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Anderson-Fabry disease with cerebrovascular complications in two Italian families

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Abstract We describe four patients with cerebrovascular complications from two unrelated Italian families with Anderson-Fabry disease. Clinical examination, neuroimaging (MRI), biochemical and genetic analyses were carried out in all the patients. α-Galactosidase A activity was detected by fluorimetric assay and genetic analysis was performed by DNA sequencing. Family 1. A male patient presented recurrent strokes when he was 34 years old, albuminuria and subsequently progressive renal failure to renal transplantation. Family 2. A male patient, aged 32 years, had diplopia for a few days and then recurrent strokes with left spastic hemiparesis and internuclear ophthalmoplegia. A female patient, aged 48 years, presented L-dopa-responsive parkinsonism, and her sister had a stroke when she was 55 years old. MRI was abnormal in all the patients and showed lacunar infarctions in the periventricular white matter, basal ganglia and pons. Lesions were detected by MRI even before stroke in a female patient. In patients with Anderson-Fabry

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F. Martinelli Nephrology Unit Careggi Hospital Florence, Italy disease, stroke is a frequent complication, and may be the first threatening clinical manifestation. In young people with undefined stroke, even without signs of renal involvement, it is important to consider the diagnosis of Anderson-Fabry disease and so to perform clinical examination and biochemical analyses. The pre-clinical stage of cerebrovascular involvement may be evaluable by MRI.

Key words Anderson-Fabry disease \cdot Stroke \cdot Leukoencephalopathy \cdot Parkinsonism \cdot Genetics $\cdot \alpha$ -Galactosidase A

Introduction

Anderson-Fabry disease is an inborn X-linked disorder of glycosphingolipid metabolism resulting from deficient activity of the lysosomal hydrolase α -galactosidase A. The disease is transmitted by the gene encoding α -galactosidase A, which has been localized to chromosome Xq22. This genetic disorder results in the deposition of glycosphingolipids in blood vessel walls. Lipid deposition leads to ischemic lesions, in the skin kidney, heart, peripheral nerves, and the brain, which account for polymorphous symptoms [1]. Central nervous system involvement in Anderson-Fabry disease is related to deposits of glycosphingolipids in the endothelial cells and in the neurons [2].

Cerebrovascular disorders (especially stroke) are frequent in Anderson-Fabry disease [3]. These have been observed in about 40% of hemizygous males, and mainly affect young subjects. A subsequent severe inability can be caused by cerebrovascular involvement. Cerebrovascular manifestations have been rarely described in heterozygous women. The onset in hemizygotes is usually about the age of 34 years, while it is slightly later in heterozygotes. There is a high risk of recurrence (in about 80% of patients). The vertebrobasilar system is often involved [4].

In patients with Anderson-Fabry disease, it is possible to detect cerebrovascular complications by T2-weighted mag-

netic resonance imaging (MRI) since the preclinical stage. No patients younger than 26 years had lesions at MRI; all patients older than 54 years had cerebrovascular involvement [5]. Proton magnetic resonance spectroscopy (MRS) permits measurement of *N*-acetylaspartate (NA), a putative neuron-specific molecule, and shows a widespread pattern of cortical and subcortical NA reduction even in hemizygotes without clinical signs of cerebrovascular disease [6].

We describe the results of biochemical investigations and molecular analyses carried out in two unrelated Italian families with Anderson-Fabry disease. In the first reported family we describe a hemizygous patient, while in the second family, we report 3 patients (one hemizygote and 2 heterozygotes); all these patients have had cerebrovascular complications. These patients are still in follow-up program at the Neurology and Psychiatry Department of the University of Florence.

Materials and methods

Activity of α -galactosidase A was measured in a fluorimetric assay. Proteins were extracted from leukocytes and the enzymatic activity assay was performed with a synthetic substrate for α -galactosidase A, 4-methylumbelliferyl- α -D-galactoside, with the addition of *N*-acetyl- α -galactosamine in the reaction mixture to inhibit the α galactosidase B activity. In our laboratory, the normal value of leukocyte α -galactosidase A activity is 33.76±14.16 nmol/mg/h (mean ± 2 SD).

For molecular analyses, genomic DNA was extracted from leukocytes. The seven exons and the small portions of flanking intron sequence were amplified by polymerase chain reaction (PCR) according to Kornreich and Desnick [7]. PCR was performed for all exons by using 1 U Amplitaq Gold (Applied Biosystems). Purified PCR products were sequenced on a 377 DNA Sequencer, and mutation analysis was performed with Sequence Navigator software (all from Applied Biosystems). With this method, mutations in exons of α -galactosidase A gene were detected [8].

Patients underwent MRI according to the following sequences: spin echo T1-weighted and fast spin echo (FSE) T2-weighted scans in sagittal and coronal planes, proton density and FLAIR.

Results

The pedigrees of two unrelated Italian families with Anderson-Fabry disease are shown in Fig. 1.

In Family 1, a point mutation was found in exon 2 of the gene encoding α -Galactosidase A. This caused the substitution of arginine to cysteine in position 112 (R112C). Proband III3, a 43-year-old man, has cerebrovascular complications. α -galactosidase A activity in this subject was 0.96 nmol/mg/h. Since childhood he has been affected by episodic, painful acroparesthesia caused by changes in temperature and from hypohidrosis. When he was 34 years old he had a cerebral stroke followed by a partially reversible right hemiparesis. He was assessed by neurological investigations including MRI. At the examination he showed mild proteinuria, angiokeratoma and cornea verticillata. Furthermore, after 3 years, he had another cerebral stroke with right hemiparesis and partial recovery, and was diagnosed with nephrotic syndrome. When he was 39 years old, he subsequently developed progressive renal failure and for that reason underwent continuous hemodialysis. Then, when he was 41 years old, he underwent renal transplantation. MRI showed lacunar infarction of the basal ganglia and white matter, and infarction in the area of the paramedian pontine arteries (Fig. 2b, c).

In Family 2, the point mutation was localized in exon 6 and caused the substitution of arginine to proline in position 301 (R301P). In this family, we also identified an A->G variant in intravening sequence (IVS)-4. Three members of the family have cerebrovascular complications (Fig. 1).

The proband is a 32-year-old man whose α -galactosidase activity was 0.01 nmol/mg/h. The patient presented episodic, painful acroparesthesia and skin angiokeratomas in adolescence. He had diplopia for a few days when he was 25 and 29 years old. Subsequently, he developed diplopia and, after a few days, had a cerebral stroke with residual left spastic hemiparesis. In addition, he had another cerebral stroke characterized by internuclear ophthalmoplegia. He presented a mild proteinuria and cornea verticillata. MRI showed lacunar

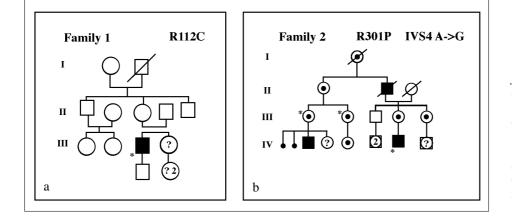


Fig. 1a, b Pedigrees of two Italian families with Anderson-Fabry disease. a Family 1 with one member affected by cerebrovascular complications. b Family 2 with three members with cerebrovascular complications. Filled squares, hemizygotes; small solid circle in circle, obligate carrier; small solid circle, miscarriage; *cerebrovascular complications; ?, not examinated

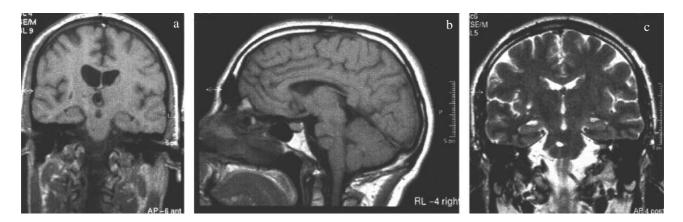


Fig. 2a-c MR images two unrelated male patients with cerebrovascular complications. a Lacunar infarction in the basal ganglia. b, c Lacunar infarction in the area of the paramedian pontine arteries

infarction in the basal ganglia and in the white matter, and infarction in the area of the paramedian pontine arteries (Fig. 2a).

A 53-year-old woman (III1) had an α -galactosidase A activity of 5.3 nmol/mg h. Her general examination was remarkable for cornea verticillata. There was neither angiokeratoma nor painful acroparesthesia. Her medical history included spontaneous miscarriages at 19 and 35 years of age. She also presented episodic thrombophlebitis at ages 46 and 48 years, the latter during estroprogestinic therapy. Then, she presented L-dopa-responsive parkinsonism and depression. She showed progressive hypo- and bradykinesia and rigidity. When she was 51 years old, she presented on-off symptoms. MRI showed lacunar infarctions in the basal ganglia and in the periventricular white matter (Fig. 3b, c). In a 55-year-old woman (III2) of Family 2, the α -galactosidase A activity was 14.2 nmol/mg/h. At the age of 30 years, the patient showed cornea verticillata and mild albuminuria, but neither angiokeratoma neither painful acroparesthesia. Multi-infarctual leukoencephalopathy was noted at T2weighted MRI when the patient was 54 years old, although she had no overt cerebrovascular symptoms (Fig. 3a). At the age of 55 years, she had a cerebral stroke with left hemiparesis.

These four patients with Anderson-Fabry disease and cerebrovascular complications did not present cardiovascular risk factors (e.g. smoking, dyslipidosis, diabetes, arterial hypertension, cardiopathy, disorders of coagulation, vasculitis). The male patients also had no significant alterations on angiography through the femoral artery or on cerebral vessel echography.

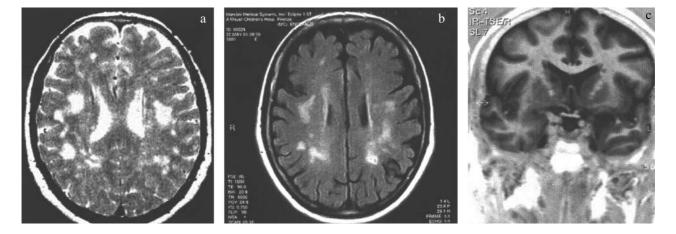


Fig. 3a-c MR image of two heterozygous sisters with cerebrovascular complications. a Periventricular leukoencephalopathy before stroke. b, c Periventricular white matter lesions and basal ganglia lacunar infarctions in the woman with parkinsonism

Discussion

Our study documents the importance for the neurologist to study the cerebrovascular complications in Anderson-Fabry disease, confirming the data in literature [3, 4].

In affected males, cerebrovascular complications present at the age of about 30 years and are severe and can prevail in the clinical picture. They are usually accompanied by painful acroparesthesia and angiokeratoma; these manifestations of the disease are not always easily detected. The painful acroparesthesia can have a modest intensity or can occur occasionally; it can mistakenly be related to rheumatic disease (elevated sedimention rate). The routine neurophysiological study (nerve conduction study, electromyography) is usually negative in our experience and in the literature [9]. Angiokeratoma can be limited in extension in small periumbelical and/or scrotous areas like in the male patient of Family 2. Renal failure can become evident or occur only after the first stroke, as in these patients.

Cerebrovascular complications of Anderson-Fabry disease are considered to be rare and usually mild in female heterozygotes, since the disease is X-linked. We know however that women develop minor symptoms of the disease. Cornea verticillata is present in 95% of females heterozygote for Anderson-Fabry disease, while painful neuropathy and angiokeratoma are often absent [1]. Some women have severe cerebrovascular complications [4]. In female heterozygotes, the values of α -galactosidase A activity range from those levels of normal subjects to those levels next to 0 of many hemizygotes [1]. The carriers not only have an α -galactosidase A activity ranging from hemizygotes to normal subject's values but they present a striking individual variability in the biochemical expression of the gene even in the same family (various women with the same mutation have different values of enzymatic activity) [1]. This individual variability is related to the random inactivation of one of the two X chromosomes at the cellular level precociously in embryogenesis (Lyon's phenomenon) [1, 10]. This mechanism characterizes most X-linked diseases. In women, our results confirm intermediate levels of activity and a strong individual variability even in the same family. The two male patients show an α -galactosidase A activity inferior to 1 nmol/mg/h while the two women have values of 5.3 and 14.2 nmol/mg/h (the normal minimum level is 19.6 nmol/mg/h in our laboratory). The condition of heterozygotes has consequences in the enzymatic activity even if at a different degree in each subject. It is not amazing that some women can have clinical consequences, for examples cerebrovascular complications. The partial enzymatic deficit in women causes a slow accumulation of glycolipids in many organs, as documented by the presence of cornea verticillata and the increase in Gb3 in plasma and urine (unpublished observations). This increase is the probable cause of cerebrovascular complications even in the heterozygotes. We do not know why only in 30% of affected males and in some heterozygotes, the cerebrovascular manifestations are precocious and severe.

The neurologist should consider Anderson-Fabry disease in the differential diagnosis of patients with stroke due to unknown causes, with a precocious onset in both genders and when there are lacunar and multi-infarctual lesions at MRI. It is important to point out that, in our experience, MRI lesions can precede the onset of cerebrovascular complications [5, 6]. The neurologist should search for a confirmation in the patient's history, in the history of painful acroparesthesia and in the presence of angiokeratomas. These signs and symptoms in the affected male can be of modest clinical evidence and they are often absent in women. The neurophysiological study of painful acroparesthesia usually shows a normal speed in the nerve conduction study and does not help the diagnosis. However, the neurologist can reach a definite diagnostic confirmation of Anderson-Fabry disease with the determination of the enzymatic activity in leukocytes. This method is sensitive and specific in males (100% of cases). In women, our experience confirms that the determination of the enzymatic activity and the evaluation of cornea verticillata help us in identifying almost all heterozygotes [1]. The analysis of molecular genetics completes the diagnostic assessment.

Today the diagnostic commitment is moreover justified for the presence of a specific replacement therapy [11, 12] with the recombinant enzyme. With the help of such a therapy, we hope in a medium-long term to prevent more severe complications in this disease. Clinical trials have shown clearance of glycolipidic accumulations in kidney, heart and skin biopsies by enzymatic replacement therapy. The impact of such a therapy in the prevention and treatment of cerebrovascular complications has not yet been studied and this may be an interesting aim to achieve.

Sommario Descriviamo quattro pazienti affetti da malattia di Anderson-Fabry con complicazioni cerebrovascolari. I pazienti provengono da 2 famiglie italiane non correlate. I pazienti e gli altri membri consenzienti delle due famiglie sono stati esaminati sul piano clinico, biochimico, delle neuroimmagini e della genetica molecolare. L'attività dell'α-galactosidase A è stata determinata con metodo fluorimetrico; è stata effettuata l'analisi della sequenza del gene dell' α -galactosidase A. Famiglia 1. Un maschio emizigote presenta, all'età di 34 anni, ictus ischemici ricorrenti, albuminuria e successivamente insufficienza renale progressiva fino al trapianto. Famiglia 2. Un maschio emizigote di 32 anni presenta diplopia per pochi giorni, seguita da ictus ischemici recidivanti con emiparesi spastica sinistra e oftalmoplegia internucleare. Una donna eterozigote di 48 anni si presenta con parkinsonismo parzialmente responsivo alla Ldopa. La sorella, pure eterozigote, ha un ictus ischemico a 55 anni. La RM è anormale in tutti i pazienti descritti, maschi e

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femmine, e mostra infarti e lacune nella sostanza bianca periventricolare, nei nuclei della base e nel ponte. Le lesioni in una paziente sono documentate dalla RM prima della comparsa delle manifestazioni cliniche. Nei pazienti con malattia di Anderson-Fabry l'ictus ischemico è una complicazione frequente, e può essere la prima minacciosa manifestazione clinica della malattia. In persone giovani con ictus da causa non definita, anche senza segni di interessamento renale, è importante considerare la diagnosi di malattia di Anderson-Fabry, e svolgere accertamenti clinici e analisi biochimiche mirate. La RM aiuta a riconoscere e valutare la fase pre-clinica delle complicanze cerebrovascolari. Sarebbe importante valutare i risultati della terapia enzimatica sostitutiva sulla prevenzione e cura delle complicazioni cerebrovascolari.

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References

- Desnick RJ, Ioannou YA, Eng CM (1995) Alpha-galactosidase A deficiency: Fabry disease. In: Scriver CR, Beaudet AL, Sly WS, Valle D (eds) The metabolic and molecular basis of inherited disease. McGraw-Hill, New York, pp 2741–2784
- 2. Tagliavini F, Pietrini V, Gemignani F, Lechi A, Pallini R,

Federico A (1982) Anderson-Fabry disease: neuropathological and neurochemival investigation. Acta Neuropathol (Berl) 56(2):93–1988

- Barbey F, Lindove O, Droz D, Grunfeld JP (2000) Maladie de Fabry: aspects cliniques et perspectives. Schweiz Med Wochenschr 130:21
- Mitsias P, Levine R (1996) Cerebrovascular complications of Fabry's disease. Ann Neurol 40:8–17
- Crutchfield KE, Patronas NJ, Dambrosia JM, Frei KP, Banerjee TK, Barton NW, Schiffmann R (1998) Quantitative analyses of cerebral vasculopathy in patients with Fabry disease. Neurology 50:1746–1749
- Tedeschi G, Bonavita S, Banerjee TK, Virta A, Schiffmann R (1999) Diffuse central neuronal involvement in Fabry disease. A proton MRS imaging study. Neurology 52:1663–1667
- Kornreich R, Desnick RJ (1993) Fabry disease: detection of gene rearrangments in the human alpha-galactosidase A gene by multiplex PCR amplification. Hum Mutation 2:108
- Torricelli F, Martinelli F, Pelo E, Minuti B, Borsini W, Battini ML, Scordo MR, Sodi A, Salvadori M (2001) Anderson Fabry disease: molecular analysis and clinical manifestations in three Italian families. Contrib Nephrol 136:216–222
- Ro LS, Chen ST, Tang LM, Hsu WC, Chang HS, Huang CC (1999) Current perception threshold testing in Fabry disease. Muscle Nerve 22(11):1531–1537
- Romeo G, Migeon BR (1970) Genetic inactivation of the alpha-galactosidase locus in carriers of Fabry's disease. Science 170:180
- Schiffmann R, Kopp JB, Austin III HA, Sabnis S, Moore DF, Weibel T, Balow JE, O'Brady R (2001) Enzyme replacement therapy in Fabry disease. JAMA 285(21):2743–2749
- Eng CM, Guffon N, Wilcox WR, Germain DP, Lee P, Waldek S, Caplan L, Linthorst GE, Desnick RJ for the International Collaborative Fabry Disease Study Group (2001) Safety and efficacy of recombinant human alpha-galactosidase A replacement therapy in Fabry's disease. N Engl J Med 345(1):9–16