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SHORT COMMUNICATION

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Proteomic analysis of the CSF in unmedicated patients with major depressive disorder reveals alterations in suicide attempters

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Abstract This is the first report on proteomic analysis of the cerebrospinal fluid (CSF) in unmedicated suicide attempters and non-attempters with major depressive disorder. Two-dimensional (2D) gel electrophoresis revealed that suicide attempters differed from non-attempters in one protein with an approximate molecular weight of 33 kD and an isoelectric point of 5.2. Proteomic analysis of the CSF is a promising non hypothesis-driven screening method for the detection of new candidate genes in neurobiological suicide research.

Key words major depressive disorder · suicide attempt · proteomic analysis · cerebrospinal fluid

Introduction

Several lines of evidence suggest a genetic influence on suicidal behavior which may be independent from the genetic susceptibility for major depression (Turecki 2001). Impulsive and aggressive behavior have been reported to be independent risk factors for suicidal behavior (Koller et al. 2002). Neurobiological suicide research has focused up to now on serotonergic candidate genes (Turecki 2001; Stefulj et al. 2004) or CSF markers of serotonergic neurotransmission (review: Brunner and Bronisch 1999; Roggenbach et al. 2002). However, since suicidal behavior is a multifactorially determined act, more complex research strategies are more appropriate (Mann 1998). Proteomics is a non hypothesis-driven exploratory screening method which enables the study of protein expression and the detection of potential protein biomarkers in a comprehensive and global manner (Hanash 2003).

In the present study, proteomic analysis of the cerebrospinal fluid (CSF) was performed in 14 unmedicated patients with major depressive disorder with the aim to detect a difference between suicide attempters (n = 7) and non-attempters (n = 7).

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Materials and methods

We studied 14 inpatients of the Max Planck Institute of Psychiatry (8 female, 6 male; age: 39 ± 15 years [mean ± SD], range: 20–64) with major depressive disorder according to DSM-IV criteria (single episode: n = 9, recurrent: n = 5). The patients had provided written informed consent to participate in the study (German Federal Research Ministry, Competence Nets in Medicine, subproject 1.5). All patients had either never been treated with antidepressants or were free of antidepressants for at least two weeks prior to lumbar puncture. Routine CSF analysis did not show any pathology in any of the patients. Cranial NMR scans were performed in 13 patients showing no abnormalities. No patient suffered currently from any severe somatic disease. Seven patients had attempted suicide prior to admission. Suicide attempters did not differ significantly from non-attempters in age and sex distribution (4 female, 3 men in each group). Five patients had used non-violent methods (intoxication: benzodiazepines [n = 2], diphenhydramine [n = 2], promethazine [n = 1]). The time interval between drug ingestion and lumbar puncture was 7 ± 2 days (range: 3–9). Two patients had used violent methods (strangulation, deep wrist cuts).

Psychopathology was assessed by means of the Hamilton Depression Rating Scale (HAMD), the Hamilton Anxiety Rating Scale (HAMA), and the Brief Psychiatric Rating Scale (BPRS). To assess the hypothalamic-pituitary-adrenocortical (HPA) system, the combined dexamethasone/CRH test was performed in 10 patients (5 suicide attempters, 5 non-attempters) as described in detail elsewhere (Zobel et al. 2001).

For the 2D gel electrophoresis CSF samples of the 7 suicide attempters were pooled and compared to the pooled CSF samples of the 7 non-attempters (3 ml of total CSF per pool). Samples were pooled because of the limited amount of material. For CSF sample preparation human serum albumin and immunoglobulins were removed by absorption of the pooled CSF samples to Cibacron Blue Sepharose and Protein G agarose, respectively. For 2D gel electrophoresis the PROTEAN IEF Cell was used for the first dimension and the PROTEAN Plus Dodeca Cell for the second dimension SDS gel electrophoresis (BioRad Laboratories, Hercules, CA). Proteins from CSF samples that had been depleted by the Cibacron Blue/Protein G were

concentrated and desalted with a Vivaspin 5 kDa cartridge (Vivascience AG, Hannover, Germany) and then dissolved in 300 µl IEF sample buffer (7 M urea, 2 M thiourea, 2 % CHAPS, 0.2 % Biolyte, pH 4–7, 100 mM DTT, 0.001 % Bromphenol Blue). IEF was carried out using IPG strips, pH 4–7 (BioRad Laboratories) until 60,000 Vh were reached. The strips were then subjected to reduction with DTT and alkylation with iodoacetamide for 10 min each and further processed by SDS electrophoresis in a 12 % gel. After electrophoresis proteins are stained with colloidal Coomassie Blue and scanned with a densitometer (BioRad Laboratories). Image analysis was carried out with the PDQUEST software 7 (BioRad Laboratories).

Results

No significant differences between suicide attempters and non-attempters were found regarding psychometric scales for severity of depression (HAMD: 22 ± 10 vs. 28 ± 12, $p = 0.35$, t-test for independent samples), anxiety (HAMA: 17 ± 8 vs. 23 ± 11, $p = 0.24$), and general psychopathology (BPRS: 29 ± 8 vs. 35 ± 10, $p = 0.22$) or cortisol and ACTH concentrations (area under the curve, corrected for baseline concentrations) in the dexamethasone/CRH test (data not shown).

Based on the image analysis of the 2D gels one protein was found as a difference between suicide attempters and non-attempters (Fig. 1). This protein has an approximate molecular weight of 33 kD and an isoelectric point of 5.2. Due to the limited amount of material this protein could not be identified by mass spectrometry.

Discussion

This is the first report on proteomic analysis of the CSF in unmedicated suicide attempters and non-attempters with major depressive disorder. No significant differences between suicide attempters and non-attempters were found regarding psychometric scales for severity of depression, anxiety, and general psychopathology or cortisol and ACTH concentrations in the dexamethasone/CRH test.

The only biological marker that differed between suicide attempters (n = 7) and non-attempters (n = 7) was the 33 kD protein in the CSF detected by 2D gel electrophoresis. Since all subjects were free of antidepressants for at least two weeks, a medication bias is not likely. Due to the limited amount of material, this protein could not be identified by mass spectrometry so that the biological function or possible mechanisms could not be elucidated in this preliminary study. A major limitation is the pooling of the CSF. However, pooling was inevitable because of the limited amount of CSF available.

Proteomic analysis of the CSF is a suitable and promising non-hypothesis-driven screening method for the detection of new candidate genes associated with altered protein formation and for the elucidation of post-genomic mechanisms contributing to proteomic differences in patients with major depressive disorder.

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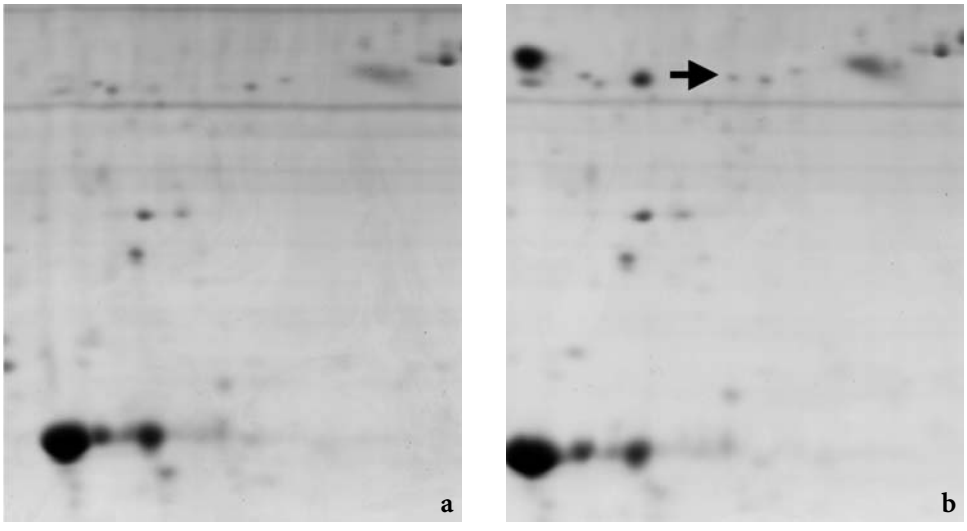

Fig. 1 2D Gel electrophoresis of CSF sample pools of unmedicated patients with major depressive disorder (n = 14) revealed a difference in a protein of approximately 33 kD and an isoelectric point of 5.2 (arrow) between **a** suicide attempters (n = 7) and **b** non-attempters (n = 7)

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