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Structure–function relationships in α -galactosidase A

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Abstract

With recent interest in the molecular mechanisms responsible for Fabry disease, the number of known mutations in the *GLA* gene which encodes α -galactosidase A has expanded considerably. Combining a large database of Fabry disease-causing mutations with the recently determined crystal structure of human α -galactosidase A allows for a new understanding of the atomic defects in the protein responsible for Fabry disease. We have conducted a systematic survey of the known Fabry disease-causing mutations and analyzed the mutations in the context of the α -galactosidase A structure. We have applied quantitative methods for identifying the plausible effect of each mutation on the α -galactosidase A protein. We present the analysis of 331 different defects in the *GLA* gene leading to non-native proteins in patients with Fabry disease. These mutations include 278 missense mutations, 49 nonsense mutations, and 4 single amino acid deletions.

Conclusion—Over half of the residues in the protein have been found to have changes in patients with Fabry disease. Most of these genetic mutations lead to the disruption of the hydrophobic core of the protein, thus Fabry disease is primarily a disease of protein folding. Further understanding of α -galactosidase A, one of the best studied members of the lysosomal storage disease family, will lead to increased understanding of other lysosomal storage diseases and other protein folding diseases.

Keywords

α -galactosidase; Fabry disease; glycosidase; lysosomal storage disease; E.C.-3.2.1.22

The degradation of macromolecules, including glycopeptides and glycolipids, occurs in the lysosome via catabolic enzymes. For example, glycosidases cleave the oligosaccharides from glycoproteins and glycolipids into smaller components used by the cell. In humans, defects in these lysosomal enzymes cause lysosomal storage diseases (LSDs). In this family of diseases, a defect in a gene leads to the loss of functional enzyme, which then leads to the accumulation of substrates in the tissues. The symptoms of the particular disease depend upon the amount, toxicity and location of the substrate that accrues. Over forty different LSDs have been identified to date, including Tay–Sachs, Gaucher, Sandhoff and Fabry diseases.

Fabry disease, an X-linked inherited disorder affecting 1 in every 40000 males, is characterized by chronic pain, vascular degeneration, cardiac and renal abnormalities, and other symptoms [1]. The disease was first reported in 1898 [2], and the defective protein responsible for the disease was first identified to be α -galactosidase A (α -gal A, also known as GLA and α -GAL) (E.C. 3.2.1.22) in 1967 [3]. α -Galactosidase A is encoded by the *GLA* gene and catalyzes the removal of terminal α -galactose groups from substrates such as glycoproteins and glycolipids. In patients with Fabry disease the loss of functional enzyme leads to the buildup of substrates, primarily globotriaosylceramide, in the tissues [1]. Most patients with Fabry disease have a single point mutation in their *GLA* gene, and several hundred different missense and nonsense mutations have been identified in the patient population.

The desired result of clinical research on LSDs is to develop treatments that would prevent disease symptoms from ever appearing. In Gaucher disease (caused by a defect in the lysosomal enzyme β -glucosidase), enzyme replacement therapy (ERT) in paediatric patients successfully prevents disease and is currently in use in over 3500 patients from 55 countries [4,5]. Gaucher disease was the first LSD approved for treatment with recombinant ERT in 1994 [4,5], and in 2001 Fabry disease became the second. We have recently determined the three-dimensional crystal structure (at 3.25 Å resolution) of the lysosomal enzyme human α -galactosidase A, the molecule which when deficient is responsible for the development of Fabry disease (Figure 1) [6]. This structure represents one of the first structures from family 27 and clan D (including over 270 proteins from different species) in the classification of glycoside hydrolases based upon similarities in sequences, active sites and mechanisms [7]. The structure was determined both alone and in complex with its catalytic product, the α -galactose monosaccharide, indicating the atomic basis for substrate recognition. The structural analysis revealed a portion of the glycoprotein's *N*-linked carbohydrate, which accounts for 5–15% of the mass of α -galactosidase A and represents over 70 different glycoforms attached to 23 different core carbohydrate structures [8]. The carbohydrate component of the glycoprotein is required for the molecule to traffic to the lysosome via the mannose-6-phosphate receptor pathway [9].

Following the recent determination of the structure of human α -galactosidase A, we are now able to ask fundamental questions about the structure and function of α -galactosidase A and its relationship to the human disease process. The α -galactosidase A structure revealed for the first time the locations and nature of the hundreds of different atomic defects responsible for Fabry disease. We are now in position to learn the details of the atomic basis for the loss of enzyme activity in patients with Fabry disease, and to tailor therapy based upon the specific defect in α -galactosidase A in different patients. In this report, we address the defects in the α -galactosidase A enzyme that lead to Fabry disease. As we are interested in the defects in the enzyme, in this work we do not include the mutations in the α -galactosidase A gene that lead to grossly altered gene products, such as insertions and deletions leading to frameshifts and splice defects. As Fabry disease represents one of the best studied LSDs, the lessons learned from the study of α -galactosidase A will lead to greater understanding of glycosidases and LSDs generally.

Materials and methods

Crystallographic structure determination

The methods associated with the X-ray crystallographic determination of the structure of human α -galactosidase A have been described previously [6]. Since that publication, we have extended our database of mutations associated with Fabry disease (see Tables I and II).

Mutation database

The database of mutations were compiled from the following sources: Online Mendelian Inheritance in Man at Johns Hopkins University (<http://www.ncbi.nlm.nih.gov/omim>) (McKusick 1998), SWISS-PROT (<http://www.expasy.org/sprot>) [10], Human Gene Mutation Database (<http://www.hgmd.org>) [11], and the references listed in Table II. Residue numbering begins with the translation initiator Methionine numbered as +1, where the mature α -galactosidase A protein spans from L32 to L429. Four trinucleotide deletions are included in the α -galactosidase A point mutation list, as they result in deletions of single amino acids from the protein.

Solvent accessibility

Solvent accessible surfaces were calculated from the human α -galactosidase A structure with crystallography and NMR Suite [12] using a probe radius of 1.4 Å. Buried residues are defined as those with less than 2 Å² solvent accessible surface area (ASA) per side chain atom, except for glycines (which have only one side chain atom) are defined as buried when all four atoms in the residue average less than 2 Å² solvent accessible surface area.

Figure preparation

Molecular figures were drawn using the program MOLSCRIPT [13].

Results

We have assembled a database of 331 mutations in the exons of the *GLA* gene, including 278 missense mutations, 49 nonsense mutations and 4 single amino acid deletion mutations [10,11,14]. The mutated residues are found in 204 different locations in the protein sequence. We describe two different mutation sets in this paper: the combined set of α -galactosidase A missense and nonsense mutations, and the smaller set of missense mutations in the sequence that codes for the mature α -galactosidase A glycoprotein. The first set of mutations includes missense mutations, nonsense mutations and single amino acid deletions in the exons coding for the full-length α -galactosidase A protein, 331 mutations in total. These affect 204 of the 429 amino acids in the α -galactosidase A protein, or nearly half of the residues in the mature polypeptide. Of the 204 residues, 152 are missense mutants, 28 are nonsense mutants, and 21 are both missense and nonsense mutants. The second set of mutations, a subset of the first, includes only the missense mutations in the exons coding for the mature 398-residue polypeptide (after removal of the signal sequence). This database contains 270 distinct missense mutations affecting 167 residues. Because this set contains only point mutations affecting the mature polypeptide, it reveals properties of the folded protein. Table I summarizes the database of Fabry disease-causing mutations.

Table II lists the point and stop mutations found in patients with Fabry disease, along with their effects on the protein. Figure 2 plots the effects of Fabry disease-causing mutations on the structure of human α -galactosidase A. Surprisingly, the affected residues are not restricted to the region around the active site, but distribute throughout the structure, with some as far as 50 Å from the substrate-binding site. A single mutation in the *GLA* gene causes Fabry disease in all cases except for in two individuals with double mutations (corresponding to E66Q/R112C and L45R/H46S in the protein sequence). In general, mutations leading to a complete loss of enzyme activity affect residues in the interior of α -galactosidase A, while mutations leading to an α -galactosidase A protein with some residual enzyme activity tend to be found in more surface exposed residues.

The database of Fabry disease mutations indicates the importance of different amino acids to the protein's fold and function. A synopsis of the Fabry disease-causing mutations by amino acid affected appears in Table III. Based upon their frequency of mutation, Trp, Cys, Gln, Tyr, and Gly are the residue types most commonly altered by Fabry disease mutations. For example, 15 of the 16 Trp residues (94%) in the mature α -galactosidase A protein have been found to be affected by Fabry disease-causing mutations. Five of those Trp residues are affected exclusively by nonsense mutations, so 10 of the 16 Trp residues (63%) in α -galactosidase A are changed by missense mutations in patients with Fabry disease. Similarly, 10 of the 12 Cys residues in the protein are found as mutants in patients with Fabry disease, highlighting the importance of this residue to the structural integrity of the folded protein. In total, 77% of the 22 Gln residues in the structure are found altered in patients with Fabry disease, but most of these mutations are nonsense mutations that convert the Gln codon to a stop codon. Over two-thirds of the 31 Gly residues are altered in patients with Fabry disease, and all of them appear in missense mutations. Glycine, unique among the amino acids for its lack of a side chain, can appear in more main chain dihedral conformations than the other residues. As expected, the unique chemical properties of glycine make it sensitive to perturbation, and it is over-represented in the database. The basic residues Arg and Lys, although chemically quite similar, appear in the Fabry disease mutation database with markedly different frequencies: more than half of the 19 arginines in the structure are affected by mutations in patients with Fabry disease, but only two of the 17 lysines are affected. Arginine is more commonly found buried or partially buried in the structure compared with lysine, which is nearly always surface exposed, so arginine plays a more critical role in the folding of the protein. Surprisingly, Phe is less likely to be found altered in patients with Fabry disease compared with the average residue, with 5 of the 15 Phe residues (33%) affected by Fabry disease-causing mutations. The dearth of Phe residues in the database is unexpected for a large hydrophobic residue typically found in the interior of a protein.

We have used the amount of buried surface area per side chain atom as a measure the degree of accessibility of a given residue. This correlates with the importance of a residue in the folded conformation of the protein. If each residue in α -galactosidase A is ranked by increasing surface accessibility, the residues found altered by Fabry disease mutations cluster among the buried residues (Figure 3). Conversely, residues that are exposed to solvent are rarely found to be affected by point mutation in Fabry disease. Thus, there is a

correlation between the extent of burial of a residue in the core of the α -galactosidase A protein and Fabry disease mutations associated with that residue. As a metric for the importance of a given residue, other researchers have used the degree of conservation of a residue in α -galactosidase A orthologues [15] and the number atoms with bad contact distances in a hypothetical model of the mutant protein [16].

Discussion

Half of residues in the protein have been found altered in the ensemble of Fabry disease patients (Table II and Figure 2). As might be expected, the active site is exquisitely sensitive to change, as all of the residues that make contact with the ligand have been affected by mutation in patients with Fabry disease. Surprisingly, most of the residues affected by Fabry disease point mutations do not cluster around the active site, but are found distributed throughout the hydrophobic core of the protein. Most of the missense mutations that cause Fabry disease produce changes in the hydrophobic core of the α -galactosidase A protein, as 65% of the missense mutations code for buried amino acid residues.

The structure of the human α -galactosidase A glycoprotein raises the possibility of tailoring the treatment of Fabry disease to the individual's specific defect in the *GLA* gene. One group of patients can in principle respond to pharmacological chaperone therapy, whereas a second group will not respond to small molecule therapy and must be treated with recombinant ERT. In the case of patients with a defective α -galactosidase A active site or with a grossly altered α -galactosidase A polypeptide (*i.e.* those with changes in the protein's active site, nonsense mutations, frameshift mutations or splice defect mutations), recombinant ERT seems more appropriate. In these patients, if the folded protein does not have catalytic capacity or if the full-length polypeptide is not even synthesized, small molecule chaperones to assist in the folding of the protein are unlikely to show efficacy, and ERT might be a preferred treatment. In the case of patients with a defect in the hydrophobic core of the molecule, small molecule chaperones might assist in the correct folding of the enzyme in the endoplasmic reticulum. This might be an appropriate treatment choice for patients who show some residual enzyme activity and for those who have amino acid changes that fall a long way from the active site of the enzyme.

Conclusion

At the amino acid level, Fabry disease is most often caused by a perturbation of the hydrophobic core of the protein. The literature on *GLA* mutations and Fabry disease prior to the availability of the three-dimensional structure presumed that the disease-causing missense mutations would produce changes in the enzyme near the active site. Using the crystal structure and an expanded database of Fabry disease mutations, we show that alterations in residues near the active site of the enzyme represent only 10% of the total. The majority of the amino acid changes responsible for Fabry disease fall in the hydrophobic core of the protein, thus Fabry disease is primarily a protein folding disease. Fabry disease, one of the best characterized members of the LSD family, might prove to be a model for other protein folding diseases, including Alzheimer's disease, Parkinson's disease, prion diseases and polyglutamine diseases such as Huntington's disease.

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Figure 1.

The structure of α -galactosidase A. The structure of the human α -galactosidase A dimer is shown in ribbon representation. The ribbon is coloured from blue to red as the polypeptide goes from *N*- to *C*-terminus. The active site is identified by the catalytic product galactose, shown in sphere Corey-Pauling-Koltun (CPK) format. Each monomer in the homodimer contains two domains, a $(\beta/\alpha)_8$ barrel containing the active site (blue to yellow) plus a *C*-terminal antiparallel β domain (yellow to red).

Figure 2.

Changes in α -galactosidase A associated with Fabry disease. The structure of human α -galactosidase A is shown in stick format in the same orientation as in Figure 1. For each missense mutation found in the Fabry disease mutation database, the affected residue is coloured by the resulting change in the glycoprotein. Perturbations of the active site are in red, changes to the buried core of the protein are shown in blue and other changes are shown in yellow. The ligand is shown in green to indicate the active site.

Figure 3.

Fabry disease-causing mutations and surface accessibility. The accessible surface area for each residue in the human α -galactosidase A structure is shown, with each residue ranked by its accessibility. The plot shows the 398 residues in the mature human α -galactosidase A sequence ordered by increasing accessibility (along the X axis) and the average accessibility of each atom in the residue (on the Y axis). Boxes identify the buried (yellow), partially buried (green), and exposed (blue) residues. Above the plot, red tick marks represent residues found altered in patients with Fabry disease and black marks represent residues not (yet) found altered in Fabry disease. The tick marks show that Fabry disease is most often caused by defects in the folding of the α -galactosidase A molecule, as nearly all of the buried residues have been found altered in patients with Fabry disease (left side and red tick marks). Conversely, exposed residues are rarely found substituted in patients with Fabry disease (right side and black tick marks). This plot shows that Fabry disease is most often caused by changes to the hydrophobic core of the α -galactosidase A protein.

Table I

Synopsis of Fabry disease-causing mutations.

	Number of mutations	Number of residues affected
In full-length α -gal A		
<i>Single amino acid substitutions</i>	278	173
<i>Nonsense mutations</i>	49	49
<i>Single amino acid deletions</i>	4	4
Total	331	204
In mature α -gal A		
<i>Single amino acid substitutions</i>	270	167
<i>Nonsense mutations</i>	49	49
<i>Single amino acid deletions</i>	4	4
Total	323	198

 α -gal A, α -galactosidase A

Table II

Details of mutations reported in patients with Fabry disease.

Residue		Mutations found in Fabry disease patients			
Number	Type	Type	ASA	Effect on protein	Category Reference
1	M	I		Initiation codon required	Other [17]
		T			[18]
		R			[19]
14	L	P		In signal sequence	Other [20]
16	L	P		In signal sequence	Other [21]
19	L	P		In signal sequence	Other [22]
20	A	P		In signal sequence	Other [23]
31	A	V		In signal sequence	Other [18]
32	L	P	7.1	Signal sequence cleavage site	Other [24]
34	N	S	1.7	Bifurcated H-bond to N224	Buried [25]
		K			[15]
35	G	R	6.3	Gly phi/psi, Arg collides with N192	Other [26]
40	P	S	0.0	Helix terminus, buried and packed on W262	Buried [27]
		L			[28]
41	T	I	0.8	Buried	Buried [15]
42	M	V	0.7	Buried, no room for beta branch	Buried [29]
		T			[19]
		L			[30]
43	G	D	0.0	No room for side chain	Buried [31]
		V			[19]
		R			[32]

Mutations found in Fabry disease patients					
Residue		Effect on protein		Category	Reference
Number	Type	ASA	Type		
44	W	0.0	X	Stop	[33]
			C	Totally buried	[34]
45	L	2.4	R	Buried	[18]
46	H	0.0	R	Buried	[18]
			S	Buried, next to W47 in active site (double mutant with L45R)	[18]
			Y		[35]
47	W	3.8	L	Active site residue	[36]
			G		[35]
48	E	0.0	K	Buried	[37]
49	R	2.9	L	Buried	[26]
			S	Mostly buried, on dimer interface	[29]
			P		[35]
			G		[32]
50	F	0.4	C	Buried	[19]
51	M	14.7	K	Active	[38]
52	C	13.0	S	Other	[39]
			R	Disulphide required near active site	[17]
			X	Stop	[40]
56	C	10.6	G	Other	[25]
			F	Disulphide bond	[39]
			Y		[29]
			X	Stop	[41]
59	E	7.9	K	Other	[39]
63	C	1.7	Y	Other	[42]
				Ion pairs across dimer interface to H406	
				Buried disulphide	

Mutations found in Fabry disease patients						
Residue Number	Type	Type	ASA	Effect on protein	Category	Reference
65	S	T	3.9	Partially buried (splice variant)	Other	[43] [44]
66	E	Q	3.1	Mostly buried in ion pair (double mutant with R112C)	Other	[45] [42]
68	L	F	0.6	Buried in hydrophobic pocket	Buried	[19]
72	M	V	0.0	Totally buried	Buried	[46] [47] [32]
78	S	X	12.0	Stop	Stop	[48]
79	E	X	17.3	Stop	Stop	[49]
81	W	X	0.2	Stop	Stop	[39]
		S		Buried	Buried	[37]
85	G	D*	3.3	Restricted phi/psi in turn	Other	[24]
86	Y	C	0.0	Totally buried	Buried	[18]
		X		Stop	Stop	[50]
88	Y	D	2.4	Partly buried in hydrophobic pocket	Buried	[36]
89	L	R	0.1	Buried in hydrophobic pocket	Buried	[39] [18]
		P				
91	I	T	0.0	Buried in hydrophobic pocket	Buried	[18]
92	D	H	0.0	Active site residue	Active	[29] [18,38]
		N				
93	D	G	0.4	Active site residue	Active	[29] [41,51] [15]
		N				
		V				

Mutations found in Fabry disease patients					
Residue		Effect on protein		Category	Reference
Number	Type	Type	ASA		
94	C	Y	0.0	Buried disulphide	[18]
		S			[35]
95	W	S	0.0	Buried in hydrophobic pocket	[28]
		X		Stop	[37]
97	A	V	2.0	Mostly buried in turn, little room for larger side chains	[18]
		P			[52]
99	Q	X	21.7	Stop	[39]
100	R	K	3.4	Ion pairs to D155, stacked on Y151	[39]
		T			[18]
103	E	Q	20.6	Ion pair to R105	[42]
107	Q	X	11.0	Stop	[29]
112	R	C	1.9	Mostly buried guanidium group in ion pair	[45]
		H			[39]
		S			[41]
113	F	L	0.0	Completely buried in packed hydrophobic core	[18]
		S			[35]
119	Q	X	18.0	Stop	[29]
120	L	P	1.5	Side chain buried, main chain in middle of helix	[39]
121	A	T	0.6	No room for larger group (double mutant with L120P)	[39]
		P			[53]
127	K	X	8.8	Stop	[15]
128	G	E	7.8	Restricted phi/psi in turn	[17]

Mutations found in Fabry disease patients					
Residue		Effect on protein		Category	Reference
Number	Type	ASA	Type		
129	L	P	0.0	Buried	[54]
131	L	P	0.0	Buried in hydrophobic pocket	[39]
132	G	R	0.0	No room for side chain	[19]
134	Y	S	0.0	Active site residue	[18]
		X		Stop	[28]
135	A	V	0.0	Buried, no room for larger side chain	[41,51]
136	D	H	0.1	Buried	[38]
138	G	R	0.0	Buried, no room for side chain	[18]
		E			[32]
141	T	I	0.1	Buried, mutation removes N139 carbohydrate	[19]
142	C	Y	8.8	Disulphide required near active site	[55]
		R			[40]
		X		Stop	[40]
		W			[42]
143	A	P	3.0	Larger side chain disrupts C52–C94 disulphide	[39]
		T			[18]
144	G	V	5.6	In turn; gly restricted phi/psi	[39]
146	P	S	3.2	Partially hydrophobic	[56]
147	G	R	1.6	In turn; gly restricted phi/psi	[42]
148	S	R	0.7	Buried, little room for larger side chain	[18]
		N			[28]
151	Y	X	8.8	Stop	[41]
152	Y	X	1.4	Stop	[19]

Mutations found in Fabry disease patients					Category	Reference
Residue Number	Type	ASA	Effect on protein	Type		
153	D	-	Deletion within helix changes register		Other	[41]
155	D	H	Buried, ion pairs to R100		Buried	[51]
156	A	T	Buried, no room for larger side chain		Buried	[57]
		V				[55]
157	Q	X	18.8	Stop	Stop	[39]
162	W	R	2.7	Mostly buried in hydrophobic pocket	Buried	[25]
		C				[58]
		X		Stop	Stop	[59]
163	G	V	2.7	In turn, restricted phi/psi	Other	[18]
165	D	V	2.8	Buried ion pair w/H125	Buried	[26]
166	L	V	0.4	Buried, little room for branch at beta carbon	Buried	[55]
		G		Creates hole in interior (2 nucleotide changes)		[15]
167	L	P	0.2	Creates bend in strand β 4 near active site	Active	[60]
168	K	R	3.3	Active site residue	Active	[19]
169	F	S	0.0	Buried in hydrophobic pocket	Buried	[38]
170	D	V	0.0	Catalytic residue in active site	Active	[18]
		H				[37]
171	G	R	0.6	Between two active site residues	Active	[51]
		D				[41]
172	C	Y	11.4	Required disulphide in active site	Active	[39]
		R				[28]
		F				[38]
		G				[61]

Mutations found in Fabry disease patients					
Residue	Effect on protein		Category		
Number	Type	ASA	Type	Reference	
W					
173	Y	12.6	Stop	Stop	[15]
177	L	10.6	Stop	Stop	[41]
183	G	0.3	Buried, no room for side chain	Buried	[40]
	S				[19]
187	M	0.0	Totally buried, little room for branched beta carbon	Buried	[28]
	T				[15]
191	L	1.6	Buried in hydrophobic pocket	Buried	[38]
	P				[36]
194	T	0.2	Buried, little room for longer side chain	Buried	[42]
199	V	0.4	buried, little room for longer side chain	Buried	[19]
201	S	3.7	Larger side chain extends into active site	Active	[41]
	Y				[15]
202	C	0.0	Buried disulphide	Buried	[56]
	Y				[18]
204	W	0.5	Stop	Stop	[28]
205	P	0.0	Buried hydrophobic	Buried	[17]
	R				[19]
	L				[62]
207	Y	6.5	Active site residue	Active	[19]
215	N	7.5	Mutation disrupts N-linked carbohydrate	Other	[63]
216	Y	0.9	Buried hydrophobic	Buried	[18]
219	I	0.3	Buried hydrophobic (2 nucleotide changes)	Buried	[39]

Mutations found in Fabry disease patients					
Residue		Effect on protein		Category	Reference
Number	Type	ASA	Type		
220	R	X	7.6	Stop	[64]
221	Q	X	13.5	Stop	[19]
222	Y	X	5.8	Stop	[65]
223	C	G	0.0	Buried	[66]
		R			[19]
		Y			[62]
24	N	D	0.0	Buried	[48]
		S			[28]
226	W	X	0.0	Stop	[67]
		R		Buried	[28]
		C			[37]
227	R	X	0.4	Stop	[63]
		Q		Active site residue	[25]
230	A	T	43.1	Affects critical D231 in active site	[28]
231	D	N	10.2	Active site residue	[68]
234	D	Y	0.1	Buried, no room for larger side chain	[19]
		E			[41]
235	S	C	3.5	Initiates $\alpha 6$ helix	[40]
236	W	C	1.9	Buried	[29]
		L			[40]
		X			[32]
		R		Stop	[15]
239	I	T	0.0	Buried	[53]

Mutations found in Fabry disease patients					
Residue	Effect on protein			Category	Reference
Number	Type	ASA	Type		
242	I	0.2	N	Buried in hydrophobic pocket	Buried [49]
243	L	0.2	F	Buried in hydrophobic pocket, no room for Phe	Buried [32]
244	D	11.6	N	Ion pairs to R356	Other [39]
			H		[40]
245	W	3.5	X	Stop	Stop [32]
247	S	2.0	P	Buried on $\alpha 6$ helix, no room for Pro	Buried [38]
			C		[32]
250	Q	4.9	X	Stop	Stop [38]
251	E	22.6	X	Stop	Stop [69]
257	A	0.1	P	Buried	Buried [36]
258	G	0.6	R	Buried, restricted phi/psi in turn	Buried [35]
259	P	11.0	L	P259 and G260 form beta turn	Other [40]
			R		[38]
260	G	0.4	A	Buried in turn, restricted phi/psi	Buried [76]
261	G	0.0	D	Buried in turn, no room for side chain	Buried [49]
262	W	0.0	X	Stop	Stop [70]
			C	Completely buried	Buried [42]
263	N	0.9	S	Buried in hydrogen bonding network	Buried [18]
264	D	4.3	V	Near active site residues	Active [25]
			Y		[41]
265	P	0.8	R	Buried, little room for Arg	Buried [58]
266	D	1.0	V	Active site residue	Active [25]

Residue		Mutations found in Fabry disease patients				Category	Reference
Number	Type	ASA	Effect on protein	Type	ASA		
267	M	I	Buried, little room for branch at beta carbon	Buried	[40]	Buried	[15]
		R					
		E					
268	L	S	0.0	Completely buried	Buried	Buried	[42]
269	V	A	0.0	Completely buried	Buried	Buried	[63]
		M					
270	I	T	0.0	Completely buried	Buried	Buried	[62]
271	G	C	4.1	In turn, restricted phi/psi, no room for large side chain	Other	Other	[19]
		S					
		V					
272	N	K	0.0	Completely buried	Buried	Buried	[39]
		S					
276	S	N	0.8	Buried	Buried	Buried	[42]
		G					
277	W	X	9.2	Stop	Stop	Stop	[40]
279	Q	E	0.3	Buried hydrogen bonding network on dimer axis	Buried	Buried	[45]
		H					
		R					
280	Q	H	3.2	Little room for larger side chain	Other	Other	[35]
		K					
282	T	N	0.0	Buried hydrophobic pocket	Buried	Buried	[38]

Mutations found in Fabry disease patients					
Residue	Type	ASA	Effect on protein	Category	Reference
283	Q	0.0	Completely buried	Buried	[15]
284	M	0.1	Buried hydrophobic pocket	Buried	[17]
285	A	0.2	Buried	Buried	[41]
	D				[15]
287	W	0.0	Stop	Stop	[63]
	G		Buried hydrophobic	Buried	[29]
	C				[18]
288	A	0.0	Buried in hydrophobic pocket on helix a7	Buried	[39]
	P				[19]
289	I	0.0	No room for Phe	Buried	[40]
290	M	0.8	Buried	Buried	[15]
292	A	0.0	Completely buried	Buried	[34]
293	P	0.3	Buried	Buried	[36]
	A				[19]
	T				[15]
294	L	0.0	Stop	Stop	[35]
296	M	0.0	Completely buried in hydrophobic pocket	Buried	[73]
	I				[23]
297	S	0.0	Completely buried, no room for larger side chain	Buried	[25]
	C				[32]
298	N	0.2	Buried, in hydrogen bonding network	Buried	[17]
	H				[29]
	S				[18]
300	L	0.0	Completely buried	Buried	[42]

Residue		Mutations found in Fabry disease patients				Category	Reference
Number	Type	Type	ASA	Effect on protein			
F							
301	R	Q	3.6	Partly buried, ion pairs with D299	Other	[33]	
		X		Stop	Stop	[39]	
		P				[38]	
		G				[19]	
303	I	N	2.7	Partially buried in hydrophobic pocket	Buried	[19]	
306	Q	X	14.5	Stop	Stop	[15]	
312	Q	H	4.4	Partially buried	Other	[15]	
313	D	Y	3.5	Partially buried, little room for Tyr	Other	[25]	
				Polymorphism with ~60% wild-type activity		[74]	
316	V	E	0.1	Buried in hydrophobic pocket	Buried	[26]	
317	I	T	1.5	Buried in hydrophobic pocket	Buried	[75]	
320	N	K	0.0	Buried hydrogen bonding network	Buried	[76]	
		Y				[28]	
		I				[42]	
321	Q	E	5.0	Ne2 of gln needed in H-bonding network	Other	[40]	
		X			Stop	Stop	[42]
		R				[15]	
325	G	D	0.6	Buried, no room for side chain	Buried	[62]	
327	Q	K	0.6	Buried hydrogen bonding network	Buried	[63]	
		E				[42]	
328	G	R	0.2	Buried, no room for side chain	Buried	[45]	
		A				[25]	
		V				[41]	

Mutations found in Fabry disease patients					
Residue	Effect on protein		Category		
Number	Type	ASA	Type	Reference	
330	Q	1.8	Stop	Stop	[51]
333	Q	16.4	Stop	Stop	[42]
338	E	0.3	Buried, H-bonds to W340	Buried	[41]
	X		Stop	Stop	[41]
340	W	0.1	Stop	Stop	[25]
	R		Buried in hydrophobic pocket	Buried	[29]
341	E	0.3	Buried hydrogen bonding network	Buried	[77]
	D				[19]
342	R	0.0	Stop	Stop	[63]
	Q		Totally buried	Buried	[56]
344	L	2.3	Mostly buried, mutation creates consecutive Pro's	Buried	[42]
345	S	14.9	Little room for Pro	Other	[42]
348	A	5.4	Pro introduces kink in strand β 1	Other	[15]
349	W	2.2	Stop	Stop	[38]
352	A	0.0	Completely buried	Buried	[60]
354	I	0.0	Completely buried	Buried	[78]
355	N	0.0	Buried hydrogen bonding network	Buried	[32]
356	R	4.6	Ion pairs to D244	Other	[79]
357	Q	9.2	Stop	Stop	[42]
358	E	6.6	Ion pair to K240 and hydrogen bonds to W236	Other	[17]
	K				[80]
	G				[32]
	A				[15]

Mutations found in Fabry disease patients					
Residue	Effect on protein			Category	Reference
Number	Type	Type	ASA		
360	G	S	0.9	Gly specific phi/psi	Other [51]
361	G	R	3.8	No room for large side chain	Other [63]
362	P	L	10.7	initiates β 12 strand	Other [19]
363	R	H	6.3	Guanidinium packs on F337	Other [35]
		C			[19]
365	Y	X	4.6	Stop	Stop [81]
373	G	S	0.0	Buried, no room for side chain	Buried [76]
		D			[82]
377	A	D	0.0	Buried in hydrophobic pocket	Buried [35]
378	C	Y	0.0	In disulphide	Buried [40]
		R			[83]
382	C	W	0.0	In disulphide	Buried [84]
		Y			[37]
383	F	-	6.8	Deletion shifts register of strand β 13	Other [85]
384	I	N	0.0	Buried in hydrophobic pocket	Buried [19]
385	T	P	7.2	Pro kinks strand β 13	Other [19]
386	Q	X	2.2	Stop	Stop [18]
		P		Pro kinks strand β 13	Other [15]
396	F	Y	12.4	Unknown; mutation not disease associated	Other [86]
398	E	X	16.2	Stop	Stop [25]
		K		Unknown	Other [19]
399	W	X	12.6	Stop	Stop [18]

Residue		Mutations found in Fabry disease patients			
Number	Type	Type	ASA	Effect on protein	Category Reference
401	S	X	8.3	Stop	Stop [19]
404	R	-	9.5	Deletion shifts register in strand β 15	Other [25]
407	I	K	0.1	Buried	Buried [37]
409	P	A	0.0	Buried; initiates strand β 16	Buried [35]
		T			[35]
		S			[32]
410	T	K	0.0	Buried; no room for Lys, Ala introduces hole	Buried [38]
		A			[65]
411	G	D**	0.0	Buried; no room for side chain (double mutant with D313Y)	Buried [48]
					[74]
414	L	S	0.0	Buried	Buried [37]

ASA: Accessible Surface Area as defined in Materials and Methods

Phi/psi: Main chain dihedral angles as seen in a Ramachandran plot

* This mutation was described in the original citation as G85N, which is not possible from the point mutation identified.

** This mutation was described in the original citation as G120Y.

Table III

Synopsis of residues in the mature α -galactosidase A protein that are affected by Fabry disease-causing mutations.

Residue type	Total no. residues in protein	% of total (and no. of) residues affected by mutations	No. residues affected by AA substitution only	No. residues affected by substitution or nonsense	No. residues affected by nonsense only	Percentage of total residues affected by AA substitution
Trp	16	94% (15)	1	9	5	63
Cys	12	83% (10)	7	3	0	83
Gln	22	77% (17)	5	2	10	32
Gly	31	68% (21)	21	0	0	68
Tyr	15	67% (10)	3	2	5	33
Glu	18	56% (10)	6	2	2	44
Met	15	53% (8)	8	N/A	N/A	53
Arg	19	53% (10)	6	3	1	47
Ile	21	52% (11)	11	N/A	N/A	52
Ala	28	46% (13)	13	N/A	N/A	46
Asp	29	45% (13)	13	N/A	N/A	45
Ser	23	43% (10)	8	0	2	35
Thr	14	43% (6)	6	N/A	N/A	43
Pro	19	42% (8)	8	N/A	N/A	42
Leu	41	41% (17)	15	0	2	37
Asn	20	40% (8)	8	N/A	N/A	40
Phe	15	33% (5)	5	N/A	N/A	33
Val	16	19% (3)	3	N/A	N/A	19
His	7	14% (1)	1	N/A	N/A	14
Lys	17	12% (2)	1	0	1	6
Total	398	50% (198)	149	21	28	43

AA, amino acid