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ORIGINAL PAPER

Structural and Quantitative Comparison of Cerebrospinal Fluid Glycoproteins in Alzheimer's Disease Patients and Healthy Individuals

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Abstract Glycoproteins in cerebrospinal fluid (CSF) are altered in Alzheimer's Disease (AD) patients compared to control individuals. We have utilized albumin depletion prior to 2D gel electrophoresis to enhance glycoprotein concentration for image analysis as well as structural glycoprotein determination without glycan release using mass spectrometry (MS). The benefits of a direct glycoprotein analysis approach include minimal sample manipulation and retention of structural details. A quantitative comparison of gel-separated glycoprotein isoforms from twelve AD patients and twelve control subjects was performed with glycoprotein-specific and total protein stains. We have also compared glycoforms in pooled CSF obtained from AD patients and control subjects with mass spectrometry. One isoform of α_1-antitrypsin showed decreased glycosylation in AD patients while another glycosylated isoform of an unassigned protein was up-regulated. Protein expression levels of α_1-antitrypsin were decreased, while the protein levels of apolipoprotein E and clusterin were increased in AD. No specific glycoform could be specifically assigned to AD.

Keywords Proteomics · Glycoproteomics · Glycoprotein · N-linked · Glycosylation · Glycoform · Isoform · CSF · Cerebrospinal fluid · Alzheimer's Disease · α_1-antitrypsin · Apolipoprotein E · Clusterin · Haptoglobin · α-1-β-glycoprotein · Zinc-α-2-glycoprotein · 2D gel electrophoresis · 2D-GE · Albumin depletion · Image analysis · Mass spectrometry · FT-ICR MS · Linear ion trap

Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disease characterized by accumulation of amyloid plaques, neurofibrillary tangles and loss of synapses and neurons in the brain. The major constituent of senile plaques in AD brain is amyloid-β peptide (40-42 residues). In neurofibrillary tangles, tau protein is abnormally hyperphosphorylated and aggregates into paired helical filaments. Glycosylation of amyloid precursor protein, tau protein, acetylcholinesterase and butyrylcholinesterase is known to be associated with AD [1–3] Protein glycosylation has many functions in the cellular processes and glycans are often capped with sialic acid residues. For example altered glycosylation of tau facilitates the phosphorylation process [2]. Because O-linked *N*-acetylglucosamine (O-GlcNAc) can compete with phosphate groups to occupy specific sites, an inverse relationship for these two modifications of tau has been found [4]. Glycosylation of acetylcholinesterase

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and butyrylcholinesterase increases with the duration of AD and in other neurodegenerative diseases [3]. Moreover, reelin is a glycoprotein proposed to be involved in signaling pathways in several neurodegenerative diseases and an increase of reelin in the brain and CSF from AD patients was recently shown [5].

Glycoproteins present in cerebrospinal fluid (CSF) are a source of biomarkers because changes in CSF composition can reflect on-going disease processes in the brain [6]. Several glycoproteins, e.g., apolipoprotein E, apolipoprotein J (clusterin), α-1-β-glycoprotein and α_1-antitrypsin were altered in a proteomic study of CSF from AD patients [7]. The alteration of glycans bound to proteins in relation to disease is a highly relevant subject and there is a great need for AD biomarkers for diagnosis and response to treatment, as well as for the understanding of the molecular pathology of the disease. Combined clinical examination and measurement of the biochemical markers beta-amyloid and tau in CSF have become valuable diagnostic tools during the recent years, predicting more than 80% of the AD cases [8–10]. Still, additional diagnostical tools would be of great importance to facilitate an early and correct clinical diagnosis.

There are two major methods of isolating glycoproteins. Lectin affinity purification can be employed to isolate multiple types of glycoproteins and hydrazide chemistry can capture N-glycopeptides in a specific manner. A combination of these methods is one way to characterize and quantify the CSF glycoproteome [11]. The limitation of this approach is that quantification only is possible at the level of tandem mass spectrometry (MS/MS) and therefore isoforms and degradation products of the proteins may be missed. An alternative approach is to use 2D gel electrophoresis (2D-GE) to build CSF maps and to study post-translational modifications. Several comparative studies as well as mapping of CSF proteins have been carried out, [7, 12–19]. We have previously characterized glycan structures in CSF glycoproteins from AD patients, and this study indicated the need for sample prefractionation [20]. The present study utilizes albumin affinity chromatography to increase the detection of disease-related glycoproteins in CSF.

Experimental Procedure

CSF Samples

CSF samples were obtained from Karolinska University Hospital in Huddinge, Stockholm, Sweden. All patients underwent a comprehensive investigation including clinical examination, routine blood and CSF laboratory tests, electroencephalography, magnetic resonance imaging, and single photon emission computed tomography. The cognitive evaluations included mini-mental state examination (MMSE) as well as comprehensive neuropsychological tests. Alzheimer's disease was diagnosed according to the DSM-IV criteria (APA 1994, WHO 1992) [21, 22]. The AD group included totally 17 patients, divided in two groups; seven men and five women, mean age 78.8 years (range 74–85) for both quantitative and qualitative glycoprotein analysis (pooled CSF) and a second group with five patients, all women, mean age 75 (range 69–81) for qualitative glycoform MS analysis.

The control group consisted of individuals with subjective cognitive impairment (SCI) and included totally 16 patients, divided in two groups; seven men and five women, mean age 57.5 (range 38–75) for both quantitative and qualitative glycoprotein analysis (pooled CSF) and a second group with four patients, one man and three woman, mean age 62 (range 62–64) for qualitative glycoform MS analysis. Those were subjects who were referred due to subjective memory problems but did not show any pathological signs in any of the tests performed above. To prevent inclusion of very early dementia patients, only individuals that did not deteriorate over a minimum of 2 years follow-up were included in this group. Samples were stored in −80°C. The local Ethics committee at Huddinge University Hospital approved the study.

Albumin Depletion

Albumin depletion was performed by affinity chromatography [23] in small spin columns after concentration and desalting of 1.5 ml CSF in 5 kDa membrane cut-off centrifuge tubes. Proteins were precipitated with aceton in −20°C for 2 h prior to 2D gel electrophoresis.

2D Gel Electrophoresis

The 2D gel electrophoresis method has previously been described in detail [23]. The protein pellet was dissolved and diluted in buffer and the two sample tubes (from the same individual) divided prior to the precipitation were pooled together. Isoelectric focusing (IEF) was performed with IPG strips, pH 4.7–5.9, 7 cm (BioRad, Hercules, CA, USA), in the Ettan IPGphor IEF System (Amersham Biosciences/GE Healthcare, UK). After equilibration of the IPG strips in buffer containing 1% DTT for 15 min and 2.5% iodoacetamide for a further 15 min, the second dimension separation was carried out by use of the NuPAGE gel system (NOVEX, San Diego, CA, USA) combined with MOPS running buffer.

In order to visualize glycoproteins, the gels were stained with Pro-Q Emerald 300 Glycoprotein stain (Molecular

Probes, Leiden, The Netherlands) scanned in a Fluor-S Multi-Imager (Bio-Rad, Hercules, CA, USA) and post-stained with SYPRO Ruby Protein Stain (Molecular Probes) according to the supplier's protocols. The gels were scanned again (2D 2920 Master Imager, Amersham Biosciences/GE Healthcare) in order to obtain an image of the protein pattern. The optical density of protein spots is proportional to protein concentration. Gels for mass spectrometry analysis were only stained with Sypro Ruby.

2D-Gel Image Analysis

Image analysis was carried out with the PD-Quest software version 7.4.0 (Bio-Rad). Protein levels were evaluated as volumes (spot area optical density) for the protein spots matched among gels representing all groups. Spot volume was normalized for each gel on total density in gel image. Data were log transformed and analyzed with Student's *t*-test with the statistics tools included in the PD-Quest software. Altered spots were selected using the average of twelve gels within one group and compared to the other group at confidential level 95%. Spots which gave significant results were verified visually to exclude artifacts. In the case of a missing isoform in any of the individual gels, the value of the missing spot was estimated and included in the average for that group. The statistical comparison was also performed without manual addition of a "missing" spot. Either way of handling missing values gave the same results.

Mass Spectrometry and Database Analysis

In-gel trypsin digestion of proteins was performed as described [24], with minor modifications [23]. After extraction, the supernatants were evaporated to dryness in a vacuum centrifuge. Tryptic digests were reconstituted in 18 µl 0.1% HCOOH.

NanoLC-MS/MS was performed as previously described in detail [23]. A C_{18}—fused silica column (3 µm porous particles, 20 cm × 50 µm i.d.) was connected to a hybrid linear ion trap-Fourier Transform Ion Cyclotron mass spectrometer (FT-ICR MS) (LTQ-FT, Thermo Electron), equipped with a 7 T magnet. 2 µl sample injections were made (HTC-PAL auto sampler, CTC Analytics AG), the gradient was 0–50% CH_3CN, starting with HCOOH 0.2% in water for 40 min and the eluent was electrosprayed (+1.4 kV) from the emitter tip into the mass spectrometer.

The mass spectrometer was operated in the data-dependent mode to automatically switch between MS (acquired in the FT-ICR) and MS/MS acquisition (in the linear ion trap). The monoisotopic precursor selection was turned off to enable selection of large, multiply protonated glycopeptides. Measured peptide masses and their CID spectra were analyzed by MASCOT database search software version 2.1.6 (Matrix Science) [25]. Swiss-Prot database was used for protein identification. Database interrogation was carried out using monoisotopic masses, 5 ppm precursor-ion mass tolerance window, 0.5 Da fragment-ion mass tolerance window, one allowed missed tryptic cleavage, oxidation of methionine and the alkylation of cysteine residues by iodoacetamide. The criteria for positive identification of proteins were two or more peptides with significant scores ($p < 0.01$).

Mass values for peptides that could not be matched to the identified protein sequence by Mascot, were examined for the presence of glycosylation by use of the GlycoMod tool (http://us.expasy.org/tools/glycomod). The SwissProt accession number corresponding to the protein identity and unmatched monoisotopic masses were entered, and a mass deviation of 10 ppm was tolerated. The number of possible hexoses (Hex) was set to 3–10, *N*-acetylhexosamines (HexNAc) 2–10, deoxyhexoses 0–3, and *N*-acetylneuraminic acids, sialic acid (NeuAc) 0–6.

Results

In order to address the question of disease-related alterations of glycosylation, we have used a focused proteomics approach including sample prefractionation, narrow range iso-electric focusing and glycoprotein specific staining followed by total protein staining. The treatment of 2D gels during the glycoprotein specific stain (Pro-Q Emerald) renders them less useful for mass spectrometric analysis because the dye binds to periodate-oxidized carbohydrate groups. Therefore, we used pooled CSF for the MS analysis representing the group of AD patients and control individuals, respectively. The outline of the experimental design of the glycoproteomic approach is shown in Fig. 1.

Differential Comparison of Glycoproteins

An individual quantitative comparison of gel-separated glycoprotein isoforms from twelve AD patients and twelve control subjects was performed by image analysis. Trains of spots were observed for different glycoforms in the 2D gel images from the two groups (typical gels shown in Fig. 2). The resulting differential two spots are the same in both cases and the significance threshold was 95%. One spot (6a in Fig. 2 and Table 1) showed decreased glycosylation of α_1-antitrypsin for AD patients. The glycopeptides (3960.670 and 3669.598) in spot 6a (Table 2), were also seen in isoforms 6b to 9 and were thus not specific for the glycoprotein up-regulation. Glycopeptide 3359.266 was

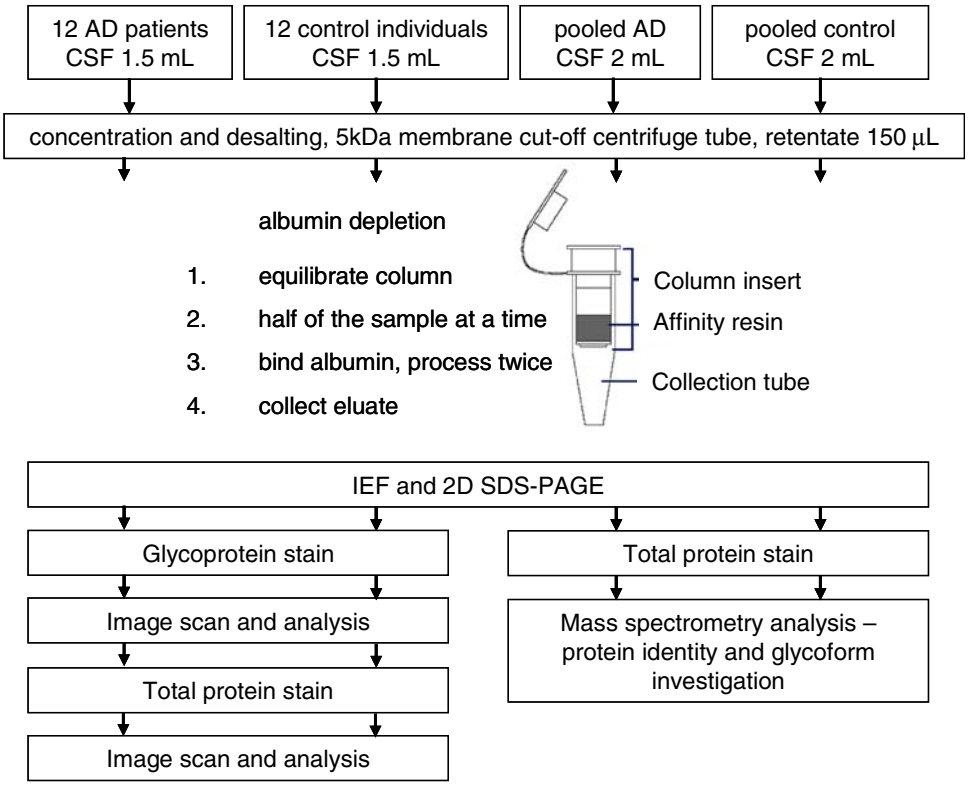

Fig. 1 Flowchart of the experimental design

found in the altered spot 10 and was continuously found in spots 11–13 for the pooled CSF from the AD patients and in spot 13 for the control individuals.

Differential Comparison of Proteins

Image analysis of the total protein pattern revealed three significantly altered protein isoforms. There was no differentially expressed isoform that was changed in both the level of glycosylation and amount of protein (Fig. 2 and Table 1). The intensity of spot 9 was decreased in the AD group and identified as a mixture of proteins where α_1-antitrypsin was identified with the highest number of peptides. Spot intensities of protein isoform 18 and 23 were both increased in the group with AD patients and were identified as apolipoprotein J (clusterin) and apolipoprotein E. Detailed analysis of spots containing these proteins was performed with pooled CSF and mass spectrometry.

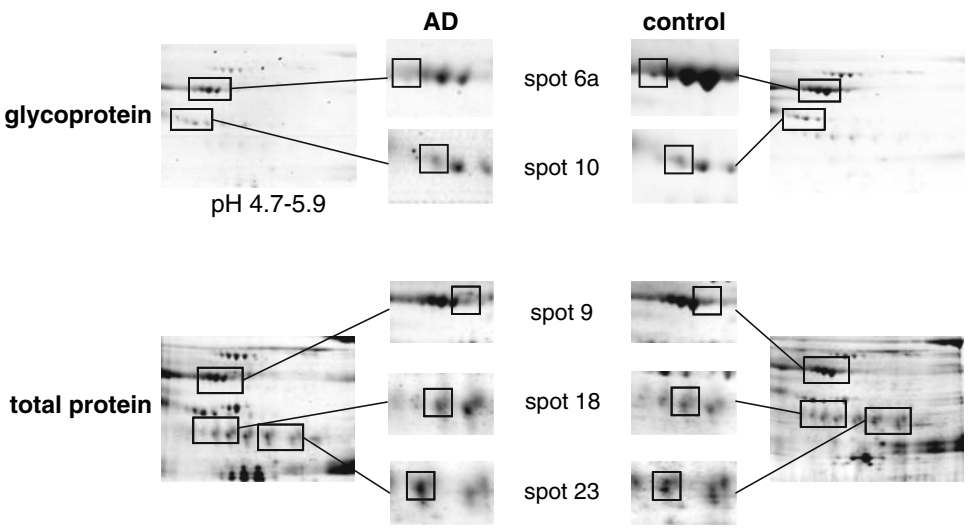

Fig. 2 Typical 2D gel images for the AD patients and the control individuals. Enlarged areas and spot numbers reflects the result from the differential protein expression or glycosylation level in the image analysis as summarized in Table 1

Table 1 Differentially altered glycoproteins found in individual comparison of twelve AD patients and twelve control subjects

Spot-group	Swissprot name short	Regulation/ AD versus control	Matched peptides	Coverage %	Mascot score	mw (kDa)/pI
6a-AD	A1AT	Glyco/ decrease	21	48	1,541	46.7/5.4
	AACT		8	21	356	47.7/5.3
	DKK3		4	12	280	53.2/5.6
	ANGT		4	6	246	38.3/4.5
	ANT3		8	12	143	52.6/6.0
6a-C	A1AT		20	51	1,239	46.7/5.4
	AACT		5	15	281	47.7/5.3
	ANGT		2	5	148	53.2/5.6
	DKK3		3	10	120	38.3/4.5
	ANT3		2	7	70	52.6/6.0
9-AD	A1AT	Protein/ decrease	13	32	1,109	46.7/5.4
	ANT3		19	39	958	52.6/6.0
	AACT		2	7	173	47.7/5.3
	ANGT		2	5	167	53.2/5.6
	NUCB1		1	2	66	53.9/5.1
9-C	A1AT		17	40	1,031	46.7/5.4
	ANT3		18	39	850	52.6/6.0
	NUCB1		7	15	238	53.9/5.1
	AACT		3	9	183	47.7/5.3
	ANGT		3	7	170	53.2/5.6
10-AD	ZA2G	Glyco/ increase	5	19	221	33.8/5.5
	HPT		3	6	92	39.0/6.1
10-C	ZA2G		3	9	149	33.8/5.5
	HPT		3	8	129	39.0/6.1
18-AD	APOE	Protein/ increase	13	27	835	36.2/5.5
	CLUS		13	39	721	53.0/5.9
18-C	CLUS		10	18	312	53.0/5.9
	APOE		8	16	215	36.2/5.5
23-AD	APOE	Protein/ increase	9	30	395	36.2/5.5
	CLUS		5	13	217	53.0/5.9
23-C	CLUS		7	17	300	53.0/5.9
	APOE		5	17	193	36.2/5.5

The 2D gels were stained for glycoprotein and total protein patterns. Protein identity was obtained from the corresponding spot in a 2D gel of pooled CSF

C Control

AD Alzheimer's Disease

A1AT P01009 Alpha-1-antitrypsin

AACT P01011 Alpha-1-antichymotrypsin

ANGT P01019 Angiotensinogen precursor

ANT3 P01008 Antithrombin-III precursor

NUCB1 Q02818 Nucleobindin-1 precursor

ZA2G P25311 Zinc-alpha-2-glycoprotein

HPT P00738 Haptoglobin precursor

APOE P02649 Apolipoprotein E precursor

CLUS P10909 Clusterin, apolipoprotein J precursor

DKK3 Q9UBP4 Dickkopf-related protein 3 precursor

Structural Comparison of Glycoproteins

For structural determination of glycoforms and protein identification by MS, we used pooled CSF representing the group of AD patients and the group of control subjects, respectively. Figure 3 shows the 2D gel of total protein stained pooled CSF with all spots used for MS analysis. Protein identification was based on unmodified peptides. High mass accuracy, below 10 ppm (typically 2 ppm) together with partial fragment information of the saccharide and database interrogations is most often enough information to predict both glycan position and glycan composition. Presence of glycosylated peptides can be confirmed, with MS/MS, with the typical diagnostic ions *m/z* 366 for Hex-HexNAc and *m/z* 657 for HexHexNAcNeuAc.

All spots were examined to find all possible glycoforms both in AD patients and control subjects. Table 2 summarizes all glycopeptides and glycan compositions together with possible protein position found in the LC-MS analysis and the database search. Some of the glycoforms from the pooled material turned out to look as AD specific (Table 2) and we tested this hypothesis with an additional set of individual 2D gels, without the non-MS compatible glycoprotein stain. CSF from additional five AD patients and four control individuals were prepared for 2D-GE for qualitative glycoform analysis and gel spots for all visible protein isoforms were excised and analyzed. The result from the individual glycoform analysis, with only those glycopeptide masses that were potentially AD specific, is shown in Table 3. Thus, there is no glycoform structure

Table 2 Structural comparison of glycoproteins from pooled CSF from AD patients and control subjects

Isoform	Glycopeptide mass MH^+ (Da)	Error ppm	Observed glycan ion/glycan composition[c]	Possible protein position[d]
1–5	3,371.561	–	$Hex_1HexNAc_1$	A1BG/ALBU/CO3/HEMO/AACT/A1AT
	3,880.674[a]	–	$Hex_1HexNAc_1$, $Hex_1HexNAc_1NeuAc_1$	A1BG/ALBU/CO3/HEMO/AACT/A1AT
	3,940.749	–	Hex_1 HexN Ac_1, Hex_1 HexN Ac_1 $NeuAc_1$	A1BG/ALBU/CO3/HEMO/AACT/A1AT
	3,979.79[a]	–	$Hex_1HexNAc_1NeuAc_1$	A1BG/ALBU/CO3/HEMO/AACT/A1AT
6–9	3,669.598	7.0	$(Hex)_2$ $(HexNAc)_2$ $(NeuAc)_1$ + $(Man)_3(GlcNAc)_2^b$	A1ATN271
	3,960.670	0.7	(Hex)2 $(HexNAc)_2$ (NeuAc)2 + $(Man)_3(GlcNAc)_2^b$	A1ATN271
10–13	3,068.177[a]	–	$Hex_1HexNAc_1$, Hex_1 HexN Ac_1 $NeuAc_1$	ZA2G/HPT
	3,252.263[a]	–	$Hex_1HexNAc_1$, Hex_1 HexN Ac_1 $NeuAc_1$	ZA2G/HPT
	3,343.276[a]	–	$Hex_1HexNAc_1$, Hex_1 HexN Ac_1 $NeuAc_1$	ZA2G/HPT
	3,359.257	–	$Hex_1HexNAc_1$, Hex_1 HexN Ac_1 $NeuAc_1$	ZA2G/HPT
	3,999.786	–	$Hex_1HexNAc_1NeuAc_1$	ZA2G/HPT
		0.7	or $(Hex)_2$ $(HexNAc)_2$ $(NeuAc)_2$ + $(Man)_3(GlcNAc)_2$	HPT N241
17–23	3,385.310[a]	3.3	$(Hex)_4$ $(HexNAc)_3$ $(NeuAc)_1$ + $(Man)_3(GlcNAc)_2$	APOJ N291
	3,743.588	5.2	$(Hex)_2$ $(HexNAc)_2$ $(Deoxyhexose)_1$ $(NeuAc)_1$ + $(Man)_3(GlcNAc)_2$	APOJ N374
	4,034.676	3.1	$(Hex)_2$ $(HexNAc)_2$ $(Deoxyhexose)_1$ $(NeuAc)_2$ + $(Man)_3(GlcNAc)_2^b$	APOJ N374

The glycoforms only found in AD patients were further analyzed and summarized in Table 3

[a] Only found in AD patients in pooled CSF but also found in controls in individual samples

[b] Confirmed in previous study, Sihlbom IJMS2004

[c] Only partial glycopeptide fragmentation obtained, indication the presence of stated components

[d] If no conclusive match from database searches, all possible proteins identified in that spot are listed

C Control

AD Alzheimer's Disease

A1BG P04217 α-1β-glycoprotein

ALBU P02768 Serum albumin precursor

CO3 P01024 Complement C3 precursor

HEMO P02790 Hemopexin precursor, β-1B-glycoprotein

AACT P01011 Alpha-1-antichymotrypsin

A1AT P01009 Alpha-1-antitrypsin

ZA2G P25311 Zinc-alpha-2-glycoprotein

HPT P00738 Haptoglobin precursor

APOE P02649 Apolipoprotein E precursor

APOJ P10909 Clusterin, apolipoprotein J precursor

that could be specifically assigned to AD patient CSF in this study.

Discussion

The comparison of intensities of glycosylated protein isoforms demonstrated that one isoform of α_1-antitrypsin (spot 6a) was differentially expressed. Spot 9 in the protein staining comparison also contained α_1-antitrypsin but was a mixture of several other proteins. α_1-antitrypsin is a serine protease inhibitor, that has been localized in neurofibrillary tangles and senile plaques [26]. The protein inhibits the activity of elastase and other proteases and possesses anti-inflammatory properties [27]. A correlation between inflammatory biomarkers in plasma and CSF might provide useful combinations with which to monitor the inflammatory processes of AD [28]. The glycoform of α_1-antitrypsin found in spot 6a was also found in the other following isoform spots and detected in both the group of AD patients and control subjects.

Zinc-α-2-glycoprotein and haptoglobin were both identified in the altered glyco-specific spot 10 showing

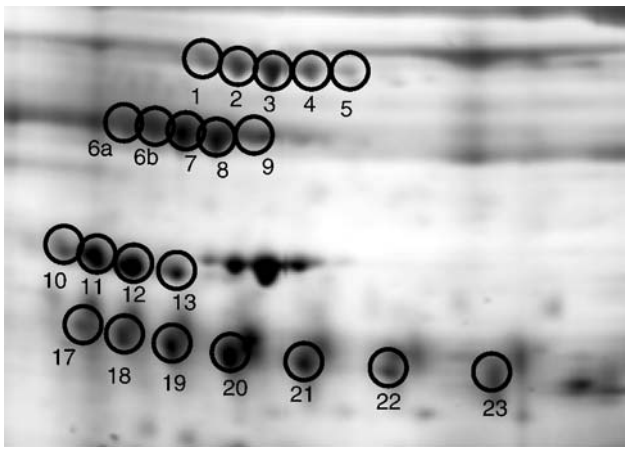

Fig. 3 2D gel electrophoresis, pH interval 4.7–5.9, of pooled CSF from AD patients showing all spots included for screening of structural glycoform differences as compared to control individuals

increased glycosylation in the group of AD patients. The glycopeptide (3359.266) in spot 10, was also seen in isoforms 11–13 and thus is not specific for the glycoprotein up-regulation. Zinc-α-2-glycoprotein stimulates lipid degradation in adipocytes and is linked to cachexia in patients with advanced stages of cancer. No connection between zinc-α-2-glycoprotein and neurodegeneration has been reported previously. Haptoglobin is an iron transporting protein as well as an acute phase protein. The function of haptoglobin is to bind free hemoglobin, to protect from oxidative damage. Two studies have demonstrated increased presence of some lower weight isoforms of haptoglobin in CSF of AD patient [29, 30].

In the differential comparison of protein expression level in specific isoforms, clusterin and apolipoprotein E were both increased in the group with AD patients. Clusterin has been shown to be present in senile plaques and increased levels have been reported in AD brain as well as increased levels of both the glycosylated and deglycosylated form in CSF from AD patients [31]. Apolipoprotein E with the specific form, ApoE4, is a major risk factor for getting sporadic AD at an early age and is found in only a small part of the population of AD patients [32]. Immunological determination of total apolipoprotein E content in CSF has earlier showed reduced levels in AD patients compared to control subjects [33]. None of the glycopeptides found in the 2D gel train spots (17–23) of clusterin or apolipoprotein E were disease-specific.

Glycoproteomic Approach

The total protein content in human CSF is about 0.3 mg/ml, which is much lower than in plasma or serum. Albumin constitutes more than 50% of the total protein. CSF also contains other compounds, such as organic and non-organic salts, various sugars, and lipids. We have previously used concentration and desalting of CSF prior to albumin depletion as a method suited for structural determination of less abundant disease-specific glycoproteins [23]. Isoelectric focusing of proteins with micro-narrow strips (pH 4.7–5.9) improves the detection and separation of different glycoprotein isomers compared to separation over a large pH range. Image analysis with targeted staining for glycoproteins allows for quantification of glycoforms. The structural comparison of glycopeptides with high mass accuracy (<10 ppm) mass spectrometry can reveal glycan differences as well as glycan positions related to disease if sufficient MS/MS data is obtained [34]. In this study, there were insufficient amounts of the glycoproteins present to perform IRMPD analysis in the FT-ICR cell. MS/MS data is necessary to confirm the glycan composition and partial fragmentation from the glycopeptides could be obtained with CID in the ion trap. However, high mass accuracy data enabled mass comparisons of glycopeptides derived from different gel spots and the partial fragmentation confirmed some of the components (Table 2).

It is very difficult to obtain CSF from age-matched control individuals resulting in the need to make tentative guiding studies comparing AD patients with healthy individuals that might not be age-matched. Discrepancy

Table 3 Results of individual analysis of 2D gel glycoprotein isoforms in five AD patients and four control subjects

Glycopeptide[a]	3,880.674	3,979.79	3,068.177	3,251.273	3,343.276
Gel number, group (spot)	Not detected	Not detected	2 AD (11) 3 AD (11,12) 7 C (12)	5 AD (13) 9 C (11)	1 AD (13) 2 AD (10, 11) 3 AD (11, 12) 7 C (10, 11, 12) 8 C (10) 9 C (11)

No glycoform analyzed could be assigned with confidence as AD specific

[a] AD specific in pooled CSF

between proteomic studies may be explained by variations in sample size, as the numbers in some experiments may have been too small for detecting relevant differences in protein expression. In addition, there are some differences in the AD diagnostic criteria used [29]. Handling of the samples may also contribute to divergent results. In the present study, the patients with probable AD were between 74 and 85 years old (median 79) and the control individuals ranged from 38 to 75 years old (median 59). To eliminate age-related differences as compared to AD related differences, the total protein 2D gel images for the control group was divided in a younger ($n = 6$) and an older part ($n = 5$), reflecting the individuals age difference, and samples were then compared within the control group. One spot of the isoforms of apolipoprotein E and J showed higher intensity among the younger part of the control individuals and this alteration was not found in the AD-related comparison.

In summary, we demonstrated the feasibility of glycoproteomic profiling with a focus on CSF glycoproteins. The study included the pH interval of 4.7–5.9, in which some glycoproteins previously linked to AD, such as clusterin, apolipoprotein E, α_1-antitrypsin, α-1-β-glycoprotein, can be detected and analyzed. No AD-specific glycoform was found for the analyzed proteins but the glycosylation level and the protein level for α_1-antitrypsin were decreased for AD patients. The glycosylation level for one specific isoform assigned to either zinc-α-2-glycoprotein or haptoglobin was increased in the CSF of AD patients and also present in the control individuals. Elucidation of the glycoform, and assignment of this isoform, would however require larger volumes of CSF material. Further studies could involve the AD- and tau-related protein cholinesterase with theoretical pI of 6.3. The study can also be extended to cover other narrow-range pH-intervals in order to include other glycoproteins. The field of glycoproteomics connected with Alzheimer's Disease is still very unexplored and knowledge of the respective glycoprotein structures in relation to clinical parameters such as duration and severity of disease, may assist in the elucidation of the pathogenesis.

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