Megadolichobasilar anomaly with thrombosis in a family with Fabry’s disease and a novel mutation in the \( \alpha \)-galactosidase A gene

Ferenc Garzuly,1 László Maródi,2 Melinda Erdös,2 János Grubits,3 Zita Varga,3 Ellen Gelpi,7 Béla Rohonyi,4 Mária Mázló,5 Anikó Molnár6 and Herbert Budka7

1Department of Neurology, Markusovszky Hospital, Szombathely, 2Department of Infectology and Pediatric Immunology, Medical and Health Science Center, University of Debrecen, Debrecen, 3Department of Neurology and 4Department of Pathology, Erzsébet Hospital, Sopron, 5Central Electron Microscopy Laboratory, Faculty of Medicine, University of Pécs, Pécs, 6Dermatological Health Centre, Dr Batthyány-Stratmann László Hospital, Kőrmend, Hungary and 7Institute of Neurology, Medical University of Vienna, Vienna, Austria

Correspondence to: Professor H. Budka, Institute of Neurology, Medical University of Vienna, Waehringer Guertel 18-20, 4J, 1097 Vienna, Austria
E-mail: herbert.budka@kin.at

Fabry’s disease is an X-linked lysosomal storage disorder. \( \alpha \)-Galactosidase deficiency leads to accumulation of globotriaosylceramide mainly in endothelial and smooth muscle cells. Cerebrovascular symptoms with predominant affection of the vertebrobasilar circulation are one of the major sources of morbidity in Fabry’s disease. We present a Hungarian family with Fabry’s disease caused by a new mutation in the \( \alpha \)-galactosidase A gene (GLA), and describe a variant expression of the disease. Megadolichobasilar anomaly was diagnosed in two male patients in the family who died of thrombosis. In another female patient who had suffered from disturbance of the vertebrobasilar circulation, a strongly dilated basilar artery without thrombosis was found at autopsy. Another three family members had basilar strokes and large and elongated basilar arteries on MRI. Genetic analysis disclosed a c.47T\( \rightarrow \)C missense mutation resulting in L16P in the amino acid sequence of the \( \alpha \)-galactosidase protein. This report suggests that megadolichobasilar anomaly is potentially life-threatening, and that L16P is a disease-causing mutation in patients with Fabry’s disease. Early enzyme replacement therapy may prevent the development of these irreversible cerebrovascular complications.

Keywords: megadolichobasilar anomaly; basilar thrombosis; Fabry’s disease; angiokeratoma corporis diffusum; GLA mutation

Abbreviations: GLA = \( \alpha \)-galactosidase A gene; GL-3 = globotriaosylceramide; MDBA = megadolichobasilar anomaly

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Introduction

Angiokeratoma corporis diffusum, or Anderson–Fabry’s disease, was independently described by Johannes Fabry and William Anderson in 1898 (Anderson, 1898; Fabry, 1898). Pompen and colleagues, in 1947, found abnormal vacuoles in the parenchyma of many organs and also in vessel walls of patients with Fabry’s disease (Pompen et al., 1947). Sweeley and Klionsky identified the storage material as globotriaosylceramide (GL-3), and Brady reported the deficient enzyme as \( \alpha \)-galactosidase (\( \alpha \)-Gal) in 1967 (Sweeley and Klionsky, 1963; Brady et al., 1967). Successful phase III clinical trials with enzyme replacement therapy were completed in 2000, and a new era in the therapy of Fabry’s disease began (Pastores and Thadhani, 2001).

An extremely dilated, elongated and S-forming basilar artery was first described by Dandy as ‘S’ aneurysm in 1947. The name ‘megadolichobasilar anomaly’ (MDBA) was recommended by Boeri and Passerini in 1964 (Boeri and Passerini, 1964). Approximately 150 cases were reported in the literature until 1985, but only single descriptions in patients with Fabry’s disease (Hegeduš, 1985; Brittig et al., 1986).

We describe a family with MDBA occurring in five men and a woman with Fabry’s disease caused by a novel mutation in the \( \alpha \)-galactosidase gene A (GLA). The c.47T\( \rightarrow \)C missense mutation resulting in L16P in the amino acid sequence of the \( \alpha \)-galactosidase protein has not been reported previously.
Case reports
Clinical phenotypes of the patients and their relatives are shown in Fig. 1.

Case II/1
In this patient with angiokeratomas, Fabry’s disease was diagnosed in 1982. He was repeatedly treated for ‘gastroenteritis’. Impaired renal function was first detected at the age of 49, followed by ataxia and depression. Sudden loss of consciousness occurred at the age of 51, and he died 3 days later. Pathological findings of dilatation of both middle cerebral arteries were reported by Brittig and colleagues in Hungarian (Brittig et al., 1986). The thrombosed basilar artery was elongated and dilated with a diameter of 24 mm, and the artery wall was extremely thin (Fig. 2B). Histological studies revealed small periventricular infarcts. In addition, storage material in inner organs, including the kidneys, the heart and the gastrointestinal tract, was detected. Similar deposits were seen in smooth muscle cells of the wall of the basilar artery (Fig. 2F and G), in the wall of the brain arteries and arterioles, with typical lamellar ultrastructure (Fig. 3), and in certain areas of the brain and spinal cord in neurons, the spinal, sympathetic and autonomic ganglia. Peripheral nerves exhibited deposition of lipids in perineurial cells and Schwann cell cytoplasm. Thin layer chromatography revealed GL-3 accumulation in the kidneys, the brain cortex and the sciatic nerve.

Case II/5
This female patient died at the age of 75. No angiokeratomas were observed. She was repeatedly hospitalized for vertebrobasilar symptoms. CT, MRI and MR angiography showed MDBA with multiple vascular lesions. The diameter of the basilar artery was 6 mm in 1995 and 10 mm in 2001. Kidney and heart involvement was also present. He is alive at age 48.

Case III/6
This male patient presented with angiokeratomas. After repeated strokes of the vertebrobasilar territory, he developed dementia and uraemia at the age of 46. CT, MRI and autopsy were not performed. He died of cardiac infarction.

Case III/13
This male patient presented with angiokeratomas in early childhood. Impairment of renal and heart function was first observed at the age of 40. CT and MRI showed MDBA with a diameter of 7 mm and multiple ischaemic lesions. He is alive at age 52.

Case III/15
This patient is the twin brother of III/14. Angiokeratomas appeared in childhood. Impairment of renal and heart function were noticed even earlier than in his twin brother. MDBA was present on MRI (Fig. 2A). He is alive at age 52.
Case III/16

This 44-year-old man was admitted for basilar territory stroke. CT and MRI revealed MDBA on admission. Angiokeratomas in the inguinal regions and renal and cardiac dysfunction were observed. He died half a year later due to thrombosis of the basilar artery and subarachnoid bleeding. The diameter of the basilar artery was 27 mm. Post-mortem examination revealed alterations similar to those observed in case II. The basilar artery was thrombosed, and its very thin wall was ruptured. In addition, subarachnoid bleedings and a small pontine infarct were seen. On histological slides, the intima of the basilar artery and the lamina elastica interna had disappeared, and storage material was seen in smooth muscle cells. Identical changes were seen in many cerebral arteries and arterioles, as well as in all other tissues examined.

Fig. 2 (A) Case III/15 MR angiography. MDBA (initial state). (B and C) Case II/1. MDBA with thrombosis. (D) Case II/5. Irregular and thinned vessel wall. (E) Ossification in the muscle layer of the basilar artery. Haematoxylin and eosin, 100×. (F and G) Case II/5. Storage in smooth muscle cells of the basilar artery. (F) Oil red O. (G) Sudan black B. Bar = 25 μm in F and G.
Investigations

**α-Galactosidase A enzyme activity**

α-Galactosidase activity was measured using the fluorogenic substrate 4-methylumbelliferyl-α-D-galactopyranoside (4MU-galactopyranoside; Sigma), essentially as described elsewhere (Ioannou et al., 1992). In brief, 25 μl of cleared medium was incubated with 100 μl reaction mixture containing the 4MU substrate (final concentration 3.5 mM) in 100 mM citrate/200 mM phosphate buffer, pH 4.6, at 37°C, for 1 h. Reactions contained 100 mM N-acetylgalactosamine to inhibit α-galactosidase B activity (Mayes et al., 1981). Reactions were terminated by the addition of 2 ml 300 mM glycine/NaOH buffer, pH 10.6, and fluorescent 4-methyl-umbelliferone was measured with a fluorimeter (Perkin-Elmer) at 445 nm.

Specific activity of the commercial α-galactosidase A preparations was determined using the protein measurement procedure of Lowry with human albumin as reference protein. Enzymatic activity was expressed as nanomoles of substrate protein hydrolysed per hour per milligram.

Enzyme activity was measured in the blood of members of the third generation (III/14, III/15, III/6) and fourth generation (IV/27) (Fig. 1). In all patients, the level of enzyme activity was below the normal range: 0.33 nmol MU/ml h (III/14); 0.42 nmol MU/ml h (III/15); 0.3 nmol MU/ml h (III/6); and 0.24 nmol MU/ml h (IV/27) (normal range 2.4–11.3 nmol MU/ml h).

In addition, patient III/15 showed increased levels of homocysteine (30.9 μmol/l), whereas concentrations of fibrinogen, antithrombin III, protein S, protein C and Leiden V factor were within the normal ranges. Analysis of the prothrombin and MTHFR genes showed no alterations.

**Molecular genetics**

EDTA blood was collected from family members of the patients and genomic DNA was isolated using the QIAamp DNA Blood Mini kit (Qiagen, Hilden, Germany). To sequence the GLA gene, genomic exons 1–7, and the flanking intron regions were amplified by PCR. The PCR products were sequenced with the BigDye Terminator Cycle sequencing kit (Applied Biosystems, Foster City, CA, USA).

We sequenced GLA in patients III/6, III/14 and III/15 (Fig. 1). We found these patients to be hemizygous for a T to C transition at nucleotide position 47 in exon 1 of the coding region. This mutation resulted in the replacement of leucine by proline at amino acid position 16 in the α-galactosidase protein.

**Discussion**

Fabry’s disease is an inherited X-linked lysosomal storage disorder. The α-galactosidase gene is located on the long arm of the X chromosome (Xq22.1). More than 200 mutations have been reported so far, and many of them have been found in single families (Sorensen, 2002). This may explain the prominent variability in clinical presentation.

We present here a large family with classical features of Fabry’s disease and megadolichobasilar anomaly, a rare disease manifestation. We have found a mutation in the α-galactosidase gene in three family members. The c.47T→C missense mutation resulting in the replacement of leucine at amino acid position 16 by proline in the amino acid sequence of the α-galactosidase protein has not been reported before in patients with Fabry’s disease. Similar enzyme activity levels were detected in these patients. This finding suggests that the type of mutation may influence expression of enzyme activity in this family.

In the absence of sufficient α-galactosidase, GL-3 accumulates in lysosomes of many cell types, mainly in the endothelium and smooth muscle. In its classical form, cerebrovascular events accompany serious renal and cardiac dysfunction in the last stage of the disease (Grewal, 1994).

Cerebral ischaemia or infarction was found in 68% of 50 classical Fabry patients by using MRI. The older the patient, the more frequently complications occurred; above the age of 54 years, MRI showed pathological signs in all patients (Crutcherfield et al., 1998). Only one family with Fabry’s disease and prominent basilar artery aneurysm has been reported (Maisey and Cosh, 1980). In the present family, however, MDBA was also pathologically verified and is an almost consistent feature.

Previous studies have described that Fabry’s disease may lead to a prothrombotic state, characterized by the presence of endothelial activation markers and leucocyte integrin...
expression in the peripheral blood (DeGraba et al., 2000). In our family, one patient had increased plasma concentrations of homocysteine, although no thrombotic events have been detected. Thus, the clinical significance of hyperhomocysteinemia remains unclear (Demuth and Germain, 2002). In the other family members no data on concomitant coagulopathy are available, which may have contributed to recurrent thrombotic cerebrovascular events.

Studies by Moore and colleagues indicated a predominance of cerebrovascular abnormalities in the posterior arterial territory in Fabry’s disease. The authors observed a significantly increased resting regional cerebral blood flow predominantly in the posterior circulation, suggesting a vascular dysfunction of arteriolar smooth muscle and endothelial cell components in early phases of disease. After enzyme replacement therapy they observed decreased blood flow velocities, suggesting a reduction of the risk of stroke in Fabry disease after treatment (Moore et al., 2001a, b, 2002a, b). Nevertheless, the exact pathogenesis of cerebrovascular complications has not been completely elucidated. Complex multifactorial mechanisms have been proposed which may lead to endothelial dysfunction, vessel wall dilatation, induce procoagulant and abnormal flow states, which in turn may increase the incidence of emboli or thrombosis (Moore et al., 2001b).

MDBA can cause various neurological manifestations, including ischaemia in the vertebrobasilar system, compression of cranial nerves (trigeminus neuralgia, paralysis of the eye muscles, hypacusis) and compression of the aqueduct and ventricle III (Herpers et al., 1983; Otterstedde et al., 1999). The basilar artery can be thrombosed in its full length, and the thin wall might rupture and cause subarachnoid haemorrhage (D’Andrea et al., 1992). The anomaly is thought to be of congenital origin (defects in the lamina elastica interna and reticular fibre deficiency in the muscular layer), but it may also occur in Marfan syndrome (Schievink et al., 1997; Croisile et al., 1988).

The normal diameter of the basilar artery is 3–4 mm. By definition, MDBA is diagnosed when the diameter of the basilar artery surpasses 1 cm. The tortuous and elongated artery causes a characteristic impression on the floor of the third ventricle. The initial phase has a diameter below 1 cm, and the full stage reaches more than 1 cm. The initial stage is a frequent phenomenon in Fabry patients: in a retrospective study, the vertebrobasilar circulation was symptomatic in 67% of the hemizygotes and 60% of the heterozygotes; elongated, ectatic tortuous vertebral and basilar arteries were the most common angiographic features (Mitsias and Levine, 1996). The full stage of MDBA is rare and has been described only occasionally, whereas thrombosis has not been reported yet.

Our cases correspond to the classical form of Fabry’s disease. The unique feature in this family was that cerebrovascular events were the leading symptoms in the majority of cases and thrombosis of the basilar artery led to the death of two patients. In addition, subarachnoid bleeding occurred in one patient, and in two family members dilatation of middle cerebral arteries was detected.

In females, expression of GLA is variable, resulting in activity ranges between normal and low levels. Accordingly, female carriers may manifest symptoms of the disease in milder form, with normal lifespan, or may appear healthy (Bird and Lagunoff, 1978; Wendrich et al., 2001).

We consider the frequent and extreme dilatation of the basilar artery with or without thrombosis to be a particularly severe phenotype of the disease that may be related to the mutation found in this family. With the availability of enzyme replacement therapy, which may reverse the abnormal cerebrovascular responses in patients with Fabry disease (Moore et al., 2001b, 2002a, b), early diagnosis is important to treat patients and prevent severe complications. Recognition of MDBA in patients with cerebral manifestations should lead to early detection and treatment of affected individuals.

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References