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PREDICTION OF RESPONSE OF MUTATED α-GALACTOSIDASE A TO A PHARMACOLOGICAL CHAPERONE

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Abstract

Objective: To examine the relationship between types and locations of mutations of the enzyme α-galactosidase (Gal) A in Fabry disease and the response to the pharmacological chaperone 1-deoxygalactonojirimycin (DGJ).

Methods: T-cells grown from normal individuals or from patients with Fabry disease were tested for response to treatment with DGJ by increased activity of α-Gal A.

Results: Cells from normal controls responded with a 28% increase in α–Gal A activity whereas response in Fabry individuals was mutation dependent ranging from no increase to fully normal activity. Nine truncation mutations (all non-responsive) and 31 missense mutations were tested. Three groups of missense mutations were categorized: responders with activity more than 25% of normal, non-responders, with less than 7% and an intermediate response group. In normal cells and in responders an increase in the mature lysosomal form of α-Gal A was observed after DGJ treatment. Non-responders showed little or no protein with or without DGJ. The intermediate response group showed an increase in band intensity but incomplete processing of the enzyme to the mature form.

Conclusions: Mapping the missense mutations to the structure of α–Gal A identified several factors that may influence response. Mutations in regions that are not in α-helix or β-sheets, nor involved in disulfide bonds nor with an identified functional or structural role were more likely to respond. However, predictability is not precise and testing each mutation for response to pharmacological chaperone therapy is necessary for Fabry disease and related lysosomal storage disorders.

Keywords

pharmacological chaperone; Fabry disease; lysosomal storage disorders; alpha-galactosidase A; molecular chaperone; molecular mechanisms of pharmacological action

Introduction

Fabry disease (Mendelian Inheritance in Man 301500) [1] is the second most prevalent hereditary metabolic storage disorder of humans. The disease is a multi-systemic disorder characterized by kidney and heart pathology secondary to a vasculopathy and small fiber neuropathy. The most common causes of death among Fabry patients are kidney failure or premature stroke [2]. The prevalence of biologically significant mutations in α-galactosidase (Gal) A from young people with cryptogenic strokes has been estimated to be 4%,

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corresponding to approximately 1.2% of all stroke patients [3;4]. Globotriaosylceramide (Gb3) storage, identified from a specimen of a patient with Fabry disease [5], subsequently shown to be primarily lysosomal, led to the demonstration of the defective enzyme α-Gal A as the cause for this disease [6]. More than 417 different mutant alleles have been identified [7]. Diagnosis is usually made by fluorimetric enzyme assay frequently followed by complete genotyping [8].

Undigested Gb3 accumulates in the vascular endothelium, the kidney and the heart in affected individuals. Enzyme replacement therapy has been approved as a treatment for Fabry disease; however, inadequate distribution of administered enzyme to various organs and tissues due to inequities in flow dynamics and receptor distribution in various tissues may limit its effectiveness [9]. Pharmacological chaperone therapy offers a promising alternative that would increase enzyme activity in all tissues and cell-types independently of these variables.

α-Gal A synthesis follows the secretory glycoprotein pathway common to most soluble lysosomal enzymes [10;11]. A 31 amino acid N-terminal signal peptide is removed as the protein enters the endoplasmic reticulum. Oligosaccharide transfer and glycan modification in the Golgi results in the 46 kDa mature lysosomal form of α-Gal A [12]. Fibroblasts from classic Fabry patients have no or low α-Gal A activity whereas patients with the late onset or cardiac phenotypes may have somewhat higher residual α-Gal A activity and may have slower processing of the mutant α-Gal A compared to the normal enzyme [11].

X-ray crystallography [13] resolved to 3.25 Å demonstrates the α-Gal A to be a glycoprotein homodimer, each monomer containing two domains. Domain 1, comprising amino acids 32 to 330, including the active site is a $(\beta/\alpha)_8$ barrel structure. Domain 2 containing amino acids 331 to 429, is composed of 8 antiparallel β sheets and is remote from the active site. Despite the absence of any known functional role, many mutations in domain 2 also result in the loss of α-Gal A activity and the Fabry phenotype [14].

Stabilization of α-Gal A by the use of sub-inhibitory concentrations of 1-deoxygalactonojirimycin (DGJ) forms the basis of pharmacological chaperone therapy (PCT) [12;15;16]. At high concentrations DGJ is an active site-specific inhibitor of α-Gal A; however, at lower concentrations DGJ has been demonstrated to stabilize many mutated forms of α-Gal A [16] resulting in higher total cellular activity despite any change in specific activity as a result of the altered configuration of the mutated enzyme. We have previously reported an assay system that demonstrates a positive response to DGJ following testing of homogeneous populations of T-cells derived from individual Fabry patients[16].

Our previous report of drug response for 11 mutations has been extended here to include 29 additional mutations. We have identified an “intermediate” response group, and discuss these results with reference to the locations of the mutations within the three-dimensional α-Gal A structure. We have mapped the sites of the mutations for the three categories of responders to highlight the importance of the location of individual mutations within the 3-dimensional structure of α-Gal A in predicting the probability that any mutation might respond to the drug. Here we identify three different response categories representing various levels of enzyme activity and differing in physical properties as demonstrated by Western blots.

Experimental Procedures

Materials

Reagents were purchased from the following sources: DGJ (Cambridge Major Laboratories, Inc., Germantown, WI); 4-methylumbelliferyl-α-D-galactopyranoside (Research Products International, Mount Prospect, IL); N-acetylgalactosamine (Sigma Chemical Co., St. Louis,

MO); RPMI-1640 medium, Dulbecco's Modified Eagle's medium (DMEM), and Phytohemagglutinin M (Life Technology, Gaithersburg, MD); Cosmic calf serum (CCS) (Hyclone Laboratories, Logan, UT); Interleukin-2 (IL-2) (PreProTECH, Rocky Hill, NJ).

Cell Cultures and DGJ Treatment

Fresh blood from hemizygous male Fabry patients and normal controls was collected and cultured as has been previously reported [16]. Lymphocytes were counted and ~2.5×10^6 cells were grown in the absence or presence of 20 μM DGJ for 3 days prior to measurement of enzymatic activity.

α-Gal A Assays

At the end of the DGJ treatment period, cells were rinsed twice with phosphate-buffered saline, centrifuged, homogenized and α-Gal A activity was assayed fluorimetrically as described [17] with modifications necessary for the 96 well microplates [16]. Protein was determined using a Micro BCA Protein Assay kit (Pierce, Rockford, IL) with bovine serum albumin (BSA) as a standard as described [18]. For each patient sample at least three normal samples were tested concurrently. Different levels of response from various mutations were calculated by comparison with the mean value of normal α-Gal A activity tested on the same day.

Western Blot Analysis

For gel electrophoresis prior to Western blotting, proteins were separated by using Novex Tris-glycine native or SDS-PAGE in 8-16% gradient gels from Invitrogen. Western blots were developed using rabbit polyclonal antibody against α-Gal A was performed as described previously [19].

Protein Structure Visualization

Protein sequences were downloaded from NCBI PubMed site and visualized by the application, Cn3D, from the same site: http://www.ncbi.nlm.nih.gov/Structure/CN3D/cn3d.shtml

Results

The mean α-Gal A activity in T-cells from normal individuals cultured in the absence or presence of DGJ was determined using a number of independently derived cultures obtained from 24 healthy control subjects. α-Gal A enzyme activity in normal controls T-cells following DGJ treatment was 128 % (n=162, $P < 1 \times 10^{-8}$) of the mean normal activity in the absence of DGJ. Included in this group were cultures from 7 females and 17 males. There were no gender specific differences in α-Gal A response to DGJ (Table 1). In some instances the residual enzyme activity in cells derived from patients with Fabry disease were near zero, resulting in excessive variability in the response ratio (RR) calculated between activity in the presence of DGJ and that in the absence of DGJ. Therefore, we elected to use percent of normal activity in the presence of DGJ compared with that in untreated normal cells as an indicator of response. This serves the dual purpose of providing a reference point for comparison with normal endogenous activity.

Table 1 reports response to DGJ of 40 mutations in the gene for α-Gal A in 55 patients. Eleven of these were previously reported and 29 mutations are new. Four of the previously reported mutations have been repeated and confirmed with cultures obtained from additional patients bearing the same mutation. Nine mutations (one splice mutation, 4 frameshift, one large (26 bp) deletion and 3 nonsense mutations) cause truncation of the protein and are characteristic of patients with the classic presentation of Fabry disease. These mutations result in polypeptide chains missing from 83 to 419 residues of the wild-type polypeptide chain, the effect of which

results in an absence of response to DGJ as determined by enzyme measurement and confirmed by the absence of additional protein on Western blots (data not shown). Quantitative results for S201F, P205T, and N272K, and 256del1 were reported with only a single experiment for each. Repeat assays were qualitatively similar but failed to yield quantitative results due to low cell numbers. Similarly R356W#1 and del26bp21 are also only reported once, but additional subjects bearing the same mutation are also reported.

Historical data on α-Gal A activity from peripheral white blood cells has established a maximum value for an affected hemizygous male Fabry patient (n= 102) of 12% of normals (Jane Quirk, Unpublished). Thus the various mutations reported here (Table 1) were assigned to three categories: Responders (R) with increases in enzyme activity > 25 % of normal include 14 mutations: T41I, M51K, A97V, R112C, R112H, A143T, S201F, P205T, N215S, P259R, F295C, L300P, R301Q, and G328A (Tables 1 and 2). The 14 mutations C94S, G128E, G132R, A143P, H225R, R227Q, W236R, G261D, G271C, N272K, W287C, R356W, ΔE358, and L415P were non-responders (NR) with activity < 7% of normal. Three mutations were in the intermediate group (IR) between 7% and 25% of normal: R49C, Y207S and S276G.

Different individuals with the identical mutation, either from the same family or from unrelated patients, were qualitatively consistent (Table 1). Among the responders, two patients were tested with each of the mutations T41I, R112C, A143T, and P259R, and three patients each with the R112H and R301Q mutations. Although untreated enzyme activity levels vary and the percentage change after treatment with DGJ varies, increased α-Gal A activity to more than 25% of normal was uniformly observed. This is also the case for the single intermediate responder (S276G) for which two patients were available for testing. Two individuals for each of the G128E, A143P, G271C, W287C, R356W, and del26bp21 mutations (NR) were available for testing and were equally non-responsive. These findings demonstrate that increases in α-Gal A activity by DGJ depends on the *α-Gal A* genotype.

Cells obtained from a single individual with the mutation R49C demonstrate increased α-Gal A activity in the intermediate response range from 2.5 % to 11 % (n=9, $P < 0.01$) of normal (Table 1). Varying the DGJ concentration demonstrated that for R49C the highest level of response was obtained using 20 μM of DGJ (data not shown) similar to the results for the responders [16].

Increased activity of α-Gal A following DGJ treatment is also consistent with the increased intensity of α-Gal A specific bands by Western blot [16]. Using extracts from normal cells and cells representing each of the three different response groups, T41I (R), R49C (IR), and R356W (NR), demonstrates differences in the distribution of molecular weights for the α-Gal A produced in the absence or presence of DGJ for each of the response groups. In extracts from normal cells, a small amount of the 50 kDa precursor form of α-Gal A was seen with a more intense 46 kDa band representing the mature, fully processed lysosomal species [11] (Fig. 1, lanes 1 and 2). In the absence of DGJ the responders and intermediate response groups, T41I and R49C, contain primarily the 50 kDa immature form of α-Gal A, (Fig. 1, lanes 3 and 5). After growth in DGJ for 3 days, both the responders and the intermediate group demonstrate an increase in the intensity of the 46 kDa mature enzyme although the band is more intense in the responders than in the intermediate group (Fig. 1, lanes 4 and 6) [11]. Cells from normal individuals grown with DGJ show greater than a 5-fold increase in band intensity but only 118 to 133% of the control activity. This discrepancy is more pronounced if band intensity is compared enzyme activities in normals and in the responders (e.g. T41I). These results may indicate some inhibitory effect of residual DGJ at 20 μM on normal α-Gal A activity masking the full effect on activity. In the non-responders, e.g. R356W, no protein bands corresponding to α-Gal A were detectable in the absence or presence of DGJ (data not shown), except by use of a longer exposure which permits detection of the 50 kDa band (Fig. 1, lane 7 and 8). We

conclude that α-Gal A produced by the R356W mutation is present at a low concentration but is not stabilized in the presence of 20 μM DGJ. Additional samples from other responders showed a consistent increase in the intensity of the 46 kDa form of α-Gal A following DGJ treatment. No α-Gal A specific bands were found in any of the non-responder samples (data not shown).

Discussion

Using a T-cell based system for the determination of the response of catalytic activity to DGJ we report results for 40 different mutations of the *α-Gal A* gene. Since the response to the pharmacological chaperone is expected to be a property of the individual mutation, multiple individuals with the same mutation resulted in qualitatively similar results.

Predicting clinical efficacy of chaperone therapy for patients with Fabry disease is imprecise. However, we suggest here that those individuals in the non-responders group, i.e. with mutations achieving a level of activity in the presence of DGJ at or below that of affected individuals with Fabry disease may be unlikely to benefit from this therapy. Responders, i.e. individuals for whom enzyme activity can be increased to twice the affected level, are more likely to develop sufficient activity for a strong therapeutic response. Patients in the intermediate response class may also benefit from the demonstrated increases in enzyme activity, however, the outcome is less certain and needs to be tested in controlled clinical trials. Finally, the increase in activity seen for normal individuals seen by us and by others [20] may be an indication that chaperone therapy may also be a useful therapy for heterozygotes.

Our conclusions are based on data generated using cells derived from individual patients. Some investigators have performed similar testing using transient expression using strong viral promoters in COS-7 cells [20;21]) which may result in artifacts due to overexpression of the mutant proteins and even near normal enzyme levels in the absence of the chaperone[16;20]. The elevated activity in the absence of chaperone compels the authors to use the enhancement ratio (response ratio) as a measure of response which may not represent the true potential efficacy *in vivo.* A response ratio of two, i.e. the doubling of enzyme activity, may be an effective enzyme level if the starting activity is quite high but may still be inadequate for most patients for whom endogenous enzyme activity is less than 10% of normal. Despite this concern, Shimotori et al. [20] have been able to demonstrate definitive increases in enzyme activity meeting our criteria for positive responders for the mutations R112H, P205T, G260A, K208N, Q312R and L403S and one mutation, M42V in the intermediate response group. One additional mutation E66Q reported by these investigators reaches 60% of normal but also shows unusually high activity in the absence of DGJ.

The nature of each mutation, i.e changes in polarity, charge, and side chain (length, surface area and volume), may all influence the nature of the response to the pharmacological chaperone but each must also be considered individually with respect to the location within the molecular structure. For instance, comparing A143T and A143P, the former is a non-conservative change (from a non-polar to a polar residue) with only one carbon atom increase in side chain length that responds positively to DGJ. In contrast, A143P is a conservative (non-polar to non-polar) substitution with a similar increase in side chain volume and is a non-responder. The introduction of a significant structural change such as a turn forced by the Pro substitution may be more significant than the small change in hydrophobicity as a result of the addition of the polar Thr residue.

Responders appear to be more likely to be conservative with respect to size. Overall 6 of 8 mutations that resulted in a significant increase in size (by 3 or more side chain atoms), were non-responders and 15 of 23 that were smaller or unchanged showed a positive response to the

drug. Thus, absence of steric influence may contribute to a positive response, but the type of mutation does not appear to be the sole determinant for response.

Mutations in the interdomain, dimer interfaces, and catalytic residues are less likely to be fully responsive i.e. 9 of 10 are either non-responders or intermediate responders (Table 2). The absence of response with mutations R356W and ΔE358 support the need to preserve the interdomain interface. R356 plays an essential role in stabilizing the interdomain interface of α-Gal A as does E358 and W236 [13] (Fig. 2a). Disruption of these bonds at the interdomain interface by mutations of either Domain 1 (W236R) or Domain 2 (R356W and ΔE358) results in the degradation of α-Gal A that cannot be rescued by DGJ treatment. In addition to the effect of ΔE358 on the disruption of the interdomain interface, deletion of a single amino acid from a protein structure changes the register of the peptide chain and may result in significant changes in the tertiary structure remote from the site of the mutation. Yam et. al.[22], using a different assay system based on a reduction of storage of Gb3 in fibroblasts, have reported that the activity of the truncated mutation Q357X responds to the presence of DGJ. Q357X would be expected to eliminate E358 and all downstream amino acids, a truncation that we would not expect to produce an enzyme responsive to DGJ treatment.

Although the intact structure of the dimer interface is clearly important for enzyme activity, a group of mutations located at or near the dimer interface [13] vary in their response to DGJ. M51K is a responder, while R49C and S276G are intermediate, and G271C and N272K are non-responders. Proximity to the active site as in M51K may explain the greater positive influence of the chaperone on activity in this mutation.

R227Q, an active site residue, and C94S (adjacent to two consecutive catalytic residues) are non-responders, however, Y207S, also at the active site, is in the intermediate response group. The loss of the polar interaction of R227 with the 2-hydroxyl group of both substrate and inhibitor[21] prevents a positive response to DGJ (Fig. 2c). Disruption of the disulphide bond by the C94S mutation, leads to a significant structural change, possibly also affecting the H-bonding of the active site aspartic acid residues D92 and D93, with DGJ resulting in the absence of response. Although Y207S (Fig. 2c) causes a change in an active site residue, the mutation results in a smaller residue, retention of the polar hydroxyl group results in an intermediate response.

Without including any other structural or functional considerations, we found that that 9 of the 14 non-responders occur within α-helical regions or β-sheets (Fig. 3 and Table 2). Additionally, 12 of the 17 mutations that are responders or intermediate responders are Non-α and Non-β (Table 2). Fig. 3 shows a 3 dimensional representation of α-Gal A with the location of mutations for both responders (Fig. 3a) and non-responders (Fig. 3b). Examining all regions of α-helices, β-sheets, interdomain, dimer interfaces, or active sites, termed collectively as structurally significant elements (SSE), we find 9 of 21 are responders or intermediate and 12 of 21 are non-responders. Eight of the ten mutations that are not involved in any of the above mentioned SSE are responders. The preponderance of responders in areas of the molecule with less restricted geometry suggests that the intervening sequences have more structural freedom and that mutations in these regions are less likely to result in a non-recoverable effect on the protein. Thus, mutations from the less structurally constrained areas may be more amenable to the stabilizing force provided by the pharmacological chaperone.

The exceptions to this generalization are of interest. The mutations T41I, S201F, N215S, F295C, and G328A, each of which occurs within the α-helices and β-sheets but have no other functional or structural role (Table 1), are responsive to the chaperone. In addition, T41I, N215S, F295C and G328A are all conservative mutations with respect to hydrophobicity and polarity. Although S201F and T41I each result in loss of enzyme activity due to the introduction

of a bulkier side chain, the proximity to the active site may allow the chaperone to counterbalance and stabilize the enzyme. Only two non-responders have no structural or functional roles. A143P introduces a severe turn in the structure as previously discussed and Gly128 occurs at a restricted psi-phi turn with little room for any increase in size of the side chain. The failure to respond in each of these mutations may thus be explained as interference with nearby important structural elements. Just as the positive response of the M51K, T41I and S201F mutations may be partially attributed to the proximity to the active site, mutations that are remote from the active site may be less likely to respond to pharmacological chaperones. The failure to respond for G128E may also be primarily due to the distance, more than 25 Å, from the substrate/chaperone binding site. L415P is unchanged in size, polarity or charge but is located in Domain 2 more than 25 Å away far from the active site and is non-responsive.

Fabry disease is an important risk factor for stroke[4] and attempts at enzyme replacement therapy have not decreased the incidence of stroke. The current study provides criteria for screening and inclusion of patients in clinical trials for pharmacologic chaperone therapy. Our observations of differing levels of response in various genotypes in combination with mapping of the non-responders onto the crystal structure of α-Gal A suggest that the response of mutated α-Gal A activity to DGJ is not easily predicted but may be more likely to occur in mutations that are located in regions outside of the α-helical regions or β-sheets in the protein.

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REFERENCES

1. McKusick VA. OMIM - Online Mendelian Inheritance in Man.
2. Desnick, RJ.; Ioannou, YA.; Eng, CM. α-Galactosidase A Deficiency: Fabry Disease. In: Scriver, CR.; Beaudet, AL.; Valle, D.; Sly, WS.; Vogelstein, B.; Childs, B.; Kinzler, KW., editors. The metabolic and molecular bases of inherited disease. McGraw-Hill; New York: 2001. p. 3733-3771.
3. Rolfs A, Bottcher T, Zschiesche M, Morris P, Winchester B, Bauer P, et al. Prevalence of Fabry disease in patients with cryptogenic stroke: a prospective study. Lancet 2005;366:1794–1796. [PubMed: 16298216]
4. Schiffmann R, Ries M. Fabry's disease--an important risk factor for stroke. Lancet 2005;366:1754–1756. [PubMed: 16298202]
5. Sweeley CC, Klionsky B. Fabry's Disease: Classification as a sphingolipidosis and partial characterization of a novel glycolipid. J Biol Chem 1963;238:3148–3150. [PubMed: 14081947]
6. Brady RO, Gal AE, Bradley RM, Martensson E, Warshaw AL, Laster L. Enzymatic defect in Fabry's disease. Ceramidetrihexosidase deficiency. N Engl J Med 1967;276:1163–1167. [PubMed: 6023233]
7. Cooper, DN.; Ball, EV.; Stenson, PD.; Phillips, AD.; Howells, K.; Mort, ME. The Human Gene Mutation Database at the Institute of Medical Genetics in Cardiff. http://www.hgmd.org/
8. Bishop DF, Kornreich R, Desnick RJ. Structural organization of the human alpha-galactosidase A gene: further evidence for the absence of a 3′ untranslated region. Proc Natl Acad Sci U S A 1988;85:3903–3907. [PubMed: 2836863]
9. Murray GJ, Anver MR, Kennedy MA, Quirk JM, Schiffmann R. Cellular and tissue distribution of intravenously administered agalsidase alfa. Mol Genet Metab 2007;90:307–312. [PubMed: 17188539]
10. Erickson AH, Ginns EI, Barranger JA. Biosynthesis of the lysosomal enzyme glucocerebrosidase. J Biol Chem 1985;260:14319–14324. [PubMed: 3932353]

11. Lemansky P, Bishop DF, Desnick RJ, Hasilik A, von Figura K. Synthesis and processing of alpha-galactosidase A in human fibroblasts. Evidence for different mutations in Fabry disease. J Biol Chem 1987;262:2062–2065. [PubMed: 3029062]
12. Fan JQ, Ishii S, Asano N, Suzuki Y. Accelerated transport and maturation of lysosomal alpha-galactosidase A in Fabry lymphoblasts by an enzyme inhibitor. Nat Med 1999;5:112–115. [PubMed: 9883849]
13. Garman SC, Garboczi DN. The molecular defect leading to Fabry disease: structure of human alpha-galactosidase. J Mol Biol 2004;337:319–335. [PubMed: 15003450]
14. Garman SC, Garboczi DN. Structural basis of Fabry disease. Mol Genet Metab 2002;77:3–11. [PubMed: 12359124]
15. Fan JQ. A contradictory treatment for lysosomal storage disorders: inhibitors enhance mutant enzyme activity. Trends Pharmacol Sci 2003;24:355–360. [PubMed: 12871668]
16. Shin SH, Murray GJ, Kluepfel-Stahl S, Cooney AM, Quirk JM, Schiffmann R, Brady RO, Kaneski CR. Screening for pharmacological chaperones in Fabry disease. Biochem Biophys Res Commun 2007;359:168–173. [PubMed: 17532296]
17. Kusiak JW, Quirk JM, Brady RO. Purification and properties of the two major isozymes of alpha-galactosidase from human placenta. J Biol Chem 1978;253:184–190. [PubMed: 201618]
18. Smith PK, Krohn RI, Hermanson GT, Mallia AK, Gartner FH, Provenzano MD, Fujimoto EK, Goeke NM, Olson BJ, Klenk DC. Measurement of protein using bicinchoninic acid. Anal Biochem 1985;150:76–85. [PubMed: 3843705]
19. Park J, Murray GJ, Limaye A, Quirk JM, Gelderman MP, Brady RO, Qasba P. Long-term correction of globotriaosylceramide storage in Fabry mice by recombinant adeno-associated virus-mediated gene transfer. Proc Natl Acad Sci U S A 2003;100:3450–3454. [PubMed: 12624185]
20. Shimotori M, Maruyama H, Nakamura G, Suyama T, Sakamoto F, Itoh M, et al. Novel mutations of the GLA gene in Japanese patients with Fabry disease and their functional characterization by active site specific chaperone. Human Mutation 2008;29:331. [PubMed: 18205205]
21. Ishii S, Chang HH, Kawasaki K, Yasuda K, Wu HL, Garman SC, Fan JQ. Mutant alpha-galactosidase A enzymes identified in Fabry disease patients with residual enzyme activity: biochemical characterization and restoration of normal intracellular processing by 1-deoxygalactonojirimycin. Biochem J 2007;406:285–295. [PubMed: 17555407]
22. Yam GH, Zuber C, Roth J. A synthetic chaperone corrects the trafficking defect and disease phenotype in a protein misfolding disorder. Faseb J 2005;19:12–18. [PubMed: 15629890]

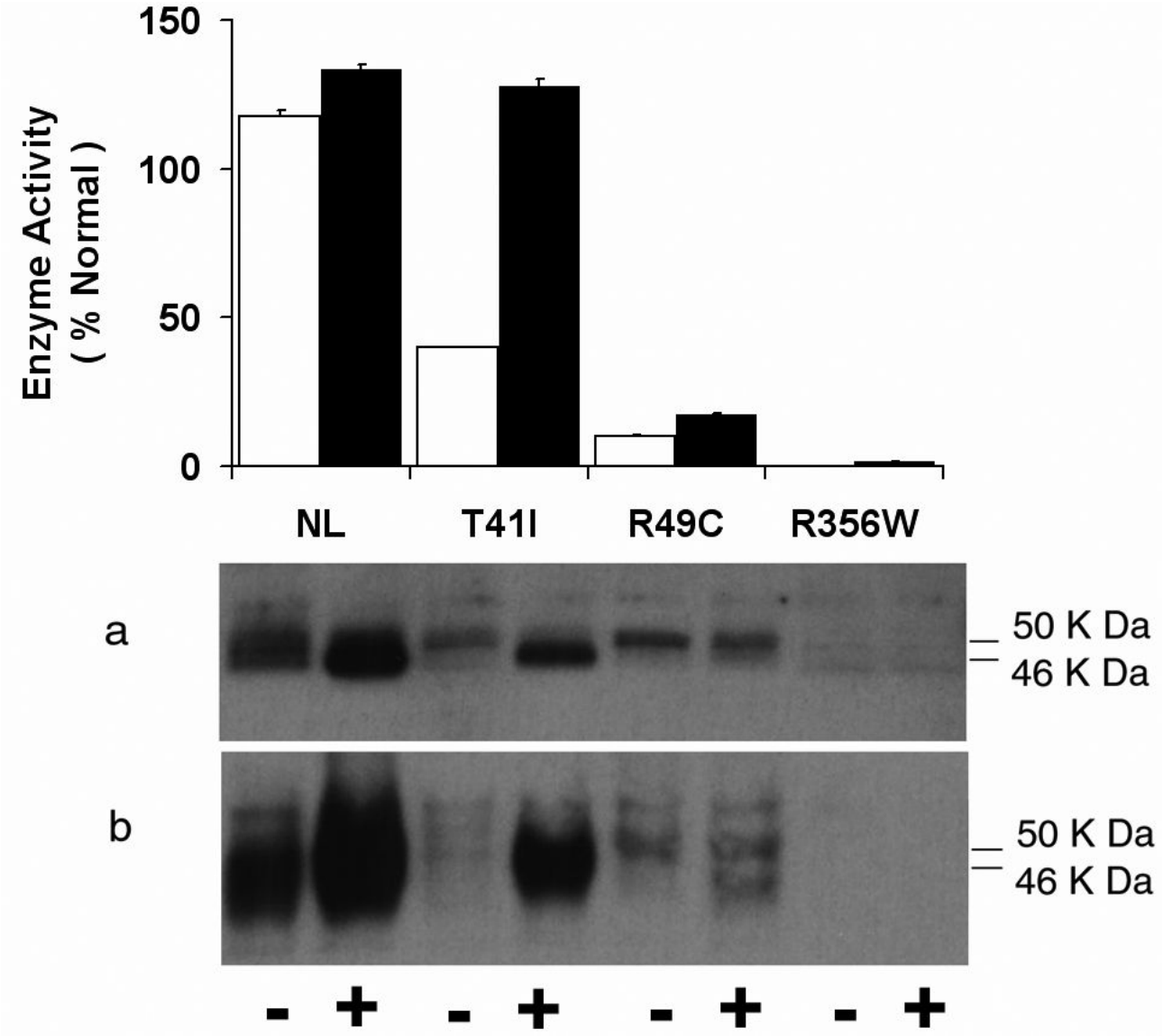

Fig. 1. Western blot of normal controls, responders, intermediate responders and non-responders
Upper panel: Measurement of α-Gal A activity. Lower panel: Western blots corresponding to upper panel data. Westerns were developed using polyclonal rabbit antisera raised against normal α-Gal A.

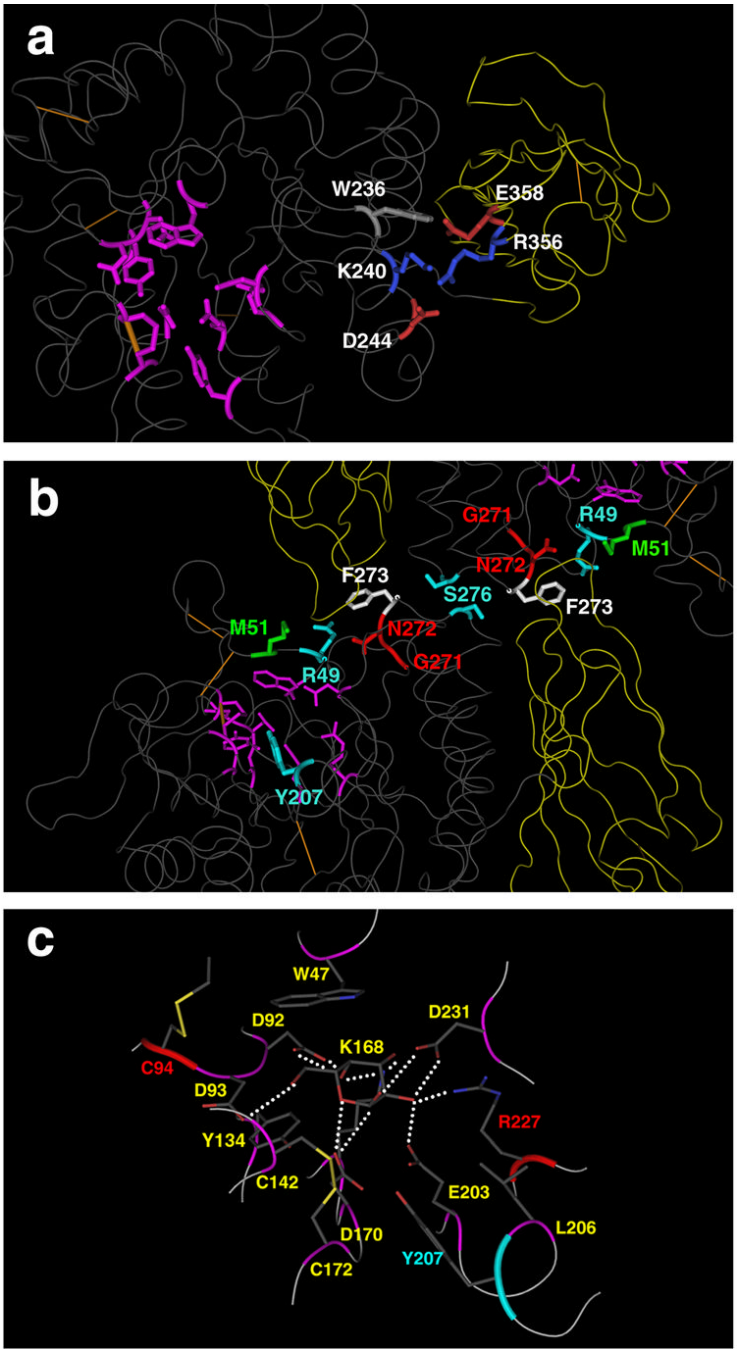

Fig. 2. Three domains important for the response of α-Gal A to DGJ

a. Mutation of any of W236, R356, or E358, disrupts the interface between Domain 1 and Domain 2. E358 (charged acidic, red) forms an ionic bond with K240 (charged basic, blue) and an H-bond with W236 (gray). R356 (charged basic, blue) forms an additional ionic bond with D244 (charged acidic, red).

b. Five mutations found in the dimer interface show variable response to DGJ treatment. M51K: Responder (green); R49C and S276G: Intermediate Responder (aqua); G271C and N272K:Non-Responder (red). Y207S: intermediate Responder (aqua) is located at the active site (not in the dimer interface). F273 (essential for dimer formation) is shown in white for reference only.

c. Mutations Y207S (aqua, Intermediate Responder), R227Q (red, Non-responder) are all located within the α-Gal A active site. Active site residues are shown in yellow. C94S (red, Non-responder) is located next to D92 and D93 which is within the active sites.

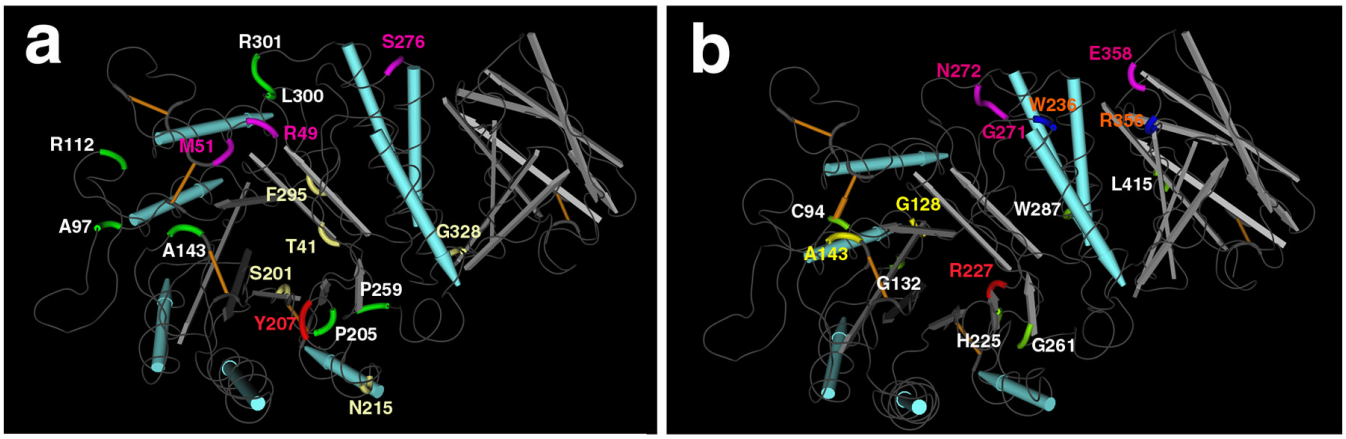

Fig. 3. Structural mapping of responders and non-responders

a. Responders are shown in monomeric α-Gal A. Eight mutations at 7 loci are located outside of α or β structures (green) and 5 mutations (pale yellow) occur within these structures. Disulfide bonds are shown in orange. M51K (purple) responded to DGJ and located in the dimer interface. Intermediate responders located outside of α or β structures are also shown: Y207S (red) in the active site and R49C and S276G (purple) in the dimer interface.

b. Non-Responders are shown in monomeric α-Gal A. Two non- responders are found outside all significant structural elements (yellow). Six non-responders are found within α or β structures (green), two are in the dimer interface, one in the interdomain interface (purple) and two are in both the interdomain interface and also located within α or β structural elements (blue with orange numbers). One non-responder is in the active site (red).

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Table 1

Response of α-Gal A activity in T-cells from normal controls and patients with Fabry disease

All enzyme assays were performed in triplicate. For normal control data, n=162 (115 males & 47 females) represents independent assay results from 24 normal individuals (17 males and 7 females). For patient samples "n" represents the number of independent repeat assays for a single individual. Where more than one patient was assayed with the same mutation the additional cultures are designated as in the example: T41I#1, T41I#2. Patients T-cells are grouped according to the response of α-Gal A activity to 20 μM DGJ. The locations of each mutation with respect to structurally significant elements including α-helices, β-sheets, dimer and domain interfaces and active site residues are noted. The two-tailed Student's t-test assuming unequal variances was used for calculation of p values. Response ratio (RR) is the ratio of the mean of α-Gal A activity with DGJ divided by that without DGJ.

Genotype	Mutation	Number of repeats (n)	Activity (% normal)		RR (ratio)	P value	Structure/ Function
			(− DGJ)	(+ DGJ)			
	All Normals	162	101 +/− 34	128 +/− 47	1.3	1.06E-08	
	Male normals	115	101 +/− 32	124 +/− 44	1.2	1.07E-05	
Normals	Female normals	47	84 +/− 30	117 +/− 49	1.4	0.0002	
Responders							
	T41I #1**	2	53 +/− 48	156 +/− 93	3.7 + 1.6	0.4	
c.122C>T	T41I #2	4	61 +/− 33	175 +/− 52	2.9	0.01	β1
c.152T>A	M51K	2	6 +/− 1	29 +/− 2	4.6	0.004	Dimer
c.290C>T	A97V*	3	14 +/− 4	75 +/− 8	5.5	0.001	
	R112C #1	2	6 +/− 5	23 +/− 18	3.7	0.4	
c.334C>T	R112C #2*	3	8 +/− 4	49 +/− 10	6.3	0.006	
	R112H #1	2	3 +/− 1	51 +/− 21	15.9	0.2	
	R112H #2	2	8 +/− 3	73 +/− 2	9.4	0.001	
c.335G>A	R112H #3*	3	3 +/− 2	60 +/− 17	20.3	0.003	
	A143T #1*	4	31 +/− 5	69 +/− 11	2.2	0.004	
c.427G>A	A143T #2**	3	46 +/− 13	62 +/− 20	1.3 +/−0.2	0.3	
c.602C>T	S201F	ψ1	9	82	9.6		β5
c.613C>A	P205T	ψ1	37	108	2.9		
c.644A>G	N215S	2	15 +/− 4	79 +/− 10	5.2	0.07	α5
c.776C>G	P259R #1	2	4 +/− 2	154 +/− 77	42.3	0.2	

Genotype	Mutation	Number of repeats (n)	Activity (% normal)		RR (ratio)	P value	Structure/ Function
			(− DGJ)	(+ DGJ)			
	P259R #2	3	4 +/− 2	138 +/− 16	47.4	0.004	
c.884T>G	F295C	3	1 +/− 1	29 +/− 5	32.3	0.009	β8
c.899T>C	L300P*	5	2 +/− 3	72 +/− 25	36.9	0.004	
	R301Q #1	3	11 +/− 6	87 +/− 11	7.9	0.002	
	R301Q #2	3	10 +/− 3	94 +/− 42	9.2	0.07	
c.902G>A	R301Q #3*	4	7 +/− 2	80 +/− 27	12	0.01	
c.983G>C	G328A	4	2 +/− 1	54 +/− 13	24.6	0.004	β9
Intermediate Responders							
c.145C>T	R49C	9	3 +/− 4	11 +/− 8	4.3	0.01	Dimer
c.620A>C	Y207S	3	4 +/− 4	15 +/− 7	3.5	0.09	Active Site
	S276G #1	2	2 +/− 4	14 +/− 6	5.5	0.2	
c.826A>G	S276G #2	3	1 +/− 1	12 +/− 2	9.8	0.01	Dimer
Non- Responders							
c.281G>C	C94S	2	2 +/− 1	2 +/− 0	ND	ND	Disulfide
c.383G>A	G128E #1	2	3 +/− 1	4 +/− 0	ND	ND	
	G128E #2	5	2 +/− 2	2 +/− 1	ND	ND	
c.394G>A	G132R*	3	1 +/− 1	2 +/− 0	ND	ND	β3
	A143P #1*	2	2 +/− 1	1 +/− 1	ND	ND	
c.427G>C	A143P #2*	2	1 +/− 1	1 +/− 0	ND	ND	
c.674.A>G	H225R	3	5 +/− 3	5 +/− 1	ND	ND	β6
c.680G>A	R227Q	3	4 +/− 5	3 +/− 4	ND	ND	β6, Active Site
c.706T>C	W236R	3	1 +/− 0	2 +/− 1	ND	ND	α6, Domain
c.782G>A	G261D	3	7 +/− 7	5 +/− 4	ND	ND	β7
	G271C #1	3	3 +/− 1	4 +/− 1	ND	ND	
c.811G>T	G271C #2	3	1 +/− 1	2 +/− 1	ND	ND	Dimer
c.816C>A	N272K	ψ1	0	0	ND	ND	Dimer
c.861G>T	W287C #1	3	3 +/− 1	3 +/− 1	ND	ND	α7

Genotype	Mutation	Number of repeats (n)	Activity (% normal)		RR (ratio)	P value	Structure/ Function
			(− DGJ)	(+ DGJ)			
	W287C #2	3	3 +/− 2	3 +/− 1	ND	ND	
	R356W #1	ψ1	1	1	ND	ND	β11, Domain
c.1066C>T	R356W #2*	4	0 +/− 0	0 +/− 1	ND	ND	
1070-1072delAGG	ΔE358	3	2 +/− 2	4 +/− 1	ND	ND	Domain
c.1244T>C	L415P	3	7 +/− 6	6 +/− 3	ND	ND	β16
Frameshift and Nonsense Mutations							
ivs4-1g/a	Splicing mutation	2	0 +/− 0	0 +/− 0	ND	ND	
30delG	L10fsX109*	6	2 +/− 1	2 +/− 1	ND	ND	
82insG	P27fsX2	2	2 +/− 2	0 +/− 0	ND	ND	
256del1	G85fsX34	ψ1	0	0	ND	ND	
1042insG	L347fsX26	3	1 +/− 1	1 +/− 1	ND	ND	
del26bp21	26bp del #1	ψ1	1	1	ND	ND	
del26bp21	26bp del #2	1	0	0	ND	ND	
c.355C>T	Q119X	3	3 +/− 2	4 +/− 3	ND	ND	
c.658C>T	R220X*	3	0 +/− 1	1 +/− 0	ND	ND	
c.901C>T	R301X	4	0 +/− 1	1 +/− 1	ND	ND	

*Mutations for which responder status has been reported previously (16) are noted by a single asterisk.

**Two exceptions were made for T41I#1 and A143T#2 for calculation of RR (denoted by double asterisks). These two mutations have large variations with high p value as a result of high α-Gal A activity without DGJ. RR was derived from the response ratio of individual reactions for these two mutations. Patients for which results reported are from a single culture (assayed in triplicate) are designated by ψ.

Table 2

α-Gal A Response Related to Structural and Non-Structural Elements

Mutations are classified by response group (R, NR, IR) and by location of the mutation with respect to significant structural elements such as α-helices, β-sheets, dimer and domain interfaces and active site residues are noted.

Response Group/ Phenotype (Number of Mutations)	Stability, Size with DGJ, Location	Functional domains	α or β	Non-α, Non-β	Mutations	Color codes in Fig. 3	
Responder/ Variant, (14)	Stable, 46 kDa, Lysosomal		5		T41I, S201F, N215S, F295C, G328A	Pale yellow	Fig. 3 a
		none		8	A97V, R112C, R112H, A143T, P205T, P259R, L300P, R301Q	Green	
		Dimer interface		1	M51K	Purple	
Intermediate Responder/ Classic, (3)	Half-Stable, 50 kDa, Various	Active site		1	Y207S	Red	
		Dimer interface		2	R49C, S276G	Purple	
Non-Responder/ Classic (14)	Unstable, 50 kDa, Undetectable		6		C94S, G132R, H225R, G261D, W287C, L415P	Green	Fig. 3 b
		Active sites	1		R227Q	Red	
		Domain interface	2		W236R, R356W	Blue with orange number	
		None		2	G128E, A143P	Yellow	
		Dimer interface		2	G271C, N272K	Purple	
		Domain interface		1	ΔE358*		

*ΔE358 is the result of a 3 base pair deletion resulting in a single amino acid deletion but is included here with missense mutations.