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High Incidence of Later-Onset Fabry Disease Revealed by Newborn Screening*

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The classic phenotype of Fabry disease, X-linked **α**-galactosidase A (**α**-Gal A) deficiency, has an estimated incidence of ~1 in 50,000 males. The recent recognition of later-onset variants suggested that this treatable lysosomal disease is more frequent. To determine the disease incidence, we undertook newborn screening by assaying the **α**-Gal A activity in blood spots from 37,104 consecutive Italian male neonates. Enzyme-deficient infants were retested, and "doubly screened-positive" infants and their relatives were diagnostically confirmed by enzyme and mutation analyses. Twelve (0.03%) neonates had deficient **α**-Gal A activities and specific mutations, including four novel missense mutations (M51I, E66G, A73V, and R118C), three missense mutations (F113L, A143T, and N215S) identified previously in later-onset patients, and one splicing defect (IVS5$^{+1G\rightarrow T}$) reported in a patient with the classic phenotype. Molecular modeling and in vitro overexpression of the missense mutations demonstrated structures and residual activities, which were rescued/enhanced by an **α**-Gal A–specific pharmacologic chaperone, consistent with mutations that cause the later-onset phenotype. Family studies revealed undiagnosed Fabry disease in affected individuals. In this population, the incidence of **α**-Gal A deficiency was 1 in ~3,100, with an 11:1 ratio of patients with the later-onset:classic phenotypes. If only known disease-causing mutations were included, the incidence would be 1 in ~4,600, with a 7:1 ratio of patients with the later-onset:classic phenotypes. These results suggest that the later-onset phenotype of Fabry disease is underdiagnosed among males with cardiac, cerebrovascular, and/or renal disease. Recognition of these patients would permit family screening and earlier therapeutic intervention. However, the higher incidence of the later-onset phenotype in patients raises ethical issues related to when screening should be performed—in the neonatal period or at early maturity, perhaps in conjunction with screening for other treatable adult-onset disorders.

Fabry disease (MIM 301500) is an X-linked lysosomal storage disorder that results from the deficient activity of the lysosomal glycohydrolase α-galactosidase A (α-Gal A).[1] The enzymatic defect leads to progressive accumulation of globotriaosylceramide (GL-3) and related glycosphingolipids, particularly in blood vessels in the skin, kidney, heart, and brain. Affected males who have little, if any, α-Gal A activity exhibit the classic phenotype with onset of angiokeratomas, acroparesthesias, hypohidrosis, and corneal opacities in childhood.[1] With advancing age, progressive lysosomal GL-3 accumulation—particularly in the vascular endothelium—leads to renal failure, cardiac and cerebrovascular disease, and premature death.[1]

During the past 15 years, later-onset cardiac and renal variants with residual α-Gal A activity due to missense and splicing mutations have been identified in individuals who lack the early manifestations of classically affected patients (i.e., angiokeratoma, acroparesthesias, hypohidrosis, and corneal/lenticular abnormalities).[2–5] Patients with the cardiac variant typically present in the 5th–8th decades of life with left ventricular hypertrophy (LVH), arrhythmias, and/or cardiomyopathy,[3,4,6] whereas patients with the renal variant who also lack the early classic manifestations develop proteinuria and later-onset end-stage renal disease (ESRD), typically after age 50 years.[5,7–11] More recently, males and females aged 18–55 years who have acute cryptogenic strokes were found to have previously undiagnosed Fabry disease, including ~30% with classic manifestations.[12]

With the availability of effective enzyme-replacement therapy[13–15] and the future prospect of pharmacologic enzyme-enhancement therapy,[16–19] efforts have been directed to develop screening methods to identify patients with Fabry disease,[20–22] which has an estimated incidence of 1 in ~40,000–60,000 males.[1,23] Recently, screening of males in hemodialysis, cardiac, and stroke clinics, by determination of plasma α-Gal A activities, detected previously undiagnosed Fabry disease in 0.25%–1% of males undergoing hemodialysis,[5,7–11] in ~3%–4% of males with LVH or hypertrophic cardiomyopathy,[4,6] and in ~5% of males with acute cryptogenic strokes[12] (table 1). Detection of patients with unrecognized Fabry disease is important for family studies, to identify other affected relatives with this X-linked disease for therapeutic intervention.

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Table 1. Estimated Frequency in Males of Classic and Later-Onset Phenotypes of Fabry Disease

Phenotype	Frequency (in Males)	
	No. Screened	%
Classic:		
Estimate[1]	…	~.0025
Based on diagnostic data[21]	…	~.0017
Later-onset variants:		
In patients in cardiac clinics:		
LVH[4]	230	3.0
Hypertrophic cardiomyopathy[6]	153	3.9
In patients in hemodialysis clinics:		
Japan (Kyushu)[5]	514	1.0
Japan (Tokyo)[7]	440	.5
United States[9]	1,903	.5
Austria[11]	2,046	.24
Italy[8]	6,378	.25
In patients in stroke clinics (acute cryptogenic strokes)[12]	432	4.9

Here, we report the screening of 37,104 consecutive newborn males for Fabry disease by determination of the α-Gal A activity in dried blood spots. Surprisingly, 12 neonates, or 1 in ~3,100 males, had deficient α-Gal A activities and α-Gal A mutations. Molecular modeling studies and in vitro overexpression of the missense mutations revealed structural characteristics and residual enzymatic activities consistent with mutations identified previously that cause the later-onset phenotype.[1,24,25] These findings provide the first estimate of the incidence of α-Gal A deficiency; emphasize the need to diagnose unrecognized later-onset Fabry disease in patients in cardiac, renal, and stroke clinics; and have important implications for mass-screening programs for newborns that presymptomatically detect this and other treatable later-onset genetic disorders.

Subjects, Material, and Methods

Newborn Screening

From July 1, 2003, to June 30, 2005, 37,104 consecutive newborn males from the Piemonte area of northwestern Italy were screened for α-Gal A deficiency with the use of dried blood spots on filter paper collected at age 3 d. This population includes individuals from northern (~50%) and central-southern (~40%) Italy, eastern Europe (~5%), and Africa (~5%). The project was approved by the Piemonte Regional Committee for Newborn Screening, and parental consent was obtained for Fabry disease testing, as well as for the other routinely screened diseases. The parents and available family members of the newborns with α-Gal A deficiency were counseled and were offered testing, medical evaluations, and medical follow-up.

Enzyme Assay, Mutation Analysis, and Microsatellite Haplotype Studies

Blood-spot α-Gal A activity was determined using a fully automated technique described elsewhere.[20] One unit (U) of enzymatic activity was equal to 1 nmol of 4-methylumbelliferyl-α-*D*-galactopyranoside cleaved per h. Infants with activities $\leqslant$20% of the normal mean $\pm$ SD (7.7 $\pm$ 3.1 U/ml whole blood; $n = 350$) were considered "screen positive," and a second blood spot was requested and assayed. Retested newborns with blood-spot α-Gal A activities $\leqslant$1.5 U/ml were recalled with their mothers for plasma α-Gal A activity determinations.[26] Plasma α-Gal A activities of $\leqslant$2.5 U/ml (normal mean $\pm$ SD = 14.3 $\pm$ 3.2 U/ml; $n = 150$) were considered diagnostic. Genomic DNAs were isolated for α-Gal A mutation analyses as described elsewhere.[27]

To determine if the neonates with the same α-Gal A mutations were related, we performed haplotype analyses with seven microsatellite markers tightly linked to the α-Gal A gene between X-chromosome positions 98424692 and 102588188, six of which (*DXS8020, DXS8034, DXS8089, DXS8100, DXS8063,* and *DXS8096*) were informative, and with 11 SNPs, 2 of which (*rs2071397* and *rs1023431*) were informative (UCSC Genome Bioinformatics). Forward primers were fluorescently dye labeled (Invitrogen Life Technologies). Genomic DNA was amplified in 10-μl volumes with 50 ng of genomic DNA, 2 mM $MgCl_2$, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 200 nM of each primer, 0.2 mM dinucleotide triphosphates, and 2 U of *Taq* DNA polymerase (AmpliTaq Gold [Applied Biosystems]). The reaction mixtures were initially incubated at 95°C for 10 min and were amplified for 27 cycles, with denaturation at 94°C for 30 s, annealing at 58°C for 30 s, extension at 72°C for 30 s, and a final extension step at 72°C for 7 min. Microsatellites were analyzed with an ABI Prism 3100 Genetic Analyzer with the use of GeneScan Analysis software (v. 3.1.2) and Genotyper software (v. 2.5) (Perkin-Elmer-Cetus).

Molecular Modeling Studies

Structural modeling of the novel missense mutations was based on the human α-Gal A crystal structure[24] and used the SYBYL/BIOPOLYMER molecular modeling software (TRIPOS), as described for classic and later-onset mutations.[25] The structural location and the number of atoms influenced by the amino acid substitution in the main and side chains of the mutant enzyme were determined.

In Vitro Overexpression Studies

In vitro overexpression and characterization of the missense mutations were performed as described elsewhere, except that the eukaryotic expression vector used was pcDNA 3.0 (Invitrogen Life Technologies).[28] Mutant constructs were generated using the GeneTailor site-directed mutagenesis kit (Invitrogen Life Technologies). Transient transfections of COS-7 cells with each α-Gal A cDNA construct (~1 μg DNA) and determination of the expressed intracellular α-Gal A activities were performed as described elsewhere.[28] COS-7 cells were incubated in the presence or absence of 20 μM deoxygalactonojirmycin (DGJ), and the intracellular α-Gal A activities were measured 72 h after transfection.[16] Results are expressed as the mean (±SD) of at least three independent transfections for each construct.

Results

Screening of Newborn Males

Initial screening of α-Gal A activity in blood spots from 37,104 consecutive newborn males detected 41 (0.11%) neonates with enzyme levels ⩽20% of the mean normal activity. The physicians and families of all 41 neonates were contacted, and a second blood spot was obtained from each neonate, as well as one from each mother. When retested, 12 (0.03%) neonates had α-Gal A activities ⩽20% of the normal mean, whereas the other 29 had higher enzyme activities ranging from 2.5 to 6.1 U/ml whole blood. Table 2 shows the enzyme activities in the blood spots and plasma from the 12 "doubly screened-positive" neonates whose α-Gal A activities ranged from 0.0 to 0.4 U/ml whole blood and from 0.2 to 1.1 U/ml plasma. Plasma α-Gal A activities of the 12 mothers ranged from 14.7% to 43.4% of the mean normal value.

Mutation Analysis and Haplotype Studies

Sequencing of the α-Gal A gene in genomic DNAs from the 12 enzyme-deficient neonates identified four novel mutations (M51I, E66G, A73V, and R118C) and four known mutations reported elsewhere (F113L, A143T, N215S, and IVS5$^{+1G\rightarrow T}$)[27,29,30] (table 2). The respective mutation was present in each neonate's mother. The four novel mutations were not detected in >400 X chromosomes from normal individuals, which indicated that they were not common sequence variants or polymorphisms.

Analyses of informative, closely linked microsatellite markers surrounding the α-Gal A gene revealed that the two neonates with the N215S and the three neonates with the A143T genotypes were not related (three markers differed in the two neonates' N215S alleles, and one marker differed in each of the three neonates' A143T alleles). In contrast, the relatedness of the two neonates with the F113L alleles could not be excluded (no markers differed). On the basis of the deficient α-Gal A activities and detection of specific α-Gal A mutations, the diagnosis of Fabry disease was predicted in these 12 neonates, for an incidence of 1 in ~3,100 males in this population. If the four novel missense mutations were excluded, the incidence

Table 2. α-Gal A Activities and Genotypes in 12 Male Neonates Detected by Newborn Screening

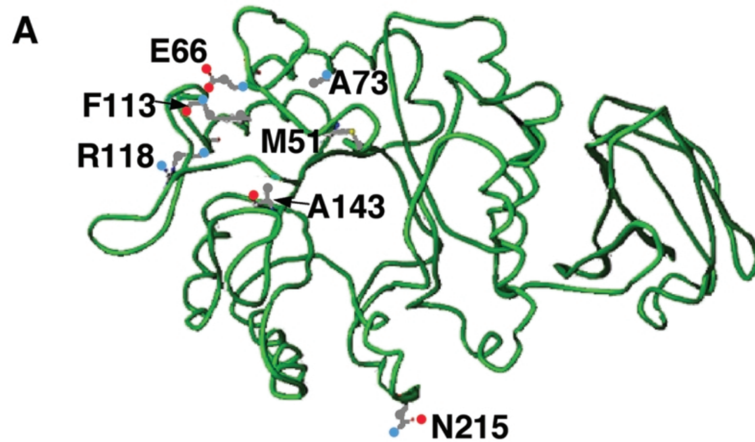
		α-Gal A Activity[a] (U/ml)		α-Gal A Mutation		No. of Atoms Influenced[b]	
Neonate	Genotype	Blood Spot	Plasma	Nucleotide Substitution	Amino Acid Substitution	Main Chain	Side Chain
1	N215S	.0	.8	AAT→AGT	215 Asn→Ser	0	1
2	E66G[c]	.2	.7	GAG→GGG	66 Glu→Gly	5	4
3	M51I[c]	.0	.6	ATG→ATA	51 Met→Ile	1	0
4	A73V[c]	.0	.8	GCA→GTA	73 Ala→Val	5	11
5	N215S	.1	.7	AAT→AGT	215 Asn→Ser	0	1
6	F113L	.0	.3	TTT→CTT	113 Phe→Leu	1	3
7	A143T	.1	1.1	GCA→ACA	143 Ala→Thr	3	8
8	A143T	.2	.6	GCA→ACA	143 Ala→Thr	3	8
9	R118C[c]	.2	.7	CGC→TGC	118 Arg→Cys	1	4
10[d]	IVS5$^{+1G\rightarrow T}$	.0	.2	AGgt→AGtt	Altered 5′ donor consensus sequence	…	…
11	F113L	.0	.2	TTT→CTT	113 Phe→Leu	1	3
12	A143T	.4	.8	GCA→ACA	143 Ala→Thr	3	8

[a] Normal mean (±SD) values: 7.7 ± 3.1 U/ml whole blood and 14.3 ± 3.2 U/ml plasma.

[b] The number of atoms in main and side chains of the α-Gal A structure influenced by a given missense mutation was calculated through molecular modeling, as described elsewhere.[25] The means (±SD) (and ranges) for main- and side-chain influences were 14.1 ± 14.9 (0–77) and 17.1 ± 15.7 (0–68), respectively, for 147 classic mutations; 1.7 ± 1.8 (0–6) and 2.9 ± 2.6 (1–8), respectively, for 15 known later-onset mutations; and 3.0 ± 2.3 (1–5) and 4.8 ± 4.6 (0–11), respectively, for the four novel mutations.

[c] Novel missense mutations: E66G resulted from an A→G transition in codon 66, predicting the substitution of a negatively charged polar Glu by a smaller nonpolar Gly; M51I resulted from a G→A transition in codon 51, predicting the replacement of a nonpolar Met by a nonpolar Ile; A73V resulted from a C→T transition in codon 73, predicting the substitution of a nonpolar Ala by a slightly larger nonpolar Val; and R118C resulted from a C→T transition in codon 118, predicting the replacement of a basic Arg with an uncharged polar, potentially sulfhydryl-binding Cys.

[d] IVS5$^{+1G\rightarrow T}$ = Intervening sequence of intron 5, g→t transition of the consensus "gt" to "tt"of the 5′ donor splice site.



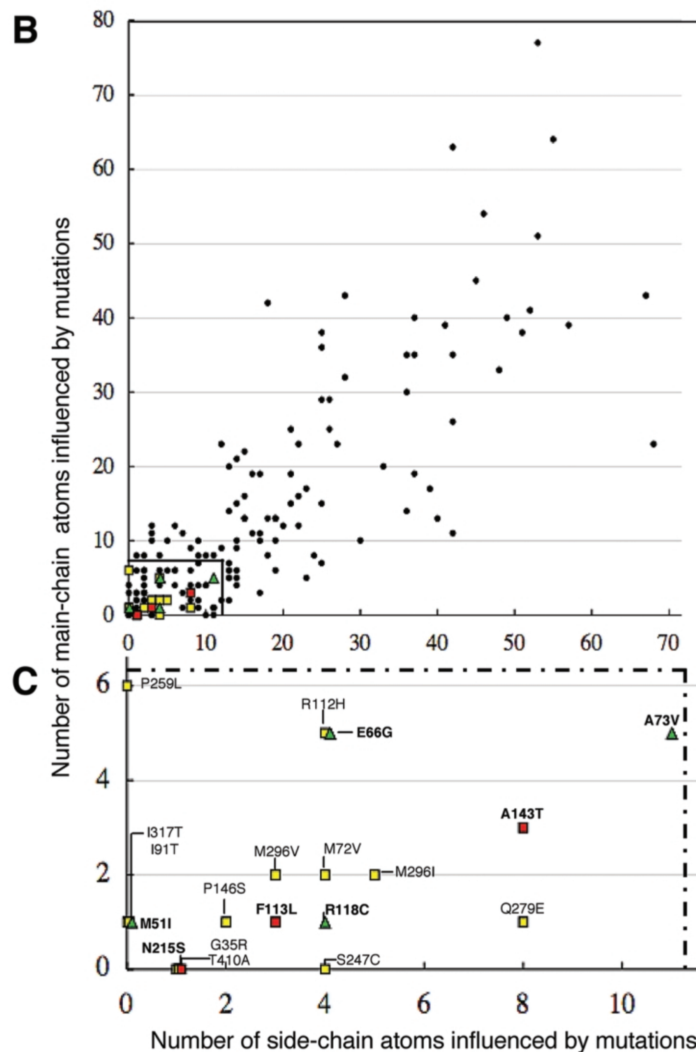


Figure 1. Structural changes and molecular modeling of α-Gal A mutations. *A,* Locations in the α-Gal A crystal structure of the seven missense mutations identified in neonates by newborn screening. *B* and *C,* Molecular modeling studies comparing the number of main-chain and side-chain atoms influenced by various amino acid substitutions in α-Gal A missense mutations that cause different phenotypes.[25] These include known mutations causing the classic phenotype (*black circles*), known later-onset mutations (F113L, A143T, and N215S) identified in the neonates (*red squares*), novel mutations (M51I, E66G, A73V, and R118C) identified in the neonates (*green triangles*), and other known later-onset mutations (*yellow squares*). Panel C is an enlargement of the boxed area in panel B that contains the known later-onset mutations and the novel mutations identified in the neonates. See table 2 for main- and side-chain values.

Table 3. In Vitro Overexpression of α-Gal A Missense Mutations in COS-7 Cells

Mutation	% of Expressed α-Gal A Wild-Type Activity[a] (No Treatment)	Fold Increase over Untreated Expressed Wild-Type Activity[b] (20 μM DGJ)
Wild type	100	1.05
Classic mutations:		
C172G	<1	ND
D264Y	<1	ND
Known later-onset mutations:		
F113L	38	1.50
A143T	36	1.61
N215S	47	1.79
Novel mutations:		
M51I	36	2.25
E66G	39	1.95
A73V	44	1.77
R118C	29	1.10
Pseudodeficiency allele:		
D313Y[28,34]	67	ND

NOTE.—Transiently transfected COS-7 cells were cultured in the presence or absence of 20 μM DGJ for 72 h, and then the intracellular α-Gal A activity was assayed. The activities are expressed as the percentage of the untreated mean expressed wild-type activity. All results are the means of at least three independent transfections.

[a] Mean activity of expressed wild-type human α-Gal A was 16.5 μmol/h/mg protein.

[b] ND = not determined.

of known disease-causing mutations would be 1 in ~4,600 males.

Molecular modeling studies of the novel missense mutations (fig. 1*A*) indicated that the substituted residues could be accommodated in the α-Gal A crystal structure and that the numbers of "influenced" main- and side-chain atoms were similar to those of known later-onset–causing mutations (fig. 1*B* and 1*C* and table 2).

In Vitro Overexpression of Novel Missense Mutations

In vitro transient overexpression in COS-7 cells with each of the seven missense mutations revealed that they all had in vitro residual activities between 29% and 47% of the mean (±SD) transiently expressed wild-type activity (16.5 ± 1.5 μmol/h/mg protein; range 14.3–18.7 μmol/h/mg protein) (table 3). The high levels of expression were consistent with the simultaneous translation and transcription of multiple copies of the mutant constructs, whereas these mutant alleles had low levels of activity (<5% of normal) in the plasma or tissues of patients with the later-onset phenotype.[28,31–33] In contrast, transiently overexpressed missense mutations from patients with the classic phenotype (e.g., C172G and D246Y) had activities that were ⩽1% of the mean expressed wild-type activity. Also, the only known pseudodeficiency allele, D313Y, had ~60%–67% of the mean expressed wild-type activity and no clinical phenotype.[28,34] Incubation of the pharmacologic chaperone, DGJ, in the media of the COS-7 cells enhanced the transiently expressed activities of the three known later-onset mutations and three of the novel missense mutations 1.50- to 2.25-fold (table 3), as demonstrated elsewhere for rescuable α-Gal A mutations with residual α-Gal A activities.[16,19]

Pedigree Studies

In each of the 12 families, genetic counseling was provided to the parents, and, after pedigree analysis, diagnostic testing was offered to at-risk family members (fig. 2). All 12 mothers, aged 23–38 years, were heterozygous for the respective α-Gal A mutations. The 34- and 32-year-old mothers of newborns 1 (N215S) and 10 (IVS5$^{+1G\rightarrow T}$), respectively, had mild proteinuria (75 and 150 mg/day, respectively); neither had cardiac involvement. Newborns 2, 3, 6, and 10 each had one sibling; only the sister of newborn 10 (IVS5$^{+1G\rightarrow T}$) inherited the family mutation.

Information on the maternal grandparents was limited because of their availability, interest, or demise. Three maternal grandfathers (families 3, 8, and 10) had the family mutation but had not received a previous diagnosis. The 63-year-old grandfather of newborn 3 (M51I) had mild hypertrophic cardiomyopathy. The 58-year-old grandfather of newborn 8 (A143T) went into renal failure of unknown etiology at age 52 years and had a stroke at age 54 years. The grandfather of newborn 10 (IVS5$^{+1G\rightarrow T}$) had hypertrophic cardiomyopathy and died at age 60; his 58- and 62-year-old sisters had cardiac disease and stroke, respectively.

Six maternal grandmothers (families 1, 2, 4, 6, 11, and 12) were heterozygous—five confirmed by mutation analysis and one by pedigree analysis (fig. 2). The 62-year-old grandmother of newborn 6 (F113L) had cardiomyopathy, a complete atrial ventricular (AV) block, and a pacemaker; her 60-year-old brother had hypertrophic cardiomyopathy and previously unrecognized Fabry disease. The deceased great-grandmother of newborn 7 (A143T) had three brothers who died from cardiac disease. In family 11 (F113L), the 57-year-old heterozygous grandmother had LVH, and her 34-year-old affected son had hypertrophic cardiomyopathy and previously unrecognized Fabry disease. In the remaining three families (5, 7, and 9), additional medical information was unavailable.

Discussion

During the past decade, the clinical spectrum of Fabry disease expanded from the classic phenotype, first described independently in 1898 by Fabry in Germany[35] and by Anderson in England,[36] to include the later-onset subtypes, which were designated the "cardiac" and "renal" variants.[1–5] Cardiac variants were first recognized in the early 1990s,[2–4] and renal variants were recently identified by the screening of male patients undergoing chronic hemodialysis.[5,7–11] Most recently, later-onset cerebrovascular variants were detected among patients with pre-

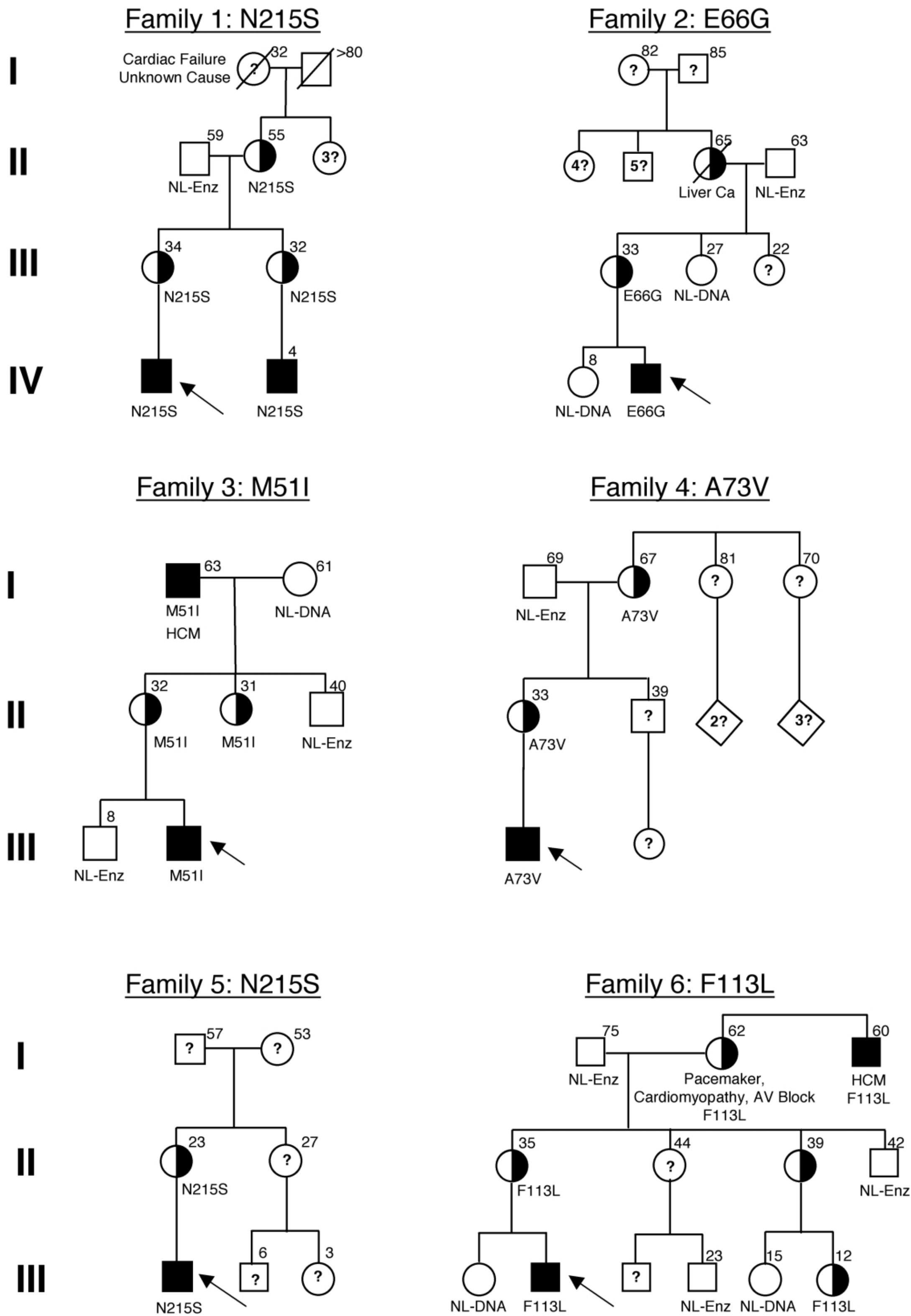
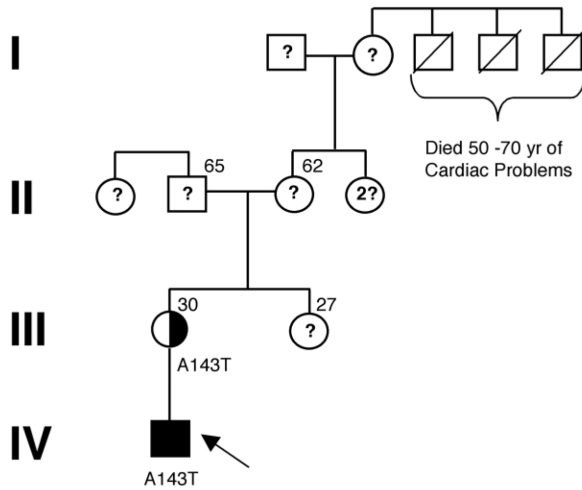
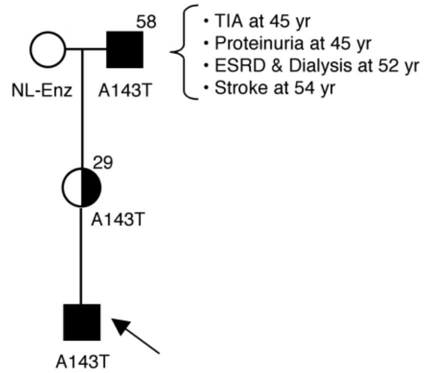


Figure 2. Family pedigrees of the 12 neonates with α-Gal A deficiency detected by newborn screening. Arrow indicates neonate proband. Males with Fabry disease (*solid black squares*) were identified by deficient α-Gal A enzyme activities, and heterozygous females (*half black circles*) were identified by α-Gal A mutation analysis. Mutations are listed below and ages are listed above the symbols. Clinical manifestations include hypertrophic cardiomegaly (HCM), LVH, complete AV block, transient ischemic attack (TIA), and ESRD. NL-Enz = males with normal α-Gal A enzyme activity; NL-DNA = males or females with no α-Gal A mutation; symbols with number and question mark = number of male or female relatives unavailable for evaluation; and diamonds = sex unknown.

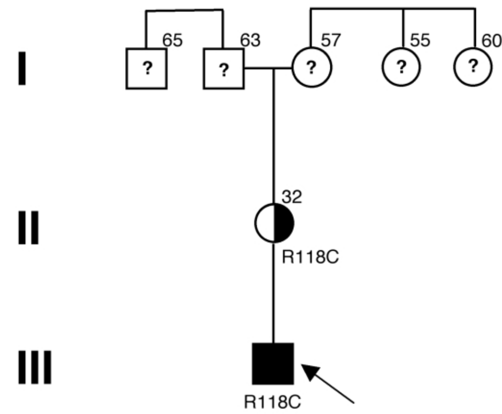
Family 7: A143T



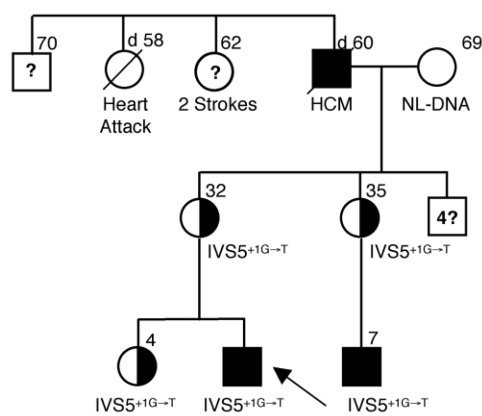
Family 8: A143T



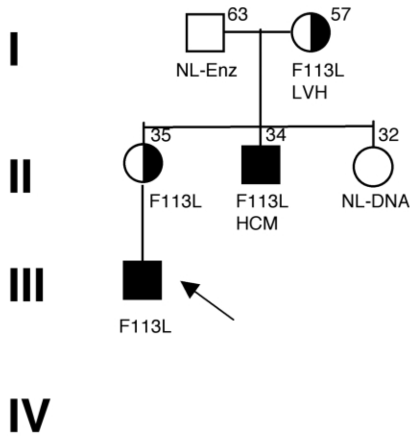
Family 9: R118C



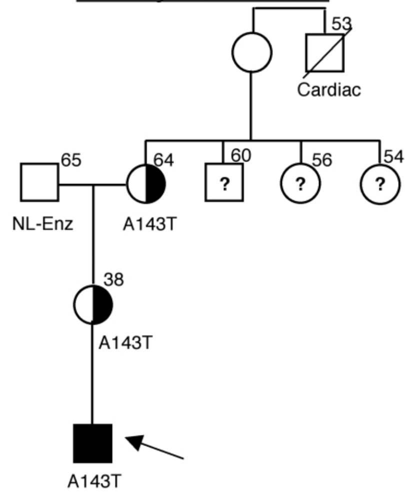
Family 10: IVS5$^{+1G \rightarrow T}$



Family 11: F113L



Family 12: A143T



mature acute cryptogenic strokes.[12] Affected males with the classic subtype have little, if any, α-Gal A activity, whereas the males with the later-onset cardiac and renal subtypes have missense or splicing mutations encoding residual α-Gal A activity, which is responsible for their lack of the early classic manifestations and for their difficult diagnoses. The specific mutations of the cerebrovascular variants were not reported, but at least 30% had the classic phenotype.[12] Although the prevalences of the cardiac, renal, and cerebrovascular variants have been estimated from pilot studies in cardiac, hemodialysis, and stroke clinics[4–6,8,11,12] (table 1), the combined prevalence of the later-onset subtypes has not been studied.

Our screenings of >37,000 consecutive newborn males in northwestern Italy found the surprisingly high incidence of α-Gal A deficiency of ~1 in 3,100 males in this population, or ~15–20 times higher than previously estimated.[1,37] In the 12 α-Gal A–deficient newborns, the enzymatic diagnosis was confirmed by detection of a specific α-Gal A mutation in each neonate and his heterozygous mother. Eight newborns had four known α-Gal A mutations, including a splice-site mutation (IVS5$^{+1G\rightarrow T}$) that occurred in a patient with the classic phenotype[30] and three missense mutations (F113L, A143T, and N215S) identified in patients with residual α-Gal A activities and later-onset phenotypes.[8,10,27,29] The A143T mutation was the most common lesion detected in males screened in hemodialysis clinics.[8–10]

Four newborns had novel missense mutations (E66G, M51I, A73V, and R118C) that had structural characteristics and in vitro overexpression levels similar to those of known later-onset missense mutations. Molecular modeling indicated that the four novel mutant enzymes did not alter the active site, could be accommodated in the crystal structure, and affected similar numbers of atoms in their main and side chains as known later-onset mutations did[25] (fig. 1 and table 2). These studies predicted that the mutant proteins encoded by the novel missense mutations would not interfere with catalysis but presumably had altered stabilities, making them excellent candidates for rescue by pharmacologic chaperones,[16,18,19] as shown when they were incubated with the α-Gal A–specific pharmacologic chaperone, DGJ (table 3). It should be noted that the in vitro overexpression of the multiple mutant constructs transfected simultaneously into COS-7 cells can be compared only with that of other overexpressed mutations and not with the in vivo activities in affected males or heterozygotes who have only one mutant gene in their cells. Thus, these studies indicated that the novel missense mutations encoded enzyme proteins with rescuable residual activities, consistent with their prediction of the later-onset phenotype.

Of the 12 neonates with α-Gal A deficiency, only one had a known mutation causing the classic phenotype, for an incidence of ~1 in 37,000 males, consistent with previous estimates for the classic phenotype.[1,23] In contrast, 11 neonates had mutations predicting the later-onset phenotype, for an 11:1 ratio of later-onset:classic phenotypes. Although it is not known if all the individuals with novel mutations will develop disease manifestations, it is notable that the affected 60-year-old grandfather of newborn 3 (M51I) had hypertrophic cardiomyopathy. Even if the novel mutations are excluded, the incidence of α-Gal A deficiency was 1 in ~4,600 males, with a 7:1 ratio of later-onset:classic phenotypes. Clearly, studies in other populations are required to confirm the high incidence of the later-onset phenotypes, since disease frequency variations may occur in different ethnic or demographic groups. Also, the finding of the same later-onset mutations (i.e., N215S and A143T) in newborns with different haplotypes indicates the absence of a founder effect for these lesions and reduces the likelihood that the high incidence of later-onset Fabry disease is an isolated occurrence. Whatever the incidence of α-Gal A deficiency and symptomatic Fabry disease in different populations, the high incidence found by newborn screening and the continued screening/identification of the later-onset phenotype in patients in cardiac, hemodialysis, and stroke clinics emphasize the fact that the later-onset phenotype of this panethnic disease is more prevalent than was previously appreciated.

General physicians and cardiologists should consider the diagnosis of Fabry disease in patients with LVH, cardiomyopathy, and conduction abnormalities, including short PR intervals and inverted ST segments without evidence of an infarction. The presence of microalbuminuria, or frank proteinuria, isosthenuria, and/or decreasing renal function should alert nephrologists to consider Fabry disease in the differential diagnosis. As demonstrated by the family studies of the 12 affected newborns identified here, relatives with cardiac or renal disease who had not received a Fabry diagnosis were affected or heterozygous for this X-linked disease. The determination of the α-Gal A activity in plasma or in isolated leukocytes can reliably identify affected males with all subtypes,[1] and subsequent genotyping can provide genotype/phenotype correlations and accurate identification of heterozygous females.[1] In addition, the availability of safe and effective enzyme replacement therapy[13–15] and the future prospect for pharmacologic chaperone therapy[16,18,19] emphasize the importance of diagnosing later-onset Fabry disease for early therapeutic intervention.

Genetic screening has proven effective for the identification of neonates with inherited metabolic diseases who require early therapeutic intervention[37,38] and for the prenatal or perinatal detection of carriers of genetic disorders in high-risk populations, such as cystic fibrosis in whites,[39] Tay-Sachs disease in Ashkenazi Jews,[40] and various hemoglobinopathies in targeted racial, ethnic, and demographic populations.[41] Newborn screening has proven so effective in identifying and treating infants with inherited metabolic disorders that efforts are under way to expand the disease menu by employing tandem mass spectroscopy, which can detect >40 disorders.[42,43] Efforts also are under way to develop high-throughput newborn screen-

ing for lysosomal storage diseases.[20–22] In our study, newborn screening revealed the surprisingly high incidence of later-onset Fabry disease of ~1:3,100 to ~4,600 (with and without the novel mutations), compared with ~1: 37,000 for the classic phenotype. The higher incidence of the later-onset phenotype raises the question of when genetic screening for this disease should be undertaken—in the neonatal period or at early maturity (e.g., age 18–21 years), with informed consent and genetic counseling and perhaps in conjunction with screening for other treatable later-onset disorders. Clearly, early detection, genetic counseling, and therapeutic intervention for the classic phenotype is beneficial,[13–15,44] but the issue of when to screen for the later-onset variants of Fabry and other treatable diseases that also present in childhood raises ethical issues for consideration and debate.

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Web Resources

The URLs for data presented herein are as follows:

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for Fabry disease)

UCSC Genome Browser, http://genome.ucsc.edu (for markers around the α-Gal A gene)

References

1. Desnick RJ, Ioannou YA, Eng CM (2001) α-Galactosidase A deficiency: Fabry disease. In: Scriver CR, Beaudet AL, Sly WS, Valle D, Kinzler KE, Vogelstein B (eds) The metabolic and molecular bases of inherited disease. McGraw-Hill, New York, pp 3733–3774
2. Elleder M, Bradova V, Smid F, Budesinsky M, Harzer K, Kustermann-Kuhn B, Ledvinova J, Belohlavek, Kral V, Dorazilova V (1990) Cardiocyte storage and hypertrophy as a sole manifestation of Fabry's disease: report on a case simulating hypertrophic non-obstructive cardiomyopathy. Virchows Arch A Pathol Anat Histopathol 417:449–455
3. von Scheidt W, Eng CM, Fitzmaurice TF, Erdmann E, Hubner G, Olsen EG, Christomanou H, Kandolf R, Bishop DF, Desnick RJ (1991) An atypical variant of Fabry's disease with manifestations confined to the myocardium. N Engl J Med 324: 395–399
4. Nakao S, Takenaka T, Maeda M, Kodama C, Tanaka A, Tahara M, Yoshida A, Kuriyama M, Hayashibe H, Sakuraba H, Tanaka H (1995) An atypical variant of Fabry's disease in men with left ventricular hypertrophy. N Engl J Med 333:288–293
5. Nakao S, Kodama C, Takenaka T, Tanaka A, Yasumoto Y, Yoshida A, Kanzaki T, Enriquez ALD, Eng CE, Tanaka H, Tei C, Desnick RJ (2003) Fabry disease: detection of undiagnosed hemodialysis patients and identification of a "renal variant" phenotype. Kidney Int 64:801–807
6. Sachdev B, Takenaka T, Teraguchi H, Tei C, Lee P, McKenna WJ, Elliott PM (2002) Prevalence of Anderson-Fabry disease in male patients with late onset hypertrophic cardiomyopathy. Circulation 105:1407–1411
7. Utsumi K, Kase R, Takata T, Sakuraba H, Matsui N, Saito H, Nakamura T, Kawabe M, Lino Y, Katayam Y (2000) Fabry disease in patients receiving maintenance dialysis. Clin Exp Nephrol 4:49–51
8. Spada M, Pagliardini S (2002) Screening for Fabry disease in end-stage nephropathies. J Inherit Metab Dis 25:S113
9. Desnick RJ (2002) Fabry disease: unregonized ESRD patients and effectiveness of enzyme replacement on renal pathology and function. J Inherit Metab Dis 25:S116
10. Spada M, Marongiu A, Voglino G, Merlino S, Alluto A, La Dolcetta M, Pagliardini S (2003) Molecular study in 20 unrelated male patients with Fabry disease: the A143T genotype correlates with the late-onset end-stage nephropathy. J Inherit Metab Dis 26:171
11. Kotanko P, Kramar R, Devrnja D, Paschke E, Voigtlander T, Auinger M, Demmelbauer K, Lorenz M, Hauser AC, Kofler HJ, Lhotta K, Neyer U, Pronai W, Wallner M, Wieser C, Wiesholzer M, Zodl H, Fodinger M, Sunder-Plassmann G (2004) Results of a nationwide screening for Anderson-Fabry disease among dialysis patients. J Am Soc Nephrol 15:1323–1329
12. Rolfs A, Bottcher T, Zshiesche M, Morris P, Winchester B, Bauer P, Walter U, Mix E, Lohr M, Harzer K, Strauss U, Pahnke J, Grossmann A, Benecke R (2005) Prevalence of Fabry disease in patients with cryptogenic stroke: a prospective study. Lancet 366:1794–1796
13. Eng CM, Guffon N, Wilcox WR, Germain DP, Lee P, Waldek S, Caplan L, Linthorst GE, Desnick RJ (2001) Safety and efficacy of recombinant human α-galactosidase A replacement therapy in Fabry's disease. N Engl J Med 345:9–16
14. Schiffmann R, Kopp JB, Austin HA 3rd, Sabnis S, Moore DF, Weibel T, Balow JE, Brady RO (2001) Enzyme replacement therapy in Fabry disease: a randomized controlled trial. JAMA 285:2743–2749
15. Wilcox WR, Banikazemi M, Guffon N, Waldek S, Lee P, Linthorst GE, Desnick RJ, Germain DP (2004) Long-term safety and efficacy of enzyme replacement therapy for Fabry disease. Am J Hum Genet 75:65–74
16. Fan JQ, Ishii S, Asano N, Suzuki Y (1999) Accelerated transport and maturation of lysosomal α-galactosidase A in Fabry lymphoblasts by an enzyme inhibitor. Nat Med 5:112–115
17. Frustaci A, Chimenti C, Ricci R, Natale L, Russo MA, Pieroni M, Eng CM, Desnick RJ (2001) Improvement in cardiac function in the cardiac variant of Fabry's disease with galactose-infusion therapy. N Engl J Med 345:25–32
18. Desnick RJ, Schuchman EH (2002) Enzyme replacement and enhancement therapies: lessons from lysosomal disorders. Nat Rev Genet 3:954–966
19. Fan JQ (2003) A contradictory treatment for lysosomal storage disorders: inhibitors enhance mutant enzyme activity. Trends Pharmacol Sci 24:355–360
20. Pagliardini S, Spada M (2003) A simple and rapid approach for screening lysosomal storage disorders. J Inherit Metab Dis 26:S1

21. Meikle PJ, Ranieri E, Simonsen H, Rozaklis T, Ramsay SL, Whitfield PD, Fuller M, Christensen E, Skovby F, Hopwood JJ (2004) Newborn screening for lysosomal storage disorders: clinical evaluation of a two-tier strategy. Pediatrics 114:909–916
22. Li Y, Scott CR, Chamoles NA, Ghavami A, Pinto BM, Turecek F, Gelb MH (2004) Direct multiplex assay of lysosomal enzymes in dried blood spots for newborn screening. Clin Chem 50:1785–1796
23. Meikle PJ, Hopwood JJ, Clague AE, Carey WF (1999) Prevalence of lysosomal storage disorders. JAMA 281:249–254
24. Garman SC, Garboczi DN (2004) The molecular defect leading to Fabry disease: structure of human α-galactosidase. J Mol Biol 337:319–335
25. Matsuzawa F, Aikawa S, Doi H, Okumiya T, Sakuraba H (2005) Fabry disease: correlation between structural changes in α-galactosidase, and clinical and biochemical phenotypes. Hum Genet 117:317–328
26. Desnick RJ, Allen KY, Desnick SJ, Raman MK, Bernlohr RW, Krivit W, Schaefer E (1973) Fabry's disease: enzymatic diagnosis of hemizygotes and heterozygotes: α-galactosidase activities in plasma, serum, urine, and leukocytes. J Lab Clin Med 81:157–171
27. Eng CM, Ashley GA, Burgert TS, Enriquez AL, D'Souza M, Desnick RJ (1997) Fabry disease: thirty-five mutations in the α-galactosidase A gene in patients with classic and variant phenotypes. Mol Med 3:174–182
28. Yasuda M, Shabbeer J, Benson SD, Maire I, Burnett RM, Desnick RJ (2003) Fabry disease: characterization of α-galactosidase A double mutations and the D313Y plasma enzyme pseudodeficiency allele. Hum Mutat 22:486–492
29. Eng CM, Resnick-Silverman LA, Niehaus DJ, Astrin KH, Desnick RJ (1993) Nature and frequency of mutations in the α-galactosidase A gene that cause Fabry disease. Am J Hum Genet 53:1186–1197
30. Sakuraba H, Eng CM, Desnick RJ, Bishop DF (1992) Invariant exon skipping in the human α-galactosidase A pre-mRNA: A g^{+1} to t substitution in a 5′-splice site causing Fabry disease. Genomics 12:643–650
31. Okumiya T, Ishii S, Kase R, Kamei S, Sakuraba H, Suzuki Y (1995) α-Galactosidase gene mutations in Fabry disease: heterogeneous expressions of mutant enzyme proteins. Hum Genet 95:557–561
32. Okumiya T, Ishii S, Takenaka T, Kase R, Kamei S, Sakuraba H, Suzuki Y (1995) Galactose stabilizes various missense mutants of α-galactosidase in Fabry disease. Biochem Biophys Res Commun 214:1219–1224
33. Ishii S, Suzuki Y, Fan JQ (2000) Role of Ser-65 in the activity of α-galactosidase A: characterization of a point mutation (S65T) detected in a patient with Fabry disease. Arch Biochem Biophys 377:228–233
34. Froissart R, Guffon N, Vanier MT, Desnick RJ, Maire I (2003) Fabry disease: D313Y is an α-galactosidase A sequence variant that causes pseudodeficient activity in plasma. Mol Genet Metab 80:307–314
35. Fabry J (1898) Ein Beitrag Zur Kenntnis der Purpura haemorrhagica nodularis (Purpura papulosa hemorrhagica Habrae). Arch Dermatol Syph 43:187
36. Anderson W (1898) A case of angiokeratoma. Br J Dermatol 10:113
37. Meikle PJ, Hopwood JJ (2003) Lysosomal storage disorders: emerging therapeutic options require early diagnosis. Eur J Pediatr 162:S34–S37
38. McCabe LL, McCabe ER (2004) Genetic screening: carriers and affected individuals. Annu Rev Genomics Hum Genet 5: 57–69
39. Grody WW, Cutting GR, Klinger KW, Richards CS, Watson MS, Desnick RJ (2001) Laboratory standards and guidelines for population-based cystic fibrosis carrier screening. Genet Med 3:149–154
40. Kaback MM (2001) Screening and prevention in Tay-Sachs disease: origins, update, and impact. Adv Genet 44:253–265
41. Henthorn JS, Almeida AM, Davies SC (2004) Neonatal screening for sickle cell disorders. Br J Haematol 124:259–263
42. Schulze A, Lindner M, Kohlmuller D, Olgemoller K, Mayatepek E, Hoffmann GF (2003) Expanded newborn screening for inborn errors of metabolism by electrospray ionization-tandem mass spectrometry: results, outcome, and implications. Pediatrics 111:1399–1406
43. Wilcken B, Wiley V, Hammond J, Carpenter K (2003) Screening newborns for inborn errors of metabolism by tandem mass spectrometry. N Engl J Med 348:2304–2312
44. Desnick RJ, Brady R, Barranger J, Collins AJ, Germain DP, Goldman M, Grabowski G, Packman S, Wilcox WR (2003) Fabry disease, an under-recognized multisystemic disorder: expert recommendations for diagnosis, management, and enzyme replacement therapy. Ann Intern Med 138:338–346