A Mutant mRNA Expression in an Endomyocardial Biopsy Sample Obtained from a Patient with a Cardiac Variant of Fabry Disease Caused by a Novel Acceptor Splice Site Mutation in the Invariant AG of Intron 5 of the $\alpha$-Galactosidase A Gene

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Abstract

We herein describe the case of a 58-year-old man who presented with dilated-phase hypertrophic cardiomyopathy (HCM) and required an implantable cardioverter defibrillator implant. Subsequently, the patient was diagnosed with Fabry disease (FD), which was suspected based on the results of an endomyocardial biopsy and diagnosed following demonstration of deficient $\alpha$-galactosidase A (GLA) activity. Molecular studies showed a novel point mutation in the 3' splice site consensus sequence of intron 5 in the gene encoding GLA that created a new splicing site, resulting in the expression of mutant mRNA. FD should be considered a cause of HCM in patients with severe tachyarrhythmia without other remarkable manifestations of FD.

Key words: Fabry disease, endomyocardial biopsy, ventricular tachycardia, intron, $\alpha$-galactosidase A

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Introduction

Fabry disease (FD) is a rare hereditary disorder characterized by systemic and vascular accumulation of globotriaosylceramide (Gb3) caused by mutations in the gene encoding the enzyme $\alpha$-galactosidase A (GLA), a lysosomal hydrolase (1). Previous studies have suggested that FD is under-diagnosed in the general population and that approximately 3% of patients with left ventricular hypertrophy of unknown origin are afflicted with FD (2-5). The classical form of FD is characterized by acroparesthesias angiokeratomas, severe skin manifestations in youth and progressive organ involvement with renal failure and cardiac and cerebrovascular disease in adulthood (6). Atypical forms of FD are characterized by symptoms affecting a single, specific organ, usually the heart, kidneys or brain (2, 7). These renal, cardiac and cerebrovascular variants manifest later in life. We herein report a mutant mRNA expression in an endomyocardial biopsy sample obtained from a patient with cardiac variant-like FD caused by a novel acceptor splice site mutation in the invariant AG dinucleotide of intron 5 in the $\alpha$-galactosidase A gene.

Case Report

A Japanese man was diagnosed with hypertrophic cardiomyopathy (HCM) and developed cardiomegaly at 51 years of age in 2005 (Fig. 1A). Thereafter, sinus bradycardia gradually developed (Fig. 2). Subsequently, echocardiogra-
Figure 1. Chest radiographs taken in 2005 (A), 2007 (B) and 2012 (C).

Figure 2. Electrocardiogram obtained in 2007.

Figure 3. Endomyocardial biopsy specimen. Optical microscopy showed vacuolar degeneration in the myocardial cells.

Figure 4. Sequencing data of the PCR product containing the intron 5/exon 6 splice junction site in the α-galactosidase A gene of this patient.

phy showed thinning of the posterolateral left ventricular wall (Table) and impaired hemodynamics due to ventricular tachycardia (VT) induced by an electrical physiological study. The patient was diagnosed with dilated-phase HCM and underwent implantation of an implantable cardioverter defibrillator (ICD) in 2007 (Fig. 1B). Thereafter, an episode of VT (cycle length: 310 msec) with presyncope and termination by a 35-J direct current shock of ICD was detected. The patient's mother had also been implanted with a pacemaker and afterwards subsequently died suddenly of a cardiac cause at 63 years of age. In 2012, at 58 years of age, chest radiographs demonstrated progressive cardiomegaly (Fig. 1C). The patient was admitted for advanced decompen-
sated heart failure and a biopsy of the right ventricular endocardium revealed severe vacuolar degeneration of myocardial cells (Fig. 3) compatible with lysosomal storage diseases such as FD. FD was diagnosed following demonstra-
tion of deficient serum and leukocyte GLA activity. Careful investigation of the patient’s clinical history revealed slight limb pain and hypohidrosis in childhood; however, no angiokeratomas, skin manifestations, cerebrovascular diseases or renal failure were found at the time of diagnosis of HCM. For the mutation analysis, genomic DNA was isolated from whole blood using standard techniques. The entire coding region, including all exons and flanking intronic regions, was amplified using polymerase chain reaction (PCR) of genomic DNA in seven fragments, as previously described (8), and each amplified genomic region was se-
quenced using an ABI Prism 373 DNA Sequencer (model 373A, Applied Biosystems). We found a G to T transition at the -1 position of the invariant AG dinucleotide of the 3’ acceptor splice site of intron 5 (IVS5-1G->T) (Fig. 4). A se-
quence analysis of the RT-PCR product using primers spanning exons 4 to 6 and RNA from leukocytes and an endomyocardial biopsy sample revealed a 4-base deletion (T802-G805) in exon 6 (Fig. 5). All intron-exon splice junctions in the genomic DNA of the GLA gene follow the “GT/AG” rule. Splice mutations are caused by intronic nucleotide ab-
normalities that alter the consensus sequence of the splice site. We demonstrated a novel point mutation in the 3’ splice site consensus sequence of intron 5 that created a new splicing site and a mutant mRNA expression with a 4-base deletion not only in leukocytes, but also in an endomyocardial biopsy sample.
Table. Echocardiographic Findings

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<tr>
<td>IVS/LVPM (cm)</td>
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<td>1.5/1.2</td>
<td>2.2/0.9</td>
<td>1.6/0.8</td>
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LVEDD: left ventricular end diastolic dimension, LVESD: left ventricular end systolic dimension, EF: ejection fraction, IVS: interventricular septum, LVPM: left ventricular posterior wall, MR: mitral regurgitation

Discussion

This case seemed to be a cardiac variant of FD. In the beginning, the patient required implantation of an ICD for severe arrhythmia, and we suspected a diagnosis of familial cardiomyopathy caused by mutations in the gene encoding sodium channel α-subunit (SCN5A) (9) or lamin A/C (LMNA) (10). However, we could not find these genetic mutations in a sequence analysis. Thereafter, the patient’s left ventricular function gradually deteriorated. We therefore performed an endomyocardial biopsy. The results suggested a lysosomal storage disease such as FD, glycogen storage disease or mitochondrial disease. We then examined the serum and leukocyte GLA activity. FD was diagnosed because the enzyme activity was found to be below the measurement sensitivity of the assay and a genomic mutation of IVS5-1G>T was also found. An analysis of RNA obtained from leukocytes and an endomyocardial biopsy sample revealed a 4-base deletion frameshift mutation (T802-G805) in exon 6 caused by the IVS5-1G>T transition. Severe vacuolar degeneration of myocardial cells can be easily explained by the mutant mRNA expression of GLA observed in the endomyocardial biopsy of this patient, which was different from that of a patient without FD. However, the reason for the dominance of cardiac symptoms remains unclear because there were no differences between the mutation found in the heart sample and that found in the leukocytes. We identified that a novel mutation in IVS5-1G>T caused a 4-base deletion (T802-G805) in exon 6 of GLA mRNA and a frameshift mutation resulting in premature termination. A previous report showed that IVS6-1G>A caused a first base pair deletion (c909del) in exon 7 in the mRNA (11). These mutations destroy the authentic splice site and seem to create new acceptor splice sites at a position downstream from the normal IVS splice sites. This patient is currently undergoing enzyme replacement therapy with agalsidase α (12).

A recent report indicated that arrhythmias are important causes of morbidity and mortality in patients with Fabry cardiomyopathy (13). In that report, the authors suggested that sudden cardiac death in patients with Fabry cardiomyopathy might be related to bradycardia. However, in this case, episodes of impaired hemodynamics due to ventricular tachycardia were observed. Of note, the patient’s mother had died suddenly after pacemaker implantation for bradycardia. These results indicated that severe tachyarrhythmia should be considered an important cause of sudden cardiac death in patients with Fabry cardiomyopathy, as reported by Shah et al. (14). In any case, FD should be considered in the differential diagnosis of hypertrophic cardiomyopathy most likely requiring implantation of an ICD for severe arrhythmia without other remarkable manifestations of FD.

The authors state that they have no Conflict of Interest (COI).
References


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