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Case Report

A novel double *GLA* gene mutation of W24R and N419D in a patient with cardiac Fabry disease

Masanori Hirose [1], Sho Okada [*,1], Yoshio Kobayashi

Department of Cardiovascular Medicine, Chiba University Graduate School of Medicine, Chiba, Japan

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ABSTRACT

Fabry disease (FD) is an X-linked lysosomal storage disorder caused by insufficient activity of α-galactosidase A (α-Gal A) encoded by *GLA*. The enzymatic defect causes the progressive accumulation of sphingolipids in various tissues and body fluids, causing systemic disorders. We report a rare familial case of inherited cardiac FD associated with a novel double mutation in the *GLA* gene: W24R and N419D.

A young man with severe obesity was admitted for heart failure (HF) with the diagnosis of dilated cardiomyopathy. Left ventricular hypertrophy was suspected during HF treatment after discharge, and in association with his mother's family history of cardiac diseases and sudden death, the etiology of the hypertrophy was re-examined. Very low α-Gal A activity confirmed the diagnosis of FD. Gene mutation analysis of *GLA* demonstrated a double mutation: W24R and N419D. Proband analysis revealed the same double mutation in his mother. Although she had no signs or symptoms of FD, we detected mild accumulation of globotriaosylsphingosine. The good laboratory practice-validated assay using HEK293 cells showed that the double mutation was amenable to migalastat, a pharmacological chaperone stabilizing α-Gal A.

This case highlights a novel double gene mutation in *GLA* (W24R and N419D) identified in a family with FD. Although clinical significance of each mutation remains unknown, its combination might work synergistically to attain or augment pathogenicity.

1. Background

Fabry disease (FD; OMIM 301500) is an X-linked lysosomal storage disorder caused by insufficient activity of α-galactosidase A (α-Gal A) encoded by *GLA*. The enzymatic defect causes the progressive accumulation of sphingolipids, such as globotriaosylceramide (GL-3 or Gb3), its deacylated derivative globotriaosylsphingosine (Lyso-Gb3), and their related glycosphingolipids, in the plasma and tissue lysosomes throughout the body [1]. FD affects various organs, including the heart, kidney, brain, nerves, eyes, ears, skin, and gastrointestinal tract [1].

Classically, dyshidrosis and acroparesthesia occur during childhood, and cardiac manifestations become evident during the fourth decade of life [2]. Left ventricular hypertrophy (LVH) is atypical and is the earliest sign of the cardiac variant of FD, which is usually identified in the fifth or sixth decades of life [3,4].

We report a rare familial case of FD associated with a novel double mutation of W24R and N419D in the *GLA* gene. To our knowledge, this is the first report on the pathogenicity of these combined mutations in FD.

2. Case presentation

A young man in his twenties was admitted to our hospital due to heart failure (HF). He was severely obese (body mass index [BMI] = 41.9 kg/m^2) but otherwise healthy and took no medications. Chest X-ray revealed an enlarged cardiac silhouette and diffuse lung congestion (Fig. 1). His electrocardiogram (ECG) indicated biatrial overload, normal PQ, short $P_{end}Q$ interval, left ventricular (LV) high voltage, and flat T waves (Fig. 2). Echocardiography revealed a markedly reduced LV ejection fraction (LVEF) to 22%. The LV was dilated to 82 mm (Fig. 3). Neither LVH nor primary valvular disease was detected. His pulmonary congestion resolved with intravenous diuretics under catecholamine support, and a diagnostic workup was performed. Coronary angiography showed no significant stenosis in the coronary arteries. Light microscopy of the endomyocardial biopsy specimen revealed only

* Corresponding author at: Department of Cardiovascular Medicine, Chiba University Graduate School of Medicine, 1-8-1 Inohana, Chuo-ku, Chiba 260-8670, Japan.
E-mail address: shookada_circ@chiba-u.jp (S. Okada).
[1] These authors contribute equally to the work.

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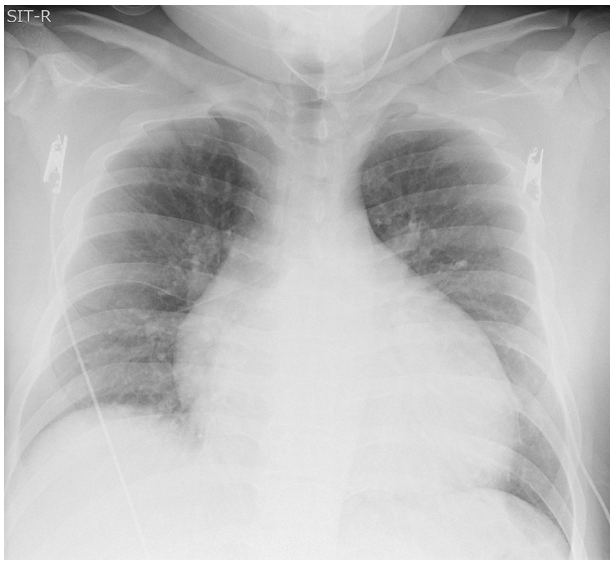

Fig. 1. A chest X-ray image.
A chest radiograph demonstrating marked cardiomegaly and lung congestion.

nonspecific myocyte hypertrophy, some vacuolar degeneration, and fibrosis (Fig. 4). Accordingly, the patient's HF was considered idiopathic dilated cardiomyopathy. He was discharged after 1 month of treatment and continued to receive optimal medical therapy for HF. After discharge, he failed to lose weight, but his heart gradually exhibited reverse remodeling. After about 1 year of treatment, his LVEF improved to 36%, and his LV diameter decreased to 62 mm. LV wall thickening was also observed during the reverse remodeling (Fig. 5). Suspected LVH in association with his mother's family history of cardiac disease and sudden death led to a re-examination of the etiology of his LVH.

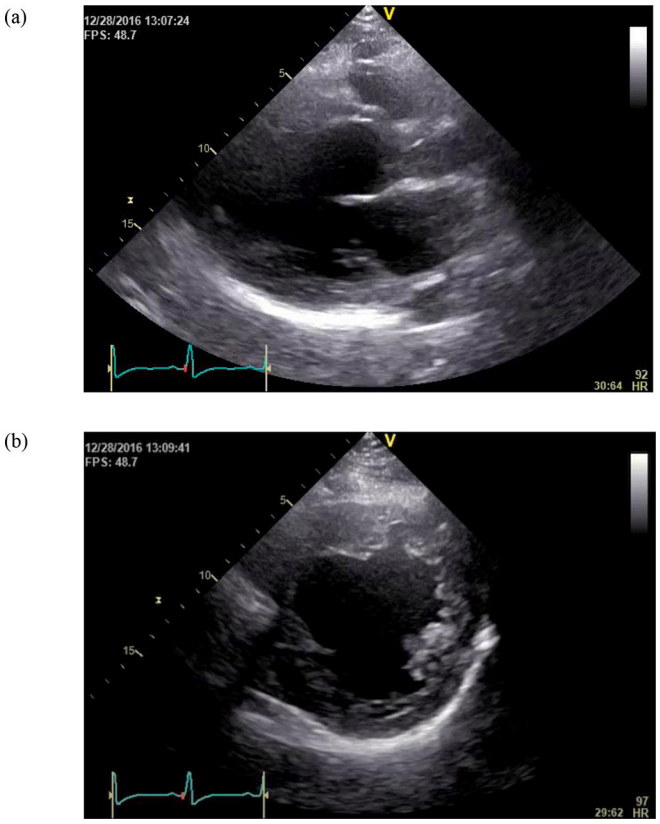

Fig. 3. Echocardiogram during hospitalization.
Parasternal long-axis (a) and short-axis (b) images showing severe left ventricular dilatation and dysfunction.

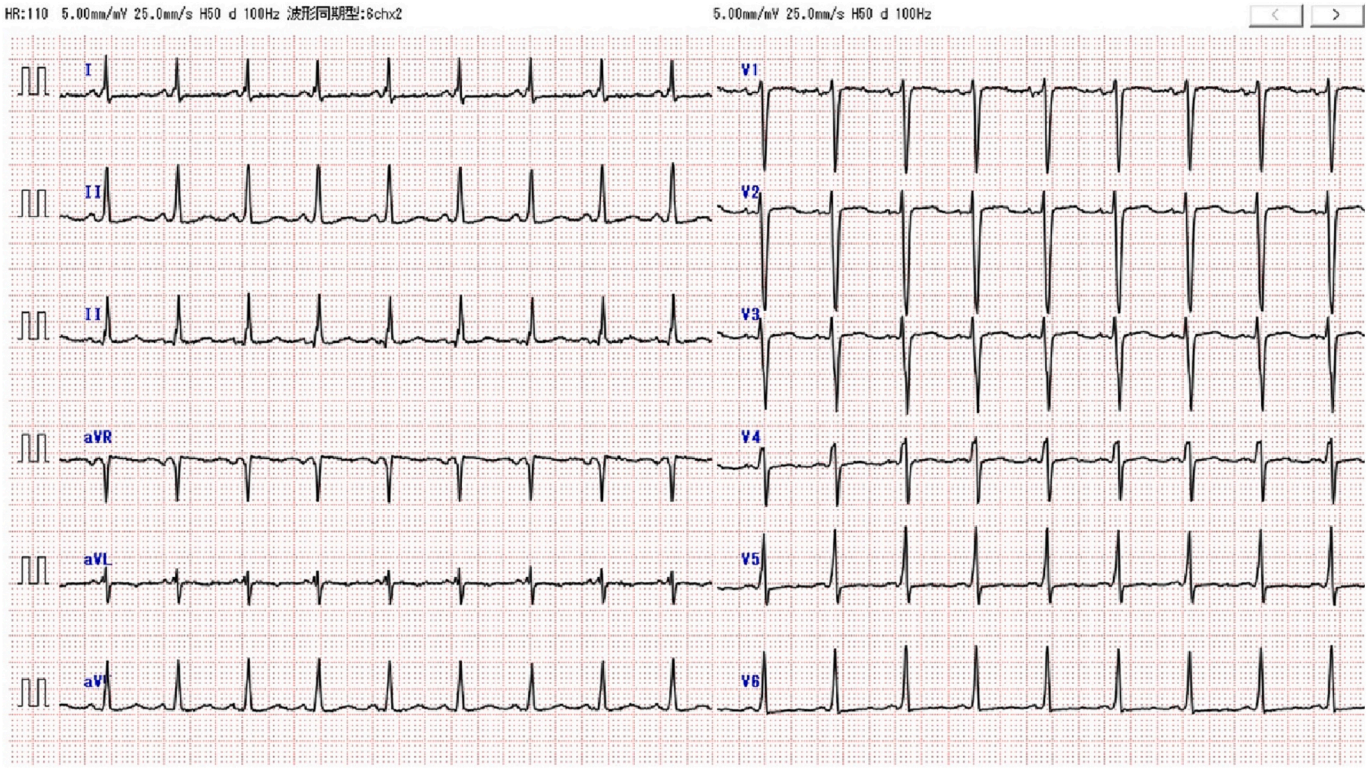

Fig. 2. Electrocardiogram.
An electrocardiogram showing biatrial overload, normal PQ, short $P_{end}Q$ interval, left ventricular (LV) high voltage, and flat T waves.

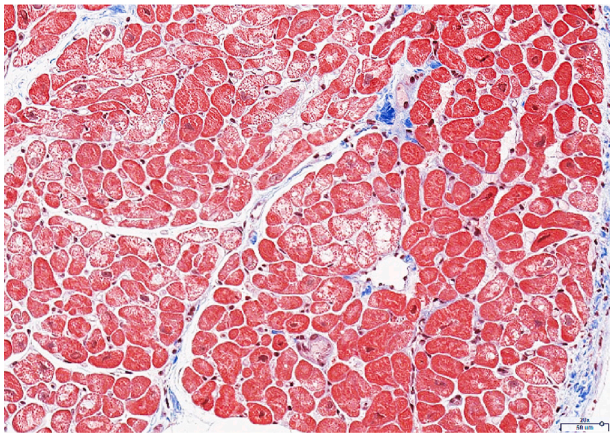

Fig. 4. Endomyocardial biopsy specimens.
Light microscopy with Masson's trichrome staining of ventricular tissues showing nonspecific myocyte hypertrophy, some vacuolar degeneration, and fibrosis. Scale bar = 50 μm.

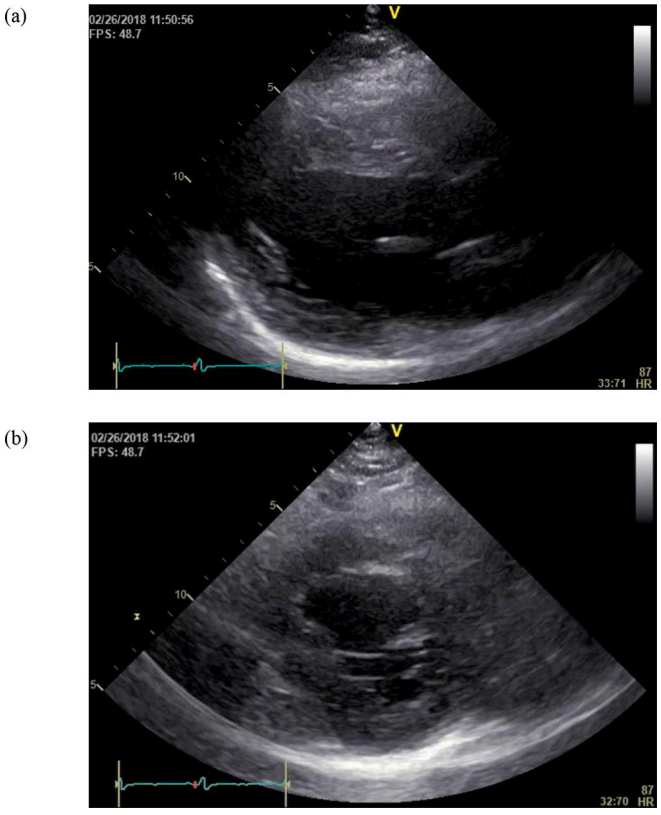

Fig. 5. Echocardiogram 14 months after hospitalization.
Parasternal long-axis (a) and short-axis (b) images showing mild reverse remodeling with thickened LV walls.

Markedly decreased α-Gal A activity in the white blood cells confirmed the diagnosis of FD (4 nmol/h/mg protein (reference value: 17–65 nmol/h/mg protein)). Plasma lyso-Gb3 concentration was elevated to 1.9 nmol/L (reference value: 0.35–0.71 nmol/L), and urine Gb3 excretion was positive (reference value: negative). Gene mutation analysis of *GLA* demonstrated a double point mutation in two distinct regions: W24R and N419D (c.[70 T > A; 1255A > G). The patient was started on agalsidase beta infusion therapy, but the drug was discontinued after 18 months due to feasibility issues such as frequent visits and difficulty accessing the vascular route. As both mutations were amenable to migalastat, oral migalastat therapy was started and he has been on it for three years without any FD-related problems. Serum lyso-GB3 had already fallen to 1.25 nmol/L just before the migalastat therapy, but after three years of treatment it fell further to 1.17 nmol/L.

3. Proband's family members

Following the diagnosis of the present case, his family members (i.e., his parents) were referred for FD screening. His ~80-year-old father had normal α-Gal A activity, without LVH on echocardiography. His mother was a ~ 60-year-old Filipina from the Philippines. Although she was healthy, with no specific symptoms; however, she had several male siblings with an unknown disease associated with sudden death, cardiac hypertrophy, syndactyly, and implantation of cardiac devices. Both her ECG and echocardiography were unremarkable (Figs. 6, 7). Genetic testing of her white blood cells revealed the same genetic mutations as her son. Enzymatic activity of α-Gal A in the white blood cells was normal (61 nmol/h/mg protein (reference value: 17–65 nmol/h/mg protein)) and urine Gb3 excretion was negative (reference value: negative). However, plasma Lyso-Gb3 was mildly elevated to 0.91 nmol/L (reference value: 0 nmol/L). Therefore, she was diagnosed as a least manifest FD. Extensive systemic examinations were performed to determine the potential organ involvement of FD in the mother; however, only aging-related cataracts were identified. Her relatives resided in the Philippines, and she was unwilling to contact them.

4. Discussion

In the present case, suspected LVH during treatment for HF with reduced ejection fraction (HFrEF) led to the diagnosis of FD. Furthermore, a rare novel double mutation was identified by *GLA* gene mutation analysis.

Considering the severe obesity and dilated heart with reduced EF in the present case, the likely principal cause of HF would be obesity rather than FD. Although the diagnosis of obesity cardiomyopathy is not always feasible and should involve the exclusion of other causes of diastolic cardiomyopathy, patients presenting with HF exclusively or predominantly due to obesity, usually with a BMI >40 kg/m^2, are often considered to have obesity cardiomyopathy [5]. In the Framingham cohort, most people who developed obesity cardiomyopathy had a body weight ≥ 135 kg, a relative weight of 175–200%, or a BMI ≥40 kg/m^2. Furthermore, the risk of HF in the population increased by 5% for each increment of BMI in men [6]. A review article reported that loss of LV contractility in FD was uncommon, with a prevalence of only 6–8% [7]. These facts suggest that obesity was the most prevalent aggravator for HF in the present case because his estimated HF risk was more than double that of patients with normal BMI, and the detailed diagnostic workup revealed no definite cause for HFrEF.

FD is a rare, inherited metabolic disease caused by mutations in the *GLA* gene, which encodes lysosomal α-Gal A [7]. >1000 genetic abnormalities have been reported to cause FD [8]. In the Japanese population, missense mutations were reported to be the most common in *GLA* gene mutations, accounting for 56.5% of the cases [9]. The present case revealed a double missense mutation of W24R and N419D in the *GLA* gene. Although there have been several reports of double mutations in FD [10,11], the double missense mutations observed in the present case are rare and thus represent a novelty.

The clinical significance of the W24R mutation is unclear. Scott et al. suggest its pathogenicity by detecting W24R in a newborn with low *GLA* enzyme activity [12], while Al-Thihli et al. consider it a variant of unknown significance (VUS) [13]. The role of the N419D mutation is undetermined as no reports exist regarding its significance. Although some double mutations have been reported to decrease, increase, or even normalize GLA enzyme activity [10,14,15], we found no definitive reports for the present mutations in the Fabry mutants list [16]. According to the good laboratory practice (GLP)-validated assay using HEK293

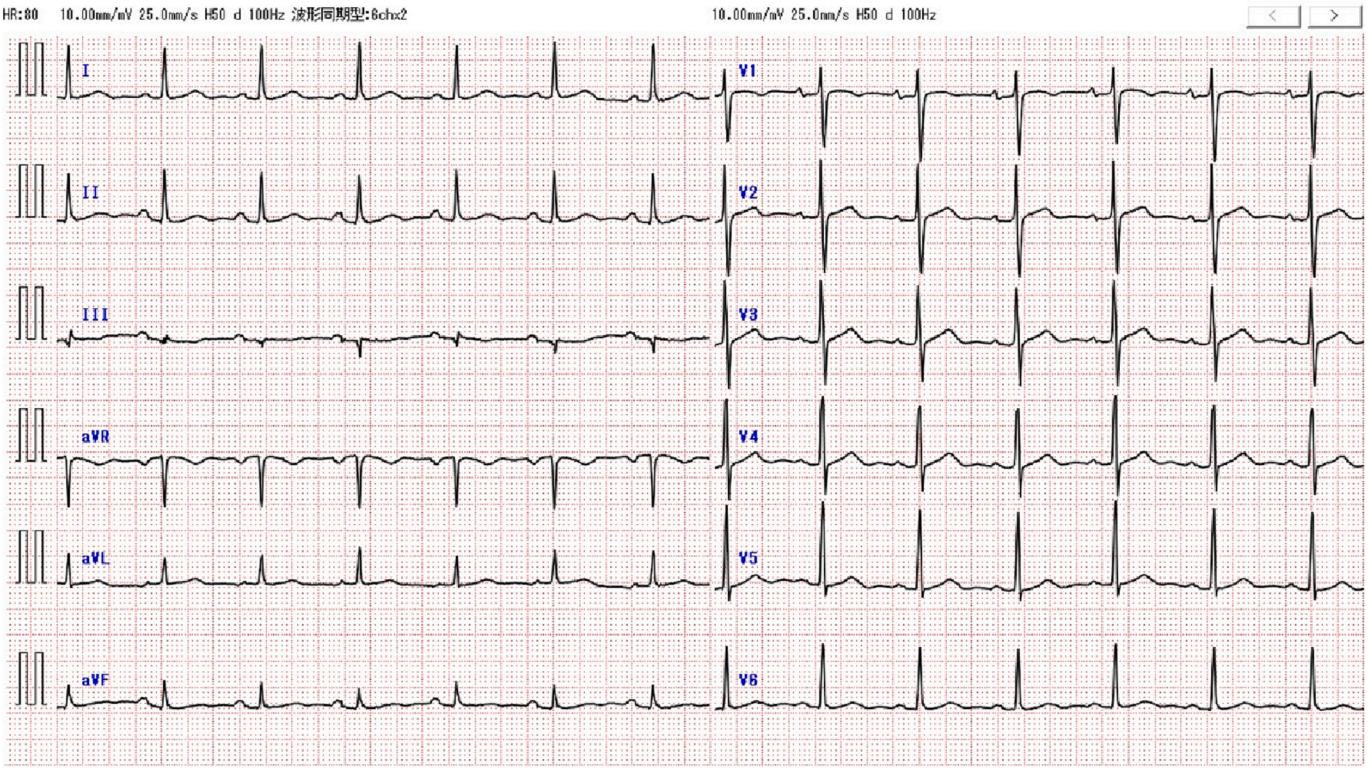

Fig. 6. Electrocardiogram of the proband's mother.
An electrocardiogram with normal $P_{end}Q$ interval, LV voltage, and T waves.

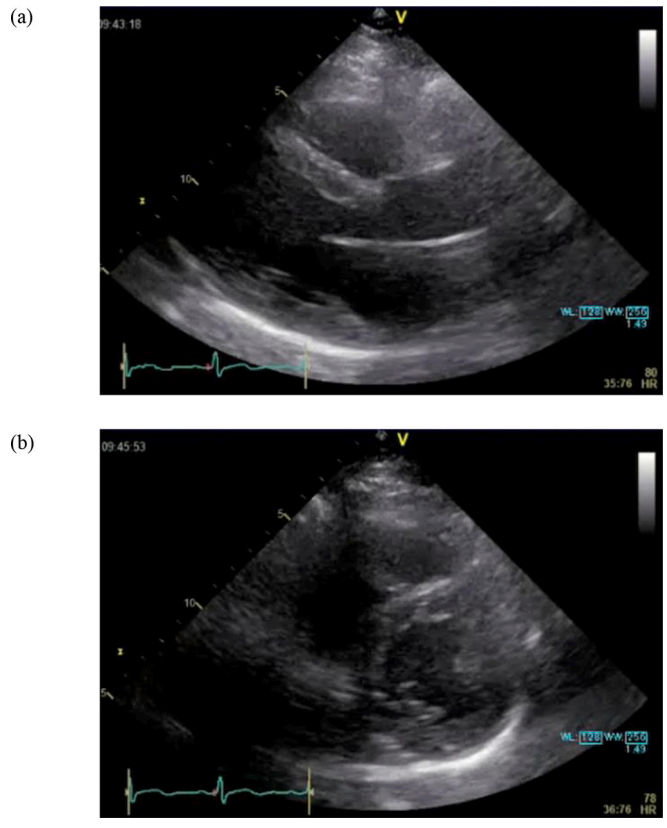

Fig. 7. Echocardiogram of the proband's mother.
Parasternal long-axis (a) and short-axis (b) images showing normal structure and function.

cells (GLP-HEK assay) provided by Amicus Therapeutics, both W24R and N419D demonstrated decreased enzyme activity (Table 1) [17,18], and combining these mutations further decreased the enzymatic activity (Table 1). Although this is only in vitro data, and clinical significance of each mutation remains unknown so far, combined mutation of W24R and N419D might only synergistically attain or potentiate the pathogenicity of FD.

Of note, both W24R and N419D mutations are amenable to migalastat treatment. Migalastat, a low-molecular-weight analog of the terminal galactose residue on GL-3, selectively and reversibly binds to the active sites of amenable mutated forms of the α-Gal A enzyme [19]. This binding allows migalastat to serve as a pharmacological chaperone, stabilizing α-Gal A in the endoplasmic reticulum and facilitating its transport to lysosomes. The intra-lysosomal environment, which has a more acidic pH and a higher concentration of substrates, enables migalastat to dissociate from α-Gal A in the lysosome and break down GL-3 [19]. In vitro amenability to migalastat does not indicate pathogenicity of the corresponding mutation(s), because their definitions are essentially different. Nevertheless, improvement of subnormal α-Gal A activity after migalastat treatment might suggest its pathogenicity. Furthermore, amenability to migalastat is of great clinical significance, because it indicates that the mutation(s) can be treatable with migalastat regardless of the degree of pathogenicity.

In the present case, a novel double gene mutation of W24R and N419D in *GLA* was identified in a family with FD. It is unknown whether these mutations each or in combination exert pathogenicity, however, biochemical analyses in vivo and vitro strongly suggests the treatable pathogenicity of the combined mutations.

CRediT authorship contribution statement

Masanori Hirose: Conceptualization, Data curation, Methodology, Investigation, Writing – original draft. **Sho Okada:** Conceptualization, Data curation, Methodology, Investigation, Visualization, Writing –

Table 1
Change in in vitro α-Gal A activity with and without treatment with migalastat in the GLP-HEK assay.

α-Gal A Mutant Form		Without Migalastat		With Migalastat (10 μM)		Change in α-Gal A Activity		Meets Amenable Mutation Criteria?
Amino Acid Change	Nucleotide Change	α-Gal A Activity (nmol/mg/h)	% WT	α-Gal A Activity (nmol/mg/h)	% WT	Absolute Increase (%WT)	Fold Increase over Baseline	
W24R	c.70 T > A	20,250 ± 1395	52.6 ± 2.3	24,519 ± 1565	63.4 ± 1.7	10.9	1.21	Yes
N419D	c.1255A > G	10,639 ± 512	31.5 ± 1.5	14,558 ± 678	44.1 ± 2.9	12.6	1.37	Yes
W24R + N419D	c.70 T > A, c.1255A > G	3765 ± 366	12.8 ± 1.3	6983 ± 472	24.4 ± 1.7	11.4	1.85	Yes

Abbreviations: GLP-HEK assay, the good laboratory practice-validated assay using HEK293 cells; WT, wild type.

review & editing. **Yoshio Kobayashi:** Writing – review & editing, Supervision.

Declaration of Competing Interest

None.

Data availability

The authors do not have permission to share data.

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