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INTERNAL MEDICINE

□ CASE REPORT □

A Novel Mutation of the *GAA* Gene in a Patient with Adult-onset Pompe Disease Lacking a Disease-specific Pathology

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Abstract

We herein report a novel compound heterozygous mutation of the acid α-glucosidase (GAA) gene in a 23-year-old man with adult-onset Pompe disease. The patient was admitted for respiratory failure and a highly elevated serum level of creatine kinase (CK). His muscle pathology did not show typical vacuolated fibers; however, globular inclusion bodies with acid phosphatase (ACP) activity was observed. A molecular genetic analysis of the *GAA* gene revealed a novel compound heterozygous mutation, c.1544 T>A (M515K), combined with a previously reported mutation, c.1309 C>T (R437C). The presence of ACP-positive globular inclusion bodies is a useful diagnostic marker for adult-onset Pompe disease, even when typical vacuolated fibers are absent.

Key words: adult-onset Pompe disease, α-1,4-glucosidase, acid phosphatase

(Intern Med 52: 2461-2464, 2013)
(DOI: 10.2169/internalmedicine.52.0311)

Introduction

Pompe disease (glycogen storage disease type II; GSD II) is an autosomal-recessive lysosomal storage disorder caused by a deficiency of acid α-glucosidase (GAA). Based on the age of onset and clinical severity, the disease is classified into infantile, childhood-onset and adult-onset forms (1-6). The *GAA* gene is located on chromosome 17q25.2-q25.3. Mutations in this gene vary widely, including missense, nonsense, large and small insertions and deletions, and frameshift mutations. In general, a good correlation between the nature of the mutation, the degree of residual enzyme activity and the severity of the clinical presentation is observed (7-9). Most of the infantile and childhood-onset forms exhibit a disease-specific skeletal muscle pathology including fibers occupied by huge vacuoles that contain basophilic amorphous material; however, diagnosing the adult-onset form is challenging due to clinical similarities with muscular dystrophy and the paucity of typical vacuolated myofibers. We herein report a novel mutation of the *GAA* gene in a patient with adult-onset Pompe disease lacking a disease-specific pathology.

Case Report

A 23-year-old man was admitted to our hospital due to respiratory insufficiency. His serum levels of creatine kinase (CK) and transaminases were found to be elevated at age 18; however, he had not noticed weakness of his extremities. He had no particular medical or family history. On admission, a physical examination revealed a thin man measuring,

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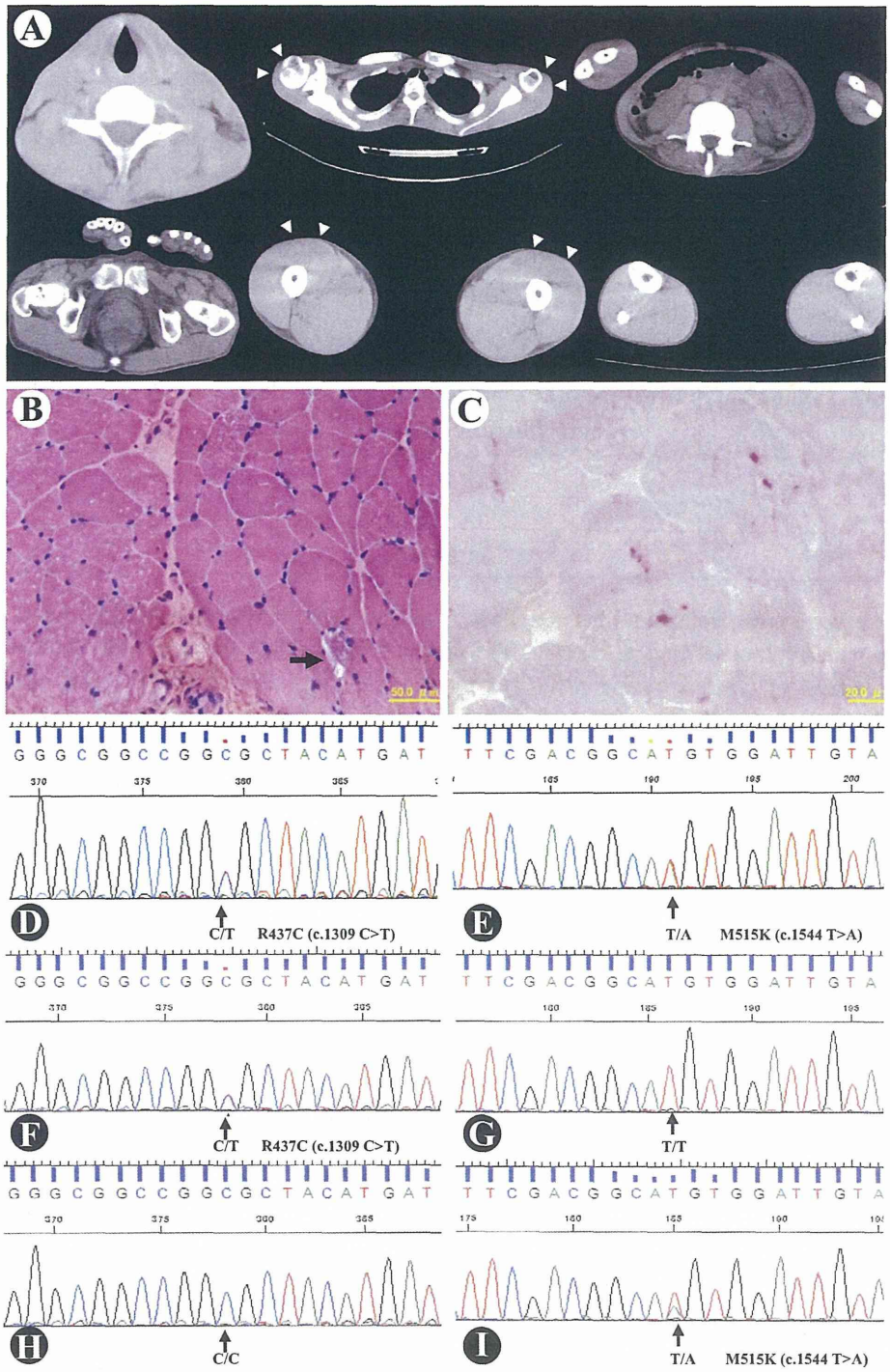

Figure. Computed tomography (CT) scan showing skeletal muscle atrophy in the extremities (A, arrowheads). A muscle biopsy of the left biceps brachii revealed fibers with nonspecific vacuoles in addition to a mild variation in fiber size (B, arrow) (Hematoxylin and Eosin staining). The globular inclusions were positive for acid phosphatase (C) (acid phosphatase). Sequence chromatograms of the two heterozygous missense mutations. C/C to C/T transversion at nucleotide 1309 in exon 8 showing a conservative amino acid change (R437C) (D), and T/T to T/A transition at nucleotide c.1544 in exon 10 resulting in a conservative amino acid change c.1544 T>A (M515K) (E). The patient's father was heterozygous for c.1309 C>T (R437C) in exon 8 (F,G). The patient's mother was heterozygous for c.1544 T>A (M515K) in exon 10 (H, I).

168.7 cm in height and 40.0 kg in weight. On neurological examination, the cranial nerves were found to be intact. A motor examination revealed 4/5 strength in the proximal portion of the four limbs with muscle atrophy in the four limbs, as delineated by the Medical Research Council of Great Britain (MRC). Gowers' sign was positive. The bi-

ceps, triceps and patellar reflexes were diminished, although the ankle jerk was normal. Bilateral Babinski reflexes were absent. The patient was able to stand and walk unaided. The rest of the neurological examination was unremarkable. The laboratory studies revealed elevated CK [807 IU/L (normal range, 62-287 IU/L)] and transaminase levels [alanine transaminase (ALT), 49 IU/L (normal range, 7-42 IU/L); aspartate aminotransferase (AST), 80 IU/L (normal range, 10-35 IU/L)]. The functional vital capacity (FVC) was markedly reduced to 26.1% of the normal predicted value for the patient's age. Electrocardiography (ECG) revealed incomplete right bundle branch block, while echocardiography demonstrated pulmonary hypertension. A computed tomography (CT) scan disclosed skeletal muscle atrophy in the extremities (Figure A, arrowheads). Brain magnetic resonance imaging (MRI) showed no abnormalities. Needle electromyography (EMG) of the upper and lower extremities demonstrated myogenic conversion with a low amplitude motor unit potential and myotonic-like repetitive discharges. A muscle biopsy of the left biceps brachii revealed fibers with nonspecific vacuoles in addition to a mild variation in fiber size (Figure B, arrow). No necrotic or regenerating fibers were observed. On periodic acid Schiff (PAS) staining, the glycogen level was found to have increased in scattered fibers. Inclusion bodies were stained only faintly on acid phosphatase [ACP (Figure C)]. A biochemical analysis of the muscle tissue confirmed the diagnosis of Pompe disease, as the α-glucosidase activity in leukocytes was found to be 11.9 nmol/mg protein/hr (control range, 13.1-46.3 nmol/mg protein/hr) and the acid α-glucosidase activity in the muscle was found to be 2.0 nmoles 4 MU/mg/30 min (control range, 14.6±4.8 nmoles 4 MU/mg/30 min).

DNA was extracted from peripheral blood lymphocytes after obtaining the patient's informed consent. Each exon and flanking sequence of the *GAA* gene were amplified via polymerase chain reaction (PCR), and the amplified fragments were directly sequenced. We identified a compound heterozygous mutation, c.1309 C>T (R437C), in exon 8 and a novel compound heterozygous mutation, c.1544 T>A (M515K), in exon 10 (Figure D, E). The patient's father was heterozygous for c.1309 C>T (R437C) in exon 8 (Figure F, G). The patient's mother was heterozygous for c.1544 T>A (M515K) in exon 10 (Figure H, I).

Non invasive positive pressure ventilation (NPPV) was performed only during the night due to the patient's respiratory failure. He received an intravenous infusion of recombinant human acid α-glucosidase (rhGAA; 20 mg/kg body weight) every two weeks. After one year of rhGAA treatment, his muscular strength remained 4/5 on the MRC scale. The FVC remained low at 27.3% of the predicted value for his age. The serum levels of CK and hepatic enzymes decreased. Thereafter, the patient's clinical manifestations did not worsen.

Discussion

We herein report a novel compound heterozygous mutation of the *GAA* gene in the present patient. Currently, 455 variants in the *GAA* gene have been described, 364 of which are considered to be disease-causing mutations (www. pompecenter.nl). The mutations are randomly spread throughout the entire gene and are typically discrete. The primary effect of the residual enzyme activity on the clinical course of Pompe disease can be confirmed (10). The residual activity observed in adult-onset Pompe disease patients correlates with a later age of onset and slower disease progression. As reported in previous studies, the most common c.-32-13 T> G mutation is associated with a milder course, although there is broad variability in the decline in the locomotive and respiratory functions (11). Our patient exhibited slowly progressive proximal muscular weakness and respiratory failure because the enzyme activity was slightly reduced. The residual activity of this enzyme is primarily determined by the severity of the pathogenic mutations in both GAA alleles and is likely controlled by unknown modifying factors.

Respiratory failure as an early symptom of neuromuscular disease is rare; however, it has been previously described, not only in patients with Pompe disease, but also those with motor neuron disease, myasthenia gravis and Werdnig-Hoffmann disease (12). Diagnosing adult-onset Pompe disease is sometimes challenging due to its clinical similarities with muscular dystrophy and the paucity of disease-specific vacuolated fibers in the skeletal muscle pathology. Importantly, 20% of patients with non-classic Pompe disease have a normal muscle glycogen content. Likewise, not all muscle biopsies disclose morphologic abnormalities. In the literature this is most often reported in patients presenting with symptoms after 18 years of age (13). Our patient did not exhibit typical vacuolated fibers, although he did demonstrate unique globular inclusion bodies with ACP activity. The presence of globular inclusions is suggestive of cytoplasmic bodies, which are nonspecific findings reflecting degeneration of the Z-disk in patients with various neuromuscular diseases. Although it remains unclear how ACP-positive globular inclusions are formed, the absence of glycogen in the globular inclusion bodies suggests that they differ from the areas of glycogen accumulation observed in lysosomes (14). The presence of ACP-positive globular inclusion bodies is a hallmark of Pompe disease and a useful diagnostic marker for adult-onset Pompe disease in patients lacking typical vacuolated fibers (14). Since enzyme replacement therapy is effective in adult-onset patients, making an early diagnosis is necessary in order to obtain a better prognosis.

The authors state that they have no Conflict of Interest (COI).

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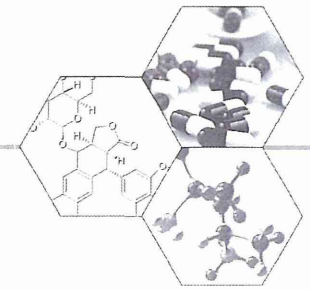

Candidate molecules for chemical chaperone therapy of G_{M1}-gangliosidosis

A growing body of evidence suggests that misfolding of a mutant protein followed by its aggregation or premature degradation in the endoplasmic reticulum is one of the main mechanisms that underlie inherited neurodegenerative diseases, including lysosomal storage diseases. Chemical or pharmacological chaperones are small molecules that bind to and stabilize mutant lysosomal enzyme proteins in the endoplasmic reticulum. A number of chaperone compounds for lysosomal hydrolases have been identified in the last decade. They have gained attention because they can be orally administrated, and also because they can penetrate the blood–brain barrier. In this article, we describe two chaperone candidates for the treatment of G_{M1}-gangliosidosis. We also discuss the future direction of this strategy targeting other lysosomal storage diseases as well as protein misfolding diseases in general.

Protein homeostasis (or proteostasis) in the endoplasmic reticulum (ER) is an essential biological process, which tightly regulates protein synthesis, folding, modification, transport and degradation. When these events are defective or unregulated, disease states, such as cancer, diabetes and neurodegeneration, can ensue, and these are referred to as protein misfolding diseases [1]. Such disorders imply dysfunction of a cellular process either as a result of a toxic gain of function due to protein aggregation, or loss of function due to protein instability, inefficient folding or defective trafficking. Because of recent advancement in understanding molecular pathophysiology, it has become evident that dysfunction and/or aggregation of a mutant misfolding-prone protein is one of the main mechanisms of many neurodegenerative diseases, including Alzheimer's disease, Parkinson's disease, Huntington's disease and lysosomal storage diseases (LSDs) [2,3].

LSDs are a group of inborn errors of metabolism that result from genetic deficiency of lysosomal functions including hydrolases, proteases, lipases, sulfatases or proteins for their synthesis or trafficking [4]. These diseases encompass more than 60 clinically different entities and are characterized by progressive accumulation of undigested substrates in the lysosomes of affected cells. The lysosome is an acidic organelle primarily engaged in degradation of various substrates in diverse processes such as endocytosis, phagocytosis and autophagy. More than half of LSDs exhibit severe progressive neurodegeneration, indicating that neurons are most vulnerable to lysosomal dysfunction, although the biological basis for the selective vulnerability remains unknown [5,6].

In the last few decades, much progress has been made in the development of therapeutic strategies for LSDs. In general, the acid hydrolases are synthesized in the ER, move to the Golgi apparatus, where the mannose-6-phosphate (M6P) recognition marker is attached. This marker is essential for targeting the acid hydrolases to the lysosome via the M6P receptor [7]. A part of newly synthesized enzyme does not bind the M6P receptor in the Golgi apparatus and is secreted to the cell milieu. A portion of the secreted enzyme is taken up via a cell-surface M6P receptor and transported to the lysosomes, where they can carry out their catalytic function. This secretion-uptake pathway is an essential mechanism for the enzyme replacement therapy (ERT) or hematopoietic stem cell transplantation in LSD treatment [8].

ERT that exploits parenteral administration of recombinant enzymes became a reality in the early 1990s. Initially developed for the type 1 Gaucher disease, ERT is now approved for six LSDs (Gaucher disease, Fabry disease, mucopolysaccharidosis type I, II and VI, and Pompe disease), and clinical trials are ongoing in others [9,10]. Efficacy of ERT on visceral symptoms has been confirmed in these diseases, but difficulties have been observed in the effects on neurological symptoms. Although extensive studies have been conducted to explore a way to transduce enzyme proteins into the brain of LSDs, such as modification of the recombinant protein and high-dose intrathecal administration, there has been little success [11–14]. LSDs owing to genetic deficiency

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10.4155/FMC.13.123 © 2013 Future Science Ltd *Future Med. Chem.* (2013) 5(13), 1551–1558 ISSN 1756-8919

of soluble lysosomal enzymes are generally considered as a promising target of gene therapy, and preclinical trials have been conducted for some of these diseases [15]. Unfortunately, its efficacy for the brain pathology has never been proven. Substrate reduction therapy (SRT) has emerged as another therapeutic approach to LSDs including G_{M1}-gangliosidosis [8,16]. The concept of SRT is to ameliorate the accumulation of undigested substrate using inhibitors against an enzyme responsible for substrate synthesis. SRT is now approved for the treatment of two disease entities, Gaucher disease and Niemann–Pick type C, but further evaluation is required to conform its efficacy, particularly on neurological phenotypes [17].

We have been in pursuit of chemical (or pharmacological) chaperone therapy as an alternative therapeutic approach for the brain pathology of LSDs [18]. This strategy employs a small chaperone molecule that has the ability to bind to and stabilize the mutant enzyme. With the help of a chaperone, the mutant enzyme escapes premature degradation in the ER, which is often the reason for reduced enzyme activities in these patients. The mutant enzyme is then transported to the lysosome, where it can exhibit its catalytic activity (**Figure 1**). Ideally, a chaperone should have a higher binding capacity to the mutant enzyme at the neutral condition in the ER than at the acidic condition in the lysosome. As a proof-of-concept, it was shown that 1-deoxygalactonojirimycin (DGJ) could stabilize mutant α-galactosidase and enhance their residual activities in cell derived from patients [19]. DGJ has now come up to Phase III of the clinical study of Fabry disease [20].

Genotype-phenotype relationship of G_{M1}-gangliosidosis

G_{M1}-gangliosidosis is a rare, fatal neurodegenerative LSD caused by mutations of the *GLB1* gene (EC 3.2.1.23) that encodes a lysosomal hydrolase acid β-galactosidase (β-gal) [21]. β-Gal cleaves the terminal β-galactose linkage in ganglioside G_{M1} and other glycoconjugates, and deficiency of this enzyme leads to accumulation of undigested substrates such as G_{M1} and its asialo derivative G_{A1}. The accumulation of these gangliosides is most prominent in the brain. Oligosaccharides derived from keratan sulfate or other glycoproteins also accumulate in visceral organs and are excreted in urine of patients with G_{M1}-gangliosidosis as well as Morquio B disease, another rare systemic bone

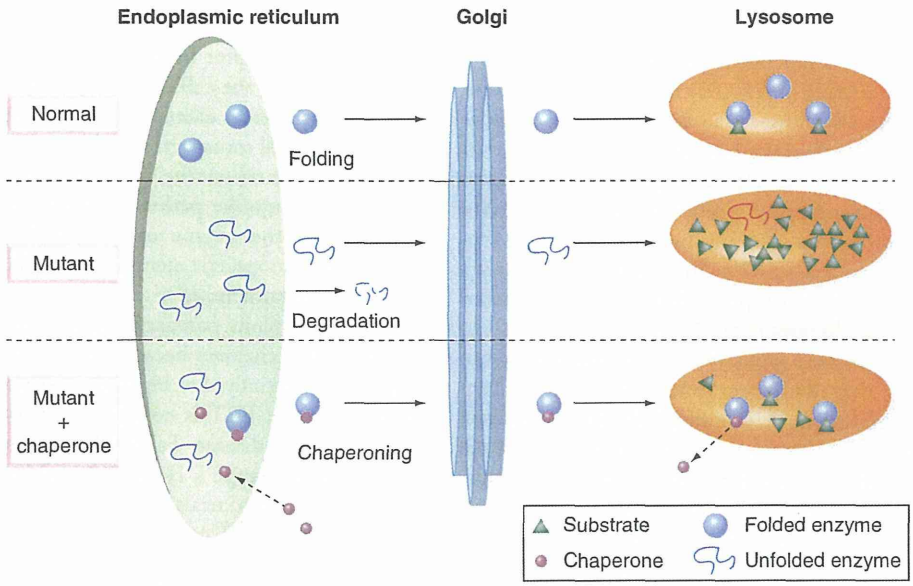

Figure 1. Mechanism of chaperone effect on mutant lysosomal enzyme. Normal lysosomal enzyme is synthesized in the endoplasmic reticulum (ER) and transported to the lysosome through the Golgi apparatus. Most of the mutant enzyme is unstable and rapidly degraded in the ER. A chaperone compound binds to the mutant protein, stabilizes its conformation (chaperoning). The mutant enzyme with chaperone escapes degradation in the ER and is transported to the lysosome, where it can exhibit its catalytic activity.

disease caused by mutations of the *GLB1* gene. The disease phenotype of G_{M1}-gangliosidosis is characterized by severe CNS involvement, visceromegaly and skeletal dysplasia. Three clinical forms (infantile, juvenile and adult/chronic) have been distinguished according to the age of onset and severity, largely determined by residual activities of the mutant enzyme. Until now, more than 150 mutations in the human *GLB1* gene have been identified as disease-causing mutations, and approximately 70% of them are missense mutations [21–23]. Among them, eight types of mutation have been found to be associated with a common disease phenotype in specific ethnic groups; R208C in American patients with infantile form, R482H in Italian patients with infantile form, c.1622–1627insG in Brazilian patients with infantile form, R59H in Argentinean gypsies and Italian patients with infantile form, Y270D in European patients with infantile form, R201C in Japanese patients with juvenile form and I51T in Japanese patients with adult form. In addition, W273L in Caucasian patients is associated with Morquio B disease [22–27].

The human *GLB1* gene, located on 3q21.33, encodes 677 amino acids of β-gal with an N-terminal signal sequence of 23 amino acids [28]. It is transcribed to two alternative splice forms of mRNAs, a major product that encodes the lysosomal β-gal and a minor product that encodes an elastin-binding protein. β-gal is synthesized as an 88 kDa precursor, which is transported to the lysosome, where proteolytic cleavage of its C-terminus yields a 64 kDa mature protein [29]. It then forms a complex with a protective protein (cathepsin A) and neuraminidase. Intracellular trafficking and turnover has been characterized in some forms of the mutants. The enzyme that carries R201C mutation, which is common in juvenile G_{M1}-gangliosidosis, is degraded in the lysosome because of its failure to interact with the protective protein. The enzyme with I51T mutation, that is common in adult G_{M1}-gangliosidosis, is not transported to the lysosome but is secreted to the cell milieu [30]. Recently, the crystal structure of human β-gal in complex with galactose or with DGJ was resolved [31]. It showed that human β-Gal belongs to the glycoside hydrolase (GH) family 35 member of carbohydrate activating enzymes. It also found that the missense mutations that cause infantile G_{M1}-gangliosidosis are segregated in the protein core regions, whereas those with milder forms are located in residues exposed to the solvent, indicating the genotype–phenotype relationship of this disease. When the residual β-gal activities in lysates from cultured skin fibroblasts from patients with the infantile form were measured using 4-methylumbelleferone-labeled artificial substrate, the enzyme activity was generally less than 3% of the control values. The same analysis revealed the residual activities of 3–6% in those with the juvenile form, and up to 9% in those with the adult form [18,21]. These results suggested that 10% of the normal enzyme activity would be enough to reduce or prevent the lysosomal storage of G_{M1}.

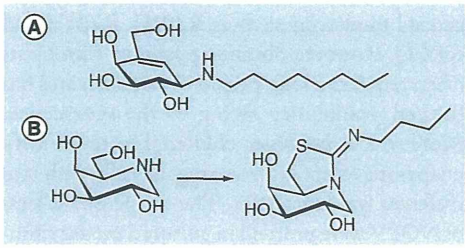

Figure 2. Chaperone candidates for human β-gal. (A) *N*-octyl-4–4epi-β-valienamine [20].
(B) 1-deoxygalactonojirimycin and its derivative 5*N*,6*S*-(*N*′-butyliminomethylidene)-6-thio-DGJ [32].

N-octyl-4-epi-β-valienamine as a pioneer chaperone compound for mutant β-gal

Initial studies on the intracellular processing of the mutant enzyme showed that thiol cysteine protease inhibitors such as E-64 enhanced residual activities of several types of mutant β-gal [30]. These findings encouraged us to search for other compounds that can specifically enhance the activity of the mutant β-gal in patients' cells. These efforts led to the identification of *N*-octyl-4-epi-β-valienamine (NOEV) as a potent chaperone molecule (**FIGURE 2A**) [32–34]. It has a molecular weight of 287.40 and its hydrochloride salt is freely soluble in water. NOEV inhibited β-gal activity in lysates from human fibroblasts with an IC_{50} value of 0.125 μM at pH 5 [35,36]. It attenuated thermodenaturation of β-gal protein *in vitro*. In addition to these properties *in vitro*, a cell-based assay is needed to test the ability of the compound to penetrate both the plasma and ER membranes to allow binding to the mutant enzyme protein. Screening of the cultured fibroblasts from patients revealed positive responses in approximately one-third of the cell lines [35]. Those with positive responses were mainly from patients with juvenile and adult forms and

carried mutations such as R201C, R201H and R457Q. However, obtaining primary fibroblasts often requires a long period to establish and has limited availability owing to the senescence. Moreover, it becomes challenging to identify chaperone effect in heterozygous cells with two different mutant alleles. The chaperone effects of NOEV were profiled in cultured mouse fibroblasts transiently expressing human recombinant enzyme and this analysis demonstrated a positive response of 22 out of 94 β-gal mutants [36]. It should be noted that relatively high residual activities were detected in the lysates from cells expressing several mutant β-gal, this is probably because of excess of gene products [26,36]. Therefore, analyses with both human skin fibroblasts and transfected cells are necessary to obtain a prospective chaperone profiling.

Subsequent assessments of chaperone effect *in vivo* warrant its potential for oral administration and CNS distribution. We developed two strains of model mice for G_{M1}-gangliosidosis, one is β-gal knockout mice and the other is R201C transgenic mice that express human mutant R201C on the null-basis of the mouse enzyme (R201C mice) [34,37]. These mice recapitulate most of the features of the brain pathology of the human disease including large G_{M1} and G_{A1} deposits in neurons. R201C mice were used as an important tool to evaluate chaperone effect *in vivo*. Oral administration of NOEV for 1 week resulted in significant enhancement of residual activities in tissues of R201C mice, including the brain [34]. It also attenuated neuronal storage of G_{M1} and G_{A1} in neurons in the cortex and brainstem. These findings suggested the ability of NOEV to cross the blood–brain barrier and its effects on CNS pathology. Administration of NOEV also suppressed impairment of tropomyosin receptor kinase signaling in R201C mice [38]. Long-term administration study revealed the ability of NOEV to attenuate neurological manifestations of R201C mice and to prolong the life span [39]. Pharmacokinetic analyses showed that NOEV was rapidly absorbed by the intestine, and was excreted in urine in mice [40].

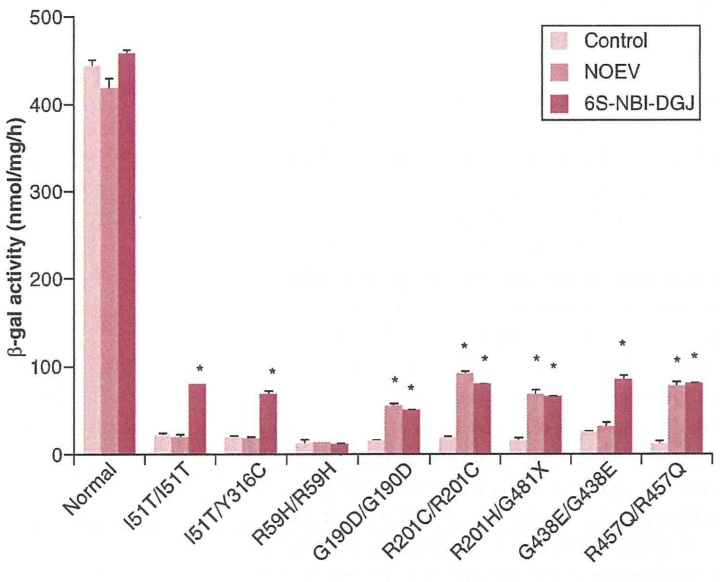

Figure 3. Chaperone effects of NOEV/6S-NBI-DGJ on cultured human fibroblasts with G_{M1}-gangliosidosis. Fibroblasts were cultured in the absence or presence of chaperone (2 μM NOEV or 80 μM 6S-NBI-DGJ) for 96 h, and β-gal activity in lysates were measured using 4-methylumbeliferone-labeled substrate. *P <0.05, statistically different from the values of the untreated controls.

A novel derivative of 1-deoxygalactonojirimycin, 6S-NBI-DGJ, as a chaperone for mutant β-gal

We designed a new family of glycosidase ligands termed sp^2-iminosugars to develop more potent and selective chaperone compounds for LSDs. These compounds are characterized by a glycone-type moiety that mimics the sugar substrate of the enzyme and a hydrophobic aglycone substituent that interacts with amino acids at the vicinity of the active site. The overall structure has amphiphilic character, imparting their ability to cross biological membranes [41]. Structure–activity relationship and x-ray studies suggested that the nature and orientation of the aglycon segment is critical for the enzyme selectivity [42,43]. Recently, we have succeeded in synthesis of selective sp^2-iminosugar inhibitors against β-gal. These inhibitors are characterized by a rigid bicyclic core derived from DGJ. Of the two compounds that showed specific inhibitory activities against human β-gal *in vitro*, we focused on 5*N*,6*S*-(*N*′-butyliminomethylidene)-6-thio-DGJ (6S-NBI-DGJ also referred to as MTD118, a code name of this compound [44–46]), because it was less toxic to cultured fibroblasts (**Figure 2B**) [44]. *In vitro* analyses showed that 6S-NBI-DGJ specifically inhibited human β-gal with an IC_{50} value of 32 μM, which was relatively higher than the IC_{50} value of NOEV. It protected human β-gal from heat-induced degradation [45]. Computational analysis confirmed that the rigid glycone bicyclic core of this compound binds to the active site of human β-gal. When tested for chaperone effects in

cultured fibroblasts from patients with G_{M1}-gangliosidosis, 6S-NBI-DGJ significantly increased the activities in cells with mutations such as R201C and R458Q. Of note, 6S-NBI-DGJ enhanced β-gal activities in cells with I51T and G438E mutations, neither of which responded to NOEV (**Figure 3**). Profiling of 6S-NBI-DGJ effects on human β-gal mutants expressed in COS7 cells revealed positive responses of 24 types out of 88. Up to 16 out of these 24 mutants were unresponsive to NOEV (**Table 1**). Noticeably, the mutations with positive responses to 6S-NBI-DGJ included four common mutations, I51T, R201C, R208C and R482H, suggesting its beneficial effects in a large number of G_{M1}-gangliosidosis patients. Oral administration of 6S-NBI-DGJ enhanced β-gal activities in the cortex and brainstem of R201C mice. It also reduced accumulation of G_{M1} as well as autophagy substrates, LC3-II and p62 proteins, which are potential culprits for neurodegeneration in this mice brain [38,47]. Since these results indicate that 6S-NBI-DGJ is a promising chaperone compound for G_{M1}-gangliosidosis, further studies are warranted such as long-term administration and pharmacokinetic analysis.

Table 1. Responses of human β-gal mutants to NOEV and/or 6S-NBI-DGJ.

NOEV	6S-NBI-DGJ	Mutation
+	+	G190D, R201C, R201H, V216A, D332N, Y444C, R457Q, R590H (n = 8)
+	-	S54N, Y83C, E131K, L173P, Y199C, R201Y, Q255H, N318H, Y324C, D332E, N484K, G494S, R590C (n = 14)
-	+	I51T, R148T, L155R, R208C, D214Y, C230Y, L264S, N266S, W273R, K346N, S434L, G438E, R482H, D491Y, E632G, D640E (n = 16)
-	-	R49C, S541I, R59C, R68W, T82M, F107L, R121S, G123R, G134V, P136S, R148C, R148S, D151V, W161G, L162S, G178R, I181K, V240M, R263S, Y270D, G272D, W273L, H281Y, Y361C, T329A, Y333H, Y347C, R351X, Q408P, T420K, T420P, L422R, V439G, D441N, R442Q, D448V, R457X, M480V, R482C, D491N, G494C, T500A, W509C, P594L, G554E, K578R, G579D, Y591N, Y591C (n = 50)

Chaperone effects were tested using transfected cultured COS7 cells transiently transfected with human mutant β-gal.

Other chaperone candidates for human β-gal

Chaperone candidates for human β-gal were also explored in the derivatives of DGJ (**Table 2**) [48–50]. Recently, Rigat *et al.* reported that *N*-nonyl-DGJ (NN-DGJ) enhanced mutant β-gal activity in feline G_{M1}-gangliosidosis fibroblasts. This naturally occurring feline G_{M1}-gangliosidosis is likely to be a good model of human juvenile form and is a suitable model for the evaluation of *in vivo* chaperone effect in the future study [50]. There is compelling evidence indicating that drug repositioning or repurposing could accelerate drug discovery including chaperones for LSDs [51–55]. We have identified several chaperone candidates for β-gal in a library of chemical compounds approved for human administration, although they enhanced human mutant β-gal activities only to the modest levels in patients' fibroblasts [Higaki et al., Unpublished Data].

Molecular mechanisms have been investigated for LSD chaperones [56–58]. Most chaperones identified are directed to the active site of the targeted enzyme and are intrinsically its competitive inhibitors. Since, ideally, chaperone should not inhibit the enzyme, novel chaperones are now being developed such as those directed to nonactive [59–61]. The systems biology approach based on the crystal structure of human β-gal will enable rational design of novel compounds. Moreover, new technologies, such as stem cell technology with induced pluripotent stem cells or next generation sequencing will provide attractive tools to evaluate this therapy [62].

Future perspective

Preclinical evaluation of NOEV and 6S-NBI-DJG is in progress in Japan [46]. Promising preclinical results have been obtained for some chaperone compounds in the treatment of several other LSDs. DGJ (or migalstat, AT1001) is currently in the Phase III study for the treatment of Fabry disease [63]. Chaperones for Gaucher and Pompe diseases have also been tested in preclinical studies. In addition, synergetic effect of

Table 2. Chaperone compounds for human mutant β-gal.

Compounds	Responsive mutations	BBB permeability	Ref.
NOEV	22 out of 88 missense mutations (including R201C and R457Q)	Yes (model mice)	[32–36,38–40]
DGJ, NB-DGJ	R201C, R201H, R457Q	ND	[32]
Galactose	R442Q	ND	[72]
DLHex-DGJ	R201C, R201H, C230R, G428E	ND	[48]
Fluorous iminoalditols	R148S, R201C, D332N	ND	[49]
NN-DNJ	R201H, W509C, R483P (feline GLB1)	ND	[50]
6S-NBI-DGJ (MTD118)	24 out of 88 missense mutations (including I51T, R201C, G438E)	Yes (model mice)	[44–46]

BBB: Blood–brain barrier; ND: Not determined.