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Clinical and Genetic Investigation of a Japanese Family With Cardiac Fabry Disease: Identification of a Novel α-Galactosidase A Missense Mutation (G195V)
(心臓Fabry病の1家族についての臨床的並びに遺伝的研究)

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Clinical and Genetic Investigation of a Japanese Family With Cardiac Fabry Disease

Identification of a Novel α-Galactosidase A Missense Mutation (G195V)

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SUMMARY

Fabry disease is an X-linked lysosomal storage disorder caused by mutations of the α-galactosidase A gene (*GLA*), and the disease is a relatively prevalent cause of left ventricular hypertrophy mimicking idiopathic hypertrophic cardiomyopathy. We assessed clinically 5 patients of a three-generation family and also searched for *GLA* mutations in 10 family members. The proband had left ventricular hypertrophy with localized thinning in the basal posterior wall and late gadolinium enhancement (LGE) in the near-circumferential wall in cardiovascular magnetic resonance images and her sister had vasospastic angina pectoris without organic stenosis of the coronary arteries. LGE notably appeared in parallel with decreased α-galactosidase A activity and increased NT-pro BNP in our patients. We detected a new *GLA* missense mutation (G195V) in exon 4, resulting in a glycine-to-valine substitution. Of the 10 family members, 5 family members each were positive and negative for this mutation. These new data extend our clinical and molecular knowledge of *GLA* gene mutations and confirm that a novel missense mutation in the *GLA* gene is important not only for a precise diagnosis of heterozygous status, but also for confirming relatives who are negative for this mutation. (Int Heart J 2011; 52: 308-311)

Key words: Left ventricular hypertrophy, Hereditary cardiovascular diseases, Glycosphingolipid deposition

Fabry disease is an X-linked lysosomal storage disorder caused by abnormalities in the α-galactosidase A gene (*GLA*) (MIM:300644), which leads to a deficiency in the activity of the lysosomal enzyme, α-galactosidase A (α-Gal A).[1)] Loss of this activity results in the intralysosomal deposition of neutral glycosphingolipids with terminal α-linked moieties primarily in the plasma and vascular endothelium, leading to angiokeratoma, acroparesthesias, and vascular diseases of the heart, kidneys, and brain.[2,3)] The clinically severe classic form usually manifests during childhood or adolescence, although an atypical variant with residual α-Gal A activity or late-onset cardiomyopathy without systemic manifestations has been reported.[4,5)] Affected hemizygous males are relatively easy to identify using a combination of pedigree analysis and measurement of α-Gal A activities in plasma or leukocytes. In contrast, such identification is more difficult in heterozygous females because many exhibit normal levels of α-Gal A.[2,6)] Thus, genetic analysis is essential to identify genotype/phenotype correlations and to discriminate heterozygotes. Moreover, early diagnosis is important and critical since recent clinical trials have shown that human recombinant α-Gal A is safe and effective for reducing accumulated intracellular globotriaosylceramide, which is thought to be the trigger for a series of pathologic processes leading to irreversible fibrotic organ damage.[7,8)]

Here, we describe a novel *GLA* missense mutation (G195V) in a family with individuals affected by cardiac hypertrophy. Confirming a novel missense mutation in the *GLA* gene associated with Fabry disease mimicking hypertrophic cardiomyopathy is important to discriminate heterozygous individuals.

METHODS

Participants and clinical evaluations: We studied 10 members of a three-generation family (Figure 1). All of these 10 family members provided written, informed consent in accordance with the Declaration of Helsinki Principles and informed consent for participation was obtained from individuals. Medical records of each participant that included interviews, physical examinations, electrocardiography, radiography, echocardiog-

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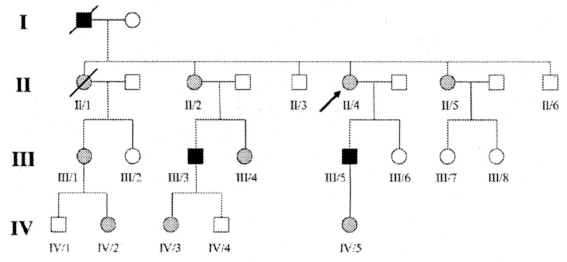

Figure 1. Pedigree of three-generation family studied in this report. Open, grey and filled symbols indicate unaffected, heterozygous and hemizygous individuals, respectively. Circles indicates females, squares; males, and arrow, proband (II/4).

Table. Exon Primer Sets for GLA

Primer	Sequence	bp
Exon 1-1 (+) sense:	5'-CCAGTTGCCAGAGAAACAA-3'	390
Exon 1-2 (+) antisense:	5'-GAGACTCTCCAGTTCCC-3'	
Exon 2 (+) sense:	5'-CTTGTGATTACTACCACACT-3'	367
Exon 2 (-) antisense:	5'-AACAAGCTTCTGTACAGAAGTGC-3'	
Exon 3-4(+) sense:	5'-TCAGCAGAACTGGGGGATT-3'	1436
Exon 3-4 (-) antisense:	5'-AGTAACGTTGGACTTTGAAGG-3'	
Exon 5-7 (+) sense:	5'-CATCTCACAAGGATGTTAGT-3'	1227
Exon 5-7 (-) antisense:	5'-AGGAAGTAGTAGTTGGCAAT-3'	

raphy, and cardiovascular magnetic resonance (CMR) findings were reviewed.

Molecular genetic analysis: Genomic DNA was extracted from peripheral leukocytes of the 10 family members according to standard procedures.[9] The human *GLA* consists of 7 exons. To determine the sequence of exons 1, 2, 3-4, 5-7 of *GLA*, we performed PCR on genomic DNA with the specific primers listed in the Table.

Results

Clinical findings: Patient II/4 (proband) was a 58-year-old woman (height, 152 cm; weight, 48 kg) with an ECG abnormality. Her father had committed suicide at the age of 50 due to severe depression, and her elder sister had suddenly died at age 54 with end-stage renal disease of unknown cause. A clinical cardiac evaluation revealed a regular heart rate of 60 bpm, normal heart sounds and no murmurs. Arterial blood pressure was 104/70 mmHg. Urinalysis showed no proteinuria and normal microscopic findings. Chest X-rays showed an enlarged heart shadow without pulmonary edema (Figure 2A). Electrocardiography displayed a normal sinus rhythm with normal PR interval and no dysrhythmias. The frontal plane QRS axis was +40°. The rSR' pattern in V1 and V2 with a QRS duration of < 120 ms was consistent with incomplete right bundle branch block. Inverted T waves presented in DI, DII, aVF, and V4-V6 (Figure 2B). Echocardiography showed left ventricular hypertrophy with localized thinning of the basal posterolateral wall of the left ventricle with preserved systolic function (left ventricular ejection fraction, 58%) (Figures 2C and 2D). Left ventricular hypertrophy with localized thinning was revealed by

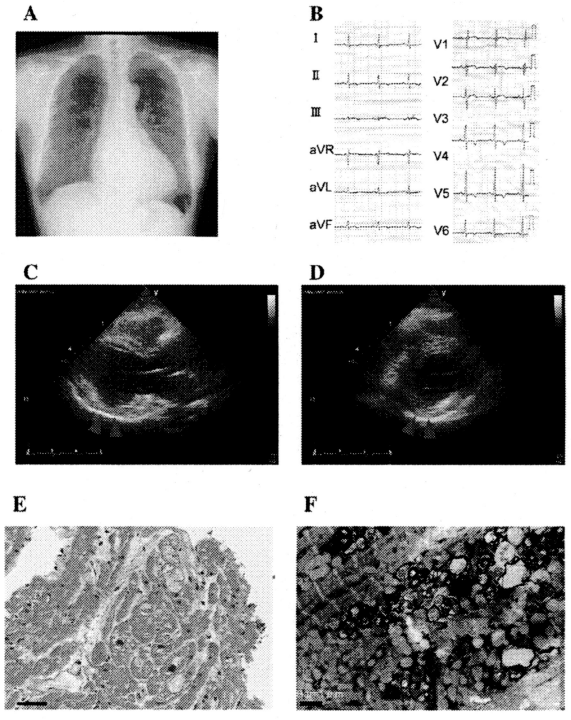

Figure 2. Chest radiography (**A**) and twelve-lead electrocardiography (**B**) findings, transthoracic echocardiography (**C** and **D**) and histological findings of the heart (**E** and **F**) of the proband. Cardiothoracic ratio is 0.58 (**A**). Twelve-lead electrocardiography shows regular sinus rhythm with complete right bundle block. Inverted T waves in DI, DII, aVF and V4-V6 (**B**). Parasternal long-axis view (**C**) and short-axis view (**D**) show left ventricular hypertrophy with interventricular septal and left ventricular posterior wall thickness of 13 mm each and localized thinning of basal posterior wall of left ventricle to 7 mm (arrows). Light microscopic findings show sarcoplasmic vacuolization of the myocardial cells (hematoxylin and eosin stain, x200) (**E**). Electron microscopic findings show typical lysosomal inclusions with a concentric lamellar configuration (arrows) (**F**). The black bar indicates 50 μm in Panel (**E**), and 1 μm in Panel (**F**).

CMR as well as late gadolinium enhancement (LGE) in the basal posterolateral wall (Figures 4A and 4D). She was tentatively diagnosed with a dilated form of hypertrophic cardiomyopathy. However, her plasma α-Gal A activity was decreased (3.2 nmol/hour/mL) compared with the normal value of 8.4 ± 2.4 nmol/hour/mL, suggesting a heterozygote of cardiac Fabry disease. Structural findings from an endomyocardial biopsy revealed lamellate deposits in the endothelial cells of the myocardial capillaries (Figures 2E and 2F).

Patient II/5 was a 56-year-old woman (height, 150 cm; weight, 53 kg) who was initially admitted at age 50 for evaluation of an episodic oppressive feeling in the chest. Clinical cardiac evaluation showed a regular heart rate of 76 bpm, normal heart sounds and no murmurs. Arterial blood pressure was 94/62 mmHg. Urinalysis showed no proteinuria and normal microscopic findings. Electrocardiography showed a normal sinus rhythm with incomplete right branch block. Inverted T waves were present in DII, DIII, aVF, V4 and V5. Echocardiography showed normal left ventricular function without hypertrophy. A coronary angiogram showed no stenotic lesions in the left coronary artery (LCA) and right coronary artery

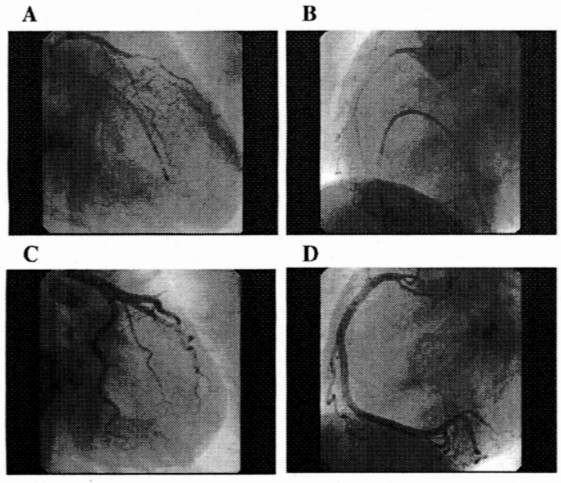

Figure 3. Coronary arteriography of LCA (RAO view; **A** and **C**) and RCA (LAO view; **B** and **D**) of patients II/5. Views after acetylcholine administration (**A** and **B**) indicate spasms in segments 7 and 11 (**A**) and in segments 1 to 4 (**B**). No stenotic lesions are evident after vasospasm was relieved by intracoronary administration of isosorbide dinitrate (**C** and **D**).

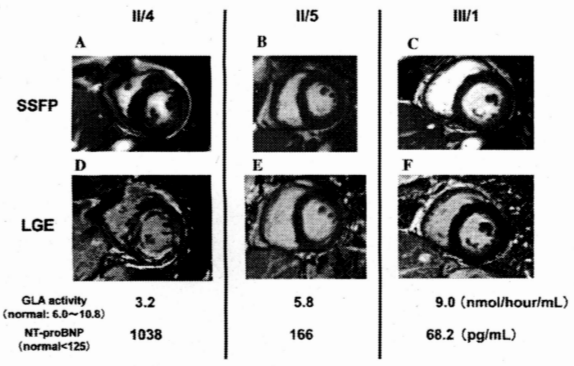

Figure 4. Cardiovascular magnetic resonance images from patients II/4 (proband) (**A** and **D**), II/5 (**B** and **E**), and III/1 (**C** and **F**). Short-axis view of steady-state free precession (SSFP) shows concentric left ventricular hypertrophy (**A**) with late gadolinium enhancement (LGE) in near-circumferential wall (**B**) of patient II/4 (proband). Thickness of left ventricle wall is normal in patient II/5 (**B**), but LGE is evident in basal posterolateral wall (arrows) (**D**). Patient III/1 had normal cardiac morphology (**C**) without LGE (**F**). The degree of LGE tends to be increased in parallel with the level of NT-pro BNP, and be inversely proportional to the degree of α-Gal A activity in these patients.

(RCA). The intracoronary administration of 25-100 μg acetylcholine revealed LCA segments 7 and 11, and RCA segments 1 to 4 (Figures 3A and 3B). She simultaneously felt the same chest oppression as that usually associated with a heart attack under these conditions and the ECG showed T-wave elevation in leads V1-V6, and ST-segment elevation in leads II, III and aVF. These symptoms were promptly resolved by the intracoronary administration of isosorbide dinitrate, indicating a diagnosis of vasospastic angina pectoris. Six years later, her sister (proband) was diagnosed with Fabry disease, and patient II/5 also had relatively low plasma α-Gal A activity (5.8 nmol/hour/mL) suggesting a heterozygote. The CMR study revealed normal wall thickness, but LGE was evident in the basal posterolateral wall (Figures 4B and 4E). She was admitted to our hospital and diagnosed with cardiac Fabry disease by endomyocardial biopsy like the proband.

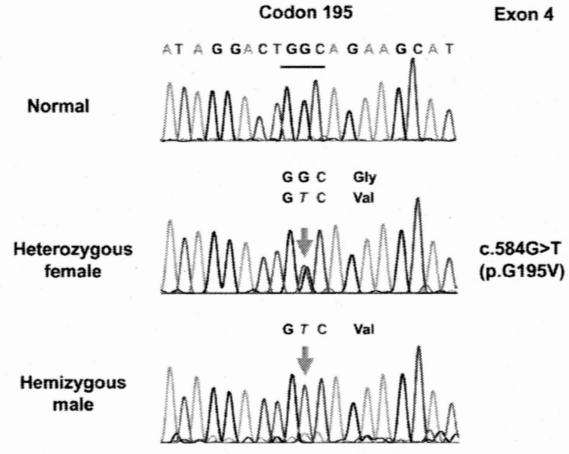

Figure 5. Chromatography of normal control (**upper panel**) and genomic DNA from patients II/4 (proband) (**middle panel**) and III/5 (**lower panel**) for *GLA* exon 7. Single base pair substitution (arrow; 584G→T) has replaced glycine with valine (G195V).

Patient III/1 was an apparently healthy, asymptomatic 42-year-old woman (height, 158 cm; weight, 53 kg). Clinical cardiac evaluation showed a regular heart rate of 76 bpm, normal heart sounds and no murmurs. Arterial blood pressure was 120/72 mmHg. Urinalysis showed no proteinuria and normal microscopic findings. Electrocardiography showed normal sinus rhythm with incomplete right branch block. Inverted T waves were present in DII, DIII, aVF, and V4-V6. Echocardiographic studies showed normal left ventricular function without hypertrophy. A CMR study revealed normal cardiac morphology without LGE (Figures 4C and 4F). Her plasma α-Gal A activity was normal (10.4 nmol/hour/mL), but molecular analysis of the *GLA* gene revealed that she had the same missense mutation as the proband. She was admitted to our hospital and diagnosed with cardiac Fabry disease by endomyocardial biopsy like the proband.

Patient III/5 was the asymptomatic, apparently healthy 35-year-old son of the proband (height, 168 cm; weight, 54 kg). Clinical cardiac evaluation revealed a regular heart rate of 70 bpm, normal heart sounds and no murmurs. Arterial blood pressure was 110/70 mmHg. Electrocardiographic findings were normal and echocardiography showed normal left ventricular function without hypertrophy. The CMR study revealed normal cardiac morphology without LGE. Urinalysis showed mild proteinuria (0.34 g/g·creatinine) but microscopic findings of kidney function were normal. Plasma α-Gal A activity was considerably below normal (0.2 nmol/hour/mL) indicating a hemizygote.

Patient IV/2 was an asymptomatic, apparently healthy 19-year-old woman (height, 160 cm; weight, 55 kg). Clinical cardiac evaluation revealed a regular heart rate of 60 bpm, normal heart sounds and no murmurs. Arterial blood pressure was 120/70 mmHg. Electrocardiographic findings were normal. Urinalysis showed no proteinuria and normal microscopic

findings. Plasma α-Gal A activity was relatively low (6.6 nmol/hour/mL) suggesting that she was a heterozygote.

Molecular genetic analyses: Molecular analysis of the *GLA* genes of patients II/4 (proband), II/5, III/1, III/5 and IV/2 showed a novel missense mutation at codon 195 in exon 4, resulting in a glycine (GGC) to valine (GTC) substitution (Figure 5). This novel mutation confirmed that they had Fabry disease. Participants III/2, III/6, III/7, III/8, and IV/1 had no genetic alterations.

Discussion

To date, approximately 500 mutations have been identified in the *GLA* gene (Human Gene Mutation Database web site, http://www.hgmd.cf.ac.uk/). We identified a novel *GLA* missense mutation (G195V) in 5 family members who had cardiac Fabry disease, but not in another 5 female members of the same family.

Precise diagnosis may be delayed or missed since the cardiac manifestation of Fabry disease often mimics hypertrophic cardiomyopathy.[10] Hypertrophic cardiomyopathy was tentatively suspected in the proband during an echocardiographic examination. However, CMR findings of left ventricular hypertrophy with localized thinning as well as LGE in the basal posterior wall, and a family history of sudden death and end stage renal disease indicated Fabry disease rather than idiopathic hypertrophic cardiomyopathy. Her α-Gal A activity was below normal and the final diagnosis of Fabry disease was confirmed by genetic analysis. Consequently, we found a Fabry heterozygote with the classic form and identified a novel missense mutation (G195V) with amino acid substitution in the *GLA* gene.

Fabry disease may not be rare in cardiac hypertrophy. Screenings have revealed Fabry disease as a cardiac variant in 7 (3%) of 230[4] and 6 (4%) of 153 male patients[5] with cardiac hypertrophy. Cardiac manifestations with hypertrophy, valvular abnormalities, and electrocardiographic abnormalities are also frequently observed in heterozygous females.[11]

LGE area corresponds to myocardial fibrosis and is associated with decreased regional functioning sites. Thus, LGE is a good index of cardiac damage even in the heart without morphological abnormality. Patient II/4 (proband) had left ventricular hypertrophy with local thinning of the left ventricular posterior wall, which is a characteristic of advanced cardiac Fabry disease.[12] Meanwhile, patient II/5 had no left ventricular hypertrophy, but LGE was evident in the basal posterolateral wall. Interestingly, the degree of LGE tends to be increased in parallel with the level of NT-pro BNP, and be inversely proportional to the degree of α-Gal A activity in these patients. (Figure 5) Therefore, CMR studies using gadolinium could be useful for evaluating cardiac damage and in the differential diagnosis of hypertrophic cardiomyopathy of unknown cause, such as Fabry disease. Furthermore, endothelial dysfunction could lead to vasospastic angina pectoris without the organic stenosis of coronary arteries[13] as in our patient II/5, suggesting that Fabry disease should be considered in the differential diagnosis of vasospastic angina pectoris.

Conclusions: We concluded that Fabry disease should be considered in the differential diagnosis of cardiac hypertrophy because early initiation of enzyme replacement therapy is required. Therefore, α-Gal A activity should be evaluated in all patients with suspected Fabry disease, especially when the family history includes relevant clinical features. Furthermore, gene mutations in heterozygote females should also be analyzed to confirm the mechanism of the intralysosomal deposition of glycosphingolipids in endomyocardial biopsy specimens. Furthermore, identification of a novel missense mutation in the *GLA* gene is very useful for recommending apparently healthy family members to undergo genetic counseling to confirm the absence of a mutation.

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