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Anti-drug antibody formation in Japanese Fabry patients following enzyme replacement therapy

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ABSTRACT

Enzyme replacement therapy (ERT) for Fabry disease (deficiency of α-galactosidase A, α-Gal) with recombinant α-Gals (agalsidase alfa and agalsidase beta) is widely available and improves some of the clinical manifestations and biochemical findings. However, recent reports suggest that recurrent administration of recombinant enzymes often induces the formation of anti-drug antibodies, which may have a negative impact on the outcome of the therapy. We examined the formation of anti-drug antibodies using blood samples from 97 Japanese Fabry patients following ERT and tried to characterize them by means of enzyme-linked immunosorbent assay (ELISA), serum-mediated α-Gal inhibition, and immunochromatographic (IC) assay, followed by *GLA* gene analysis and measurement of plasma globotriaosylsphingosine (lyso-Gb3). ELISA revealed that 20/35 (57%) classic Fabry males were antibody (Immunoglobulin G, IgG) -positive (Ab+) at 6 months after the initiation of ERT, although only two of the seventeen (12%) later-onset Fabry males and none of the 45 Fabry females were. The Ab+ state was maintained at least until 24 months after the initiation of ERT in most of the cases, the exceptions being two patients who acquired immune tolerance during ERT. As many Ab+ patients have nonsense mutations, attention should be paid to the formation of anti-drug antibodies in Fabry patients harboring such gene mutations, who hardly produce α-Gal protein. Serum-mediated α-Gal inhibition was seen in most of the Ab+ patients and the antibodies affected the reduction of the plasma lyso-Gb3 level following ERT, suggesting that the antibodies inhibit the enzyme activity. There was a correlation between the results of the IC test and those of the ELISA. As the former is easy and rapid, it should be useful as a bed-side test.

1. Introduction

Fabry disease (OMIM 301500) is an inherited metabolic disorder caused by deficient activity of α-galactosidase A (α-Gal, EC 3.2.1.22), and characterized by systemic accumulation of α-galactosylconjugates including globotriaosylceramide (Gb3) and globotriaosylsphingosine (lyso-Gb3) [1]. Classically affected male patients with Fabry disease usually exhibit pain in the peripheral extremities, angiokeratoma, hypohidrosis, and corneal opacities in childhood or adolescence, and develop life-threatening renal, cardiac and cerebrovascular involvement in adulthood, although some later-onset type Fabry males, who usually have low residual α-Gal activity, develop clinical manifestations limited to the heart and/or a renal disorder in adulthood [1,2]. Heterozygous Fabry females exhibit a wide spectrum of clinical manifestations, in general milder, according to random X-chromosomal inactivation [3].

Enzyme replacement therapy (ERT) with recombinant human α-Gals produced in human fibroblasts (agalsidase alfa) and Chinese hamster ovary cells (agalsidase beta) has been introduced and successfully used [4–8]. ERT improves some of the clinical manifestations and biochemical findings in Fabry patients or prevents their deterioration [4–8]. However, recent reports demonstrated that recurrent infusions of recombinant α-Gals often induce the formation of anti-drug antibodies, especially in Fabry males, and such antibodies may lead to various allergic reactions and a negative impact on the outcome of the

Abbreviations: ERT, enzyme replacement therapy; α-Gal, α-galactosidase A; ELISA, enzyme-linked immunosorbent assay; IC, immunochromatographic; Gb3, globotriaosylceramide; lyso-Gb3, globotriaosylsphingosine; PBS, phosphate-buffered saline; AP, alkaline phosphatase; LC-MS/MS, liquid chromatography-tandem mass spectrometry

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therapy [9–15]. In the previous study [16], we measured the plasma lyso-Gb3 levels in Fabry patients under ERT with agalsidase alfa, and the results suggested that antibodies against the enzyme had a negative effect on the reduction of plasma lyso-Gb3, a biomarker for monitoring ERT [17–19].

As there have been few reports describing the formation of anti-drug antibodies in Japanese Fabry patients who received ERT, in this study we tried to characterize them by means of enzyme-linked immunosorbent assay (ELISA), serum-mediated α-Gal inhibition, and immunochromatographic (IC) assay, followed by *GLA* gene analysis and examination of the effect of antibodies on the plasma lyso-Gb3 concentration.

2. Materials and methods

2.1. Study subjects and samples

Blood samples were obtained from 97 Japanese Fabry patients (classic males 35, later-onset males 17, and females 45; the phenotypes of the patients were determined according to the diagnoses of clinicians who examined them) who had been treated with recombinant α-Gals for at least six months, including 59 cases previously reported [16]. Of the 35 classic Fabry males, twenty-seven were treated with agalsidase alfa (0.2 mg/kg/2 weeks), seven with agalsidase beta (1 mg/kg/2 weeks), and one with both (with agalsidase beta for the first 3 months and then with agalsidase alfa thereafter); and of the 17 later-onset Fabry males, 13 were treated with agalsidase alfa and one with agalsidase beta. As to the treatment for the other three, detailed information could not be obtained from the clinicians and of the 45 Fabry females, 33 were treated with agalsidase alfa and 8 with agalsidase beta. Unfortunately, detailed information of the treatment for the other four was not available.

This study involving human samples was approved by the Ethics Committee of Meiji Pharmaceutical University, and was performed according to the ethical guidelines of the 1975 Declaration of Helsinki. Informed consent for the analyses involving anti-drug antibody formation and measurement of lyso-Gb3 was obtained from all the participants. That for *GLA* gene analysis was obtained from 68 Fabry patients, and it was performed only for them.

2.2. ELISA for detection of anti-drug antibodies in serum

To detect anti-drug antibodies (Immunoglobulin G, IgG) in serum from Fabry patients, ELISA was performed. A 96-well microtiter plate (Nunc, Roskilde, Denmark) was coated with 100 μL of 1 μg/mL agalsidase beta (Fabrazyme, Sanofi Genzyme, Cambridge, MA; as there is immune cross-reactivity between agalsidase alfa and agalsidase beta [20,21], we used the latter here as an antigen) overnight at 4C°. Then the wells of the microtiter plate were washed three times with phosphate-buffered saline (PBS) and blocked with 2% bovine serum albumin in PBS for 1 h at room temperature (RT). Subsequently, the plate was washed three times with PBS containing 0.1% Tween-20. Then, a 100 μL aliquot of a diluted serum sample (1:100 to 1:1000) was applied, followed by incubation for 1 h at RT. After incubation, the plate was washed three times with PBS containing 0.1% Tween-20. Then, a 100 μL aliquot of 1:10,000-diluted peroxidase-conjugated goat anti-human IgG antibodies (Invitrogen, Carlsberg, CA) was applied, and the plate was incubated for 1 h at RT. After incubation, the plate was washed three times with PBS containing 0.1% Tween-20, and then 100 μL of peroxidase substrate (0.4 mg/mL *o*-phenylenediamine and 0.016% hydrogen peroxide in 0.1 mol/L citrate phosphate buffer, pH 5.0) was applied and reacted for 15 min at RT. The reaction was stopped by adding 50 μL of 2 mol/L H_2SO_4, and finally the absorbance at 490 nm was measured using a microplate reader (Wallac 1420 ARVO MX Multilabel Counter; Perkin-Elmer, Waltham, MA). Measurement of the blank value was performed with a non-coated plate, and the absorbance was determined for each sample. Then, the difference in absorbance (ΔOD 490 nm) at each time point as to the baseline in individuals was essentially calculated based on three measurements. To determine the cut-off point for the ELISA analysis, the mean and standard deviation (SD) values for ΔOD 490 nm in the group comprising the later-onset Fabry males and Fabry females (62 patients; number of time points, $n = 95$) were calculated, as these subjects were expected to have residual GLA protein and to hardly form anti-α-Gal antibodies. The mean and SD values in this group were determined to be 0.048 and 0.067, respectively. Based on the data, the cut-off value was calculated to be 0.250 (mean + 3SD), according to the previous report [16], and it was determined that a subject exhibiting a value of ≥0. 250 was antibody-positive (Ab+) and one of < 0.250 antibody-negative (Ab-), respectively.

2.3. Serum-mediated α-Gal inhibition

The serum-mediated α-Gal inhibition test was performed to examine neutralizing activity toward purified recombinant α-Gal by means of a modification of the method of Linthorst et al. [22], using serum samples from the Fabry patients obtained at 6 months after the initiation of ERT. Briefly, five μL of serum from a Fabry patient or a healthy subject (control) was mixed with 10 μL of 1 μg/mL agalsidase beta in PBS for 10 min at RT. Subsequently, α-Gal activity was fluorometrically measured with 4-methylumbelliferyl α-D-galactopyranoside (Calbiochem, La Jolla, CA) as a substrate and *N*-acetyl-D-galactosamine (Sigma-Aldrich, St. Louis, CA) as an inhibitor of α-galactosidase B (α-*N*-acetylgalactosaminidase), as described previously [23]. Finally, the fluorescence released on the enzyme reaction was measured with a fluorometer (Wallac 1420 ARVO MX Multilabel Counter) at excitation and emission wavelengths of 355 nm and 460 nm, respectively. To evaluate serum-mediated α-Gal inhibition, PBS was used instead of a serum sample, and the inhibition rate was calculated as [α-Gal activity (+PBS) – α-Gal activity (+ serum)] /α-Gal activity (+PBS) × 100 (%) based on triplicate measurements. The mean and SD values for the inhibition rate for healthy controls ($n = 110$) were 34 and 5%, respectively. Based on these data, the cut-off value for the inhibition rate was calculated to be 49% (mean + 3SD); and it was determined that a subject exhibiting a value of ≥49% was inhibition-positive (Inh+) and one of < 49% inhibition-negative (Inh-), respectively.

2.4. IC test for detection of anti-drug antibodies in serum

IC test for detection of anti-α-Gal antibodies was performed to examine the utility of the method as a bed-side test, according to the method described previously [24]. Briefly, serum samples were 1:10-diluted with sample buffer (50 mmol/L Tris-HCl, pH 7.2, 150 mmol/L NaCl, and 1% Triton X-100), and then applied on an IC chip (Synthera Technologies, Tokyo, Japan), agalsidase beta having been immobilized on the membrane. Then, the reservoir unit containing conjugation buffer (50 mmol/L Tris-HCl, pH 7.2, 150 mmol/L NaCl, 1% Triton X-100, 1 mmol/L $MgCl_2$, and alkaline phosphatase (AP) labeled-goat anti-human IgG) was opened to initiate the immune reaction, and the substrate of AP (nitro-blue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate) fixed on the membrane was mixed with the conjugation buffer after opening of the reservoir unit. Thus, anti-α-Gal antibodies in serum samples were captured by the agalsidase beta immobilized on the membrane, and detected with AP-conjugated goat anti-human IgG. The formation of an antigen/antibody complex was visualized as the enzyme reaction of AP (total reaction time: 20 min). The color strength (score) was evaluated, from level 0 (no color) to level 8 (maximum density), by visual determination based on the attached color paper. According to the validation study previously performed [24], it was determined that a subject was IC test-positive (IC+) when the score was 2 or more, pseudopositive (IC ±) when it was one, and negative (IC-) when it was zero.

2.5. GLA gene analysis

GLA gene analysis was performed for 68 Fabry patients whose informed consent was obtained. Genomic DNA was extracted from their blood samples, and seven exons, intron/exon boundaries, and the specific intronic region containing IVS4 + 919 of the *GLA* gene were amplified by polymerase chain reaction, followed by direct sequencing, as described previously [23].

2.6. Measurement of plasma lyso-Gb3

Lyso-Gb3 in plasma was measured by means of liquid chromatography-tandem mass spectrometry (LC-MS/MS) using stable-isotope labeled lyso-Gb3 as a standard, as described previously [16].

2.7. Statistical analysis

The experimental value for each group is basically expressed as the mean ± SD [n: number of samples]. The differences among the targeted groups were assessed by means of Welch's *t*-test, it being taken that there was significant difference if $p < 0.05$. The correlation coefficient between the targeted groups was calculated using Excel 2016 (Microsoft, Redmond, WA).

3. Results

3.1. Anti-α-Gal antibodies detected by means of ELISA and GLA gene mutations in Ab + Fabry patients

The anti-α-Gal antibodies in serum from Fabry patients was semi-quantitatively measured at 6 months after the initiation of ERT by means of ELISA, and the results are shown in Fig. 1. In the classic Fabry male group, 20/35 (57%) were Ab+ (15/27 treated with agalsidase alfa, 5/7 with agalsidase beta, and 0/1 first with agalsidase beta and then with agalsidase alfa). In the 17 later-onset Fabry males, two cases (one treated with agalsidase alfa, the treatment in the other one being unknown) were Ab+ (12%). All the Fabry females were Ab-.

Table 1
The results of *GLA* gene analysis in the Fabry patients.

	Classic males	Later-onset males	Females
Nonsense mutation	8/8	0/0	0/12
Deletion	1/1	0/0	0/5
Splicing defect	1/2	0/2	0/3
Missense mutation	1/8	2/12	0/15
Not examined	9/16	0/3	0/10
Total	20/35	2/17	0/45

Number of Ab+ patients/Total number of patients.

GLA gene analysis was performed for 19 classic Fabry males (Ab+/Ab-, 11/8), 14 later-onset Fabry males (Ab+/Ab-, 2/12), and 35 Fabry females (Ab+/Ab-, 0/35), whose permission could be obtained. The results of gene analysis are summarized in Table 1. Gene analysis revealed eight nonsense mutations (W204X; R227X; W236X; W245X, 2; G258X, 2; and W340X), one deletion (c.85delG), one splicing defect (IVS3-1 G > A), and one missense mutation (C378F) in the 11 Ab+ classic Fabry males examined. In the two Ab+ later-onset Fabry males, two missense mutations (L300P and R301Q) were detected. In the 20 Ab- Fabry males, various gene mutations were found (classic, P40S; R112C; R227Q; M296I; W340S; N355K; L417P; IVS5-1G > C, later-onset, G35E; A37T; R112H, 3; F113I; N215S, 2; M296I; R301Q; IVS4 + 919G > A, 2), but no nonsense mutations were detected.

The time course of the antibody formation following ERT was examined for the 13 Ab+ Fabry cases from whom serum samples could be obtained at 24 months after the initiation of ERT. The results are shown in Fig. 2. In six of them, high ΔOD 490 nm values were maintained, and one of them developed anaphylactic shock at 21 months and thus ERT was stopped for him thereafter. In five cases, the ΔOD 490 nm values decreased during ERT, although the values were more than the cut-off one at 24 months after the initiation of ERT. In the two cases exhibiting apparently lower ΔOD 490 nm values than those in the other Ab+ patients at 6 months, the values became less than the cut-off one at 24 months, suggesting the acquisition of immune tolerance.

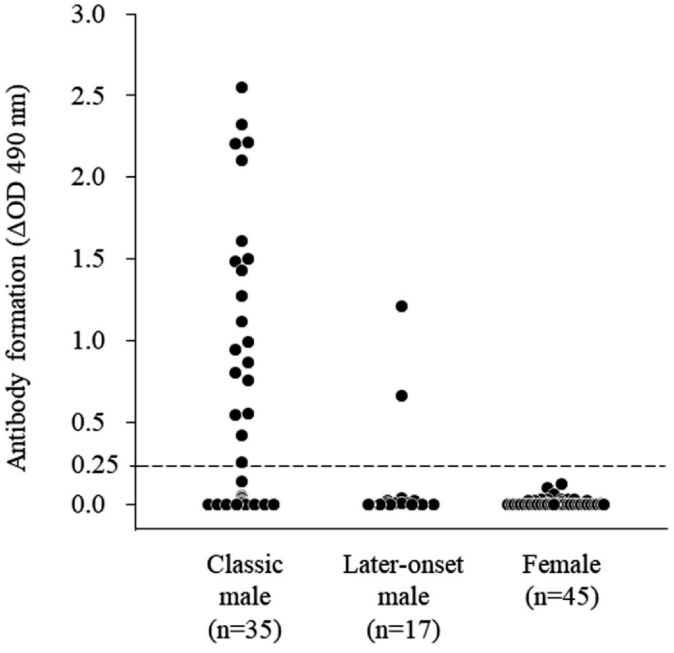

Fig. 1. Measurement of anti-α-Gal antibodies (IgG) in serum samples from Fabry patients by means of ELISA. The antibody formation in individual classic Fabry males, later-onset Fabry males, and Fabry females is presented as the absorbance value (ΔOD 490 nm). The cut-off value is represented as a dotted line (ΔOD 490 nm = 0.250).

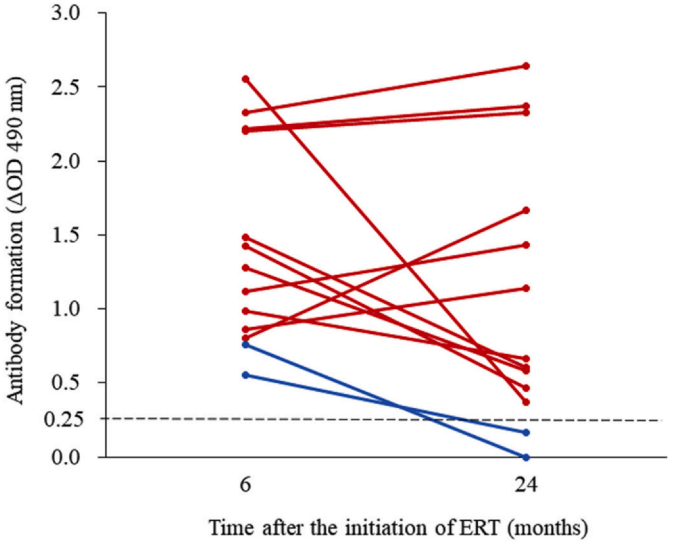

Fig. 2. Longitudinal profiles of anti-α-Gal antibodies (IgG) in serum samples from the antibody-positive (Ab+) Fabry patients following ERT. The ΔOD 490 nm values at 6 and 24 months after the initiation of ERT are presented. Blue lines: Subjects who acquired immune tolerance by 24 months after the initiation of ERT. Red lines: Subjects who exhibited an immune reaction (Ab+) at 24 months after the initiation of ERT. The cut-off point is represented by a dotted line (ΔOD 490 nm = 0.250).

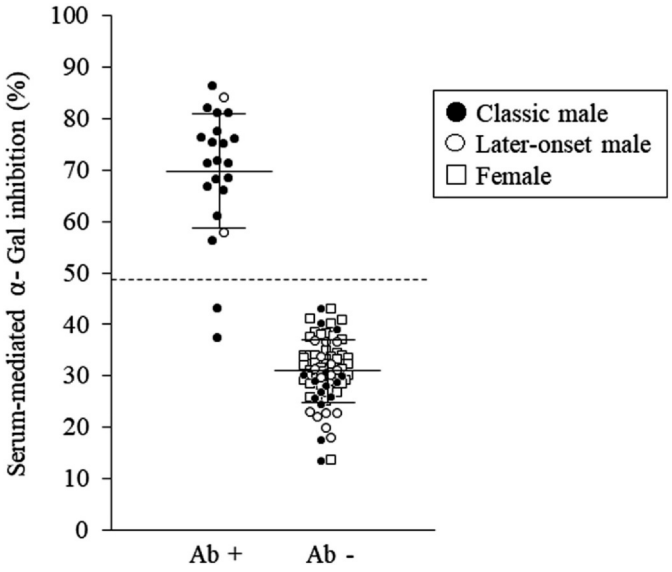

Fig. 3. Measurement of serum-mediated α-Gal inhibition in antibody-positive (Ab+) and antibody-negative (Ab-) Fabry patients. The dotted line represents the cut-off value for the inhibition rate (49%). Closed circles: classic Fabry males, open circles: later-onset Fabry males, and open squares: Fabry females.

3.2. Inhibition of α-Gal activity by serum from Fabry patients

The serum-mediated α-Gal inhibition test was performed using serum samples obtained at 6 months from the Fabry patients after the initiation of ERT. The results are shown in Fig. 3. The inhibition rates for the Ab+ and Ab- groups were calculated to be 70 ± 12% [n = 22] and 31 ± 6% [n = 75], respectively. There was a significant difference between these two groups (p < 0.05), although no statistical difference was found between the Ab- group and the healthy control one (p ≥ 0.05). Of the 22 Fabry patients that were Ab+, 20 were Inh + (91%), suggesting the existence of neutralizing antibodies in most of the Ab+ patients, although the other two cases were Inh-.

3.3. Impact of antibody formation on plasma lyso-Gb3 reduction following ERT

To examine the impact of antibody formation on the effect of ERT, the lyso-Gb3 concentration in plasma samples in the classic Fabry males was measured at the baseline, and 6 and 24 months after the initiation of ERT. The time courses of the plasma lyso-Gb3 concentration in each case (Fig. 4a), and in the Ab+, Ab-, and immune tolerance groups (Fig. 4b) were summarized. The mean ± SD values for plasma lyso-Gb3 in the Ab+ group at the baseline, and 6 and 24 months after the initiation of ERT were 170 ± 71, 63 ± 28, and 79 ± 41 nmol/L [n = 11], respectively (reference interval: 0.31–0.75 nmol/L [16]). On the other hand, those in the Ab- group were 144 ± 40, 39 ± 14, and 35 ± 13 nmol/L [n = 8], respectively. The mean values for plasma lyso-Gb3 at the baseline, and 6 and 24 months after the initiation of ERT in the two patients who acquired immune tolerance were 124, 48, and 35 nmol/L, respectively. The plasma lyso-Gb3 concentration in the Ab+ group was decreased at 6 months after the initiation of ERT compared with the baseline, but thereafter increased. On the other hand, that of the Ab- group continued to decrease from 6 months after the initiation of ERT. There was a statistical difference (p < 0.05) in the plasma lyso-Gb3 level at 24 months after the initiation of ERT between the Ab+ and Ab- groups. The relationship between the titer of anti-drug antibodies and the plasma lyso-Gb3 concentration is shown in Fig. 4c. There was a correlation between the ΔOD490 nm values on ELISA and the plasma lyso-Gb3 levels at 24 months after the initiation of ERT (r = 0.855).

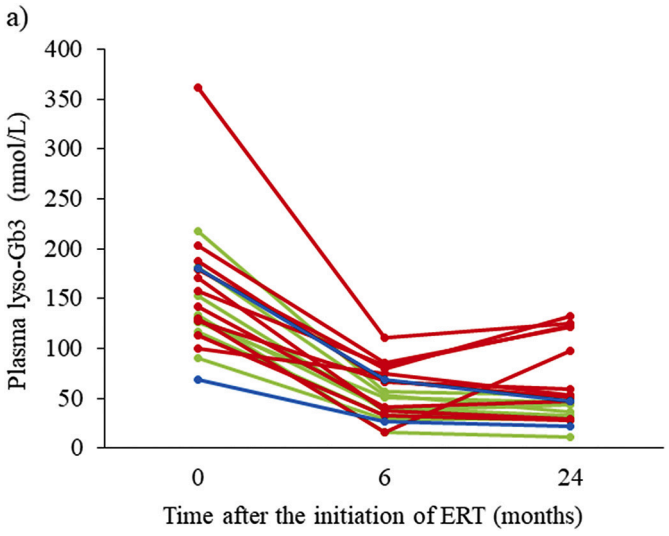

Fig. 4. Longitudinal profiles of the plasma lyso-Gb3 concentration in classic type of Fabry individuals **a**), and the antibody-positive, antibody-negative, and immune tolerance groups following ERT **b**), and the relationship between the anti-drug antibody titer and the plasma lyso-Gb3 concentration at 24 months after the initiation of ERT **c**). The plasma lyso-Gb3 value in each group is basically represented as the mean (± SD). Antibody-positive (Ab+) individuals and group (red lines and dots), antibody-negative (Ab-) ones (green lines and dots), and immune tolerance ones (blue lines and dots). There is a statistical difference (*: p < 0.05) in the plasma lyso-Gb3 level at 24 months after the initiation of ERT between the Ab+ and Ab-groups. There is a correlation between the ΔOD 490 nm values on ELISA and the plasma lyso-Gb3 levels of the Fabry patients (r = 0.855). The dotted line represents the cut-off value for the absorbance value (ΔOD 490 nm) on ELISA. Reference interval for the plasma lyso-Gb3 concentration: 0.35–0.71 nmol/L [16].

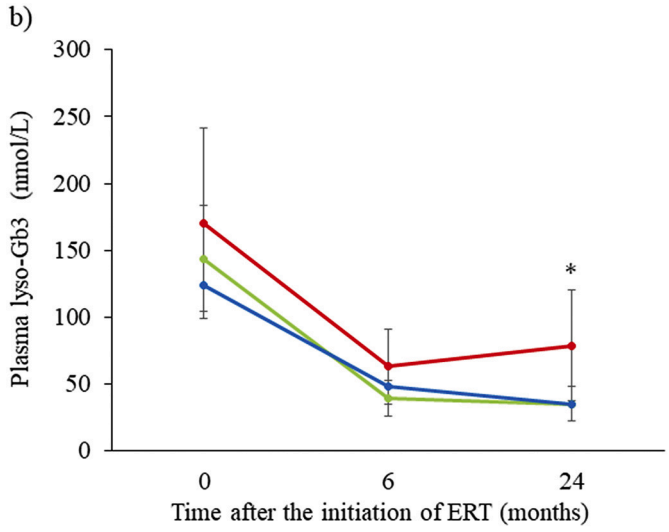

Fig. 4. (*continued*)

3.4. Comparison of IC test versus ELISA as to serum from Ab+ Fabry patients

To allow easy and rapid detection of anti-drug antibodies, we developed an IC kit and confirmed that Ab- cases were IC- in the previous study [24]. In this study, we compared the ΔOD 490 nm values on conventional ELISA and the visual scores with the IC test using serum from Ab+ Fabry patients as samples. The results are summarized in Fig. 5. The visual scores were correlated with the ΔOD 490 nm values (r = 0.759), although some cases exhibited the highest visual score value (Fig. 5a). The results of comparative analysis using the serum

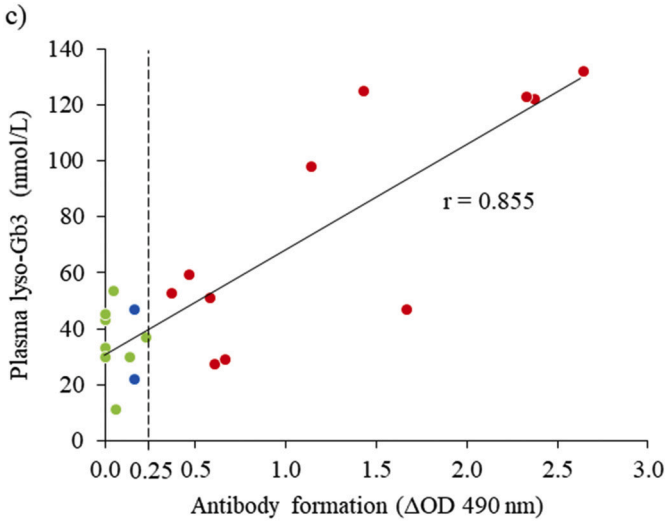

Fig. 4. (*continued*)

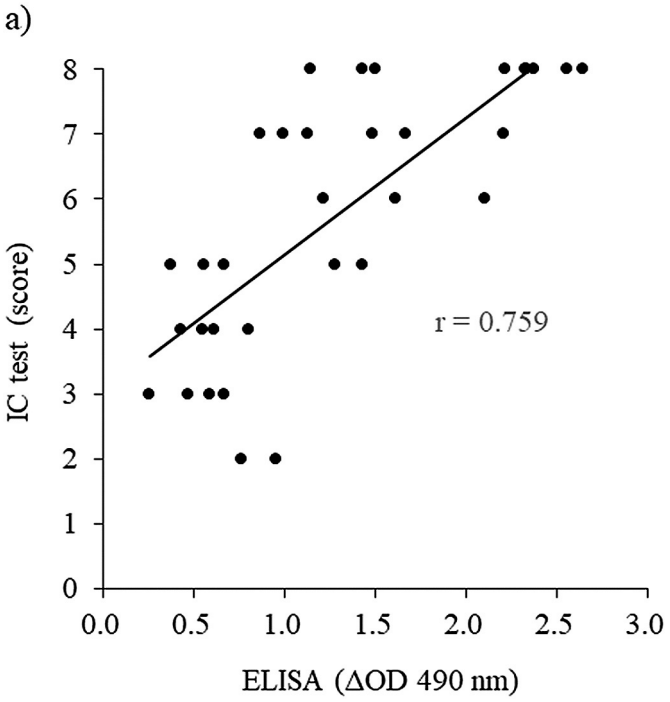

Fig. 5. Relationship between the results of IC test and ELISA as to the anti-α-Gal antibodies **a**), and comparison of the antibody level at 6 and 24 months after the initiation of ERT in the Fabry patients using the IC test and ELISA **b**). The antibody level determined on ELISA is represented as the absorbance at 490 nm (ΔOD 490 nm), and that on IC test as the visual score (levels 0–8). Closed columns represent data at 6 months and open columns those at 24 months after the initiation of ERT. The cut-off value in the ELISA test is represented as a dotted line (ΔOD 490 nm = 0.250).

samples obtained at 6 months and those at 24 months are shown in Fig. 5b. The scores on IC test for the two cases that acquired immune tolerance changed from IC+ at 6 months to IC- at 24 months after the initiation of ERT (Fig. 5b, Cases 12 and 13), this result being well correlated with that on ELISA.

4. Discussion

Since the introduction of ERT with agalsidase alfa and beta, many investigators have reported that it is biochemically and clinically effective for treatment of Fabry disease [4–8]. However, recent reports suggest that the anti-drug antibody formation during ERT adversely affects the safety and outcome of the therapy, and this has attracted the attention of clinicians and researchers as to ERT for Fabry disease [9–15]. Systematic reviews reported that the incidence of antibody (IgG) formation reached 20–56% of male patients treated with agalsidase alfa and 68–91% of those treated with agalsidase beta [7,15]. Although there were methodological differences between these studies involving detection of the antibodies (i.e., the dilution rate of samples, the timing of detection, the definition of seropositivity, etc.) and it is difficult to simply compare them, the outcome of ERT in some Fabry males is very likely affected by the formation of anti-drug antibodies. Thus, it is important to know the antibody formation status in Fabry patients following ERT. However, there have been few reports of study of the anti-drug antibody formation in a Japanese Fabry cohort following ERT and thus we tried to characterize the anti-drug antibodies by means of ELISA and serum-mediated α-Gal inhibition assay.

In this study, we used serum from Fabry patients for ELISA and serum-mediated α-Gal inhibition assaying as samples, and there was no problem: on the ELISA, the mean and SD values for ΔOD 490 nm in the group comprising the later-onset Fabry males and Fabry females were sufficiently small, and there was no statistical difference in the inhibition rate between the Ab- group and the healthy control one, all the serum samples being available for these assays. On the other hand, Lenders M., et al. recommended using purified IgGs for the serum-mediated inhibition test instead of serum as samples to minimize the background signal [25].

ELISA revealed that anti-drug antibodies often develop in classic type Fabry males during ERT, regardless of whether agalsidase alfa or agalsidase beta was used. Especially for Fabry males harboring *GLA* gene mutations leading to a deficiency of α-Gal protein, such as nonsense mutations, attention should be paid during ERT.

Considering the results of the follow-up analysis, the Ab+ state seems to be maintained for a long time in patients who exhibit a high ΔOD 490 nm value at 6 months after the initiation of ERT. On the other hand, tolerance might develop during continuing ERT in some Fabry patients whose antibody titers gradually decrease. Actually, two patients who had exhibited a low ΔOD 490 nm value acquired immune tolerance at 24 months after the initiation of ERT. In such cases, recurrent infusions of recombinant α-Gals may accelerate neutralization of the antibodies.

Serum-mediated α-Gal inhibition was seen in most of the Ab+ patients, as other investigators reported for non-Japanese Fabry cohorts [22,26–28], suggesting that most of these anti-drug antibodies were neutralizing ones. The results of the comparative study between the Ab + and Ab- groups clearly revealed that the anti-drug antibodies had a negative effect on the reduction of the plasma lyso-Gb3 level following ERT.

Unfortunately, detailed information about adverse drug reactions in the Fabry patients examined in this study is not available except in one severe case who exhibited anaphylactic shock, and the relation between the formation of anti-drug antibodies and the adverse drug reactions has not been elucidated. However, Sasa H., et al. had summarized the results of post-marketing surveillance of 493 Japanese Fabry patients including 59 of our 97 cases who received ERT with agalsidase alfa [29]. According to the report, the percentage of patients with adverse drug reactions (rash, malaise, erythema, and extremity pain) was 24%, and 12.6% had infusion-related reactions (malaise, nausea, extremity pain, urticaria, chills, rash, headache, and chest discomfort). The outcome was “resolving” or “resolved” either without any treatment or with treatment (steroid and/or antihistamines).

As the formation of anti-drug antibodies is likely to have an impact on the safety and effect of ERT, establishment of an antibody detection method available as a bed-side test is important. We here tested an IC kit previously developed [24], and checked whether it can be used. The results revealed that there was a correlation between the absorbance on

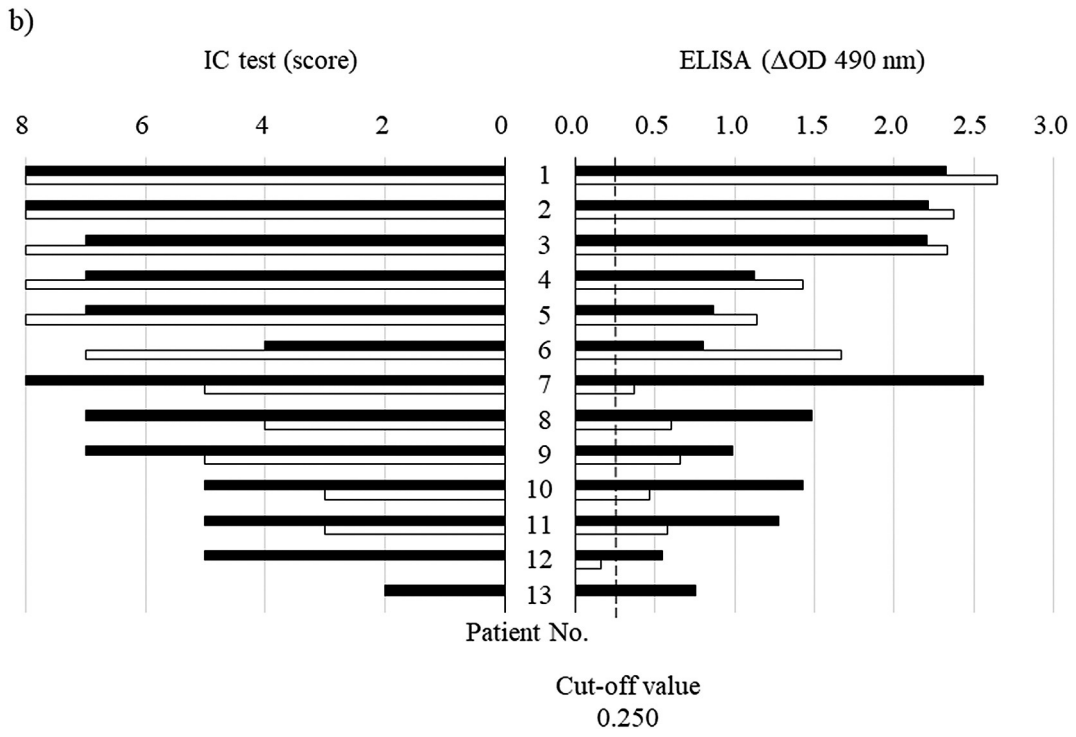

Fig. 5. (*continued*)

ELISA and the score on IC test. As the IC test is very easy and rapid, and does not need the sending of samples to a laboratory, it should be useful as a bed-side test, although further improvement is necessary, i.e., preparation of a proper standard for the assay. The IC test followed by ELISA and the serum-mediated inhibition test may be used as a tool for early detection of anti-drug antibodies and an early response to immune reactions when any adverse drug reaction or inhibition of the reduction of plasma lyso-Gb3 concentration is found in Fabry patients during ERT. Furthermore, efforts to overcome immune responses would be required, i.e., immunotitration [15], use of immunomodulatory drugs [30–32], and development of a new enzyme expected not to induce the formation of anti-drug antibodies in Fabry patients [21].

This study has several limitations. First, we have no proper standard to measure anti-α-Gal antibodies in serum, and the detection of antibodies in this study was performed semiquantitatively. In the near future, we would like to prepare a human-type monoclonal antibody against α-Gal and use it as a standard for measurement of antibodies in the next study. Second, we could not obtain information regarding adverse drug reactions, genotypes, and drugs used in some Fabry patients. Detailed clinical information is required.

5. Conclusion

In this study, we characterized anti-drug antibodies formed in Japanese Fabry patients following ERT. Anti-α-Gal antibodies were detected in 57% of the classic Fabry males and in 12% of the later-onset ones. No Fabry females exhibited an antibody-positive reaction. In most of the antibody-positive cases, serum-mediated inhibition of α-Gal activity was found and the reduction of the plasma lyso-Gb3 concentration following ERT was inhibited. IC test is easy and rapid, and thus useful as a bed-side test.

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Declaration of Competing Interest

We declare that none of the authors have any competing interests.

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