American Journal of Case Reports

ISSN 1941-5923
© Am J Case Rep, 2016; 17: 315-319
DOI: 10.12659/AJCR.897024

Received: 2015.12.08
Accepted: 2016.02.06
Published: 2016.05.09

p.R301X Mutation and Variable Phenotypic Appearance of Fabry Disease

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
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Funds Collection G

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Conflict of interest: None declared

Patient: Male, 39
Final Diagnosis: Fabry disease
Symptoms: Acropareshesia • fatique
Medication: —
Clinical Procedure: Gene analysis
Specialty: Metabolic Disorders and Diabetics

Objective: Rare disease

Background: Fabry disease is an X-linked disorder. Due to deficiency of the enzyme α-galactosidase A, neutral glycosphingolipids (primarily globotriaosylceramide) progressively accumulate within lysosomes of cells in various organ systems, resulting in a multi-system disorder, affecting both men and women. Misdiagnosis and delayed diagnosis are common because of the nature of Fabry disease.

Case Report: We report a case of Fabry disease with a p.R301X (c.901 C>T) mutation in a 39-year-old man who was being treated for chronic sclerosing glomerulonephritis for 2 years. Family screening tests showed that the proband's mother, sister, and daughter had the same mutation with different phenotypes. Levels of α-galactosidase A were low in the proband and his mother and sister. Cornea verticillata and heart involvement were present in multiple family members. Agalsidase alfa treatment was started in patients where indicated.

Conclusions: Pedigree analysis is still a powerful, readily available tool to identify individuals at risk for genetic diseases and allows earlier detection and management of disease.

MeSH Keywords: Codon, Nonsense • Fabry Disease • Heart Diseases • Kidney Failure, Chronic

Full-text PDF: http://www.amjcaserep.com/abstract/index/idArt/897024

Background

Fabry disease (FD) (OMIM 301500) (*http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM*) is an X-linked disorder. It is caused by deficiency of the enzyme α-galactosidase A (EC 3.2.1.22) (*http://www.chem.qmul.ac.uk/iubmb/enzyme/*). The GLA gene produces enzyme α-galactosidase A and is present on the long arm of chromosome Xq22.1. Due to deficiency of the enzyme α-galactosidase A, neutral glycosphingolipids (primarily globotriaosylceramide) progressively accumulate within lysosomes of cells in various organ systems. Globotriaosylceramide gradually accumulates in endothelial cells, vascular smooth muscle cells, pericytes, renal epithelial cells, myocardial cells, and neurons, which leads progressively to cellular dysfunction, necrosis, apoptosis, inflammation, and fibrosis [1].

Demonstration of deficient activity of α-galactosidase in plasma or leukocytes and showing GLA mutation are the primary diagnostic tools in men [1,2]. Female patients may have enzyme activity within the normal range; therefore, they should have their status determined by genotyping (analysis of the GLA gene mutation) [3].

The first clinical manifestations of the disease appear in childhood, and renal, cardiac, and cerebrovascular complications usually arise in adulthood. The clinical features of FD are acroparesthesia and pain crises, angiokeratomas, gastrointestinal symptoms, and corneal dystrophy. In later stages of disease, proteinuria, renal failure, left ventricular hypertrophy, arrhythmias, and stroke develop [1].

Misdiagnosis of the FD is an important problem. Sometimes it takes years between beginning of the symptoms and correct diagnosis. The Fabry Outcome Survey study showed that the mean time was 14.5 years for men and 16.8 years for women [4]. In another study, the recognition of the underlying diagnosis was delayed by 14 years in men and 19 years in women [5]. Therefore, it is important to examine the index cases. There are cases in which the clinical indicators of FD overlap with those of some rheumatic disorders, such as familial Mediterranean fever [6,7]. Retrospective studies found a significant delay in diagnosis of Fabry disease in ~40% of men and ~70% of women [7].

Phenotypic variability in the same family, in terms of involved organs and severity, was previously defined for M51I mutation [8]. It has been defined in 8 Italian patients aged 22–58 years. Six patients were female. The proband was a 22-year-old female patient who had been suffering from recurrent fever of unknown origin, burning pain located in the hands and feet, and gastrointestinal disturbances. It took 10 years after the appearance of the first clinical manifestations to diagnose FD. The other female patient had cardiac involvement with dyspnea and arrhythmias. A 28-year-old man with no enzymatic activity showed serious cerebrovascular involvement, although the other patient, a 58-year-old man, was completely asymptomatic. One of the daughters of the 58-year-old man had no enzymatic activity. She showed cerebral involvement and was also diagnosed with multiple sclerosis based on the presence of lesions of the corpus callosum on MRI.

This study reports the clinical, biochemical, and molecular characterization of 4 members of the same family. The proband was being treated for chronic sclerosing glomerulonephritis for 2 years. Four members of the family showed the exonic p.R301X (c.901 C>T) nonsense GLA gene mutation, which is a disease causing mutation associated with the atypical phenotype (Figure 1). The p.R301X (c.901 C>T) mutation has previously been reported in families with FD, but here we report the clinical history of a family that highlights a phenotypic variability in terms of involved organs and severity [9].

Case Report

The proband was a 39-year-old man who was treated for chronic sclerosing glomerulonephritis over 2 years. Recently, he began to complain of fatigue, hypohidrosis, and Raynaud's phenomenon. His physical examination showed angiokeratoma, grade 3/6 systolic murmur on apex, and cornea verticillata. Laboratory evaluation showed hemoglobin 13.9 g/dl, white blood cell count 6.73×10^3/μL, platelet count 1.96×10^5/μL, erythrocyte sedimentation rate 6 mm/h, blood urea nitrogen 51 mg/dL, creatinine 3.21 mg/dL, 24-h urinary protein 3.3 g/day, and other parameters were in the normal ranges. Echocardiography showed left ventricular hypertrophy, mitral regurgitation (grade 1–2/4), and tricuspid regurgitation (grade 1/4). Electrocardiography was in normal range. At the time of intake his family history was unremarkable. We evaluated the patient with suspicion of FD. The patient had a pathogenic p.R301X (c.901 C>T) mutation and showed enzymatic activity of α-galactosidase 6.56 nmol/mg·h (normal range: 32–60 nmol/mg·h), which was below the normal value. Agalsidase alfa treatment was started.

Genetic and biochemical tests were extended to the proband's family members. These analyses showed that the proband inherited the p.R301X (c.901 C>T) mutation from his mother. She was a 63-year-old woman with enzymatic activity of 5.8 nmol/mg·h (normal range: 32–60 nmol/mg·h) and with symptoms not clearly related to FD. She was taking 50 mg/day L-thyroxine for hypothyroidism and 50 mg/day metoprolol for palpitations. Her past medical history showed that she had acroparesthesia and neuropathic pain in her thirties. She began to have cardiac symptoms in the third decade. Physical examination showed blood pressure of 160/90 mmHg, grade 2/6 systolic murmur on apex, angiokeratoma, and cornea verticillata. Echocardiography showed significant left ventricular

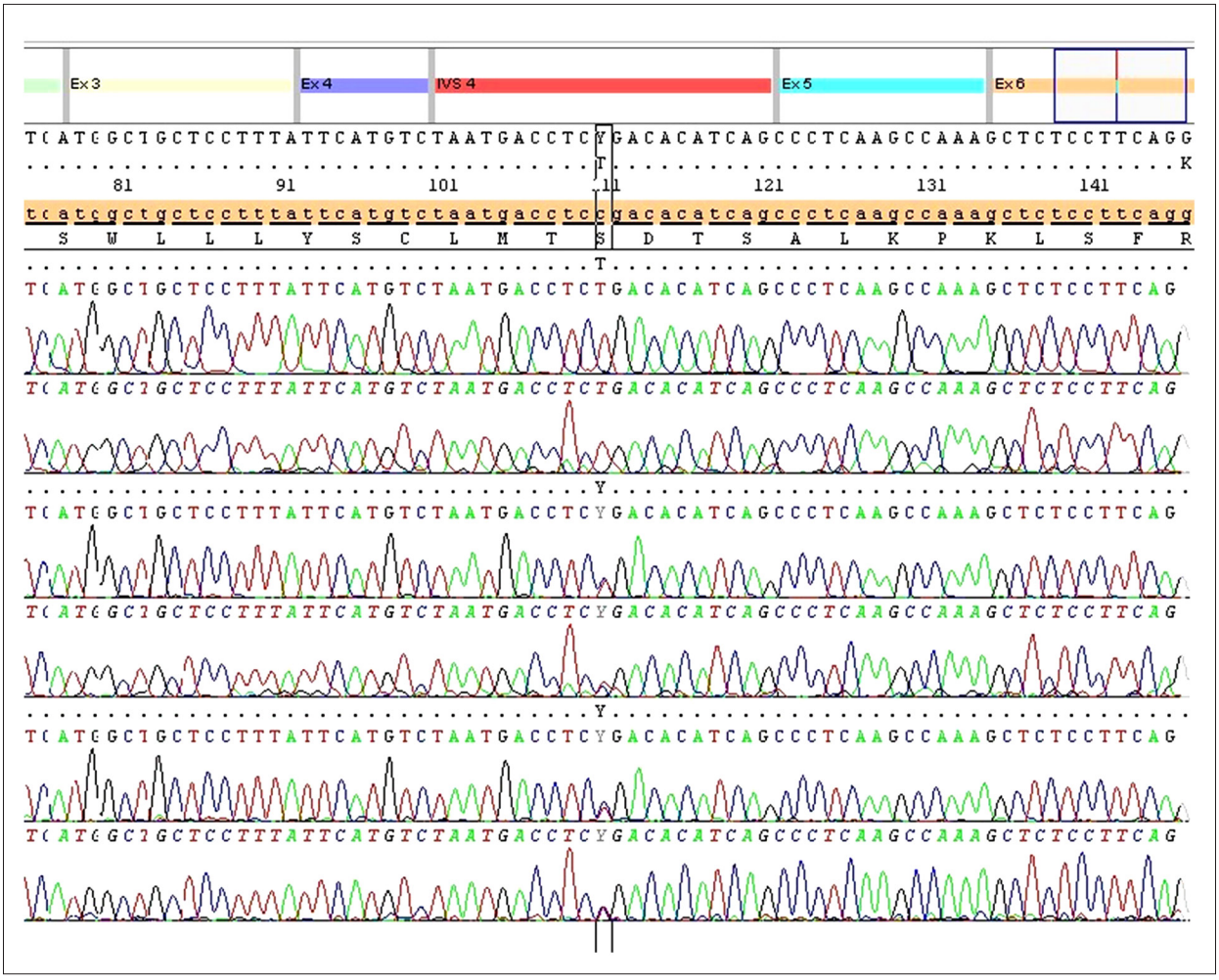

Figure 1. Exonic p.R301X (c.901 C>T) nonsense GLA gene mutation.

hypertrophy, electrocardiography showed normal sinus rhythm, and 24-h urinary protein was 31 mg/day. There was no neurological or renal involvement.

The proband's sister's enzymatic activity was 12.8 nmol/mg·h (normal range: 32–60 nmol/mg·h). She began to complain of hyperhidrosis, palpitations, and neuropathic pain in her third decade of life. Physical examination showed blood pressure of 140/90 mmHg, grade 2/6 systolic murmur on aorta, angiokeratoma, and cornea verticillata. Echocardiography showed significant left ventricular hypertrophy and mitral regurgitation, and 24-h urinary protein was 712 mg/day.

Cerebral magnetic resonance imaging (MRI) was normal in all patients. Agalsidase alfa treatment were started in the mother because of heart involvement, and in the sister for heart and kidney involvement in addition to low enzymatic activity.

The proband's 6-year-old daughter's genetic test showed that she had the same mutation and her enzymatic activity was in the normal range – 46.20 nmol/mg·h (32–60 nmol/mg·h). She does not have any symptoms associated with FD.

All patients gave written informed consent to participate in this study.

Peripheral blood samples were collected, using EDTA as an anticoagulant, for detection of α-galactosidase A activity and genetic analysis. α-Galactosidase A activity was measured by fluorometric assay, as described by Blau et al. (2008) in an external laboratory (Duzen Laboratory, Ankara) [10]. Fabry disease was indicated when α-galactosidase A activity in blood was <32 nmol/mg·h (normal range 32–60 nmol/mg·h). We also performed mutation analysis with an automated sequencing method that screened all 7 exons of GLA. The MiSeq next generation sequencing (NGS) platform (Illumina, San Diego, CA, USA) was used for GLA gene sequencing analysis. The manufacturer's standard procedure was used while extracting genomic DNA using the QIAamp DNA Blood Midi Kit (Qiagen, Hilden, Germany). IGV 2.3 (Broad Institute) software was used for visualization of the data.

Table 1. Clinical presentation of patients.

Kinship	Proband	Mother	Sister	Daughter
Age (year)	39	63	43	6
Mutation	p.R301X	p.R301X	p.R301X	p.R301X
Enzyme activity (nmol/mg·h)	6.56	5.8	12.8	46.2
Acroparesthesia	+	–	+	–
Angiokeratomas	+	–	+	–
Cornea verticillata	+	+	+	–
Kidney involvement	+	–	+	–
Heart involvement	+	+	+	–
Heat/cold intolerance	+	–	+	–

Relevant enzymatic and molecular data of the family are given in Table 1.

Discussion

The estimated incidence of FD in the general population is 1 in 117 000 [11]. Newborn screening initiatives have found a high prevalence of the disease, as high as 1 in ~3100 newborns in Italy [12].

To date, >600 mutations have been described in the exons and introns of the GLA gene, and discrimination between pathological and neutral mutations is difficult (*http://fabry-database.org/mutants/september* 2015). Classically, clinical manifestations are more severe in male hemizygous subjects than in female heterozygotes. Recently, it was found that FD can also severely affect women, but progression of the disease to organ failure generally takes longer and the severity of symptoms are more variable than in men [4,5]. For this reason, accurate follow-up is required for female patients, independent of enzymatic activity levels.

Misdiagnosis of the FD is common; it takes years (14–19 years) between beginning of the symptoms and correct diagnosis [4,5]. In our case, the patient was being treated for chronic sclerosing glomerulonephritis for 2 years, which is a relatively short time. Fabry disease may not be diagnosed until development of end-stage renal failure in patients with proteinuria. The frequency of FD was 0.04% (2/5408) in men and 0% (0/3139) in women, and then 0.02% (2/8547) in all patients undergoing dialysis in the Japan Fabry Disease Screening Study [13]. In Spain, 3650 samples from hemodialysis patients (18.5% of all patients undergoing hemodialysis in Spain) were tested and 11 new unrelated cases of Fabry disease (4 males and 7 females, 0.003%) were diagnosed [14]. The prevalence of FD was detected as 0.17% in Turkish hemodialysis patients [15].

Although the average presentation age is 6–8 years in males, the proband's symptoms began in his late thirties. His mother's and sister's symptoms also began at about the same age. His 6-year-old daughter does not have symptoms. Suspicion, careful physical examination, and past medical history (including acroparesthesia, heat and cold intolerance, and hypohidrosis) are very valuable.

In the M51I mutation, there were reports of cardiac, cerebral, and gastrointestinal involvement, but the kidneys are protected [8]. Another study evaluating intrafamilial phenotypic variability in 4 families (GLA gene mutations; p.R220X, p.C52Y, p.C172Y, p.R342X) with FD showed that renal and cardiac involvements, angiokeratoma, and acroparesthesia are common findings [16]. Cerebral involvements are found only in p.C52Y and p.R342X mutations. In our patient's p.R301X mutation, cerebral and gastrointestinal involvement were absent.

European renal best practice recommends screening patients for Fabry disease when there is unexplained chronic kidney disease in men younger than 50 years and women of any age. In men, the activity of α-galactosidase A can be measured in plasma, blood cells, or dried blood spots. In women, mutation analysis is necessary, as enzyme measurement alone can miss over one-third of female Fabry patients [17]. Our proband patient was a 39-year-old man who was being treated for chronic sclerosing glomerulonephritis. His sister had renal involvement.

Cardiac symptoms, including left ventricular hypertrophy, arrhythmia, angina, and dyspnea, are reported in 40–60% of patients with FD [1]. The prevalence of Fabry disease in primary cardiology practice with strictly defined otherwise unexplained LVH was 4% in males. Systematic screening is recommended in all men older than 30 years with LVH of unknown etiology for FD, even in the absence of obvious extracardiac manifestations [18]. Many characteristics of Fabry disease cardiomyopathy, with regard to electrocardiography and cardiac imaging,

have been claimed. None of the criteria were specific enough (90%) to be used for definitive diagnosis of Fabry disease.

Cerebrovascular manifestations result from multifocal small-vessel involvement and may include thromboses, seizures, paroxysmal hemiplegia, or hemianesthesia. Sensory neurons in spinal ganglia and small myelinated and unmyelinated fibers are preferentially affected. As a result of these features and the frequent presence of lesions in MRI scans, FD is often misdiagnosed as multiple sclerosis [19]. Fabry disease may explain ~1% of all strokes in young people, including 3–5% of cryptogenic strokes. Early recognition of FD may help to initiate appropriate treatment to decrease the risk of subsequent complications [20].

The European Fabry Working Group consensus recommends enzyme replacement therapy as soon as there are early clinical signs of kidney, heart, or brain involvement for classically affected men. Classically affected women and men with non-classical FD should be treated as soon as there are early clinical signs of kidney, heart, or brain involvement, while treatment may be considered in women with non-classical FD with early clinical signs that are considered to be due to FD [2].

Conclusions

In conclusion, our findings confirm the intrafamilial phenotypic variability in patients who have the same mutation. In the light of previous studies and results of the present report, it appears that heterozygous women could be affected as severely as man and should not be considered as asymptomatic carriers. Pedigree analysis remains a powerful, readily available tool to identify individuals at risk for genetic diseases.

Acknowledgements

α-Galactosidase A activity was measured by Dr. Murat Oktem at Duzen Laboratory, Ankara. Genetic analysis was performed by Serdar Ceylaner, MD, Associate Professor Medical Geneticist at Intergen Genetic Centre, Ankara, Turkey.

Conflict of interest

Dr. Ruya Ozelsancak has declared no competing interest. Dr. Bulent Uyar has declared no competing interest.

Statement

Financial Support: No. No experimental investigation on human subjects.

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