Molecular Genetics and Metabolism Reports 14 (2018) 3–9

Contents lists available at ScienceDirect

Molecular Genetics and Metabolism Reports

journal homepage: www.elsevier.com/locate/ymgmr

A molecular analysis of the GAA gene and clinical spectrum in 38 patients with Pompe disease in Japan

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ARTICLE INFO

Keywords:
Lysosomal disease
Pompe disease
Acid α-glucosidase
Genotype-phenotype correlation

ABSTRACT

Pompe disease is an autosomal recessive disorder caused by acid α-glucosidase (GAA) deficiency, which results in the accumulation of glycogen in lysosomes in multiple tissues, including cardiac, skeletal, and smooth muscle cells. Thus far, 558 sequence variants of the GAA gene have been published in the Pompe Disease Mutation Database, and some mutations appear with considerable frequency in particular ethnic groups, such as Caucasians, Taiwanese, Chinese, and Koreans. However, the GAA mutation pattern in Japanese patients remains poorly understood. We analyzed the relationship between the genetic and clinical features of 38 mostly Japanese patients with Pompe disease from 35 unrelated families. We identified 28 different GAA gene mutations, including 7 novel mutations, by a GAA gene analysis. c.546G > T (22.9%) and c.1857C > G (14.3%) were the most common mutations and accounted for 37.1% of the total mutant alleles. In the six patients with infantile-onset Pompe disease (IOPD), c.1857C > G was also the most common mutation. In addition, there were 13 homozygotes (5 with the c.546G > T) among the 35 families, which is the highest frequency reported thus far. Regarding the initial symptoms, cardiomegaly was the most common (3/6 = 50%) in IOPD patients, while muscle weakness was observed the most frequently in patients with late-onset Pompe disease (LOPD) (15/30 = 50%). Notably, all IOPD patients who showed respiratory distress at the time of onset require respiratory assistance at present (4/4 = 100%). Regarding the presenting symptoms, cardiomegaly (6/6 = 100%) and hepatomegaly (4/6 = 66.7%) were more commonly seen in IOPD, and muscle weakness (24/29 = 82.7%) was observed more frequently in LOPD. Respiratory assistance is required at present in 33.3% of IOPD patients and 50% of LOPD patients, and 20% of IOPD patients and 29.6% of LOPD patients are wheelchair users. These individual clinical courses may be influenced by the timing of the diagnosis and treatment; for example, in 2007, an ERT orphan drug for treatment of Pompe disease, Alglucosidase alfa, was made available in Japan, and there were 5 (5/6 = 83.3%) wheelchair users diagnosed from 2008 to 2009 (cases 32–38) and 4 (4/27 = 14.8%) from 2010 to 2015 (cases 1–31). These findings underscore the importance of the early diagnosis and treatment.

1. Introduction

Pompe disease, also known as glycogen storage disease type II (OMIM232300), is an autosomal recessive lysosomal disorder caused by deficiency of acid α-glucosidase (GAA; OMIM 606800), leading to the progressive accumulation of glycogen in lysosomes in skeletal, cardiac, and smooth muscles [1,2]. Pompe disease is classified into infantile-onset Pompe disease (IOPD) and late-onset Pompe disease (LOPD) based on the age of onset, organ involvement, and rate of progression [3].

IOPD is diagnosed in individuals with an onset before the age of 12 months who have cardiomegaly. IOPD may be apparent in utero, but more typically, the onset is at a median age of four months with hypotonia, generalized muscle weakness, feeding difficulties, failure to thrive, respiratory distress, and hypertrophic cardiomegaly. Without appropriate therapy, such as enzyme replacement therapy (ERT), such patients may die by two years of age due to cardiorespiratory failure.

LOPD includes (a) individuals with an onset before the age of

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http://dx.doi.org/10.1016/j.ymgmr.2017.10.009
Received 26 September 2017; Accepted 25 October 2017
Available online 31 October 2017
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Table 1
Clinical, enzymatic, and molecular information of 38 patients with Pompe disease.

Case	cDNA mutation	Gender	Present age (years)	GAA activity (%)	Age of onset	Initial symptoms	Cardiomegaly	Muscle weakness	Respiratory distress	Hepatomegaly	Respiratory assist	Wheel chair	Type
1	R608X Homozygote	F	7	2.5[a]	4 m	Hypotonia	+	+	+	+	−	NA	Infant
2	S566P, K162Q	F	10	4.6	4 m	Respiratory distress	+	−	+	+	+	−	Infant
3	S566P, K162Q	F	6	2.1	0 m	Hepatomegaly	+	+	−	+	−	−	Infant
4	S619R Homozygote	M	5	3.5	1y	Cardiomegaly	+	−	−	−	−	−	Infant
5	R600C, S619R	F	4	2.4	10 m	Cardiomegaly	+	+	−	+	−	−	Infant
6	R600C, S619R	M	5	6.8	1 m	Cardiomegaly	+	+	+	−	+	+	Infant
7	R437C, P726R	F	NA	1	1y	Hepatomegaly	−	−	−	−	−	−	Late
8	R437C, R600C	F	NA	3.2[a]	8y	Muscle weakness	−	+	−	−	−	−	Late
9	R600C, c.2481 + 1G > A	M	NA	NA	1y <	NA	NA	NA	NA	NA	NA	NA	Late
10	S619R Homozygote	M	39	9.3	17y	Respiratory failure	+	+	+	+	+	+	Late
11	S619R Homozygote	M	10	16.9	4y	Muscle weakness	−	+	−	−	−	−	Late
12	R190H, c.756insT	M	19	9.9	13y	Muscle weakness	−	+	−	−	−	−	Late
13	V723M, c.547-1G > C	M	NA	4.9	1y	Respiratory distress	−	+	+	−	+	+	Late
14	c.546G > T Homozygote	M	59	6.9[a]	40y	Muscle weakness	−	+	+	−	+	−	Late
15	S251L + S254 L Homozygote	M	21	4.7[a]	2y	Post encephalopathy	−	−	−	−	−	−	Late
16	c.546G > T, R660C	F	36	1[a]	28y	Muscle weakness	−	+	−	−	−	−	Late
17	c.546G > T, W367G	M	75	4.8	72y	Muscle weakness	−	+	+	−	+	−	Late
18	c.546G > T Homozygote	F	48	8.1	42y	Muscle weakness	−	−	−	−	−	−	Late
19	R437C, Y766C	M	21	9.3	14y	Muscle weakness	−	+	+	−	+	−	Late
20	M439K Homozygote	M	22	11.6	12y	Respiratory distress	−	+	+	−	+	−	Late
21	c.546G > T, P266S	M	35	NA	1y <	Muscle weakness	NA	+	NA	+	NA	NA	Late
22	c.546G > T, P266S	M	30	1.1	23y	Muscle weakness	−	+	+	−	−	+	Late
23	c.546G > T Homozygote	M	68	9.8	2.5y	Muscle weakness	NA	+	+	−	+	NA	Late
24	D860N Homozygote	F	17	4.6	15y	Muscle weakness	−	+	+	−	+	−	Late
25	c.546G > T, Q827H	M	15	5.6	2y	Easy to fall	−	−	−	−	−	−	Late
26	D860N Homozygote	M	14	5.1	12y	Carrier diagnosis	−	+	−	−	−	−	Late
27	D860N Homozygote	F	9	5.5	7y	Carrier diagnosis	−	−	−	−	−	−	Late
28	c.546G > T Homozygote	M	NA	6.7	70y	Muscle weakness	−	+	+	−	+	−	Late
29	c.546G > T Homozygote	F	NA	4	30y	Gait problem	−	+	−	−	−	−	Late
30	R40X c.546G > T	F	NA	3	1y	Development delay	−	+	−	−	−	−	Late
31	R600C, R437C	NA	NA	NA	1y <	NA	NA	NA	NA	−	NA	NA	Late
32	S619R, R437C	F	NA	NA	19y	Liver function abnormality	+	+	+	+	+	+	Late
33	Q57X, G219R	F	24	9.6	3y	Muscle weakness	−	+	−	−	−	−	Late
34	M1T, Y609X	M	13	NA	16 m	Easy to fall	+	NA	NA	−	NA	NA	Late
35	R608X,unknown	F	NA	0	11y	Fatigue	−	+	+	−	+	+	Late
36	R437C, R600C	M	NA	5.7	3y	serum CK	−	+	+	−	+	+	Late
37	N314K Homozygote	M	25	9	8y	Muscle weakness	−	+	+	−	+	+	Late
38	E579K, S619R	M	20	2.7	2y	Easy to fall	−	+	+	−	+	+	Late

Case 4 and 5, and case 24, 26, and 27 are siblings, respectively. Case 27 was diagnosed pre-symptomatically.
[a] GAA activity in blood from a dried blood spot.

12 months without cardiomegaly, and (b) all individuals with an onset after the age of 12 months. Patients with LOPD are characterized by proximal muscle weakness and respiratory insufficiency [3].

ERT with recombinant human GAA has been used for patients with Pompe disease and has demonstrated clinical efficacy [4–6]. Indeed, an early diagnosis and the early initiation of ERT are expected to bring about a better clinical outcome, so newborn screening (NBS) using dried blood spots (DBSs) has recently been attempted, with subsequent outcomes improving [5–14].

The *GAA* gene is located on chromosome 17q25.3 and spans approximately 20 kb and contains 20 exons. The first exon is noncoding [15]. The Pompe Disease Mutation Database lists all GAA variants and describes their effects, and the May 2016 edition (https://www.erasmusmc.nl) shows a list of 558 sequence variants in the *GAA* gene. Some mutations are frequently found in certain ethnic groups. For example, c.-32-13T > G is the most common mutation in Caucasian individuals, with a frequency as high as 34%–47% [16–21]. c.1935C > A (p.D645E) and c. 2238G > C (p.W746C) are common in Taiwanese individuals, [22] c. 2238G > C (p.W746C) is the most common mutation in mainland Chinese late-onset individuals, [23] and c. 1316T > A (p. M439K) and c.1857C > G (p. S619R) are the most common mutations in Korean individuals, accounting for 36.6% of the total mutant alleles [24].

In Japan and Taiwan, the pseudodeficiency allele c.1726G > A(p.G576S) is seen more frequently than in other ethnic groups [25,26]. The substitution c.1726G > A may reduce the *GAA* activity to the level of patients with Pompe disease. Although it will never cause any symptoms, it sometimes invites confusion when evaluating the findings of NBS [27].

We previously reported the *GAA* activity and frequency of the c.1726G > A allele in 530 DBSs from 400 healthy Japanese newborns, 96 healthy adults, 29 patients with Pompe disease, and 5 obligate carriers. However, the GAA mutation pattern in Japanese patients remains poorly understood.

In the present study, we analyzed the clinical features, GAA activity, and genotypes of 38 mostly Japanese patients with Pompe disease, making this the largest report from Japan. Direct sequencing of the complete GAA open reading frame revealed seven novel mutations, including four novel missense mutations, related to Pompe disease etiology.

2. Materials and methods

2.1. Patients and samples

Thirty-eight patients with Pompe disease were analyzed from 2008 to 2016 at our institution. They were diagnosed by confirming the residual GAA enzyme activity in the leucocytes among 148 individuals suspected of having Pompe disease based on their clinical features, such as cardiomegaly, or were screen-positive participants in our center's NBS [27].

Informed consent from the patients was obtained, as approved by the Institutional Review Board of the National Center for Child Health and Development. All of the samples were prepared and analyzed in accordance with the protocols approved by the ethics committee of the National Center for Child Health and Development.

2.2. Mutation analyses

A GAA mutation analysis was performed in the 38 patients with Pompe disease. Genomic DNA was extracted from peripheral blood leukocytes using the QIAamp DNA Blood Midi Kit (Qiagen, Hilden, Germany). All exons and intron/exon boundaries of the GAA gene were amplified and assessed by polymerase chain reaction (PCR) and direct sequencing (primer pairs' sequences are available upon request). The obtained sequences were compared with the reference sequences NM_000152.4 to identify pathogenic mutations. All nucleotide differences between the patients and the reference sequence were compared to the Erasmus MC University Medical Center Rotterdam and to the dbSNP database of the National Center of Biological Information. Variants that were described as polymorphisms or assured disease-causing mutations in the GAA mutation database were not further assessed.

The pathogenic nature of novel missense mutations was verified by direct sequencing of 300 alleles of unaffected individuals. To predict the effect of newly identified missense mutations, we used the Mutation taster (http://www.mutationtaster.org/) and Polyphen2 software programs (http://genetics.bwh.harvard.edu/pph2/index.shtml). Mutation taster was also used to assess conservation during the evolution of residues at mutated sites (Fig. 2).

3. Results

3.1. Disease manifestations in IOPD and LOPD

The clinical manifestations, laboratory data, and genotypes of the 38 patients from 35 unrelated families in our center are summarized in Table 1. The population was 59.5% (22/37) male and 40.5% (15/37) female. There were 35 patients from Japan and 3 patients from Pakistan. Six patients (6/38, 15.8%) had IOPD, and 32 (32/38, 84.2%) had LOPD.

The median age of onset of IOPD patients was 4 months (range: 0–12 months). The first symptom was most commonly cardiomegaly (3/6, 50%), followed by hypotonia of the muscles (1/6, 16.7%), respiratory distress (1/6, 16.7%), and hepatomegaly (1/6, 16.7%). The presenting symptoms were cardiomegaly (6/6, 100%), muscle weakness (4/6, 66.7%), respiratory distress (3/6, 50%), and hepatomegaly (4/6, 66.7%). A wheelchair and respiratory assistance were required for 1 and 2 patients, respectively (Table 2).

The median age of onset of LOPD patients was 11 years (range: 1–72 years). The first symptom was most commonly muscle weakness (15/30, 50%), followed by respiratory distress or fatigue (3/30, 10%); 2 patients were discovered based on their familial history. The presenting symptoms were cardiomegaly (3/28, 10.7%), muscle weakness (24/29, 82.8%), respiratory distress (15/28, 53.6%), and hepatomegaly (3/31, 9.7%). A wheelchair and respiratory assistance were required for 8 of 27 (29.6%) and 14 of 28 (50%) patients, respectively.

3.2. GAA activity assay

A total of 38 patients with Pompe disease, 40 carriers, and 38 people with pseudodeficiency were included among the 148 people who were registered with our study, and no mutations were detected in the other 32 people. The average GAA activity in the carriers and those with pseudodeficiency was 14.5% (n = 14, range: 4.4%–63.2%), and 16.2% (n = 28, range: 6.4%–28%), respectively. In 26 of the 38 Pompe disease patients, the GAA activity was analyzed in the isolated lymphocytes of a fresh blood sample. The median GAA activity of 5 patients with IOPD was 2.4% (n = 5, range: 0.0%–4.6%), and that of 21 patients with LOPD was 5.5% (n = 21, range: 0.0%–16.9%) (Fig. 1).

Table 2
The correlation between the disease course and the presenting symptoms.

Presenting symptoms	IOPD	LOPD
Cardiomegaly	6/6 (100%)	3/28 (10.7%)
Muscle weakness	4/6 (66.7%)	24/29 (82.8%)
Respiratory distress	3/6 (50%)	15/28 (53.6%)
Hepatomegaly	4/6 (66.7%)	3/31 (9.7%)
Respiratory assistance	2/6 (33.3%)	14/28 (50%)
Wheelchair	1/5 (20%)	8/27 (29.6%)

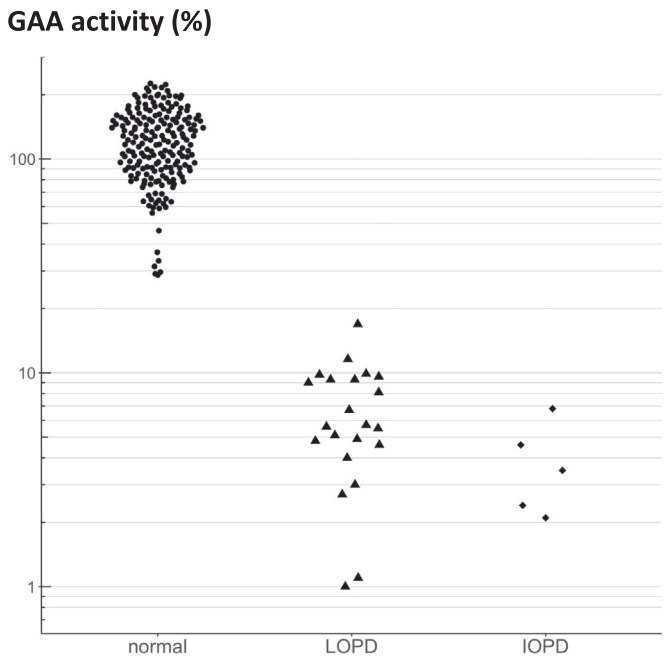

Fig. 1. The correlation between the disease course and the GAA activity in lymphocytes (%). The GAA activity in lymphocytes (%) in normal controls (n = 200) and patients with IOPD (n = 5) and LOPD (n = 23) is shown. The normal control value is 30.7 ± 10.3 nmol/mg protein/h, and the GAA activity (%) is expressed as a ratio of 30.7.

3.3. GAA mutations

The 38 patients with Pompe disease in our report consisted of 35 Japanese and 3 Pakistani patients. Cases 2 and 3 were siblings, as were cases 24, 26, and 27, resulting in 35 unrelated families in total. None of the patients had consanguineous parents. Among the 35 families, 21 were compound heterozygotes, and 13 were homozygotes. In one family (case 35), one of two mutations could not be identified.

The 28 total mutations detected in our patients with Pompe disease are shown in Table 3. The 76 alleles from our 38 patients consisted of 48 missense mutations (48/76, 63.2%), 6 nonsense mutations (6/76, 7.9%), 3 deletions or insertions (3/76, 3.9%), 18 splice cite errors (18/76, 23.7%), and 1 unknown (1/76, 1.3%). The most common mutation was c.546G > T, and 16 alleles were detected in our patients. Five patients were homozygotes (10 alleles), and 6 were compound heterozygotes (6 alleles); the allele frequency of the mutation was 16/70 (22.9%). The next most-common mutation was c.1857C > G (p.S619R), and 10 alleles were detected in our patients. Three patients were homozygotes (6 alleles), and 4 were compound heterozygotes (4 alleles); the allele frequency of the mutation was 10/70 (14.3%). c.1857C > G (p.S619R) was also the most frequent mutation in the six patients with IOPD, followed by c.1822C > T (R608X) and c.1798C > T (R600C).

Of the 28 different mutations, 21 have been already reported as pathogenic (http://www.pompecenter.nl), while 7 have not yet been registered. These 7 mutations were not encountered among 300 alleles of unaffected persons. Four novel missense mutations were included among these seven novel mutations. All four were highly conserved across the examined species (Fig. 2), suggesting that these are probably pathogenic. Indeed, the Polyphen-2 bioinformatics tool predicted that all four missense mutations were probably damaging. Similarly, Mutation taster indicated that all of these mutations were disease-causing.

Of the 38 patients with Pompe disease, 4 (10.5%) had c.1726A/A (A/A). As we reported previously, this was significantly higher than in the control population (3.3%–3.9%) [27]. However, there were no marked differences in any clinical or enzymatic features between the four Pompe disease patients with A/A and the others.

Table 3
Types and frequency of GAA mutations in patients with Pompe disease.

Nucleotide change	Structural effect	Location	Predicted severity	Incidence
Missense mutation				
c.2T > C	p.M1T	Exon2	Potentially less severe	1
c.569G > A	p.R190H	Exon3	Less severe	1
c.655G > A	p.G219R	Exon3	Potentially less severe	1
c.[752C > T, 761C > T]	p.[S251L, S254L]	Exon4	Unknown	2
c.796C > T	p.P266S	Exon4	Potentially mild	2
c.942C > A[a]	p.N314K	Exon5	[b]	2
c.1099T > G[a]	p.W367G	Exon7	[b]	1
c.1309C > T	p.R437C	Exon8	Less severe	6
c.1316T > A	p.M439K	Exon8	Potentially mild	2
c.1696T > C	p.S566P	Exon12	Potentially less severe	2
c.1735G > A	p.E579K	Exon12	Potentially less severe	1
c.1798C > T	p.R600C	Exon13	Less severe	6
c.1857C > G	p.S619R	Exon13	Less severe	10
c.1978C > T	p.R660C	Exon14	Potentially less severe	1
c.2167G > A	p.V723M	exon15	unknown	1
c.2177C > G	p. P726R	Exon15	Unknown	1
c.2297A > G	p.Y766C	Exon16	Potentially less severe	1
c.2481G > A[a]	p.Q827H	Exon17	[b]	1
c.2578G > A[a]	p.D860N	Exon18	[b]	6
nonsense mutation				
c.118C > T	p.R40X	Exon2	Very severe	1
c.169C > T[a]	p.Q57X	Exon2	[b]	1
c.1822C > T	p.R608X	Exon13	Very severe	3
c.1826dup	p.Y609X	Exon13	Very severe	1
Deletion or duplication				
c.483dup	p.K162QfsX15	Exon2	Very severe	2
c.756insT[a]	–	Exon4	[b]	1
Splicing variant				
c.546G > T	p.T182T	Exon2	Potentially mild	16
c.547-1G > C[a]	–	Intron2	[b]	1
c.2481 + 1G > A	–	Intron17	Very severe	1

[a] Novel mutation.
[b] Not described.

4. Discussion

The relationship between the genotypes and phenotypes in Pompe disease has been studied in many different ethnic groups including Caucasians, Taiwanese, Chinese, and Columbians [16–24,27–32]. This is the largest report from Japan, consisting of 35 Japanese patients (34 unrelated families) and 3 Pakistani patients (1 family) with Pompe disease.

4.1. Clinical features and courses

Regarding the initial symptom, cardiomegaly was the most common (3/6, 50%) in IOPD, while muscle weakness was observed the most frequently in LOPD (15/30, 50%). Two LOPD patients were diagnosed based on their family histories. Interestingly, all who displayed respiratory distress at the time of onset require respiratory assistance at present (4/4, 100%). Cases 4 and 5 are siblings, and both had IOPD. Case 4 showed respiratory distress initially and now requires a respiratory device; her sister's initial symptom was hepatomegaly, and she does not need such a device at all at present. This suggests that respiratory distress as an initial symptom and the need for respiratory

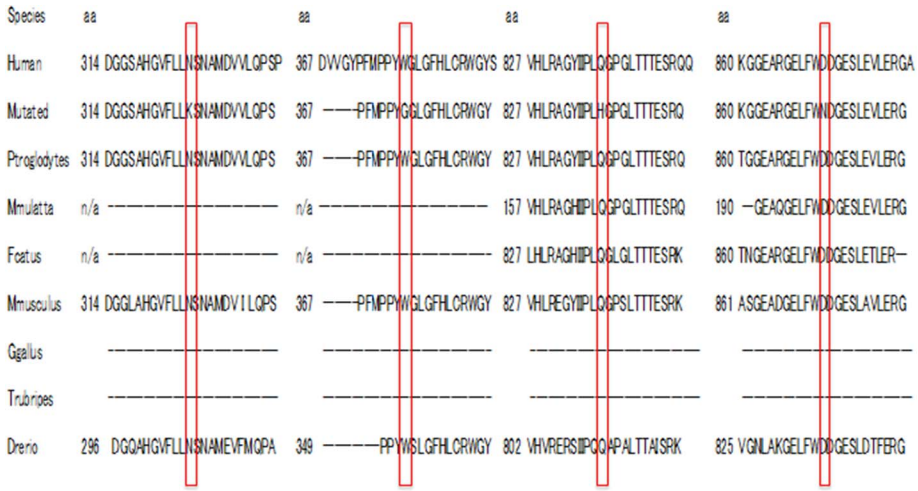

Fig. 2. The conservation of four novel missense mutations in different species.

assistance are not associated with the genotype.

On comparing presenting symptoms between IOPD and LOPD, cardiomegaly (100% vs. 10.7%) and hepatomegaly (66.7% vs. 9.7%) were more commonly seen in IOPD, and muscle weakness (66.7% vs. 82.8%) was observed more frequently in LOPD (Table 2). Respiratory assistance is required at present in 33.3% of IOPD patients and 50% of LOPD patients, and 20% of IOPD patients and 29.6% of LOPD patients are wheelchair users. These individual clinical courses may be influenced by the timing of the diagnosis and treatment; for example, in 2007, an ERT orphan drug for the treatment of Pompe disease, Alglucosidase alfa, was made available in Japan, and there were 5 (5/6 = 83.3%) wheelchair users diagnosed from 2008 to 2009 (cases 32–38) and 4 (4/27 = 14.8%) from 2010 to 2015 (cases 1–31). These findings underscore the importance of the early diagnosis and treatment.

4.2. GAA activity

The clinical findings and courses of Pompe disease basically depend on the residual GAA activity, as determined the genotype [17,33,34]. However, the genotype may not be reflected in the GAA activity alone but also in the clinical signs, particularly in patients with non-classic forms [35].

The GAA activities measured in leucocytes are shown in Fig. 1, and the average activity in patients with IOPD was markedly less than that in patients with LOPD. However, some patients with LOPD presented with extremely low activity, almost equal to that in IOPD patients. This made it difficult to distinguish IOPD from LOPD based on the GAA activity alone.

There were five kinds of mutations (seven patients) rated as very severe in our patients. Of these seven patients, only case 2 presented with homozygous ‘very severe’ mutations (R608X). Case 2 had IOPD, but the residual GAA activity was 2.5%. Regarding the other six patients, two had IOPD, and four had LOPD, with no correlation between the clinical courses and the residual GAA activities.

Two families had siblings with Pompe disease, so their clinical courses and GAA activities were comparable. Cases 2 and 3 were described the above. Cases 24, 26, and 27 were siblings from Pakistan, and they all had LOPD. Case 24 was 15 years old at the time of the diagnosis and presented with muscle weakness and respiratory distress, requiring respiratory assistance. Case 26 was 12 years old at the time of the diagnosis and only has muscle weakness at present. Case 27 has no symptoms at present.

The GAA activities were similar among members of each family (case 2: 2.9% and case 3: 2.4%; and case 24: 4.6%, case 26: 5.1%, and case 27: 5.5%). Variability in clinical findings, even between patients with the same mutation, has been reported [36]. Our data showed that the clinical course (IOPD or LOPD) and the residual GAA activity did not markedly differ within a given family.

4.3. GAA mutations

GAA mutations are reported to cluster in three critical regions: exon2, which includes a start codon; exon10 and 11, which include an enzyme catalytic site; and exon14, which includes a highly conserved region of the protein [37]. As shown in Fig. 3, the mutations are basically distributed throughout the entire gene, like previously reported [16,18,38–40]. However, while six GAA mutations were detected in exon2, no mutations were detected in exon10 and 11, and only one mutation was observed in exon14.

We detected 13 homozygotes among our 35 families, including 5 with c. 546G > T, and 3 with S619R. The frequency of homozygotes is known to be not very high in Caucasian people, [6,7] and Asian people, including Koreans and Chinese, are also reported to have a low frequency of homozygotes [3,4]. As such, the rate of 37.1% (13/35) is the highest frequency of homozygotes reported so far. Given that not all of our patients were involved in a marriage between close relatives, the reason for the high frequency of homozygotes is unclear.

The most common mutation, c.546G > T, was identified in 11 patients (16 alleles), and the allele frequency of the mutation in our patients was 16/70 (22.9%). The c.546G > T mutation is classified as potentially mild, and all 11 patients had LOPD. c.1857C > G (p.S619R), the second-most common mutation, was found in 7 patients (10 alleles). Three patients were homozygotes (6 alleles), and 4 were compound heterozygotes (4 alleles); the allele frequency of the mutation was 10/70 (14.3%). Of the seven patients with c.1857C > G(p.S619R), three had IOPD (one homozygote and two compound heterozygotes), and four had LOPD (two homozygotes and two compound heterozygotes).

Looking at only IOPD, c.1857C > G (p.S619R) was observed the most frequently, followed by c.1822C > T (R608X) and c.1798C > T (R600C). c.1857C > G and c.1316T > A were also the most common mutations, accounting for 36.6% of the total mutant alleles in Koreans [24]. c.-32-13T > G, which is the most common mutation among Caucasians, at a frequency of 34%–79%, was not observed in any of our patients [16–20,41]. c.2238G > C and c.1935C > A have a high frequency in Taiwan and along the coast of China. These mutations were also not detected in our patients [23]. c.1726G > A and c.2065G > A sequence variations are often seen on the same allele, and 3.3%–3.9% of Asian populations are homozygous for these variants. [42,43] c.1726G > A in particular reduces the amount of GAA and the catalytic capacity, prompting confusion when evaluating the findings of NBS in

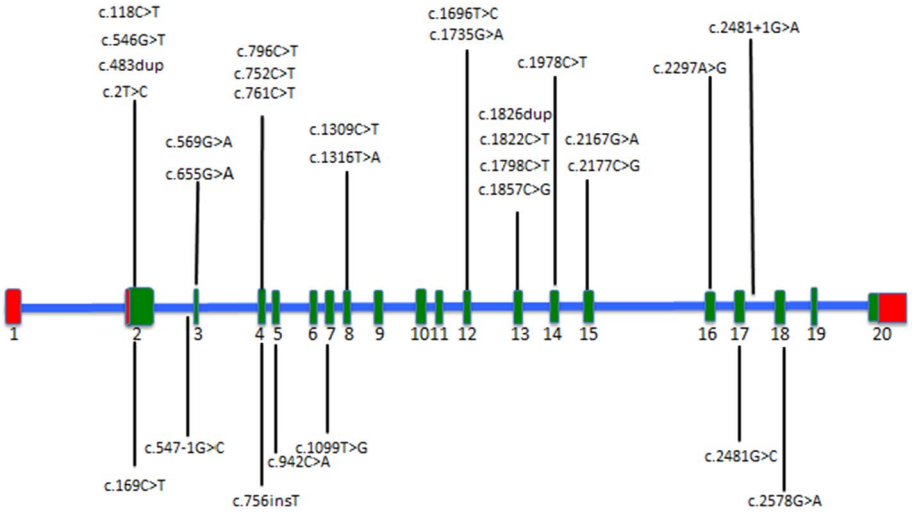

Fig. 3. Mutation spectrum in 38 patients with Pompe disease. Previously described mutations are shown above, and new mutations are shown below the diagrammed GAA gene.

Japan. [42,44,45].

Of the 38 patients with Pompe disease, 4 (10.5%) had c.1726A/A (A/A). This is similar to our previous report (10%) [27]. The A/A allele was again found to occur at a significantly higher frequency in the patient population than in the control population (3.3%–3.9%). However, there were no marked differences in any clinical or enzymatic features between the four Pompe disease patients with A/A and the others.

In our patients, there were seven novel mutations. These 7 mutations were not encountered among the 300 alleles of unaffected persons. Four novel missense mutations were included among these seven novel mutations, and all four were highly conserved across the examined species. The effect of these novel mutations should be assessed by functional studies.

5. Conclusion

In this study, we described the first genetic and clinical analyses of Japanese patients with Pompe disease. Twenty-eight different GAA gene mutations, including 7 novel mutations, were identified in 38 patients from 35 unrelated families. c.546G > T (22.9%) and c.1857C > G (14.3%) were the most common mutations and accounted for 37.1% of the total mutant alleles. Among IOPD patients, c.1857C > G (p.S619R) was the most common mutation. In addition, there were 13 homozygotes (5 with the c.546G > T) among 35 families, which is the highest frequency reported so far. The most common initial symptom was cardiomegaly for IOPD and muscle weakness for LOPD. Notably, all of the IOPD patients who displayed respiratory distress at the time of onset require respiratory assistance at present. Regarding the presenting symptoms, cardiomegaly and hepatomegaly were more commonly seen in IOPD, and muscle weakness was more frequently observed in LOPD. The requirement for respiratory assistance and a wheelchair was not associated with the clinical classification or the residual GAA activity. These results may be influenced by the timing of the diagnosis and treatment. Our findings underscore the importance of the early diagnosis and treatment.

Acknowledgements

This work was supported by the grants in aid from the Japan Agency for Medical Research and Development (17gk0110016h0002) to T.O. We would like to thank all of the patients, their families, and the many medical doctors, including Norio Sakai, Sachiko Nakaoka, Hiroki Takano, Rumi Sagawa, Maki Mitani, Homare Banba, Keiko Ishigaki, Misa Matsui, Yasuhiko Tanaka, Manami Akasaka, Ryoko Koike, Kazumasa Akiyama, Tomomi Tanaka, Junko Hanakawa, Kazushi Miya, Syuhei Arai, Ayami Okuzumi, Masafumi Sanefuji, Shiro Ozasa, and Mayumi Kawamura for their assistance.

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