ORIGINAL ARTICLE

Prevalence of Anderson–Fabry disease in patients with hypertrophic cardiomyopathy: the European Anderson–Fabry Disease Survey

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ABSTRACT

Objectives The prevalence of Anderson–Fabry disease (AFD) in patients presenting with unexplained left ventricular hypertrophy (LVH) is controversial. The aim of this study was to determine the prevalence of AFD in a large, consecutive cohort of patients with hypertrophic cardiomyopathy (HCM) using rapid mutation screening.

Design, Setting and Patients A European multicentre cross-sectional study involving 13 referral centres. Inclusion criteria for the study were: men aged at least 35 years and women aged at least 40 years with unexplained LVH (maximum left ventricular wall thickness ≥1.5 cm). All patients were screened using a denaturing high-performance liquid chromatography protocol for rapid mutation screening of the α-galactosidase A (α-Gal A) gene and, if a sequence variant was found, direct sequencing was performed. 1386 patients (63.9% men, mean age 57.9±12.0 years) were enrolled in the study.

Results Seven (0.5%) patients (age 57.4±9.0 years (45–72); three (43%) men) had pathogenic α-galactosidase A mutations. Polymorphisms were identified in 283 patients (20.4%). Maximal left ventricular wall thickness in patients carrying a disease-causing mutation was 18±2 mm (range 15–22); four patients had concentric LVH and the remainder had asymmetric septal hypertrophy.

Conclusions The prevalence of AFD gene mutations in a large, consecutive cohort of European patients with unexplained LVH is 0.5%.

Lysosomal storage disorders are a group of more than 40 diseases caused by a deficiency of lysosomal enzymes, membrane transporters and other proteins involved in lysosomal biology. Anderson–Fabry disease (AFD)[1 2] is an X-linked lysosomal disorder caused by mutations in the gene coding for the lysosomal enzyme α-galactosidase A that leads to intralysosomal accumulation of neutral glycosphingolipids, predominantly globotriaosylceramide, in various organ systems. Patients with AFD die prematurely from renal disease, stroke and cardiac disease.[3] Enzyme replacement therapy is available and has been shown to be beneficial in patients with AFD and cardiac involvement.[4 5]

Over the past decade, a number of studies have suggested that AFD can present without other classic features of the disease in middle age with symptoms and signs of hypertrophic cardiomyopathy (HCM), a common myocardial disorder characterised by the presence of left ventricular hypertrophy (LVH) in the absence of abnormal loading conditions such as hypertension or aortic stenosis.[6–11] However, published rates for the prevalence for AFD in patients with HCM vary from 0% to 12%, reflecting possible referral and gender bias, small sample size and differences in screening methodology.[6–12] The primary aim of this multicentre study was to determine the prevalence of AFD in a large series of consecutively evaluated patients fulfilling conventional diagnostic criteria for HCM using denaturing high-performance liquid chromatography (DHPLC) as a rapid screening tool and exon sequencing for genetic confirmation.

METHODS

Primary hypothesis

AFD accounts for 1–3% of late-onset HCM in referral centre populations.

Study design

Patient population

Between 2007 and 2010, patients were recruited from 13 European centres specialising in the clinical management of HCM. The inclusion criteria for the study were: LVH (maximum left ventricular wall thickness ≥15 mm) in the absence of abnormal loading conditions; age at least 35 years for men and at least 40 years for women.

Genetic screening

Centres were asked to submit stored DNA samples from consecutively evaluated patients and to collect samples prospectively on new patients seen during the study period. Male and female patients were screened using a DHPLC protocol for rapid mutation screening of the α-galactosidase A gene at the Lysosomal Storage Diseases Unit in the Royal Free Hospital, London, UK. All exons exhibiting an anomalous DHPLC profile were sequenced to include intron/exon boundaries. DNA extraction and PCR were performed at the Royal Free Hospital. Leucocyte and plasma α-galactosidase A activities were measured in all patients found to have sequence changes in the α-galactosidase A gene.

DNA samples received for retrospective screening were diluted to optimal concentration, while samples for prospective screening had DNA extracted using a commercially available DNA extraction kit (ViennaLabs, Vienna, Austria). Each

Table 1 Clinical characteristics of study population

	n=1386
Demographic	
Men	885 (63.9)
Age, years	57.9±12.0
Symptoms*	
NYHA class III–IV (n=1255)	206 (16.4)
Chest pain (n=1314)	530 (40.3)
Syncope (n=1348)	269 (20.0)
Echocardiogram*	
MLVWT (mm)	20.1±4.0
MLVWT ≥30 mm	34 (2.5)
LVOT obstruction (n=1326)	438 (33.0)

*In parenthesis the actual number of patients with available clinical data.
ICD, internal cardioverter defibrillator; LVOT, left ventricular outflow tract; MLVWT, maximal left ventricular wall thickness; NYHA, New York Heart Association.

exon of the α-galactosidase A gene was amplified separately by PCR. The PCR primers were designed so that the splice region for each exon was amplified as well as the exon itself. Each PCR product was then analysed by DHPLC using previously described methods.[13] Any samples in which a mutation was identified were sequenced using an automated sequencer (ABI 3130xL genetic analyser, Applied Biosystems, Warrington, Cheshire, UK). Sequence variations found in the α-galactosidase A gene were compared with published data on known pathogenic mutations and non-disease-causing polymorphisms.

Enzyme assay

When an AFD-causing mutation was identified in a retrospective sample, plasma α-galactosidase A activity was assayed using previously described methods.[14] When an AFD-causing mutation was identified in a prospective sample, both plasma and leucocyte α-galactosidase A activities were assayed.

Data capture

All participating centres were required to fill in a brief case record form containing basic demographic data, ECG and echo findings. The results of enzyme analysis and genetic evaluation were recorded on the same document and electronically. All data were collated and entered into a dedicated database.

RESULTS

In total, 1601 blood samples were collected. Two hundred and fifteen were excluded from the study for the following reasons: one sample was unlabelled; in 10 patients DNA extraction failed; 204 patients were excluded because they did not fulfil the entry criteria (by age or wall thickness) or because no clinical details were provided. The final study population comprised 1386 patients (mean age 57.9±12.0 years, range 35–90). Table 1 shows the main clinical characteristics of the study cohort.

Seven (0.5%) of the 1386 patients had mutations in the α-galactosidase A gene (table 2).[15–17] One was a novel variant, T410A, which was predicted to be pathogenic on the basis of reports of three other disease-causing substitutions in the same codon (T410I, T410K and T410P).[17] In addition, 283 (20.4%) polymorphisms were identified (data not shown).

Table 2 shows the clinical characteristics of the seven patients with AFD. They were aged 45–72 years (mean 57.4±9.0 years); three (43%) were men and four (57%) were women. The left ventricular maximal wall thickness was 18±2 mm (range 15–22); four patients had concentric LVH, and the remainder had asymmetric septal hypertrophy. Only one patient had evidence of left ventricular outflow tract obstruction at rest and underwent myectomy before enrolment in the study. Three patients had signs or symptoms of AFD. Five patients had evidence of renal involvement, although patient number 7 also had diabetes. α-galactosidase A levels were low in all patients, except for two women (patients 2 and 5) (table 2).

DISCUSSION

Prevalence estimates for AFD in the general population range from 0.02 to 0.09 per 10 000.[18] These figures are, however, extrapolations of data obtained from reference laboratories,

Table 2 Clinical characteristics of the seven patients with AFD

ID	Age (years)	Sex	MLVWT (mm)	Pattern of LVH	LVOTO	Conduction disease	AFD signs/ symptoms	Renal function	Enzyme activity*	Mutation	Clinical events
1	58	M	20	Concentric	–	–	–	Normal	(p) 0.2†	c. 644 A→G N215S	
2	45	F	18	Concentric	–	–	Angiokeratoma	Albuminuria normal GFR	(p) 5.9†	c. 351C→T p.R118C	
3	52	F	22	Concentric	+	LBBB	Angiokeratoma, Hypohydrosis, Acroparaesthesia, hypoacusis, cornea verticillata	Albuminuria normal GFR	(l) 35.9 ±2.7‡	c. 730G→A D244N	Myectomy
4	53	M	17	ASH	–	–	Angiokeratoma, abdominal discomfort	Proteinuria; GFR 52 ml/min/ 1.73 m^2	(p) 2.6†	c. 427G→A p.A143T	AVN ablation and PPM due to fast AF
5	56	F	15	ASH	–	–	–	GFR 58 ml/min/ 1.73 m^2	(p) 4.3§ (l) 35.1¶	c. 427G→A p.A143T	
6	66	F	18	ASH	–	–	–	Normal	(p) 1.5§ (l) 12.0¶	c. 1228A→G p.T410A	
7	72	M	18	Concentric	–	–	–	Proteinuria GFR 40 ml/min/ 1.73 m^2	(p) 0.1†	c. 644 A>G N215S	Type II diabetes mellitus

*(p) indicates plasma levels, (l) indicates leucocytes levels.
†Normal range 4–21.9 nmol/h per ml.
‡Normal range 51.4–74.9 nanomol/mg of protein/hr.
§Normal range 2.3–9.9 nkat/l.
¶Normal range 22–36 ukat/kg protein.
A minus (–) sign means absence; a plus (+) sign means presence.
AF, atrial fibrillation; AFD, Anderson–Fabry disease; ASH, asymmetric septal hypertrophy; AVN, atrioventricular node; GFR, glomerular filtration rate; LBBB, left bundle brunch block; LVH, left ventricular hypertrophy; LVOTO, left ventricular outflow tract obstruction; MLVWT, maximal left ventricular wall thickness; PPM, permanent pacemaker.

Table 3 Summary of previous studies examining the prevalence of AFD in patients with HCM

Authors	Year	Screened population	Screening method	Prevalence
Nakao *et al*[6]	1995	230 Male patients with echocardiographic evidence of LVH (septum or left ventricular posterior wall thickness ≥13 mm) from a cohort of 1603 male subjects	Plasma α-galactosidase A activity	3.0%
Sachdev *et al*[7]	2002	79 Consecutive men with HCM (unexplained LVH with a MLVWT ≥13 mm) first diagnosed ≥40 years of age and 74 HCM men first diagnosed <40 years of age	Plasma α-galactosidase A activity	6.3% in patients diagnosed ≥40 years 1.4% in patients diagnosed <40 years
Ommen *et al*[12]	2003	100 Consecutive HCM patients (44 men) who underwent septal myectomy	Transmission electron microscopy of myectomy tissue	0.0%
Chimenti *et al*[8]	2004	34 Consecutive female patients with HCM (unexplained LVH with a MLVWT ≥13 mm)	Biventricular endomyocardial biopsy and leucocyte α-galactosidase A activity	11.8%
Arad *et al*[9]	2005	75 Consecutive patients with HCM (30 women, 45 men) (unexplained LVH with a MLVWT ≥13 mm)	Genetic analysis	0.0%
Morita H *et al*[10]	2006	50 Patients (18% women) with echocardiographic evidence of unexplained LVH (MLVWT >13 mm) from a cohort of 1862 subjects	Genetic analysis	2.0%
Monserrat *et al*[11]	2007	508 Consecutive patients (328 men, 180 women) with HCM diagnosed according to the WHO/ESC criteria	Plasma α-galactosidase A activity	1.0%
Hagege AA *et al*[22]	2011	392 patient with HCM (unexplained LVH with a MLVWT ≥ 15 mm) (278 men) aged 18–79 years	α-galactosidase A assay on dried blood spot using a filter paper test	1.0%

AFD, Anderson–Fabry Disease; ESC, European Society of Cardiology; HCM, hypertrophic cardiomyopathy; LVH, left ventricular hypertrophy; MLVWT, maximal left ventricular wall thickness.

which fail to account for undiagnosed cases in the general population. Screening of consecutive newborns suggests a much higher prevalence of AFD due to the detection of both classic and later-onset variants, which might present at a much later age.[19 20] Recent screening studies have concentrated on patient populations at risk of having AFD.[21] For example, in patients with end-stage renal disease on haemodialysis, the reported prevalence of AFD is 0.2–1.2%.[1] In men and women with cryptogenic stroke, the numbers are much higher at 4.9% and 2.4%, respectively.[13]

Various strategies have been used to estimate the prevalence of AFD in patients presenting with HCM, each yielding different results (table 3). The first major study to do so examined 1603 men undergoing routine echocardiography.[6] The measurement of α-galactosidase A activity in plasma samples revealed that seven (3%) out of 230 patients with otherwise unexplained LVH had clinically unsuspected AFD. In a second retrospective analysis of male patients attending a referral clinic for patients with HCM, the prevalence of AFD using plasma α-galactosidase A activity was 4%, rising to 6% in those first diagnosed over 40 years of age.[7] Most recently,[11] screening of α-galactosidase A activity in the plasma of 508 consecutive unrelated patients (328 men) with HCM from three regional centres in Spain demonstrated low α-galactosidase A levels in 15 patients (2.9%); however, subsequent genetic analysis demonstrated disease-causing mutations in only 0.6% of men and between 1.2% and 2.4% (including two with an intronic deletion) of women. Histology consistent with AFD has been reported in 12%[8] of myocardial biopsy specimens of 34 female patients with HCM and in none of 100 myocardial histological sections taken at the time of septal myotomy–myectomy.[12] Most recently, the prevalence of AFD in patients with LVH of 13 mm or greater was determined using an α-galactosidase A assay on dried blood spots using a filter paper test. The overall prevalence in men was 1.5%. No female heterozygotes were detected, but this method has a low sensitivity in women, and no systematic genetic sequencing was performed.[22]

The data obtained in this study suggest that previous estimates for the prevalence of AFD may have been too high. On the other hand, the choice of an age cut-off (35 years for men and 40 years for women) and the threshold of 15 mm as left ventricular maximal wall thickness as the definition of LVH (instead of 13 mm) may have led to an underestimation of the prevalence of AFD in patients with unexplained hypertrophy, although it is rare that LVH manifests before the third decade in patients with AFD.[23 24] Nevertheless, even at 0.5%, the findings indicate that there may still be thousands of patients (given that HCM has a population prevalence of one in 500) with AFD who remain undiagnosed.

As patients with AFD are potential candidates for enzyme replacement therapy, this study supports the case for routine screening of adult patients with LVH in the absence of abnormal loading conditions. The identification of probands with AFD also has implications for family members who harbour disease-causing mutations as many relatives have subclinical or early disease and may, therefore, be ideal candidates for enzyme replacement therapy in order to prevent irreversible organ damage.[23]

Exon screening and sequencing of the α-galactosidase A gene detects both pathogenic mutations and benign sequence variation (polymorphisms) occurring in the normal population. The clinical significance of pathogenic mutations occurring in association with one or multiple polymorphisms is unknown and will be the subject of future studies including in silico modelling and expression analysis.

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Patient consent Obtained.

Ethics approval All participating centres obtained local ethical approval for the study.

Contributors All authors contributed to the study in accordance with authorship guidelines. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication. All authors have seen and approved the final manuscript.

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