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LETTER TO THE EDITOR

Differential phosphorylation of serum proteins reflecting inflammatory changes in schizophrenia patients

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As we have argued recently [4], despite the innovative aspects of large-scale proteome studies in schizophrenia research, efforts are still required to investigate the role of post-translational modifications of proteins, such as phosphorylation. Protein phosphorylation acts as a switching mechanism in the regulation of a range of cellular processes such as cell signalling and protein transport. Its importance is supported by the fact that at least one-third of human proteins are predicted to be phosphorylated [9]. However, many of these have yet to be confirmed experimentally. Therefore, the study of phosphoproteins could lead to an increase in our understanding about schizophrenia aetiology and the mechanism of action of antipsychotic medications [6]. In addition, differentially phosphorylated proteins in diseased or treated tissues can serve as biomarkers, especially if detectable in easily accessible peripheral tissues. These biomarkers can be further incorporated in multiplex systems, which could be applied clinically such as the first blood-based multiplex immunoassay test to aid in the diagnosis of schizophrenia [7].

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Here, we would like to communicate the first large-scale mass spectrometry–based analysis of phosphoproteins in serum from anti-psychotic-naïve first-onset paranoid schizophrenia patients ($n = 22$) and controls ($n = 33$)—schizophrenia patients: male/female = 15/7, age = 29.0 ± 11; controls: male/female = 18/15, age = 28 ± 7. A statistical power analysis [2] showed that 19 samples per group would be sufficient for a power of 0.8 and confidence level of 0.05. The mass spectrometry methods were described previously in detail [3]. Chromatograms and mass spectral processing as well as database searching were performed using the ProteinLynx Global Server (PLGS; v.2.4; Waters Corp; Milford, MA, USA). Peaks were aligned and quantitation obtained by integration of time, mass/charge (m/z) and intensity volumes after normalization to the total ion current. The resulting data were searched against the SwissProt human database (version 57.4, 20,235 entries). The maximum false identification rate was set at 4%, and peptides had to be detected in 2 out of 3 technical replicates per sample and in >80% of samples to ensure biological reproducibility. The criteria for protein identification were set at $\geq$3 ion fragments per peptide, $\geq$7 fragments per protein and $\geq$2 peptides per protein. The modifications considered were carbamidomethylation of cysteine, oxidation of methionine and phosphorylation of serine, threonine and tyrosline residues. Phosphorylation was detected by the experimentally determined loss of an 80-Dalton phosphate residue. Quantitation and statistical analyses of processed data from the PLGS analysis were performed subsequently using the Rosetta Elucidator© system (v.3.3.0.1.SP3.19; Rosetta Inpharmatics; Seattle, WA, USA). Student's unpaired t tests were used to determine significant differences ($P < 0.05$).

The analysis led to the identification of 710 phosphopeptides corresponding to 164 non-redundant proteins. Ten

phosphopeptides were detected with differences in abundance, and these corresponded to 8 distinct proteins (Table 1). Complement factor H presented two peptides with decreased phosphorylation and one peptide with increased phosphorylation. Inter-alpha-trypsin inhibitor heavy chain H1 showed increased phosphorylation while the remaining proteins showed decreased phosphorylation in schizophrenia patients compared to controls. Five of the identified proteins are involved in inflammation, two are serine-type endopeptidase inhibitors and one is a structural protein. In silico pathway analysis of these proteins was carried out using the Ingenuity Pathways Knowledge Base (Ingenuity Systems; Redwood City, CA, USA) that identified inflammation as the most affected pathway (Supplementary Material 1).

One of the decreased phosphoproteins was alpha-2-HS-glycoprotein (AHSG). We found that the overall levels of this protein were decreased in our previous study [3], reflecting effects on the acute-phase inflammatory response. This also suggests that the reduced phosphorylation may reflect the overall decreased levels of this protein. Interestingly, AHSG is associated with insulin resistance [8], consistent with our previous findings of increased levels of insulin-related peptides in first-onset schizophrenia patients [1].

Three of the proteins were components of the complement system (complement factor H, C5, C8 b-chain). This is the first report that shows these proteins undergo differential phosphorylation in schizophrenia and demonstrates the power of this approach to detecting novel post-translational modifications of proteins. The complement pathway is known to vary dynamically in schizophrenia and also involved in neurogenesis and synaptic function [5], which are important in schizophrenia pathogenesis.

Changes in the phosphorylation state of the presented set of proteins in schizophrenia patients warrant further investigation and validation in different cohorts. The results could lead to new insights into the aetiology of schizophrenia and may also lay the groundwork for future studies aimed at stratification of patients based on alterations in the immune profile at the onset of the disease. Moreover, the measurement of phosphoproteomic changes can also be used for monitoring antipsychotic drug treatment responses.

Table 1 Altered phosphorylation of peptides corresponding to 8 proteins in schizophrenia patients compared to controls

Accession	Protein Description	FC	p-value	Modified Peptide Sequence	Biological Role
ANT3_HUMAN	Antithrombin-III	-1.28	0.0253	EQLQDMGLVDLFSPEK**S**K	Inflammation
CFAH_HUMAN	Complement factor H	1.12	0.0224	NGFYPA**T**RGN**T**AK	Inflammation
CFAH_HUMAN	Complement factor H	-1.24	0.0304	GKEGWIH**T**VCINGR	Inflammation
CFAH_HUMAN	Complement factor H	-1.48	0.0425	WDPEVNCSMAQIQLCPPPPQIPNSHNM**TTT**LNYRDGEK	Inflammation
CO5_HUMAN	Complement C5	-1.14	0.0480	VV**T**EADVYITFGIREDLKDDQK	Inflammation
CO8B_HUMAN	Complement component C8 beta chain	-1.35	0.0227	Q**T**QGK**Y**EFILK	Inflammation
DYH17_HUMAN	Dynein heavy chain 17	-1.15	0.0470	EG**SY**VYGLFMEGAR	Structural
FETUA_HUMAN	Alpha-2-HS-glycoprotein	-1.20	0.0040	HTFMGVV**S**LGSPSGEVSHPR	Inflammation
ITIH1_HUMAN	Inter-alpha-trypsin inhibitor heavy chain H1	1.14	0.0122	ASGRTMEQFTIHL**T**VNPQSK	Hyaluronan metabolism *(Serine-type endopeptidase inhibitor)*
KAIN_HUMAN	Kallistatin	-1.11	0.0308	F**S**ISGSYVLDQILPRLGFTDLFSK	Proteolysis regulation *(Serine-type endopeptidase inhibitor)*

Indicated are the accession number, protein description, fold change (*FC*), significance (*P* value), the phosphorylated peptide sequence (modified amino acids highlighted in black) and the biological role (determined by Ingenuity Pathways Knowledge Base). For complement factor H (in italics), 3 peptides were found differentially phosphorylated

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Conflict of interest The authors declare no conflict of interest.

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