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Proteomic Analysis of Cerebrospinal Fluid in Suicidal Patients - A Pilot Study

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Short Communication

Despite the fact that "omic" technologies (including genomic, epigenomic, transcriptomic, proteomic and metabolomic technologies) are becoming widely used in various medical fields, their use in psychiatry is still very limited. Assessing suicidal behavior in psychiatric practice consists mostly of semi-structured questionnaires or various self-assessing scales. Information obtained this way is rather subjective. Therefore, our proteomic approach may provide more valid and objective way how to assess suicidality in daily clinical practice by finding possible candidates for biomarkers of suicidal behavior. In the present short communication, we present and discuss the results of our pilot proteomic study of cerebrospinal fluid (CSF) in two adult suicidal patients post-mortem (males, average age: 55, cause of death: hanging, no concomitant medication, no medical history), two adult controls post-mortem (males, average age: 55, cause of death: heart attack, no concomitant medication, no medical history) and two adult controls in-vivo (females, average age: 55, diagnosis: hydrocephalus, no concomitant medication – samples were drawn before the medication was taken). Samples of CSF in-vivo were included in this study to confirm the presence of identified proteins in living subjects and also to define their levels in CSF. Per subject, 5 ml of CSF was collected and post-mortem interval (PMI) did not exceed 32 hours. The protocol and informed consents for this study were approved by local ethical committee.

Proteomic analysis using trypsin digestion was performed on Bruker AmaZon speed ETD Ion Trap mass spectrometer (Bruker Daltonics, Germany) connected with UltiMate 3000 UHPLC Thermo Scientific system. HPLC analysis was performed on trap column Acclaim PepMap 100 (Thermo Scientific, 100 µm x 2 cm, 5 µm) with loading solvent water-acetonitrile containing 0.1% formic acid in isocratic mode. Peptides were separated on Acclaim PepMap RLSC analytical column (Thermo Scientific 75 µm x 15 cm, 3 µm) with mobile phase composition of acetonitrile - water with 0.1 % formic acid in gradient elution. For protein identification the Mascot 2.4 search engine was used (taxonomy: homo sapiens, variable modification: oxidation of methionine, fixed modification: carbamidomethylation of cysteine, MS tolerance: 0.6 Da, MS/MS tolerance: 0.9 Da, FDR ≤ 1 %). For protein identification and label-free quantification the Scaffold 4.8.6 (Proteome Software Inc., Portland, OR, USA) software was used to validate MS/MS based peptide and protein identifications. Peptide identifications were accepted if they could be established at greater than 95.0% probability by the Peptide Prophet algorithm with Scaffold delta-mass correction. Protein identifications were accepted if they could be established at greater than 99.0% probability and contained at least 2 identified peptides. Protein probabilities were assigned by the Protein Prophet algorithm. The label-free quantitative proteomic analysis utilizing the Total Spectral Count quantification method (data were normalized) showed statistically significant changes in 69 proteins, i.e., those passing ANOVA test ($p<0.05$) (Supplementary Table S1). Analysis of protein-protein interactions using STRING database in 69 significantly changed proteins was performed (Figure 1). From these 69 significantly changed proteins, Enolase 1 (ENO1), Enolase 2 (ENO2), Glyceraldehyde-3-phosphate (G3P) and Triose-phosphate isomerase (TPI) were significantly downregulated ($p<0.05$) and Aldolase *C* (ALDOC) significantly upregulated ($p<0.05$) in glycolysis/gluconeogenesis pathway, while protein 14-3-3 eta (YWHAH) and 14-3-3 theta (YWHAQ) had significantly higher levels in 14-3-3 mediated signaling pathway in CSF of suicidal patients versus control subjects.

Glycolytic proteins had significantly lower concentrations in suicidal patients compared to controls, what might indicate decreased utilisation of glucose in central nervous system (CNS), while the significantly higher concentration ($p<0.05$) of ALDOC in suicidal patients versus controls might be interpreted as an example of compensation mechanisms of the already decreased utilisation of glucose in CNS. We therefore suggest, that dysregulation in glucose utilisation may play a role in patients with suicidal behavior as well as in other psychiatric diseases e.g. schizophrenia. Some proteomic evidence using post-mortem brain tissue of schizophrenic patients also found dysregulations in glycolysis/gluconeogenesis metabolism. This finding was interpreted as possible increase in glucose demand and/or cellular hypoxia. For example, enzyme ALDOC was both found upregulated [1-3] and downregulated [4,5] in several studies. Prabakaran et al. [6] also identified dysregulations in several glycolytic enzymes (ENO1, ENO2, ALDOC and GP3) in prefrontal cortex of schizophrenic patients post-mortem. Peroxiredoxine 2 (PRDX2) and ENO2 play a role in oxidative stress response, while ENO2 is also considered as a marker of nervous tissue damage. In our pilot study, significantly lower concentrations of both enzymes were observed in suicidal patients versus controls, what might be considered as possibly decreased oxidative stress response. PRDX2 was also found downregulated in schizophrenic patients [7].

Furthermore, statistically significant upregulation ($p<0.05$) of protein 14-3-3 eta (YWHAH) and 14-3-3 theta (YWHAQ) in 14-3-3 mediated signaling pathway was found in suicidal patients compared to control subjects in our pilot study. Kékesi et al. [8] also identified elevated levels of YWHAH, but not of YWHAQ in prefrontal cortex and amygdala in suicidal attempters compared with control group

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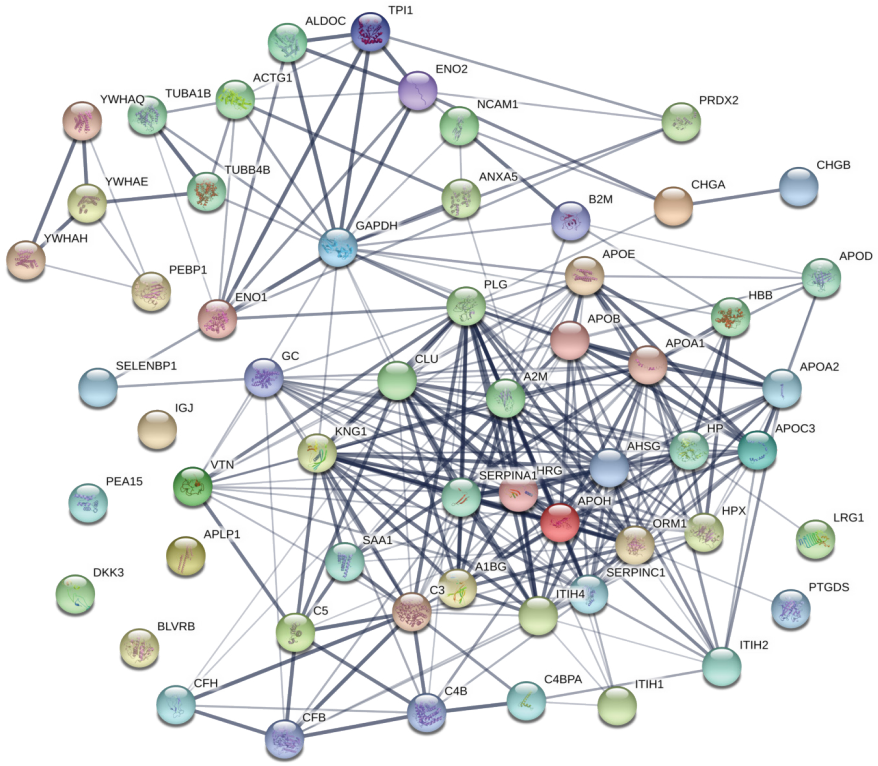

Figure 1: Protein-protein interactions of statistically significant (ANOVA-Test, p<0.05) proteins (58) with their 3D structure (if known) in suicidal patients versus controls using database STRING detected by LC-MS analysis. Each node of this network presents a protein, edges present protein-protein associations and line thickness indicates the strength of data support (STRING database did not identify 11 from 69 proteins: IGHG1_HUMAN, IGHG2_HUMAN, IGHG3_HUMAN, IGHG4_HUMAN, IGHM_HUMAN, IGKC_HUMAN, IGHA1_HUMAN, HV353_HUMAN, LAC2_HUMAN, HS71B_HUMAN and KV320_HUMAN).

Protein No.	Protein name	Accession Number	Fold change	Quantitative Profile
1.	Amyloid-like protein 1	APLP1_HUMAN	<0.01	↓
2.	Beta-2-microglobulin	B2MG_HUMAN	<0.01	↓
3.	Chromogranin-A	CMGA_HUMAN	<0.01	↓
4.	Neural cell adhesion molecule	NCAM1_HUMAN	<0.01	↓
5.	Apolipoprotein D	APOD_HUMAN	0.01	↓
6.	Tubulin beta-4B chain	TBB4B_HUMAN	0.02	↓
7.	Dickkopf-related protein 3	DKK3_HUMAN	0.03	↓
8.	Clusterin	CLUS_HUMAN	0.1	↓
9.	Apolipoprotein E	APOE_HUMAN	0.1	↓
10.	Astrocytic phosphosprotein PEA-15	PEA15_HUMAN	0.1	↓
11.	Annexin A5	ANXA5_HUMAN	0.1	↓
12.	Fibrinogen alpha chain	FIBA_HUMAN	0.2	↓
13.	Selenium-binding protein 1	SBP1_HUMAN	0.2	↓
14.	Actin, cytoplasmic 2	ACTG_HUMAN	0.2	↓
15.	Alpha-enolase	ENOA_HUMAN	0.3	↓
16.	Peroxiredoxin-2	PRDX2_HUMAN	0.4	↓
17.	Glyceraldehyde-3-phosphate dehydrogenase	G3P_HUMAN	0.4	↓
18.	14-3-3 protein theta	1433T_HUMAN	4.1	↑
19.	Complement C3	CO3_HUMAN	6.1	↑
20.	Alpha-2-macroglobulin	A2MG_HUMAN	9.4	↑

Table 1: List of 20 significantly changed proteins (T-Test, p<0.05) in suicidal CSF post-mortem samples versus control CSF post-mortem samples.

post-mortem. While protein YWHAE was found upregulated in both suicidal patients and controls in our study, the study of Yanagi et al. [9] described the gene coding protein YWHAE as a possible gene of suicidality. On the other hand, Shimada et al. [10] emphasized the neuroprotective characteristics of 14-3-3 proteins in neurodegenerative diseases. Many studies point out the vast and still not entirely known interaction network of 14-3-3 proteins with more than 200 other proteins, such as protein kinases, enzymes, receptors, cytoskeletal and structural proteins, small G-proteins, proteins included in the regulation of cell cycle and proteins regulating apoptosis [11-13]. Upregulation of YWHAH and YWHAQ proteins in our pilot study could be understood as a possible neuroprotective mechanism to

a specific type of neurodegeneration, that cannot be excluded in patients with suicidal behavior. Interestingly, comparison of CSF post-mortem samples showed 20 significantly changed proteins ($p < 0.05$) in suicidal patients versus controls (Table 1). All proteins from Table 1 (beside alpha-enolase, tubulin beta 4B chain and selenium-binding protein 1) were part of biological process pathway cellular response to stimulus. Upregulation of YWHAQ and downregulation of G3P supports our findings of possible alterations in glycolysis/gluconeogenesis pathway and 14-3-3 signaling pathway in suicidal patients. Based on these results, dysregulation of glucose metabolism (especially utilisation of glucose and altered response to oxidative stress) together with alterations in 14-3-3 mediated signaling pathway may play a role in the complex neurobiological basis of suicide. However, further proteomic research with a greater sample size is needed to clarify this hypothesis and elucidate the molecular mechanisms of this disorder. It is important to identify potential biomarker candidates for earlier diagnosis of suicidal behavior, evaluation of high or low suicidal intent/risk and finally for effective and safe therapy as a long-term suicide prevention.

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