiatric centres can only enlist around 20-30 of  
304 these subjects each year and few centres follow strict standard operating procedures for collection of  
305 samples. We have overcome this problem by obtaining samples from first onset antipsychotic naïve  
306 and minimally-treated SCZ patients from multiple clinical centres over a 10 -year time span. All of the  
307 patients and controls underwent extensive clinical characterization, and sera were collected and  
308 stored according to strict standard operating procedures. In addition, all protocols involving clinical  
309 subjects, samples and test measurements were carried out in compliance with the Standards for  
310 Reporting of Diagnostic Accuracy (STARD) initiative (16) to maximize reliability and accuracy of the  
311 results.

312 Many of the proteins and small molecules identified as SCZ biomarkers have been implicated  
313 previously in acute or chronic inflammatory conditions, endothelial cell dysfunction, cardiovascular  
314 disease, type II diabetes mellitus and metabolic disorder (23-25). This may be important as recent  
315 studies have explored the possibility of using either anti-inflammatory agents such as cyclo-  
316 oxygenase-2 (COX-2) inhibitors (26) or anti-diabetic compounds such as insulin-sensitizing agents  
317 (27) for improving cognitive deficits or other symptoms of schizophrenia. In this regard, a recent study  
318 has demonstrated that administration of the insulin-sensitizing agent pioglitazone resulted in  
319 increased localized cerebral blood flow in Alzheimer's disease patients and significant improvement in  
320 cognitive testing (28). Such compounds have only been tested as an adjunct to anti-psychotic  
321 treatment with the aim of minimizing the frequently-occurring metabolic side effects (29, 30). Thus far,  
322 they have not been tested for any benefits on cognition or psychopathology as a monotherapy.

323 Altered inflammatory response or dysregulation of the adrenal cortex hormone cortisol has  
324 been associated with a number of psychiatric disorders (31). We found a significant increase in  
325 cortisol levels across all SCZ cohorts and a non-significant trend for increase across all other non-

326 SCZ cohorts ( $p=0.188-0.268$ ). Hypercortisolemia and hypothalamic-pituitary-adrenal hyperactivity  
327 may also be linked to the increased levels of MIF in the SCZ cohorts. MIF has been shown to play a  
328 central role in the progression of immunological disturbances associated with atherosclerotic plaque  
329 development (32, 33), oxidative stress and endothelial cell dysfunction (34). Inflammatory disorders  
330 and schizophrenia also share an increased prevalence of insulin resistance, metabolic syndrome and  
331 type II diabetes (35-38). This has been supported by our recent studies showing hyperinsulinaemia  
332 and insulin resistance in a subset of first onset antipsychotic naïve SCZ subjects (27, 39), and by our  
333 findings of abnormalities in glucose regulation and brain vasculature in *post-mortem* SCZ brain tissue  
334 (9, 27, 40).

335         Recent studies have demonstrated ultrastructural abnormalities of capillaries and the  
336 pericapillary environment, supporting the concept that blood-brain barrier dysfunction might contribute  
337 to the pathogenesis of SCZ (41). The effects on brain endothelial cells are known to be mediated by  
338 alterations in prostaglandin signalling. It is interesting in this regard that we identified alterations in  
339 endothelial cell biomarkers including endothelin 1 and CTGF as part of the SCZ signature. In addition,  
340 it has been known for decades that niacin can induce a visible skin flush response caused by  
341 prostaglandin-mediated vasodilatation (42, 43). Recent studies have demonstrated that the  
342 attenuated skin flushing in response to niacin administration in SCZ subjects may be secondary to the  
343 increased oxidative stress, alterations of nonspecific immune-response or inflammation-like  
344 processes (44). Taken together, these findings suggest that conditions such as inflammation and  
345 metabolic disorder may be converging pathophysiological processes in SCZ and potentially other  
346 psychiatric and non-psychiatric disorders (45).

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

361         These results highlight the importance of evaluating biomarkers in larger studies with explicit  
362 assessment of the ability to classify subjects. The future success of biomarker strategies may depend  
363 on the discovery of new molecules to complement the most robust existing biomarkers, perhaps with  
364 the help of state-of-the-art targeted and non-targeted approaches. In addition, it should be noted that  
365 tests for disorders with a low incidence such as SCZ require exceptionally high specificities if used in

366 the general population. For this reason, the most effective use of such tests would be as a  
367 confirmatory diagnostic aid by a psychiatric specialist in conjunction with a clinical assessment. In this  
368 way, the test would be used in populations already enriched for schizophrenia with the purpose of  
369 establishing and confirming a diagnosis more rapidly, as compared to the requirement for 6 months  
370 duration of continuous symptoms using the current DSM IV-based diagnosis. Such an application of a  
371 biomarker test would help to initiate treatment of patients more rapidly and, therefore, reduce the  
372 duration of untreated psychosis and, in turn, improve patient outcomes (46). This would be an  
373 important breakthrough by helping clinical psychiatrists to identify vulnerable patients early in the  
374 disease process, allowing for earlier or even preventative therapeutic intervention.

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## 585 **ACKNOWLEDGEMENTS**

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

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## 601 **FIGURE LEGENDS**

602

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**.



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

635

636 **TABLES**

637

638 **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

639 M/F = male/female, BMI = body mass index, Y/N = yes/no, na = not available. Values are shown as mean ± sd. Control groups  
 640 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
Angiotensin 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	0.039*	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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663 FC = fold change (average intensity of analyte in SCZ divided by the average intensity in controls of  
 664 cohort 1).

665 p-values were calculated using ANCOVA based on log-transformed data from SCZ patients and  
 666 controls in cohort 1 (gender, age, BMI, smoking and cannabis consumption used as covariates),

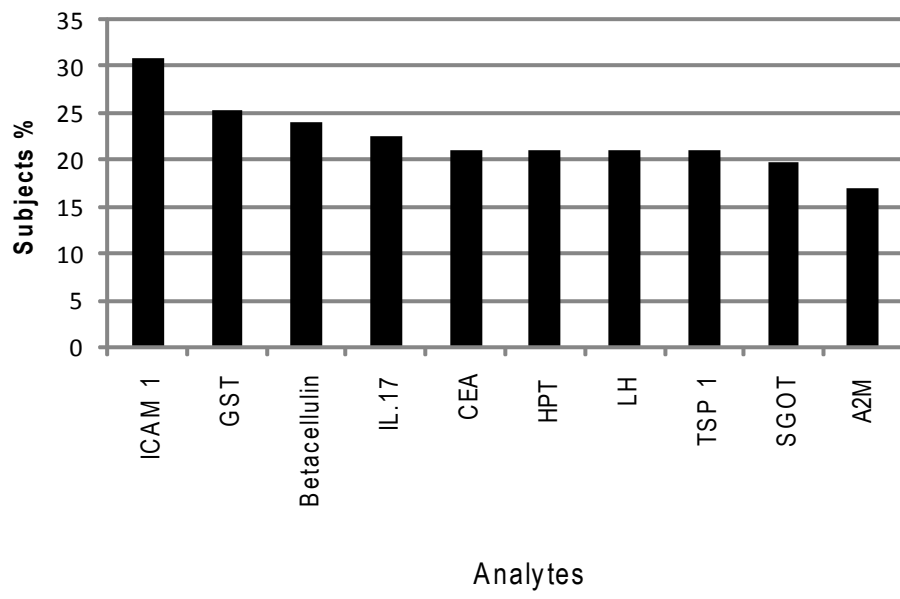
667 \*Diagnosis-covariate interaction was significant and p-value was determined using non-parametric  
 668 Wilcoxon Rank sum test instead.

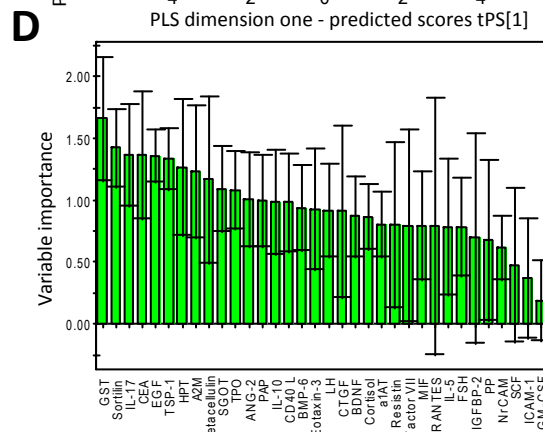
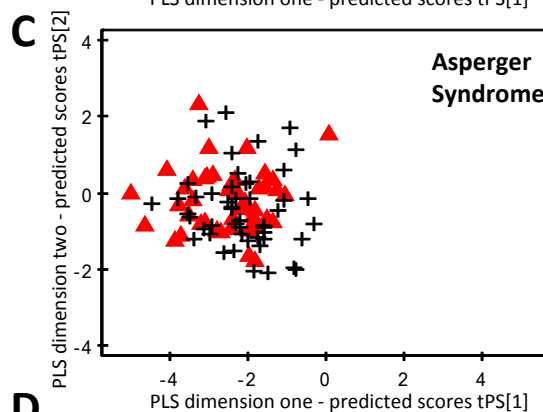
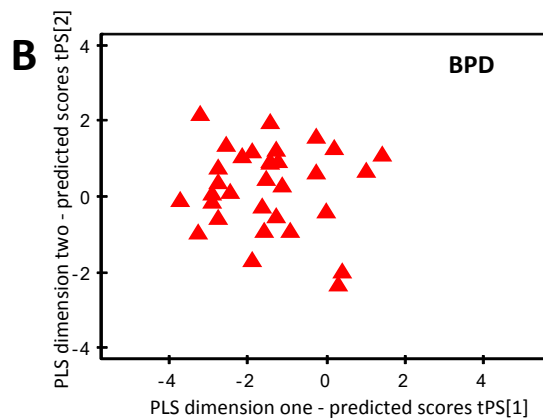
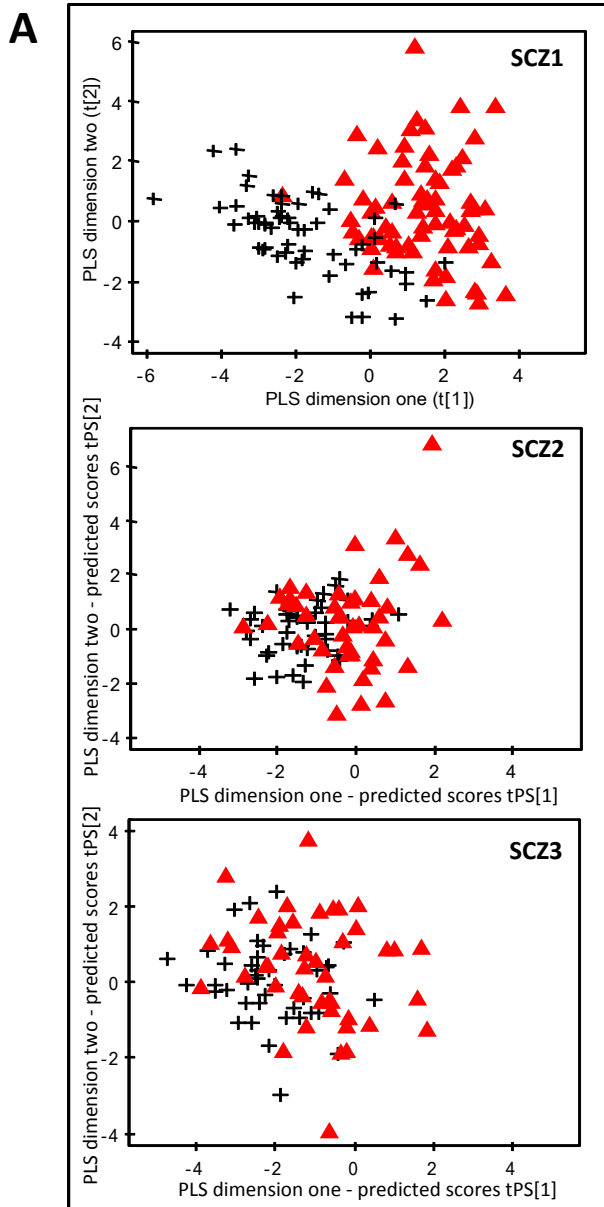
669 q values represent FDR adjusted p-values derived from Wilcoxon Rank sum tests comparing SCZ  
 670 patients and controls in cohort 1

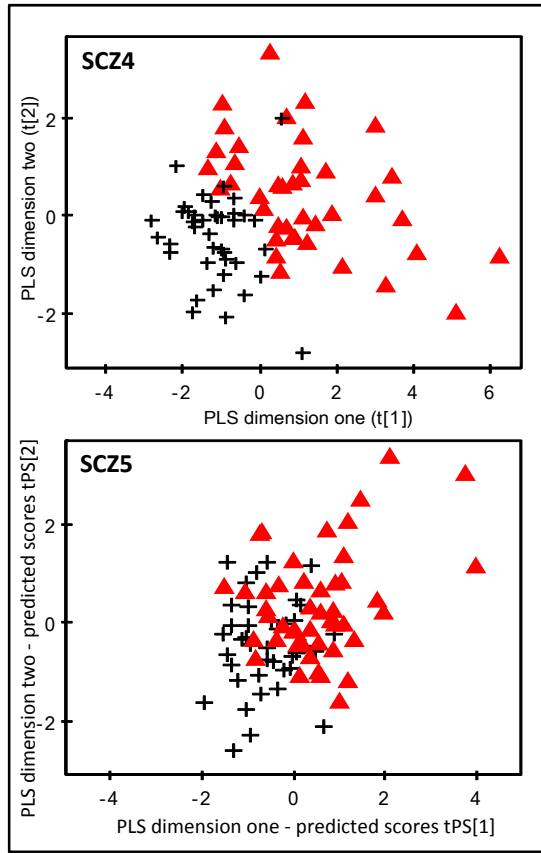
671 GM-CSF = granulocyte macrophage colony stimulating factor, IGFBP2 = insulin-like growth factor  
 672 binding protein 2, MIF = macrophage migration inhibitory factor, SGOT = serum glutamic oxaloacetic  
 673 transaminase.

674 The weight represents the PLS weight for the first latent variable of a PLS model comparing SCZ and  
 675 controls in cohort 1.

676





**A****B**