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Mucopolysaccharidosis IVA: Identification of a Common Missense Mutation I113F in the N-Acetylgalactosamine-6-Sulfate Sulfatase Gene

Shunji Tomatsu,[1] Seiji Fukuda,[1] Alan Cooper,[2] James E. Wraith,[2] Golam Md. Maruf Rezvi,[1] Atsushi Yamagishi,[1] Naoto Yamada,[1] Zenichiro Kato,[1] Kouji Isogai,[1] Kazuko Sukegawa,[1] Naomi Kondo,[1] Yasuyuki Suzuki,[1] Nobuyuki Shimozawa,[1] and Tadao Orii[1]

[1]Department of Pediatrics, Gifu University School of Medicine, Gifu; and [2]Royal Manchester Children's Hospital, University of Manchester School of Medicine, Manchester

Summary

Mucopolysaccharidosis IVA is an autosomal recessive lysosomal storage disorder caused by a deficiency of N-acetylgalactosamine-6-sulfate sulfatase. The recent isolation and characterization of cDNA and genomic sequences encoding GALNS has facilitated identification of the molecular lesions that cause MPS IVA. We identified a common missense mutation among Caucasian MPS IVA patients. The mutation was originally detected by SSCP, and successive sequencing revealed an A→T transversion at nt 393. This substitution altered the isoleucine at position 113 to phenylalanine (I113F) in the 622 amino acid GALNS protein and was associated with a severe phenotype in a homozygote. Compound heterozygotes with one I113F-allele mutation have a wide range of clinical phenotypes. Transfection experiments in GALNS-deficient fibroblasts revealed that the mutation drastically reduces the enzyme activity of GALNS. Allele-specific oligonucleotide or SSCP analysis indicated that this mutation accounted for 22.5% (9/40) of unrelated MPS IVA chromosomes from 23 Caucasian patients, including 6 consanguineous cases. Of interest, the I1e 113→Phe substitution occurred in only Caucasian MPS IVA patients and in none of the GALNS alleles of 20 Japanese patients. These findings identify a frequent missense mutation among MPS IVA patients of Caucasian ancestry, that results in severe MPS IVA when homoallelic, and will facilitate molecular diagnosis of most such patients and identification of heterozygous carriers. In addition to this common mutation, 10 different point mutations and 2 small deletions were detected, suggesting allelic heterogeneity in GALNS gene.

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Address for correspondence and reprints: Dr. Shunji Tomatsu, Department of Pediatrics, Gifu University School of Medicine, 40 Tsukasa-machi Gifu 500, Japan.

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Introduction

Mucopolysaccharidosis IVA (MPS IVA) is an autosomal recessive disorder resulting from a deficiency in the activity of the lysosomal hydrolase, N-acetylgalactosamine-6-sulfate sulfatase (GALNS; EC3164) (Matalon et al. 1974), and the accumulation of glycosaminoglycan, primarily in lysosomes. Since the first description of Morquio syndrome (a classical, severe form) (Morquio et al. 1929), many cases have been reported. Severe MPS IVA (classical Morquio disease) is a disease with progressive systematic bone involvement, manifestated by short trunk dwarfism, coxa valga, odontoid hypoplasia, corneal opacities, hepatosplenomegaly, and, if untreated, demise by 20–30 years of age. A milder form of MPS IVA is characterized by mild bone involvement and mild visceral organ involvement and survival to 50–60 years of age with a normal quality of life (Glössl et al. 1981; Orii et al. 1981; Fujimoto and Horowitz 1983; Hecht et al. 1984; Beck et al. 1986). We cloned the full-length cDNA of human GALNS and the entire genomic gene (Tomatsu et al. 1991; Nakashima et al. 1994), and two groups have assigned the gene to chromosome 16q24 (Tomatsu et al. 1992; Baker et al. 1993; Masuno et al. 1993). Recently, we characterized the molecular defects in three families with GALNS deficiency: a 2-bp deletion that predicts a truncated GALNS protein with a severe form, a missense mutation in two siblings with a mild form (Fukuda et al. 1992), and a splice-site mutation in two siblings with a severe form (Tomatsu et al. 1994*c*). Successive mutation analysis revealed allelic heterogeneity in a series of Japanese MPS IVA patients (Ogawa et al. 1995) and in some Caucasian patients (Tomatsu et al. 1995*a*; and in press). Preliminary evidence for various kinds of mutations underlying the broad clinical phenotypes was obtained in haplotyping studies with several RFLPs (Tomatsu et al. 1994*a*, 1994*b*, 1994*c*, 1995*b*, 1995*c*; Iwata et al. 1995). Here we report the molecular basis of MPS IVA patients in a group of 23 unrelated patients of different Caucasian ancestries and compare findings with those for subjects of Japanese ancestry.

Table 1

Summary of Patients with the I113F Mutation

Patient	Sex	Age at Initial Symptom	Age at Diagnosis	Population	Genotype	Clinical Phenotype
M10[a]	F	10 wk	4 years	British	I113F/I113F	Severe, talipes deformity of feet, height 103 cm at 10 years
M11	M	9 mo	9 mo	British	I113F/888del 27	Severe, cervical fusion at 12 years, height 101.5 cm at 15 years
M13	F	8 mo	8 mo	British	I113F/1374 del T	Severe, cervical fusion at 8 years, height 103 cm at 9 years
M14	M	Unknown	Unknown	British	I113F/M391V	Severe, height 120 cm at 17 years, cervical fusion at 17 years
M15	M	4 mo	12 mo	British	I113F/-	Severe, height 97 cm at 6 years
M16	M	Unknown	Unknown	Irish	I113F/-	Severe, cervical fusion at 16 years, height 90 cm at 16 years
M17	M	Unknown	Unknown	Irish	I113F/-	Severe, cervical fusion at 8 years, height 90 cm at 8 years
M18	F	12 mo	18 mo	Irish	I113F/-	Severe, operation on both lower limbs at 11 years, height 90 cm at 17 years
M20	M	20 mo	6 years	Irish	I113F/-	Intermediate, mild joint laxity, absence of cord compression, height 144 cm at 13 years

[a] Consanguineous case.

In subjects of Caucasian origin, we found a missense mutation that results in substitution of phenylalanine for isoleucine at amino acid 113 (I113F) in 9 of the 40 unrelated mutant alleles and that was not present among Japanese patients.

Material and Methods

Material

Restriction enzymes and DNA modifying enzymes were from Takara Shuzo, Toyobo, Boehringer Mannheim, Pharmacia, or BRL. [α-^{32}P]dCTP (specific activity, 3,000 Ci/mmol) was purchased from Amersham. Dideoxy cycle sequencing kits were from Applied Biosystems. PCR primers and specific oligonucleotide primers were synthesized with an automated DNA synthesizer (model 380A; Applied Biosystems). Hybond N membrane was from Amersham.

DNA Preparation

All the MPS IVA DNA samples were prepared from peripheral blood at the Royal Manchester Children's Hospital, according to standard protocols. GALNS deficiency was demonstrated in all cases by enzyme assay. A description of each patient with I113F mutation is given in table 1.

PCR Amplification, Screening by SSCP, and DNA Sequencing

Localization of mutations was obtained by screening amplified genomic fragments for each of 14 exons by SSCP as described elsewhere (Nakashima et al. 1994) (the GALNS gene is split into 14 exons and 13 introns). Here we present findings in exon 4, and detailed information of primer sequences for other exons has been reported elsewhere (Ogawa et al. 1995). PCR was carried out using primers as follows: the forward primer OMF 147 (5′-AATCTTGGGAAGTGCCATGCCCTGT-3′), the reverse primer oligonucleotide Morquio Fukuda 148 (5′-CTGGGCAGGCGTGGCCAGGAGACTT-3′).

SSCP analysis of PCR products was done according to Orita et al. (1989), with minor modifications (Tomatsu et al. 1994*a*). The PCR mixture in 10 μl of total volume contained 1 pmol of each unlabeled primer, 12.5 μM each of the four deoxynucleotide triphosphates, 50 ng of genomic DNA, 0.25 U of *Taq* DNA polymerase, and 0.1 μCi [α-^{32}Pl dCTP (Amersham). The PCR condition for exon 4 was 2 min at 94°C (denaturing), followed by 30 cycles of 1 min at 94°C), 1 min at 72°C. A portion of the reaction mixture (1 μl) was used and was mixed with 9 μl of SSCP loading buffer (95% formamide, 10 mM NaOH, 0.05% bromophenol blue, and 0.05% xylene cyanol), and was heated at 95°C for 2 min; 2 μl of sample was loaded onto a mutation detection enhancement (MDE) gel (AT Biochem). Electrophoresis was performed at 30 W for 12 h at room temperature. The gel was dried onto filter paper and exposed to x-ray film at −80°C for 16 h. Following SSCP screening, the positive products were reproduced by PCR. The amplification

mixture contained 1 μg of genomic DNA, 50 mM KCl, 10 mM Tris·HCl, pH 8.3, 1.5 mM $MgCl_2$, 200 μM concentration of dNTPs, and 50 pmol of each sense and antisense primer in a reaction volume of 100 μl. Each reaction mixture was incubated at 94°C for 2 min, as a pretreatment after the addition of 1–2.5 U of *Taq* polymerase. Forty cycles of denaturation at 94°C for 1 min, annealing at 60°C for 1 min, and extension at 72°C for 1 min were carried out. The PCR fragments were isolated from agarose gels, were subcloned into T-vector (Stratagene), and were sequenced in both directions by fluorescent dideoxy cycle sequencing. For each product, at least five clones were sequenced.

Construction of Mutant GALNS Expression Plasmid and Transient Transfection into Deficient Fibroblasts

The wild-type GALNS cDNA (Tomatsu et al. 1991) was subcloned into pUC 13, and in vitro mutagenesis was performed with mutant allele-specific oligonucleotides (ASOs) corresponding to mutation I113F, according to the supplier's recommendations (Clontech). The mutations were introduced into the GALNS-expression vector by subcloning DNA restriction fragments from pUC 13 clones carrying the respective mutation. DNA sequence of the resulting mutant GALNS expression plasmids of pMF I113F was confirmed for the entire subcloned mutant fragments. Mutant GALNS-expression plasmid was transfected into GALNS-deficient fibroblasts by the liposome-mediated transfection method (Gene Transfer; Tomatsu et al. 1991). One hundred twenty hours after transfection, the fibroblasts were collected and assayed for GALNS activity, as described below. Other expression vectors for each mutation were also made by the same procedure.

GALNS Enzyme Assays

GALNS activity was determined in cultured fibroblasts obtained from MPS IVA patients and transfected fibroblasts, using as substrate, the trisaccharide 6-sulfo-N-acetylgalactosamine-glucuronic acid-6-sulfo-N-acetyl-[1-^{3}H] galactosaminitol, as described elsewhere (Glössl and Kresse 1978; Sukegawa and Orii 1982). For these assays, cultured or transfected fibroblasts were harvested, were resuspended in ddH_2O, and were lysed by sonication on ice. Cell extracts were centrifuged (10,000 g) at 4°C for 10 min, and each supernatant was used for each assay. The standard reaction mixture contained 1.0 nmol substrate (~60,000 disintegrations per minute); 10 μl 120-mM sodium acetate buffer, pH 3.8; 5–20 μg protein (supernatant); and 10 μg bovine serum albumin in a final volume of 60 μl. After incubation for 1 h at 37°C, the reaction was stopped by the addition of 500 μl ice-cold water and was loaded onto a 0.6 ml-column of Dowex 1 × 2, 200–400 mesh, Cl^- form prepared in Pasteur pipets. After the column was washed with ddH_2O, it was eluted with 5.0 ml of 0.4 M NaCl, and the monosulphated trisaccharide product was collected in a scintillation vial. The remaining substrate was eluted by applying 5.0 ml of 0.9 M NaCl into a second scintillation vial. The sum of the radioactivity in the two vials was used to calculate the enzyme activity.

Haplotyping

GALNS gene haplotypes for the mutant allele were constructed by analysis of six RFLPs. The first polymorphism consists of a *Sty*I RFLP (CCA/TA/TGG) within the fifth intron (IVS 5 + 134; CCAAGG [allele A] or CCGAGG [allele a]) (Tomatsu et al. 1995*c*). The second polymorphism is located within exon 7 (763 nt from A of the ATG initial codon on the cDNA). This polymorphism (GCACGC [allele B] or GCATGC [allele b] affects a *Sph*I restriction endonuclease site (GCATGC) (Tomatsu et al. 1994*a*). The third polymorphism of a *Rsa*I restriction enzyme site (GTAC) is positioned within the seventh intron (IVS7 nt 90; GTAC [allele H] or GAAC [allele h]). The fourth polymorphism is positioned at 1,232 nt from A of the ATG initial codon within exon 11. This polymorphism (GTCC [allele C] or GGCC [allele c]) changes a *Hae*III site restriction site. The fifth polymorphism is located at 1,487 nt from A of the ATG initial codon within exon 13. This polymorphism (AAGCCT [allele D] or AGGCCT [allele d]) changes a *Stu*I restriction endonuclease site (AGGCCT) (Tomatsu et al. 1995*c*). The sixth polymorphism (CCAG [allele E] or CCGG [allele e]) consists of a *Hap*II RFLP (CCGG) found in exon 14 (Tomatsu et al. 1994*c*). These six RFLP products of PCR amplification of each exon and its boundary were digested with the appropriate restriction enzyme and subsequently were separated on a 8.0% acrylamide gel or 4% NuSieve gel.

ASO Hybridization

ASO hybridization for I113F was used to confirm the mutation. ASO analysis was performed using the PCR-amplified fragments of genomic DNA from patients, and 1–2 μl of the product was denatured in 100 μl 10-mM Tris-HCl, pH 7.5, containing 0.2 M NaOH, for 10 min at room temperature. This mixture was neutralized with 100 μl 2-M NH_4OAc and was transferred onto Hybond N nylon membrane (Amersham), using a dot-blot apparatus (Bio-Rad). The resultant membrane fixed by illuminator for 5 min was prehybridized at 37°C for 3 h in 10 ml buffer made from hybridization buffer tablet (Amersham) and then was hybridized at 37°C overnight in the same buffer containing [γ-^{32}P] ATP-labeled oligonucleotides. Oligonucleotides used were OMF I113F (N)-5′-ACCGCAGGAGATTGTGGGCGG-3′ and OMF I113F (M)-5′-ACCGCAGGAGTTTGTGGGCGG-3′. After hybridization, the membrane was washed twice in

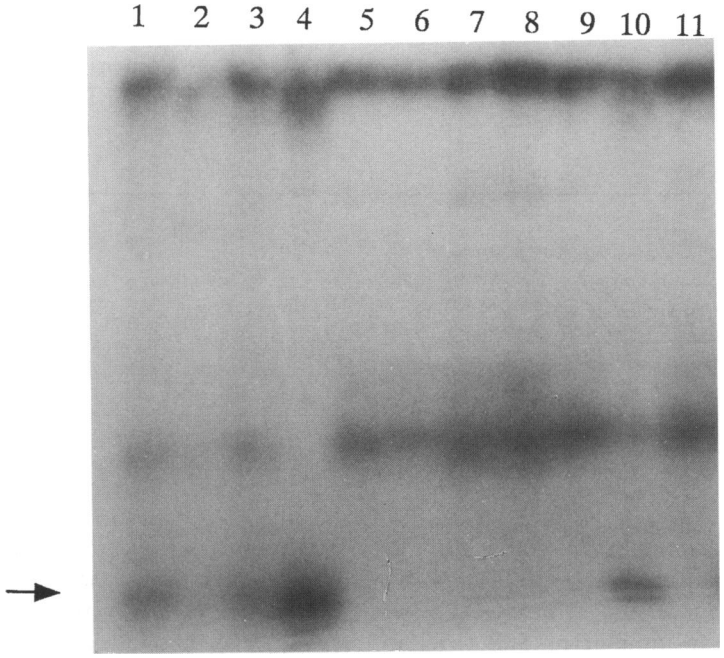

Figure 1 SSCP analysis of exon 4 of the GALNS gene. The SSCP pattern for heterozygotes or homozygote with a I113F mutation is shown (lanes 1–10 indicate samples of MPS IVA patients, and lane 11 indicates control individuals. Arrow indicates samples with a I113F mutation (lanes 1, 3, and 10, heterozygotes; lane 4, homozygote). The SSCP condition for this exon is listed in the text.

2× SSC containing 0.1% SDS at 37°C for 15 min, followed by two washings in 2× SSC containing 0.1% SDS for 30 min at 58°C, to distinguish between perfect matches and single-base mismatches. After being washed in 2× SSC, the membrane was dried and autoradiographed for 2 h at room temperature.

Results

Screening by SSCP analysis revealed the common abnormal mobility in exon 4 from Caucasian MPS IVA patients, compared with normal individuals (fig. 1). Sequence analysis of mutant PCR products from one of these subjects led to identification of a homozygous mutant allele which contained a novel A-to-T transversion at nt 392, according to Tomatsu et al. (1991). This mutation (I113F) changes the ATT codon 113 for isoleucine to a TTT codon for phenylalanine (data not shown). The A-to-T substitution was detected in all the clones expanded, and no other DNA alteration was observed in any exon. To confirm the authenticity of this candidate mutation and to screen MPS IVA patients' samples (43 patients, including 12 British, 4 Irish, 5 Pakistani, 1 Italian, 1 Indian, and 20 Japanese), a 299-bp region of genomic DNA from patients, 100 normal Caucasians and 100 normal Japanese individuals, was PCR-amplified and hybridized with normal and I113F-specific radiolabeled oligonucleotides. The PCR-amplified genomic DNA from a homozygous proband, patient M10 of British ancestry, hybridized to the mutation-specific but not to the normal ASO. This result confirmed authenticity of the transversion and indicated that this proband was homoallelic for the Ile113Phe mutation (data not shown). The I113F mutation occurred in a total of 9 (29%) of 31 alleles in Anglo-Saxon Caucasians, but was not found in other Caucasian alleles (8 Pakistani and 1 Italian) or Japanese alleles (table 2).

Of the 17 patients from Britain and Ireland, one consanguineous case was homozygous and 8 were heterozygous for this mutation. The patient M10 who was homozygous for I113F was the product of consanguineous parents, and was referred for orthopedic care because of talipes deformity. At age 12 mo, she presented with dislocated hips and was treated surgically for bilateral subluxation of the hips. She also had a skeletal dysplasia. At age 10 years she was 103 cm and her phenotype was that of MPS IVA severe form (table 1). Furthermore, it was linked to the a/b/h/c/D/E haplotype, which was also common in normal controls (~25% among Caucasian and Japanese). We identified three additional mutations in three patients heteroallelic for I113F, including two small deletions (in-frame 27-bp deletion [888del27] in a highly conserved region of the sulfatase family and frame-shift 1-bp deletion [1374delT]) and one missense mutation (M391V; ATG→GTG at nt 1226). Furthermore, nine different point mutations were detected in severe Caucasian patients in exons 2, 3, 4, 5, 7, 8, 11, and 13 (fig. 2). Nine nucleotide exchanges were as fol-

Table 2

Frequencies of I113F Mutation between Caucasian and Japanese Populations

Population	Number of Alleles[a]	Number (Percent) of Alleles with I113F Mutation[a]	χ^2	df	P
British	23	5 (21)			
Irish	8	4 (50)			
Pakistan	8	0			
Italian	1	0			
Total Caucasian	40	9 (22.5)	8.013	1	<.01
Japanese	40	0			
Total	80	9 (11)			

[a] Only unrelated alleles are compiled statistically.

lows: a C-to-G transversion at nt 286 (P77R; CCT→CGT), a C-to-T transition at nt 324 (R90W; CGG→TGG), a G-to-T transversion at nt 343 (G96V; GGC→GTC), a C-to-T transition at nt 508 (P151L; CCC→CTC), a T-to-G transversion at nt 744 (W230G; TGG→GGG), a G-to-A transversion at nt 927 (A291T), a C-to-T transition at nt 1211 (R386C), a C-to-T transition at nt 1473 (Q473X; CAG→TAG), and an A-to-G transition at nt 1515 (N487S; AAC→AGC).

To study effects of the I113F change on the activity of human GALNS, substitutions were introduced into the GALNS cDNA by site-directed mutagenesis, and the cDNA was transiently expressed in GALNS-deficient fibroblasts: a wild-type cDNA (pMF N) was compared to a mutant cDNA containing the I113F mutation (pMF I113F). As shown in table 3, the activity in cells transfected with pMF I113F was significantly lower than the activity in pMFN-transfected cells. These data clearly show that the I113F substitution is the disease-causing mutation. Although not all the mutations detected in these patients have been introduced into expression and studied for expression, mutant P151L, R386C, and M391V have been studied, and none of the three increased enzyme activity after transfection,

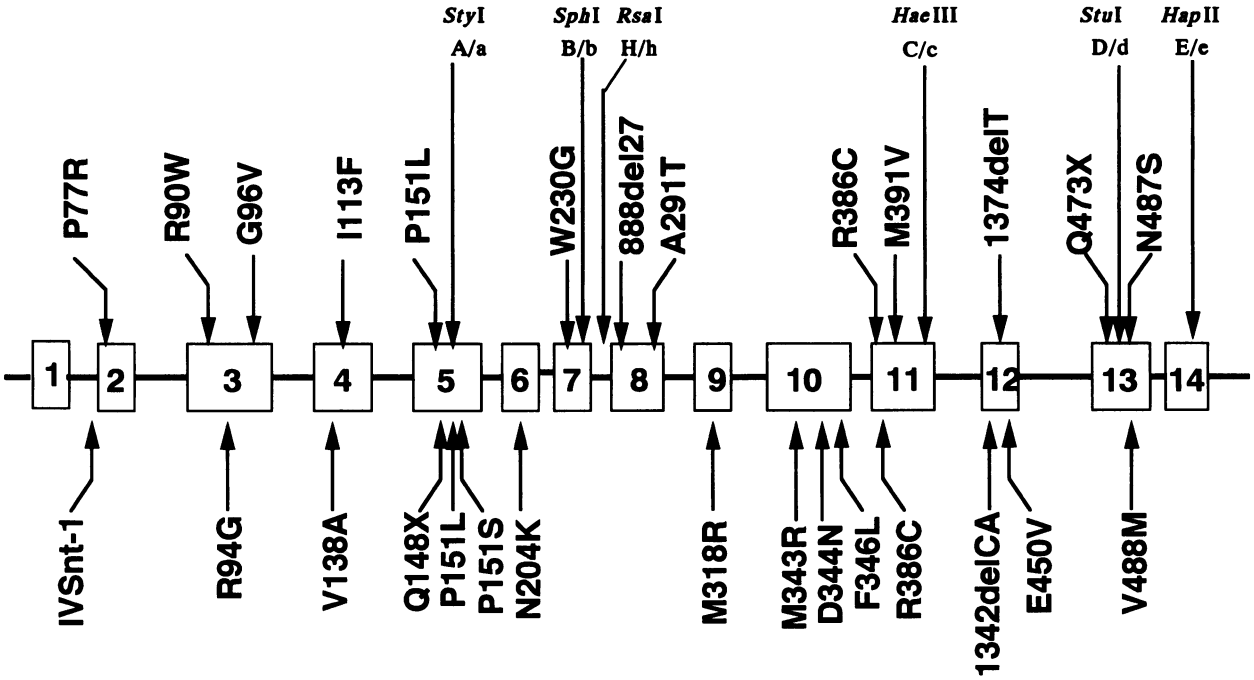

Figure 2 Schematic illustration of deletions, mutations, and polymorphisms identified in the human GALNS gene. Mutations listed were identified in Caucasian MPS IVA patients (*above*) and in Japanese MPS IVA patients (*below*).

Table 3

Expression of GALNS in Transfected Enzyme-Deficient Fibroblasts

Plasmid	GALNS Activity (nmol/mg protein/h)
Control fibroblasts	18.5 ± 5.2 (n = 5)
Deficient fibroblasts	0.4 ± 0.2 (n = 5)
pMF N	20.4 ± 3.5 (n = 5)
pMF I113F	1.0 ± 0.7 (n = 5)

suggesting that these substitutions are also causative of Morquio type IVA in patients with these mutations (data not shown).

Discussion

In 1929, Morquio described the first patient with classical Morquio disease with severe spondylodysplasia. In 1974, Matalon et al. reported the primary enzyme defect to be a deficiency of GALNS activity. Subsequently, Orii et al. (1981) and Glössl et al. (1981) found that GALNS activity was also markedly decreased in patients with the milder form of MPS IVA. Subsequent biochemical analyses suggested that the remarkable clinical heterogeneity observed in MPS IV A patients could be due to different mutations in the GALNS gene. Partly because of the inability to assay very low levels of GALNS activity in affected patients reliably, it was impossible to predict the severity of the disease. The recent cloning and characterization of GALNS cDNA and the genomic gene facilitated identification of the first mutations that result in severe and mild phenotypes. One common large rearrangement producing a severe phenotype has been found in the Japanese population. Thus, insight into the molecular nature of the remarkably distinct severe and mild phenotypes has, to some extent, been gained by identification of several mutations.

In the present study, we characterized the first common missense mutation that is associated with the severe phenotype of MPS IVA. This I113F mutation, due to a single A-to-T transversion, accounted for 22.5% of all unrelated MPS IVA alleles among our 23 Caucasian patients (29% of British and Irish [Anglo-Saxon] ancestries) with GALNS deficiency and bone dysplasia. The I113F mutation in the GALNS gene is in a nonconserved region of the sulfatase family. It is not readily evident why the change from a neutral-nonpolar aliphatic amino acid, isoleucine, to a neutral-nonpolar aromatic residue, phenylalanine with a larger side chain, is deleterious. In fact, this change does not alter the predicted secondary structure. However, direct evidence for Ile113Phe being the disease-causing mutation was provided by transient expression in GALNS-deficient fibroblasts, which showed a significant reduction in catalytic activity. Thus, our results unequivocally show that the I113F substitution severely impairs GALNS activity and is the crucial disease-causing mutation in a significant proportion of GALNS-deficient Anglo-Saxon patients. It is interesting that this I113F mutation is present in MPS IVA patients of Anglo-Saxon origin but is absent in Japanese patients investigated to date. Conversely, a large common rearrangement (6 [15%] of 40 alleles) has been seen only in Japanese populations (Hori et al. 1995; Tomatsu et al. 1995*b*). Further investigation revealed that only two mutations (R386C and P151L, shown in fig. 2) were common to both populations.

For several decades, genetic mechanisms responsible for the high frequency of mutations causing genetic diseases such as Gaucher disease, Niemann-Pick disease, medium-chain acyl-CoA dehydrogenase deficiency, Metachromatic leukodystrophy, and MPS I have been the subject of debate (Tsuji et al. 1987; Levran et al. 1991; Polten et al. 1991; Scott et al. 1992; Zhang et al. 1993). Investigators have suggested common selective pressures, higher mutation rates (mutation hotspots), and a founder effect with genetic drift, for the high gene frequencies observed. If tight associations between a specific mutation and a specific haplotype can be demonstrated in genetic disorders, a frequent mutation can be explained by the founder effect. *Haplotype analysis* of our homozygous subject revealed that the I113F mutation occurred in the haplotype a/b/h/c/D/E, the frequent haplotype in normal individuals. The haplotype of eight other patients heteroallelic for I113F could not be determined since DNA samples were not available from each parent. Further screening in diverse ethnic populations and accumulation of haplotyping data for the I113F mutant allele should reveal whether this mutation is common in Caucasians because of a founder effect, a recurring mutation, or selective pressure. Five other mutations detected in our series of patients were linked to the same haplotype, indicating that the most prevalent haplotype harbors multiple different mutations that contribute to allelic heterogeneity in MPS IVA, in some cases with different clinical phenotypes.

Patients who are homoallelic for the I113F mutation appear to have a severe phenotype. The phenotype of patients heteroallelic for I113F will depend on the genetic lesion present on other GALNS alleles. Seven of our patients heteroallelic for I113F presented with the classical severe phenotype, while the ninth has a less severe intermediate type. One patient (M11) with severe disease has an in-frame 27-bp deletion (888del27; 9 amino acids deletion) in a highly conserved region of the sulfatase family. Another patient (M13) with a severe form has a frame-shift 1-bp deletion (1374delT) resulting in truncation of the protein at the methionine of codon 494. The severe patient, M14 has a missense

mutation M391V (there has been no increase of enzyme activity, by transient expression study). These data suggest that these two deletions and the point mutation M391V are all associated with a severe phenotype. Undefined mutations in five other patients also appear to be associated with a severe phenotype, while the undefined mutation in patient M20 is probably associated with a mild phenotype.

The I113F mutation reported to be common in MPS IVA patients in Caucasians was not observed in 50 unrelated Caucasians and Japanese control individuals. Ten other point mutations (nine misense and one nonsense) were found in single patients. It is not certain whether the remainder of MPS IVA alleles have not been elucidated because the SSCP screening lacks sufficient sensitivity or because some patients have mutations in control regions or intronic sequences that are not screened by the SSCP assays. It is of interest that most of the small mutations have only been seen in Caucasian patients, while the large rearrangement has only been observed in the Japanese population (6 [15%] of 40 alleles; Tomatsu et al. 1995*b*). Only two mutations, P151L and R386C, were detected in both populations, illustrating the racial differences in the GALNS gene mutations. These present mutations have not been observed in 50 unrelated Caucasian and Japanese control individuals.

Five of the 11 amino acid substitutions described in the present study involve amino acids in a conserved region of eukaryotic sulfatases (Pro77, Arg90, Gly96, Pro151, and Asn487). Amino acid changes in this area are likely to cause a major alteration in the overall structure of the enzyme. Since all the patients in this study had a clinically severe presentation (except M20, who had an intermediate phenotype), we could expect that these mutations would profoundly reduce enzyme expression. However, such careful studies of mutant enzyme function as in vitro transfection studies have so far been limited to the P151L, M391V, and R386C mutations.

The findings reported here considerably expand the record of allelic heterogeneity in MPS IVA among Caucasians. The identification of a common mutation, I113F, in the GALNS gene provides useful information on genotype-phenotype correlations. It should also facilitate more accurate genetic counseling of newly diagnosed cases and their family members.

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