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Letter to the Editor

Clinical and Genetic analysis of Fabry disease: report of 6 cases including 3 heterozygous females

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Fabry disease is an X-linked inherited disorder (OMIM 301500) caused by defects or loss of activity in the lysosomal enzyme alpha-galactosidase A (GLA) [1]. Most hemizygous male children with either absent or remarkably reduced GLA activity develop a classic Fabry disease phenotype characterized by angiokeratoma corporis diffusum, acroparesthesias (abnormal sensations in the extremities), hypohidrosis (reduced sweating), and ophthalmic changes, and they have a greater risk of lethal renal, cardiac or cerebrovascular disease during the fourth or fifth decade [2, 3]. Importantly, cardiovascular, renal and even skin abnormalities can also be observed in heterozygous deficient females [2, 4]. Recently, enzyme replacement therapy using recombinant human GLA has been used to treat patients with Fabry disease [5]. Therefore, making a precise diagnosis in the early stages of this disease is important for affected males as well as in asymptomatic heterozygous females [2, 4, 5]. The diagnosis of Fabry disease is made based on clinical manifestations, GLA activity, and mutation analysis of the GLA gene (*GLA*) localized on Xq 22 [2]. *GLA* spans 12kb of DNA and comprises 7 exons varying in size from 92 to 291bp corresponding a 1.45kb mRNA [2]. So far, in Fabry patients, various *GLA* mutations have been identified in each of the 7 exons including involvement of

the exon-intron borders [6]. We report here clinical and genetic analysis of 6 patients in 4 unrelated Japanese families with Fabry disease. This study includes 3 heterozygous females, and we had analyzed the precise clinical and genetic findings in each case.

All of the cases tested here were consulted from different clinical departments in Japan, and we had at first analyzed the clinical findings from these cases (Table 1). All 3 male Fabry patients (cases 2b, 3b and 4) showed disseminated angiokeratoma corporis diffusum, acroparesthesias and hypohidrosis, which are all characteristic features of the “classic phenotype” [2, 3]. On the other hand, all female heterozygous Fabry patients (cases 1, 2a and 3a) were free from skin lesions, and 2 out of the 3 individuals presented with proteinuria. Case1 (a 43-year-old female), without family history indicating Fabry disease, had been suffering from slight proteinuria of an unknown origin for several years. Unexpectedly this relates to case 1 only, renal electron microscopic findings revealed concentric lamellar inclusions in the glomerulus podocytes (Fig. 1f), a characteristic finding in Fabry disease [2]. Leukocyte or plasma GLA enzyme activity from other heterozygous female cases except for Case 3a was not

significantly reduced, but urinary globotriaosylceramide (GL-3) in Case 1 and 3a was increased (Table 1). GL-3 is an intermediate metabolism product which increases in various fluid and cellular sources in response to decreased GLA activity [2]. GL-3 is metabolized into glycosphingolipid by GLA, and GL-3 levels have also been suggested to be a diagnostic marker that definitely that suggests Fabry disease [2, 7]. Reduced GLA activity is one of the most important laboratory findings that leads to a diagnosis of Fabry disease for affected males [2]. In contrast, GLA activity in heterozygous females can be variable and a female Fabry patient with normal GLA activity has even been reported [8]. This study also indicates that residual GLA activity as a major diagnostic marker alone may not be sufficient to diagnose heterozygous female Fabry patients as well as asymptomatic carriers.

To determine their genetic diagnosis in the present cases, we performed genetic analysis of *GLA*. Genomic DNA was first extracted from peripheral blood mononuclear cells obtained from patients in addition to 100 unrelated healthy volunteers as controls, which was followed by PCR amplification and subsequent direct sequencing. Oligonucleotide primers to amplify all exons

including *GLA* exon-intron borders, and PCR conditions were based on a previous report [9]. As summarized in Table 1, we found 3 missense mutations comprising of single base nucleotide substitutions (cases 1-3) and 1 nonsense mutation due to a single base nucleotide deletion (case 4), all of which could not be detected in any of the control samples and were therefore unlikely to be polymorphisms (Table 1 and Fig. 1). Among them, c.758delT, p.Q250P and p.G171C were novel mutations, whereas p.R301Q, has been previously reported [7, 10].

Case 2a (a 48-year-old female) who had p.G171C *GLA* mutation showed a more severe clinical phenotype including acroparesthesias triggered by fever, proteinuria, corneal opacity and left ventricular hypertrophy. Her son, case 2b (a 22-year-old male) demonstrated a markedly more severe classic phenotype, which was characterized by angiokeratoma corporis diffusum, acroparesthesias, hypohidrosis and proteinuria. Therefore, this novel mutation, p.G171C might be related to a more severe clinical phenotype observed in Fabry male patients as well as heterozygous females. Another deletion mutation in *GLA* has been reported to be associated with a classic Fabry disease phenotype [2]. Similarly,

the c.758delT deletion mutation detected in case 4 (a 41-year-old male) led to a premature termination codon 45 bp downstream from the site of deletion, which was also associated with the classic, severe phenotype.

Lastly,the p. R301Q mutation detected in Case 1 (a 43-year-old female) was previously reported in hemizygous males with cardiomyopathy and renal dysfunction but no skin abnormalities [7, 10]. Interestingly heterozygous females in this family did not have any clinical abnormalities but slightly reduced leukocyte GLA activity [7]. Our case (Case 1, a 43-year-old female) was also asymptomatic but renal electron microscopy findings revealed concentric lamellar inclusions in the glomerulus podocytes, and her urine GL-3 level was increased.

In summary, we have analyzed the genetic basis of defects in *GLA* from 4 Fabry disease families; including 3 symptomatic heterozygous females. It will be important to collect more extensive clinical information from unrelated patients with the same genotype to establish whether a more definitive correlation between genotype-phenotype in Fabry disease exists.

Figure legends

Fig.1 *GLA* mutations identified in the Fabry disease cases and electron microscopic findings of the renal-biopsy specimen (Case1). (a)-(d) Direct nucleotide sequencing of *GLA* demonstrated various mutations (* indicates novel mutation). (e) The positions of detected mutations in *GLA* cDNA (* indicates novel mutation). (f) Electron microscopy of the renal-biopsy specimen taken from a heterozygous female Fabry patient (case 1) demonstrated numerous concentric lamellar inclusions in the podocytes and the epithelium (arrows).

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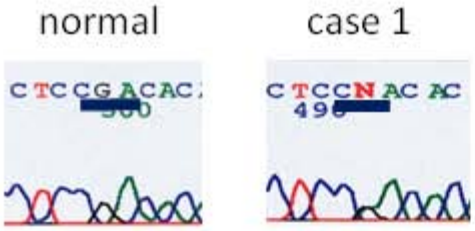
Figure 1

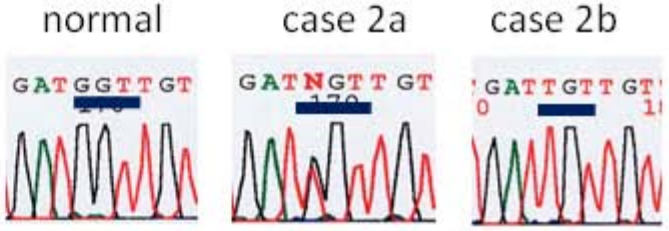
(a) case 1

ex6: p.R301Q (c.902G>A)



(b) case 2

ex3: p.G171C (c.511G>T)*

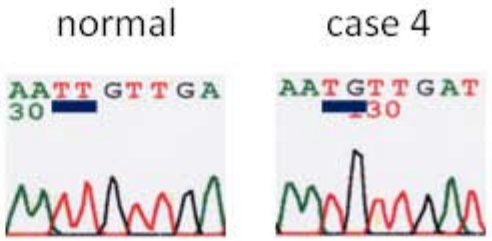


(c) case 3

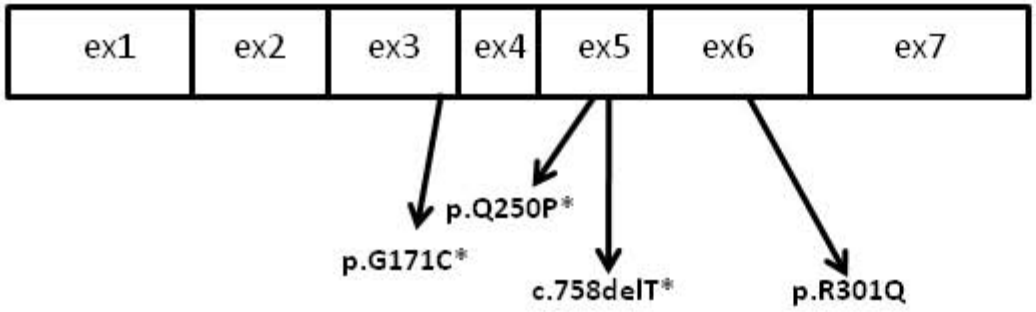
ex5: p.Q250P (c.749A>C)*

(d) case 4

ex5: c.758delT*



(e) ***GLA*** **cDNA**



(f)

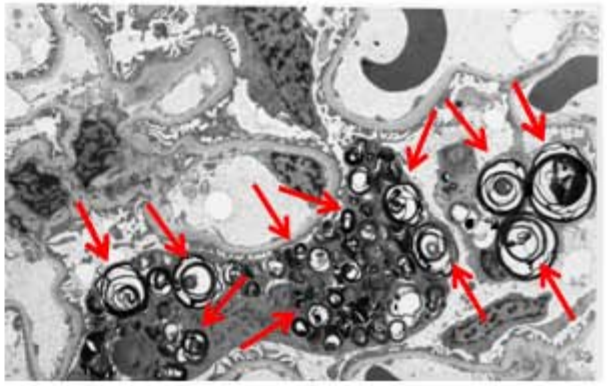


Table 1 The oligonucleotide primers were chosen on the basis of exon-intron borders. Forward primers were indicated by (+), and reverse by (-).

Exon 2L	5'- CTT GTG ATT ACT ACC ACA CT -3'	(+)
Exon 2R	5'- AAC AAG CTT CTG TAC AGA AGT GC -3'	(-)
Exon 3L	5'- CTG CTA CCT CAC GAT TGT -3'	(+)
Exon 3R	5'- ATT GGT TCT TTG GCT CAG -3'	(-)
Exon 4L	5'- ACT TGA ACC TGG GAA ACA -3'	(+)
Exon 4R	5'- CCT TGG TTG GTT TGT TG -3'	(-)
Exon 5-6L	5'- CTC ACA AGG ATG TTA GT -3'	(+)
Exon 5-6R	5'- CAT CAA GAG CAA GGG AAA -3'	(-)
Exon 7L	5'- GAA TGC CAA ACT AAC AGG -3'	(+)
Exon 7R	5'- CAG GAA GTA GTA GTT GG -3'	(-)

Table 2 Clinical manifestations and detected mutations in *GLA*.

Case	Age/Sex	Angiokeratoma	Acroparesthesias	Hypohidrosis	Corneal opacity	Proteinuria	Left ventricular hypertrophy	GLA enzymatic activity	GL-3 in urine sediment	Mutation
1	43Y/F	-	-	-	-	+	-	60.4*	1.19***	p.R301Q (c.902G>A) exon6 [7, 10]
2	63Y/F	-	-	-	-	+	-	Not done	Not done	p.M296I (c.902G>A) exon6 [★]
3a	48Y/F	-	+	-	+	++	+	5.7**	Not done	p.G171C (c.511G>T) exon3, novel
3b	22Y/M	+	+	+	-	+++	-	0.1**	Not done	p.G171C (c.511G>T) exon3, novel
4a	55Y/F	-	-	-	-	-	+	Not done	1.14***	p.Q250P (c.749A>C) exon5, novel
4b	27Y/M	+	+	+	-	±	+	1.5*	7.87***	p.Q250P (c.749A>C) exon5, novel
5	41Y/M	+	+	+	-	+++	+	0.5*	Not done	c.758 del T exon5, novel

* nmol/mg protein/hr in leukocyte (normal range: 49.8-116.4)

** nmol/hr/ml in plasma (normal range: 6.0-10.8)

***ug/mgCr in urine sediment (normal range: 0.10-0.40)