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ORIGINAL ARTICLE

High incidence of autoantibodies in Fabry disease patients

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Summary Fabry disease (FD) is an X-linked disorder of glycosphingolipid catabolism that results from a deficiency of the lysosomal enzyme α-galactosidase A. This defect leads to the accumulation of its substrates, mainly globotriaosylceramide, in lysosomes of cells of different tissues. Different studies have shown the involvement of immunopathologies in different sphingolipidoses. The coexistence of FD and immune disorders such as systemic lupus erythematosus, rheumatoid arthritis and IgA nephropathy, has been described in the literature. The aim of this study was to evaluate the prevalence of a group of autoantibodies in a series of Argentine FD patients. Autoantibodies against extractable nuclear antigens (ENAs), double-stranded DNA, anticardiolipin and phosphatidylserine were assayed by ELISA. Lupus anticoagulants were also tested. Fifty-seven per cent of the samples showed reactivity with at least one autoantigen. Such reactivities were more frequent among males than among females. Antiphospholipid autoantibodies were detected in 45% of our patients. The high rate of thrombosis associated with FD could be related, at least in part, to the presence of antiphospholipid autoantibodies in Fabry patients. We found the presence of ENAs, which are a characteristic finding of rheumatological diseases, previous a frequent misdiagnosis of FD, in around 39% of the cases. The detection of a high level of autoantibodies must be correlated clinically to determine the existence of an underlying autoimmune disease. With the recent development of therapy, the life expectancy in FD will increase and autoimmune diseases might play an important role in the morbidity of FD.

Abbreviations

CL	cardiolipin
dsDNA	double-stranded DNA
ENAs	extractable nuclear antigens
FD	Fabry disease
LA	lupus anticoagulant
PS	phosphatidylserine

Introduction

Fabry disease (FD) (OMIM 301500) is an X-linked disorder of glycosphingolipid catabolism that results from a deficiency of the lysosomal enzyme α-galactosidase A (α-D-galactoside galactohydrolase, EC 3.2.1.22) (Brady et al 1967; Kint 1970). This defect leads to the accumulation of its substrates, mainly globotriaosylceramide (Galα1-4Galβ1-4Glcβ1-1Cer) in lysosomes of cells of different tissues such blood vessels, eye, heart, kidney, nervous system and gastrointestinal tract (Desnick et al 1995). FD is a pan-ethnic disorder with an estimated frequency of 1 in 117 000 male births (Meikle et al 1999).

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Clinical manifestations in males with absent or highly reduced levels of α-galactosidase A activity begin in the first decade of life, and include pain in the distal extremities (acroparaesthesias), skin and mucosal angiokeratomas, hypo/anhidrosis, corneal opacities and gastrointestinal symptoms. Around the fourth to fifth decade of life, renal failure, stroke and ischemic heart disease are the leading causes of death (McDermot et al 2001). In contrast, heterozygous females are either asymptomatic or may have mild to severe clinical manifestations (Whybra et al 2001). In adults, unexplained left ventricular hypertrophy, arrhythmias, and stroke-like symptoms such as hemiparesis, vertigo and diplopia should raise the index of suspicion for FD.

Different studies have shown the involvement of immunopathologies, such as gammopathies, lymphomas and leukaemia (Burstein et al 1985; Fox et al 1984; Marie et al 1982; Marti et al 1988) in different sphingolipidoses, especially in Gaucher disease (Costello et al 2006). In addition, glucocerebrosidase-deficient mice demonstrated multisystemic inflammation with evidence of B cell hyperproliferation (Mizukami et al 2002). Autoantibodies may play an important role in the pathogenesis of GM_2 gangliosidoses (Yamaguchi et al 2004). The coexistence of Fabry disease and immune disorders such systemic lupus erythematosus, rheumatoid arthritis and IgA nephropathy has been described in the literature (Arias Martinez et al 2003; Rahman et al 1998; Rosenmann et al 1983; Whybra et al 2006).

The aim of this study was to evaluate the prevalence of a group of autoantibodies in a series of Argentine FD patients.

Methods

Patients

We studied the prevalence of autoantibodies in 33 patients with FD (16 male, 17 female) from five different families from Argentina. Mean age of the group was 34 ± 16 years (range 13–70 years). Diagnosis as established by clinical examination, reduced enzymatic activity and/or genetic testing. Females were obligate carriers as demonstrated by pedigree analysis. The protocol was approved by the scientific committee of Asociacion para la Difusion de Enfermedad de Fabry en Argentina according to provisions of the Declaration of Helsinki in 1995. All patients gave their informed consent prior to participation in the study.

Blood samples

All blood samples were collected after overnight fasting (8 h) by venepuncture into a Vacutainer containing 0.129 mol/L sodium citrate and a Vacutainer SST (Becton Dickinson, Franklin Lakes, NJ, USA). Platelet-poor plasma was obtained by double centrifugation at room temperature for 15 min at 2000 *g*. Plasma and serum aliquots were immediately frozen at −20°C until use.

Autoantibody determination

Autoantibodies against the following antigens were assayed by ELISA: extractable nuclear antigens (ENAs) (BINDAZIME, The Binding Site, Birmingham, UK), SSA/RO, SSB/La, Sm, RNP, Jo-1, Scl-70, Ribosomal P and Histones (INNO-LIA ANA Update, Innogenetics, Ghent, Belgium), double-stranded DNA (dsDNA) (BINDAZIME, The Binding Site), anticardiolipin (CL) (BINDAZIME, The Binding Site) and phosphatidylserine (PS) (AUTOSTAT II, Hycor Biomedical Ltd, Garden Grove, CA, USA). Results of autoantibodies positive by the screening test but negative or borderline by confirmation assays were considered negative.

Results of anti-cardiolipin and anti-phosphatidylserine antibodies are expressed in units of MPL and GPL, where one unit of MPL and GPL represents the binding capacity of 1 μg/ml of affinity-purified antiphospholipid antibodies IgM and IgG respectively, from a reference serum. This assay was considered positive when the value was greater than 30 GPL or MPL.

Lupus anticoagulant (LA) assays were performed using diluted Russell's viper venom (LA1) and phospholipid-rich reagent (LA2) (Dade Behring, Marburg, Germany). LAs were detected by screening assays and were first identified by mixing studies using pooled plasma from healthy donors, and then confirmed by neutralization procedures according to the recommendations of the Subcommittee on Lupus Anticoagulant/Antiphospholipid Antibody of the Scientific and Standardization Committee of the International Society of Thrombosis and Haemostasis. In a given sample, LA was considered positive if screening, identification and neutralization were all positive. Final results were expressed as normalized ratio (NR) of LA1/LA2 clotting times. LA was considered strongly present if NR was greater than 2.0, moderately present between 1.5 and 2.0, and weakly present between 1.3 and 1.5.

Table 1 Results of autoantibody determination in the cohort of Fabry patients analysed in this study

No.	Sex	Age (years)	Autoantibody results against the following antigens[a]				
			ENAs	dsDNA	LA	CL	PS
1	M	33	−	−	−	+	−
2	M	17	−	−	−	−	−
3	M	18	−	−	+	−	−
4	M	18	−	−	−	−	−
5	M	24	−	−	−	+	−
6	F	46	−	−	+	−	−
7	F	51	+	−	−	−	−
8	M	31	−	−	+	−	−
9	F	17	−	−	+	−	−
10	M	23	+	−	−	−	−
11	F	29	−	−	−	−	−
12	F	55	−	−	+	−	−
13	F	37	−	−	−	−	−
14	M	32	+	−	−	−	−
15	F	9	−	−	−	−	−
16	F	70	+	−	+	−	−
17	F	31	−	−	−	−	−
18	F	60	−	−	−	+	−
19	M	38	+	−	+	−	−
20	M	28	−	−	+	−	−
21	F	31	−	−	−	−	−
22	F	59	−	−	+	−	−
23	M	46	−	−	−	−	−
24	M	26	+	−	−	−	−
25	F	21	−	−	−	−	−
26	F	52	−	−	−	−	−
27	F	13	−	−	−	−	−
28	F	19	−	−	−	−	−
29	M	25	+	−	−	+	−
30	M	62	−	−	−	−	−
31	M	21	−	−	+	−	−
32	F	44	−	−	−	−	−
33	M	48	−	−	+	−	−

[a] ENAs, extractable nuclear antigens; dsDNA, double-stranded DNA; LA, lupus anticoagulants; CL, cardiolipin; PS, phosphatidylserine.

Results

The prevalence of autoantibodies related to systemic autoimmunity (ENAs and anti-DNA) and with antiphospholipid syndrome was studied in a series of Argentine FD patients (Table 1).

Nineteen samples (57.5%) showed reactivity with at least one autoantigen, and three of them reacted with two of the autoantigens analysed. Such reactivities were more frequent among males (12/16; 63%) than among females (7/17; 37%).

In the group of systemic autoimmunity-related antibodies tested in this study, autoantibodies against ENAs were found in 7 patients (21%), including 5 hemizygous and 2 heterozygous individuals, but none of the samples assayed showed reactivity with dsDNA.

Antiphospholipid syndrome-associated antibodies assayed in this study were LA, CL and PS. LA was the autoantigen with the highest record of positive results, being positive in 11 out of 33 patients (33%). Of these 11 patients, 5 were male (45%) and 6 female (55%). Moreover, a positive result for anticardiolipin autoantibodies was found in 4 (12%) patients, 3 male and 1 female. However, no positive result was obtained with PS in any of the samples tested.

Discussion

Few studies have reported the coexistence of systemic autoimmunity with FD. In this study, 21% of the Fabry patients tested had anti-ENAs antibodies, which are a characteristic finding of rheumatological diseases and a useful predictor of the diagnosis of systemic lupus erythematosus (Sanchez-Guerrero et al 1996). Moreover, rheumatological diseases were often a misdiagnosis of FD in around 39% of cases (Mehta et al 2004). The high frequency of anti-DNA antibodies in Fabry patients could be the cause of the high percentage of errors in the clinical diagnosis of FD or could indicate the coexistence of both disorders.

Stroke and thrombophilia have been associated with FD (Utsumi et al 1997). Moreover, mice deficient in α-galactosidase A are more susceptible to vascular thrombosis (Eitzman et al 2003). For these reasons, we analysed the presence of autoantibodies associated with a risk of arterial thrombosis such as lupus anticoagulant and anti-cardiolipin (anti-phospholipid syndrome-related autoantibodies). (Cines and McCrae 1995). Anti-phospholipid autoantibodies were detected in 45% (15 out of 33) of our patients. We detected a high prevalence (12%) of anti-cardiolipin autoantibodies in our sample, higher than that observed in the general population (Soloninka et al 1991). The association between these antibodies and clinical findings in our patients is still unclear. The mechanism of action of anti-phospholipid autoantibodies is to bind to phospholipid–protein complexes, interfering with the pro- or anticoagulant reactions that occur in the cell membranes and the vascular endothelial cells (Simmelink et al 2001; Zhao et al 1999). The high rate of thrombosis associated with FD

could be related, at least in part, to the presence of anti-phospholipid autoantibodies in Fabry patients.

Autoimmune diseases are more common in women than in men (Lahita 1996). This difference was attributed to the difference in sex hormones (Beeson 1994). However, in our group of Fabry patients, we obtained comparable levels or slightly elevated level in males compared with females. Hormones may not be playing a significant role in the development of autoantibodies in FD.

Susceptibility to systemic lupus erythematosus is strongly influenced by genetics, with a concordance rate in twins between 28% and 57% (Leslie and Hawa 1994). Our cohort consisted of 33 patients from five different families, but most patients from a given family were not closely related; rather, they were third- or fourth-order relatives, diminishing the effect of genetic links on the high rate of autoantibodies detected. Studies with a larger number of patients from different families should be done to rule out this effect.

The detection of a high incidence of autoantibodies in this group of Fabry patients must be correlated clinically to determine the existence of an underlying autoimmune disease. With the recent development of enzyme replacement therapy, the life expectancy in FD will increase and autoimmune diseases might play an important role in the morbidity of FD.

In this report, we observed a high prevalence (57%) of autoimmune markers in patients with FD. The pathophysiological mechanism underlying the development of autoantibodies could be explained, at least in part, by the suggestion that immunogenic galactocerebrosides accumulating in FD represent a continuous stimulus inducing autoimmune disease (Hamers et al 1978). This could be a common phenomenon found in the glycolipid disorders, in which the deposits of lipids are continuously and chronically stimulating the immune system. More studies must be done to determine the origin of this immune disorder observed in sphingolipidosis.

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