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Increased CSF sulfatide levels and serum glycosphingolipid antibody levels in healthy siblings of multiple sclerosis patients

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ABSTRACT

A proportion of healthy siblings of multiple sclerosis (MS) patients have an oligoclonal immunological reaction in their cerebrospinal fluid (CSF) termed the "MS oligoclonal trait". The CSF levels of the major myelin glycosphingolipid sulfatide and serum antibodies against the glycosphingolipids sulfatide and galactosylceramide were recently reported to be increased in MS patients. We studied the levels of these substances in pairs of 46 patients and their 46 healthy siblings and 50 unrelated healthy blood donors (HBD). The sulfatide concentration in CSF was assayed by thin layer chromatography and immunostaining, and the concentration of galactosylceramide by densitometry after thin layer chromatography. Anti-glycosphingolipid antibody levels were assayed by ELISA. In the healthy siblings, the CSF sulfatide concentrations were markedly increased ($p<0.001$, age adjusted $p=0.025$), and the serum IgM anti-GalCer antibodies were increased in healthy siblings compared with HBD ($p=0.02$). The increased sulfatide or antibody levels did not co-segregate with the "MS oligoclonal trait" or the HLA-DR15 phenotype. In conclusion, a proportion of healthy siblings of MS patients have increased CSF sulfatide and anti-glycosphingolipid antibody levels, which may, analogous to the "MS oligoclonal trait", constitute an "MS glycosphingolipid endophenotype". Endophenotypes could potentially simplify the genetics of complex disorders.

1. Introduction

Healthy first degree relatives (FDR) of patients with complex autoimmune disorders carry, to a variable degree, autoantibodies identical to those of their affected relatives [1,2]. The frequency of such asymptomatic individuals may be higher than the frequency of affected relatives [3]. Many FDR of MS patients harbor antiviral antibodies with specificities identical to those known to occur in MS [4] or a cerebrospinal fluid (CSF) enriched oligoclonal IgG reaction with undefined specificity, which (in the relevant clinical context) supports an MS diagnosis, a condition termed "MS trait" [5,6]. Haghighi et al. found measles antibodies and an "MS oligoclonal trait" in approximately 20% of FDR [7,8].

Immunoreactivity against several glycolipids was previously demonstrated in MS [9,10], and we recently reported that the CSF levels of the myelin glycosphingolipid (GSL) sulfatide and serum antibodies against the GSL sulfatide and galactosylceramide (GalCer) are increased in relapsing-remitting and progressive MS patients [7,11]. In the present study, we further investigate the levels of these GSLs and their corresponding antibodies in the healthy siblings of MS patients compared with healthy blood donors (HBD) as controls. We observed that CSF sulfatide levels and serum anti-GSL antibodies are increased in a large proportion of healthy siblings of MS patients. We examined whether these features in healthy siblings fulfill the criteria for an MS endophenotype, and whether they co-segregate with the previously reported "MS oligoclonal trait" [7] and an established genetic risk factor for MS, the HLA-DR15 genotype [12].

2. Materials and methods

Case ascertainment procedures and clinical examinations were described [7,11]. Briefly, MS patients were recruited from the Gothenburg MS register [13] and local MS societies, yielding a sample size of approximately 400 accessible MS patients. A letter was sent explaining the study. From 200 responses received, we initially included 50 consecutive eligible pairs of siblings of Scandinavian descent. When a patient with clinically definite MS (CDMS) had more than one sibling who consented to participate, we invited the sibling whose age was closest to the patient's age. Three pairs were excluded; one pair of half-siblings, one pair of monozygotic twins, and one pair with symptomatic neuroborreliosis revealed during the present investigation. Additionally, one pair of samples was lost. A request for healthy blood donors (HBD)

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free of current symptomatic disease was made at the Sahlgrenska University Hospital Blood Bank. Except for one volunteer who was of southern European origin, all participants were of Scandinavian descent. The final analysis was based on 46 pairs of patients and their healthy sibling, and 50 unrelated HBD (Table 1). Age was equally distributed between the 46 patients and their siblings, whereas the 50 healthy volunteers were younger. Sex was unequally distributed between the sibling pairs and the HBD, where the proportion of men was higher in the HBD group (Table 1). Of the 46 neurologically healthy siblings, three experienced migraine, three had had a hysterectomy, two were on antidepressive treatment, two were on antihypertensive treatment, and the remaining six had previous transient cerebrovascular disease, hip operation, ileus, orally treated diabetes, and well-controlled hypothyroidism, respectively. HBD were examined with ophthalmoscopy and blood pressure measurement. In the group of healthy volunteers, two persons were on antidepressive treatment, one had been operated on for lumbar disc hernia in 1988, and one had a previous syncopal episode. CSF and blood samples were obtained from all groups as an outpatient procedure. Fractions of CSF and serum were stored at −80 °C until they were analyzed for GSL levels and anti-GSL antibody titers. Results of total IgG and albumin assays, isoelectric focusing (IEF) with immunoblot, CSF cell counts, and blood–brain-barrier damage as defined by the CSF/serum albumin ratio for viral IgG antibody titers [14] were available from previous studies of this material [7].

The Medical Ethics Committee at the University of Gothenburg approved the study, and informed consent was obtained from all participating family members after written and verbal information had been given.

2.1. Follow-up of healthy siblings

A search for the healthy siblings (n = 46) was performed for neurological in- and outpatient contacts with the Sahlgrenska University Hospital in the electronic patient records database for the years 1996–2012. In 2012, a telephone interview was accomplished with the surviving patients (n = 36) concerning the health of their healthy siblings.

2.2. Analysis of GalCer and sulfatide in the CSF

The CSF concentration of GalCer was determined using a modified version of the method of Svennerholm [15] described in detail previously [11]. Briefly, lipids were extracted from 1 ml CSF with chloroform–methanol–water (4:8:3 by volume), and purified by silica gel column and saponification, after which GalCer was visualized with orcinol and quantified by densitometry after TLC separation (CAMAG TLC Scanner II, 515 nm). The concentration of sulfatide in CSF was determined using thin layer chromatography and immunostaining, as previously described [16]. Analyses of CSF levels of GalCer and sulfatide were performed blindly.

Table 1
Demographic data.

	Number	Age, median (years)	Age, range (years)
CDMS patients	46	46	21–59
Females	30	44	21–59
Males	16	46	27–58
Healthy siblings	46	44	21–66
Females	28	45	21–66
Males	18	42	26–65
Healthy blood donors	50	31	18–57
Females	15	31	22–57
Males	35	30	18–56

CDMS, clinically definite multiple sclerosis.

2.3. Analysis of antibodies to GalCer and sulfatide

Determination of anti-GalCer antibodies was performed with an ELISA procedure as described previously [11]. Determination of anti-sulfatide antibodies was performed with an ELISA procedure similar to that described previously [17]. Analysis of serum and CSF antibodies to sulfatide and GalCer was performed blindly. The serum antibody levels against sulfatide and GalCer were intercorrelated. We defined a combined GSL serum antibody titer as the highest serum anti-sulfatide or anti-GalCer antibody titer in an individual.

2.4. Statistical methods

GSL levels between healthy siblings and patients were compared with paired *t*-test and those between siblings and HBD with ANCOVA, adjusting for age. Antibody levels between healthy siblings and patients were compared with Wilcoxon signed rank test and those between siblings and HBD with Mann–Whitney *U* test. Pearson correlation was used for sulfatide and GalCer levels, and Spearman's correlation for antibody titers in the study of correlation between sibling pairs. Proportions were compared with Fisher's exact test.

3. Results

Search of any record of the healthy siblings (n = 46) in the neurology in- and outpatient databases from 1996 to 2012 produced 5 records, one with each of the following diagnoses: syncope, myalgia, cervical disc hernia with rhizopathy, cerebral trauma, and migraine. By 2012, 10 patients had died. Telephone interview with the surviving patients concerning the health of their siblings whom we included with a normal neurological examination in 1996 revealed no symptoms suggestive of MS. One had acute cerebral symptoms attributed to stroke. Thus, we have no indication that any of the 46 siblings classified as healthy in the present study has developed MS at a later stage.

3.1. CSF GSL levels

The CSF sulfatide concentrations were significantly increased in the healthy siblings compared with the HBD ($\Delta = 62.8$, $p < 0.001$) and were close to those of MS patients (Table 2, Fig. 1). Since CSF sulfatide levels increased slightly with age ($p = 0.027$) in the healthy siblings, we also adjusted for age ($\Delta = 41.3$, $p = 0.025$). The CSF GalCer concentrations in healthy siblings were similar to those of the HBD, but were significantly lower when compared with the MS patients (Table 2, Fig. 2). Using the upper 95th percentile from healthy unrelated controls as the cut-off level (CSF sulfatide 312 nmol/l and CSF GalCer 239 nmol/l), 10/46 healthy siblings and 10/46 patients had increased CSF sulfatide levels, and 4 of the 46 healthy siblings and 8/46 patients had increased CSF GalCer levels.

Table 2
CSF glycosphingolipid levels.

Groups	Siblings(n = 46)	Patients(n = 46)	Healthy controls (n = 50)
CSF Sulfatide		NS	**
Mean (median)	259.3 (254.0)	253.5(250.5)	196.5 (199.5)
Min–Max	52–530	76–446	89–325
CSF GalCer		*	NS
Mean (median)	126.0 (110.5)	159.5 (144.5)	139.7 (136.0)
Min–Max	22–357	10–407	10–326

Levels of two GSL in the CSF of patients, their siblings, and healthy controls.
p values for comparison with siblings.
*$p < 0.05$.
** $p < 0.001$.
NS, not significant; CSF, cerebrospinal fluid; Min–Max, minimum and maximum values.