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BRIEF COMMUNICATION

Mutation spectrum of α-Galactosidase gene in Japanese patients with Fabry disease

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Abstract

The efficacy of pharmacological chaperone therapy for Fabry disease depends on the type of α-galactosidase A (GLA) mutations. Here, we examined the mutation spectrum of the GLA gene among patients from 115 Japanese families with Fabry disease. Of these, no pathogenic mutations were identified in six families (5.2%). In total, 73 different disease-causing mutations were identified: 41 missense (56.2%), 11 nonsense (15.1%), four in frame deletion (5.5%), 10 frameshift (13.7%), six splice site (8.2%), and one intronic (1.4%) mutations. The GLA mutations detected in later-onset phenotype patients with end-stage renal disease overlapped with those seen in classical patients, indicating that it is difficult to differentiate between these two phenotypes from gene mutations. Additionally, 33 families (28.7%) had amenable mutations to the pharmacological chaperone migalastat. In conclusion, our study is informative when considering genetic counseling and pharmacological chaperon therapy for Fabry disease.

Introduction

Fabry disease is an X-linked lysosomal storage disorder resulting from a deficiency of α-galactosidase A activity (GLA) [1]. The deficiency of GLA leads to progressive accumulation of globotriaosylceramide (Gb3) in vascular endothelial cells, renal cells, dorsal root ganglia, cardiac myocytes, and cornea.

Fabry disease is classified into three clinical phenotypes from gender and manifestations of the patients. Typical male patients with "classic" Fabry disease present with acroparesthesias, hypohidrosis, angiokeratoma and corneal opacities during childhood or adolescence, and develop progressive renal impairment, cardiac hypertrophy and cerebral vascular events in adulthood. The male patients with "later-onset" form of the disease show milder phenotypes limited to the cardiac and or renal involvement in adulthood. The "female" patients with Fabry disease exhibit heterogeneous clinical severities ranging from asymptomatic to severe manifestations such as end-stage renal failure (ESRD).

Recently, pharmacological chaperone migalastat was approved in Japan. The pharmacological chaperone stabilizes specific mutant GLA protein structure and promotes the trafficking of abnormal GLA protein to lysosomes [2], resulting in elevation of patients own enzyme activities. The mutations of GLA gene encoding these specific mutant GLA forms are defined as amenable mutations [3–5]. It is important to analyze the GLA gene mutations to evaluate the efficacy of pharmacological chaperone therapy.

Here, to elucidate the mutation spectrum of GLA gene in Japan, we studied the GLA gene mutations in Japanese families with Fabry disease.

Material and methods

Study patients

We enrolled 176 Japanese patients with Fabry disease (88 male and 88 female) from 115 families. They were referred to us for diagnosis of Fabry disease between 1998 and 2017. The cases with common functional polymorphism in Japan and Korea (p.E66Q) were excluded [6, 7]. These study patients included those in our previous study [8].

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They were diagnosed with Fabry disease based on enzyme assay in male patients and gene analysis in female heterozygotes.

This study was performed under the approval of the ethical committee of The Jikei University School of Medicine.

Enzyme assay

The GLA activity in leukocytes was studied using the fluorogenic substrate, 4-methylumbelliferyl-α-D-galacto-pyranoside, as described previously [9].

Gene analysis

Genomic DNA was extracted from leukocytes. Each exon and flanking intronic sequence of the GLA gene was amplified by PCR and directly sequenced [8]. We examined IVS4 + 919 G > A mutation using previously reported method [10]. The amenability to migalastat of the GLA gene mutations was evaluated by previous report [5].

Results

In 109 of the 115 families, we identified 73 different disease-causing mutations of following types: 41 missense, 11 nonsense, four in frame deletion, 10 frameshift, six splice site, and one intronic. Although the other six families (six male patients) were diagnosed with Fabry disease from enzyme assays, their gene mutations could not be detected (Table 1).

Thirteen mutations (p.L16H, p.D33Y, p.R49H, p.Y152C, p.D155E, p.V339E, p.G361E, p.W209*, c.151_152delinsG, c.176delA, c.350_355delinsA, c.564delC, and c638delA) were novel. All seven novel missense mutations were not detected in SNP analysis and their amino acid substitutions were predicted to damage GLA activities by PolyPhen-2 test [http://www.ncbi.nlm.nih.gov/snp/, http://www.genome.med.kyoto-u.ac.jp/SnpDB/, http://genetics.bwh.harvard.edu/pph2/index.shtml].

Of the 88 male patients, 11 (12.5%) were classified as later-onset phenotype. Of the 73 detected mutations, eight were identified in later-onset male patients: four missense (p.A37V, p.Q279E, p.M296I, and p.G373S), one nonsense (p.Y173*), one in frame deletion (p.V254del), one splice site (IVS6 + 1 G > T), and one intronic mutations (IVS4 + 919 G > A). However, two mutations (p.V254del and IVS6 + 1 G > T) were detected in both classical and later-onset male patients with ESRD. The later-onset male patients with p.V254del or IVS + 1 G > T were diagnosed by the screening for Fabry disease among hemodialysis patients and lacked classical manifestations in childhood [11].

Of the 115 study families, 33 (28.7%) had amenable mutations to migalastat (Fig. 1). All nonsense, frameshift, splice site, and intronic mutations were non-amenable or unknown amenability.

Discussion

More than 900 disease-causing mutations on the GLA gene have been reported [12]. We could not identify the pathogenic mutations in six families (5.2%) and there are limitations in the conventional direct sequence targeting the exons and intron/exon boundaries. Sakuraba et al. reported the rate of undetectable mutation was 3.4% and their results were similar to this study [13].

The functional polymorphism (p.E66Q) was common in Japan and Korea [6, 7]. The common intronic mutation in Taiwan (IVS4 + 919 G > A) was identified in Japanese Fabry patients including this study [13, 14]. Because IVS4 + 919 G > A mutation was not identified in Korea [15], it is possible that Japanese origin was independently related with Korea and Taiwan.

The mutations associated with later-onset phenotype are less damaging to GLA activities, because the residual GLA activities of later-onset male patients are higher than classical male patients [16]. Therefore, most of the mutations linked to later-onset phenotype were missense mutations [12]. In this study, three later-onset male patients had severe phenotype mutations such as nonsense, in frame deletion and splice site mutations
(p.Y173*, p.V254del, IVS6 + 1 G > T) and they developed ESRD. Furthermore, two mutations (p.V254del and IVS6 + 1 G > T) were detected in both classical and later-onset male patients with ESRD. The functional polymorphism (p.E66Q) was not detected in the patients with p.V254del or IVS6 + 1 G > T. Therefore this polymorphism did not to affect their different phenotypes. It is difficult to differentiate between classical and later-onset phenotype with ESRD from gene mutations because of overlapping their mutations. The mechanism of the phenotypic variability in the patients with same mutations is not understood. Several hypotheses have been suggested such as actions of environmental factors or the modifier genes that affect the severity of Fabry disease [17].

Most of the amenable mutations are missense, because the structure of enzyme produced by this type of mutation are less damaged and might allow binding to the pharmacological chaperone [5]. In this study, 22 of 41 missense mutations (53.7%) and one of four in frame deletion mutations were amenable to migalastat. Of the 115 examined families, 33 (28.7%) had amenable mutations. These results are informative when considering genetic counseling and pharmacological chaperon therapy for Fabry disease.

Table 1 The mutations detected in this study

	Protein change	Nucleotid change	Exon	Phenotype	Number of families	Number of patients		
						Male	Female	Amenability
Misssense								
1	p.M1I	c.3 G > A	Ex 1	C	1	1	0	N
2	p.L16H	c.47 T > A	Ex 1	C	1	2	0	N
3	p.D33Y	c.97 G > T	Ex 1	C	1	1	1	Ame
4	p.A37V	c.110 C > T	Ex 1	L	1	1	0	Ame
5	p.P40S	c.198 C > T	Ex 1	C	1	1	1	N
6	p.M42I	c.126 G > C	Ex 1	Fe	1	0	1	Ame
7	p.W44C	c.132 G > T	Ex 1	C	2	1	1	N
8	p.H46L	c.137 A > T	Ex 1	C	1	1	1	N
9	p.R49H	c.146 G > A	Ex 1	Fe	1	0	1	Unknown
10	p.M76T	c.227 T > C	Ex 2	Fe	1	0	1	Ame
11	p.D93V	c.278 A > T	Ex 2	Fe	1	0	1	N
12	p.M96I	c.288 G > A	Ex 2	C	1	1	0	Ame
13	p.R112C	c.334 C > T	Ex 2	C	7	5	4	N
14	p.R112H	c.335 G > A	Ex 2	Fe	1	0	1	Ame
15	p.F113S	c.338 T > C	Ex 2	C	1	1	0	N
16	p.G147E	c.440 G > A	Ex 3	C	1	1	1	N
17	p.S148N	c.443 G > A	Ex 3	C	1	1	0	N
18	p.Y152C	c.455 A > G	Ex 3	Fe	1	0	2	Ame
19	p.D155E	c.465 T > A	Ex 3	C	1	2	1	Ame
20	p.C202Y	c.605 G > A	Ex 4	C	1	1	1	N
21	p.N215S	c.644 A > G	Ex 5	C	1	1	1	Ame
22	p.C223S	c.668 G > C	Ex 5	Fe	1	0	1	N
23	p.I239T	c.716 T > C	Ex 5	Fe	1	0	1	Ame
24	p.Q250P	c.749 A > C	Ex 5	C	2	2	1	Ame
25	p.Q279E	c.835 C > G	Ex 6	L	2	1	7	Ame
26	p.G280H	c.840 A > C	Ex 6	Fe	1	0	1	Ame
27	p.M296I	c.888 G > A	Ex 6	L	5	3	3	Ame
28	p.L300P	c.899 T > C	Ex 6	Fe	1	0	2	Ame
29	p.R301Q	c.902 G > A	Ex 6	C	1	1	0	Ame
30	p.R301P	c.902 G > C	Ex 6	C	1	1	0	Ame
31	p.N320K	c.960 T > G	Ex 6	Fe	1	0	1	N
32	p.G328R	c.982 C > G	Ex 6	C	2	3	2	N
33	p.V339E	c.1016 T > A	Ex 7	C	1	1	0	Ame
34	p.W340S	c.1019 G > C	Ex 7	C	1	1	0	N
35	p.R342Q	c.1025 G > A	Ex 7	C	4	5	2	N
36	p.E358K	c.1072 G > A	Ex 7	Fe	1	0	1	N
37	p.I359T	c.1076 T > C	Ex 7	Fe	1	0	1	Ame
38	p.G361E	c.1082 G > A	Ex 7	C	1	1	0	Ame
39	p.G373S	c.1117 G > A	Ex 7	L	2	1	1	Ame
40	p.C382Y	c.1145 G > A	Ex 7	C	1	1	0	N
41	p.L403S	c.1208 T > C	Ex 7	C	2	1	3	Ame
Nonsense								
42	p.W24*	c.71 G > A	Ex 1	Fe	1	0	1	N
43	p.Y173*	c.519 C > A	Ex 3	L	1	1	0	N

Table 1 (continued)

	Protein change	Nucleotid change	Exon	Phenotype	Number of families	Number of patients		
						Male	Female	Amenability
44	p.E203*	c.607 G > T	Ex 4	Fe	1	0	1	N
45	p.W209*	c.627 G > A	Ex 4	C	1	1	1	Unknown
46	p.R220*	c.658 C > T	Ex 5	C	4	5	4	N
47	p.R227*	c.679 C > T	Ex 5	C	1	1	2	N
48	p.R301*	c.901 C > T	Ex 6	C	3	2	3	N
49	p.R306*	c.916 C > T	Ex 6	Fe	2	0	3	N
50	p.Q321*	c.961 C > T	Ex 6	Fe	1	0	1	N
51	p.R342*	c.1024 C > T	Ex 7	C	1	1	0	N
52	p.Y365*	c.1095 T > G	Ex 7	C	1	1	0	N
Deletion in frame								
53	p.D234del	c.700-702 delGAT	Ex 5	C	1	1	0	Unknown
54	p.V254del	c.760-762 delGTT	Ex 5	C, L	3	3	0	Ame
55	p.D255del	c.763-765 delGAT	Ex 5	C	1	1	0	Unknown
56	p.E358del	c.1072-1074 delGAG	Ex 7	C	2	1	1	Unknown
Frameshift mutation								
57		c.151_152delinsG	Ex 1	C	1	1	0	Unknown
58		c.176 delA	Ex 1	C	1	1	1	Unknown
59		c.350_355delinsA	Ex 2	C	1	1	3	Unknown
60		c.564 delC	Ex 4	C	1	1	1	Unknown
61		c.638 delA	Ex 4	Fe	1	0	1	Unknown
62		c.718_719 delAA	Ex 5	C	4	3	4	N
63		c.803_806 delTAGT	Ex 6	C	2	1	3	Unknown
64		c.1033_1034 delTC	Ex 7	C	2	1	1	N
65		c.1235_1236 delCT	Ex 7	C	1	1	0	N
66		c.1277_1278 delAA	Ex 7	C	1	1	4	N
Splice site								
67		IVS1-1G > T		C	1	1	1	Unknown
68		IVS2 + 1 G > A		Fe	1	0	3	Unknown
69		IVS3-1G > A		C	2	2	0	Unknown
70		IVS3 + 1 G > A		C	1	1	0	Unknown
71		IVS5-2A > G		C	1	1	0	Unknown
72		IVS6 + 1 G > T		C, L	2	3	1	Unknown
Intronic mutation								
73		IVS4 + 919 G > A			2	2	2	Unknown
	not detected				6	6	0	Unknown
			Total		115	88	88	

C and L indicate the mutations linked to classical form and later-onset phenotype male patients, respectively. Fe indicates the mutations detected in female patients only. Ame and N denote amenable and non-amenable mutations, respectively

Compliance with ethical standards

Conflict of interest TO has active research support from Sanofi Genzyme Corporation, Dainippon Sumitomo Pharma and AVROBIO Inc. H.I has active research support from Sanofi Genzyme Corporation and Dainippon Sumitomo Pharma. The remaining authors declare that they have no conflict of interest.

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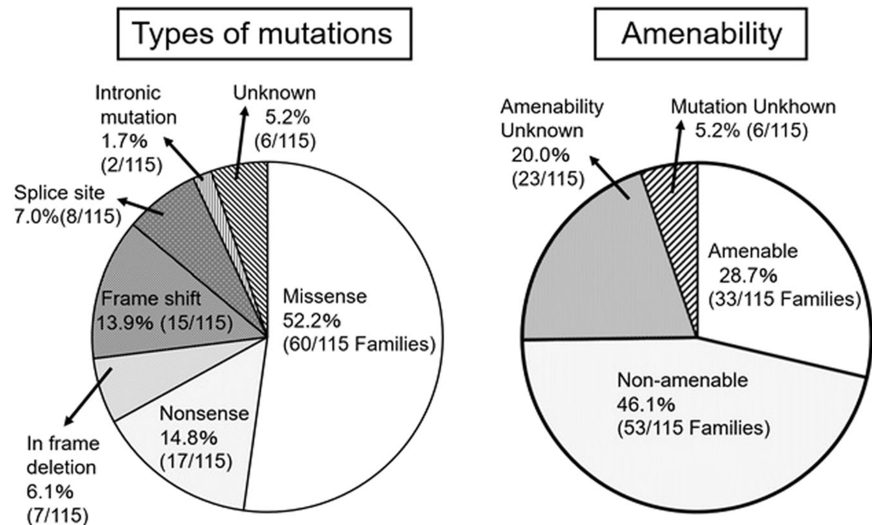

Fig. 1 The mutation types detected in this study and rate of amenability to migalastat

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