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Candidate biomarkers of chronic inflammatory demyelinating polyneuropathy (CIDP): Proteome analysis of cerebrospinal fluid

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ABSTRACT

We aimed to identify disease-related biomarkers in CIDP. Using the two-dimensional difference in gel electrophoresis (2-D-DIGE), we compared CSF from patients with CIDP (n = 11) and controls (n = 11). Protein spots that showed a significant difference were further analyzed by MALDI-TOF mass spectrometry. We identified 10 proteins that were upregulated in CIDP (two transferrin isoforms, alpha-1 acid glycoprotein 1 precursor, apolipoprotein A IV, two haptoglobin isoforms, transthyretin (TTR), retinol binding protein and two isoforms of proapolipoprotein) and 1 protein that was downregulated (integrin beta 8). The pathophysiological role of these proteins remains to be clarified by further studies.

1. Introduction

Biochemical markers reflecting specific aspects of disease pathology could support the differential diagnosis of chronic inflammatory demyelinating polyneuropathy (CIDP) and could help the identification of beneficial drugs in clinical trials (Tumani et al., 2008). Cerebrospinal fluid (CSF) is a promising source of biomarkers in CIDP since the proximal nerve roots which are affected early in the disease are in close anatomical contact with the CSF space (Reiber, 2001). In a previous study, we analyzed the CSF proteome (e.g. the total protein content) of patients with GBS using the two-dimensional fluorescence differential in gel electrophoresis (2-D-DIGE) (Lehmensiek et al., 2007a), which is known for a high sensitivity and a high reproducibility as compared to classical 2-D electrophoresis techniques (Marouga et al., 2005). So far, there is no study analyzing the proteome of patients with CIDP. Based on the findings of our previous study, we proceeded to analyze the CSF proteome of patients with CIDP. We aimed to identify a characteristic CSF protein pattern that could provide new candidate biomarkers to support clinical diagnosis in CIDP and allow further insight into the pathomechanisms underlying the disease.

2. Materials and methods

CSF samples were collected in a prospective study from 11 patients with CIDP according to criteria of the European Federation of Neurological Societies (EFNS) Task Force (2005) (Table 1). None of the CIDP patients showed a serum monoclonal gammopathy. Disability was rated using Medical Research Council sumscore (MRCS) (Kleyweg et al., 1991). At time of lumbar puncture, none of the patients was treated with corticosteroids, plasma exchange or intravenous immunoglobulins. The control group consisted of 11 age- and sex-matched patients who got lumbar puncture to rule out

Table 1
Demographic data and CSF/serum albumin quotient (Q_{alb}) of the subjects included in this study. MRCS is Medical Research Council Sumscore.

	n (female/male)	age [years] Median (Range)	disease duration [days]	MRCS	Qalb [x 0.001]
CIDP*†	11 (3/8)	64 (51-77)	360 (65-1460)	52 (22-59)	15.1 (5.5-48.2)
CTRL*†	11 (3/8)	61 (24-80)			10.7 (9.0-16.0)
GBS†	19 (7/12)	60 (17-67)	5 (2-22)	51 (14-58)	28.2 (4.8-53.5)

* Patients included in 2-D DIGE.
† Patients included in validation experiments.

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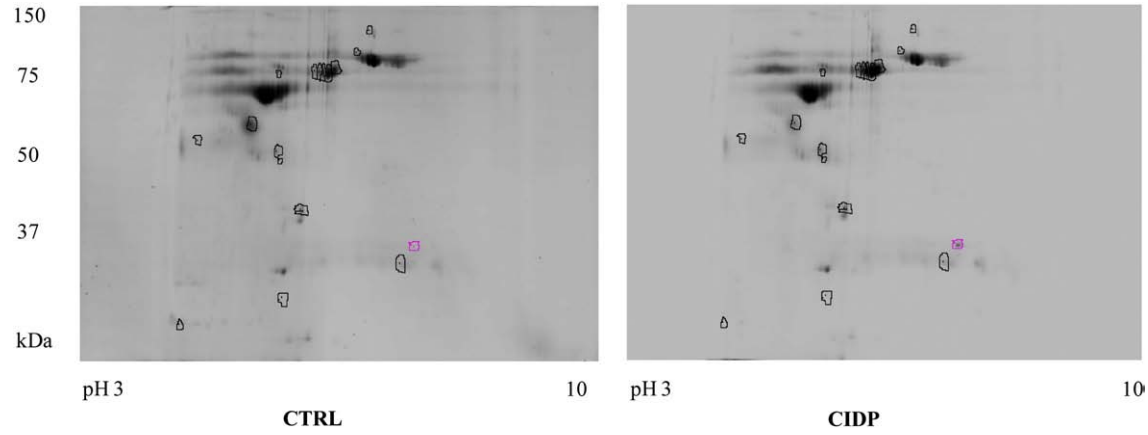

Fig. 1. Difference in-gel analysis of CSF from patients with CIDP versus controls analyzed with 2-D DIGE and DeCyder Difference Analysis Software. Left side: control sample pool (CTRL), right side: CIDP sample pool. Marked spots showed a significant difference between patients and controls over three independent 2-D-DIGE gels. Spot with pink rim is coincidentally selected in the analysis program.

subarachnoid hemorrhage (SAH) and showed no evidence of a structural, hemorrhagic or inflammatory lesion. For validation experiments on one candidate protein, 19 patients with GBS (Asbury and Cornblath, 1990) were included as inflammatory disease controls. Informed consent was obtained from all patients. The study was approved by the local ethics committee.

Details on handling and storage of CSF samples as well as the experimental procedure have been published before by our group (Lehmensiek et al., 2007a; Lehmensiek et al., 2007b). For each of the three 2-D-DIGE experiments we labeled 50 µg protein of each sample pool with 400 pmol of the appropriate CyDye. Gels were analyzed with DeCyder software (version 5.0, Amersham Biosciences) using DIA (Difference In Gel Analysis) module and BVA (Biological Variation Analysis) module. Protein spots that showed a significant difference between CIDP and controls over three independent gels were further analyzed using MALDI-TOF mass spectrometry by TOPLAB GmbH (Martinsried, Germany). Differentially expressed spots of interest were picked and trypsinated. For MALDI-TOF, trypsin peptide solutions were spotted on stainless-steel MALDI sample plates, mixed with matrix solution and analyzed with Voyager DE-STR (Applied Biosystems) MALDI-TOF mass spectrometer. Spectra were analyzed by searching the protein database of the National Centre for Biotechnology Information (NCBI).

Table 2
Proteins in CSF in CIDP identified by 2-D DIGE and mass fingerprinting analysis, with a significant difference in CSF protein concentration as compared to controls. Factor of regulation describes the relation of the spot volumes to another (CIDP vs. controls). TTR = transthyretin, ITGB8 = integrin beta 8.

Spot ID	Factor of regulation	Protein name	Accession number	Mw / Pi* [kDa / pH]	Sequence coverage*
721	2,06	transferrin	gi|37747855	79.3 / 7.1	42%
879	2,07	transferrin	gi|37747855	79.3 / 7.1	48%
1331	1,99	α-1-acid glycoprotein 1 precursor	gi|1197209	23.6 / 5.0	25%
1448	-1,5	ITGB8 protein	gi|12803591	49.4 / 5.8	26%
1564	2,63	apolipoprotein A-IV	gi|178759	45.3 / 5.2	55%
1564-2	0,46	haptoglobin	gi|3337390	38.7 / 6.1	24%
1247	0,38	haptoglobin	gi|3337390	38.7 / 6.1	42%
3151	2,04	TTR	gi|48145933	16.0 / 5.5	73%
1426	4,87	retinol binding protein	gi|225862	21.3 / 5.3	81%
2403	1,78	proapolipoprotein	gi|178775	29.0 / 5.4	55%
3151	1,68	proapolipoprotein	gi|178775	29.0 / 5.4	65%

*as obtained by ProFound (Genomic Solutions Inc., UK, Version 2004.01.26) searching the National Centre for Biotechnology Information (NCBI) non-redundant protein database.
** not available due to very low protein concentration.

To validate the 2-D DIGE findings one selected candidate protein (transthyretin) was further analyzed using nephelometry (BN ProSpec®System, Behring-Siemens, Erlangen, Germany). Data analysis was performed using SPSS (Version 15.0 SPSS Inc., Chicago, IL, USA). Because of non-normal data distribution, medians and interquartile ranges are shown. Correlations were studied using Spearman's rank correlation. Multiple correlations were corrected using the Bonferroni method. Differences between groups were compared using the two-sided Wilcoxon two-sample test. P-values <0.05 were considered significant.

3. Results

3.1. 2-D DIGE of CIDP and controls

A total of 2122-2473 spots could be detected (difference due to three independent 2-D-DIGE runs) (Fig. 1). Analysis of only those spots with a significant difference over three gels between CIDP and controls revealed 11 spots, corresponding to 11 proteins or their isoforms. We identified 10 proteins that were upregulated in CIDP as compared to controls (two transferrin isoforms, alpha-1 acid glycoprotein 1 precursor, apolipoprotein A IV, two haptoglobin isoforms, transthyretin (TTR), retinol binding protein and two isoforms of proapolipoprotein) and 1 protein that was downregulated in CIDP vs. controls (integrin beta 8) (Table 2).

3.2. Validation experiments

We observed a significant difference of CSF TTR between patients with CIDP, GBS and controls ($p=0.005$). CSF TTR correlated with blood-CSF barrier function measured by Q_{alb} ($p<0.001$). To allow for the dependency of CSF TTR from blood-CSF barrier function, CSF/serum quotients of TTR and TTR index (CSF TTR/serum TTR/Q_{alb}) were determined. We found a significant difference of CSF/serum TTR between CIDP, GBS and controls ($p=0.009$), with post-hoc analysis showing CSF/serum TTR to be higher in CIDP as compared to controls ($p<0.05$, Fig. 2). We observed a significant difference of the TTR index between the groups ($p=0.03$, Fig. 2), with post-hoc analysis showing a significant difference between CIDP and GBS. We observed no correlation of TTR index with clinical parameters like duration or s2everity of disease as measured by MRCS (data not shown).

4. Discussion

4.1. Methodological considerations

While 2-D DIGE is known for a high sensitivity and reproducibility as compared to classical 2-D electrophoresis (Marouga et al., 2005; Tumani