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Molecular Analysis of Hurler Syndrome in Druze and Muslim Arab Patients in Israel: Multiple Allelic Mutations of the *IDUA* Gene in a Small Geographic Area

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Summary

The mutations underlying Hurler syndrome (mucopolysaccharidosis IH) in Druze and Muslim Israeli Arab patients have been characterized. Four alleles were identified, using a combination of (*a*) PCR amplification of reverse-transcribed RNA or genomic DNA segments, (*b*) cycle sequencing of PCR products, and (*c*) restriction-enzyme analysis. One allele has two amino acid substitutions, Gly_{409}→Arg in exon 9 and Ter→Cys in exon 14. The other three alleles have mutations in exon 2 (Tyr_{64}→Ter), exon 7 (Gln_{310}→Ter), or exon 8 (Thr_{366}→Pro). Transfection of mutagenized cDNAs into Cos-1 cells showed that two missense mutations, Thr_{366}→Pro and Ter→Cys, permitted the expression of only trace amounts of α-L-iduronidase activity, whereas Gly_{409}→Arg permitted the expression of 60% as much enzyme as did the normal cDNA. The nonsense mutations were associated with abnormalities of RNA processing: (1) both a very low level of mRNA and skipping of exon 2 for Tyr_{64}→Ter and (2) utilization of a cryptic splice site for Gln_{310}→Ter. In all instances, the probands were found homozygous, and the parents heterozygous, for the mutant alleles, as anticipated from the consanguinity in each family. The two-mutation allele was identified in a family from Gaza; the other three alleles were found in seven families, five of them Druze, residing in a very small area of northern Israel. Since such clustering suggests a classic founder effect, the presence of three mutant alleles of the *IDUA* gene was unexpected.

Introduction

Deficiency of the lysosomal enzyme α-L-iduronidase causes mucopolysaccharidosis I (MPS I), a group of autosomal recessive disorders that is divided into three clinical subtypes (Neufeld and Muenzer 1989). The best-known and the most severe is the Hurler syndrome (MPS IH). It is manifested from late infancy by dysostosis multiplex, coarse facies, corneal opacities, cardiovascular disease, hepatosplenomegaly, joint stiffness, and mental retardation; death usually occurs in the first decade of life. The Hurler/Scheie syndrome (MPS IH/S) is somewhat milder, permitting normal intelligence and survival to adulthood. The Scheie syndrome (MPS IS) is the mildest of the α-L-iduronidase–deficiency diseases, with normal intelligence and potentially normal life span. These three subtypes represent a continuum of clinical severity rather than rigid categories.

The cDNA and gene encoding α-L-iduronidase have recently been cloned from human (Scott et al. 1991, 1992*a*; Moskowitz et al. 1992*a*, 1992*b*) and canine (Menon et al. 1992; Stoltzfus et al. 1992) tissues. Availability of these cloned reagents permits the analysis of mutations underlying MPS I both to understand the relationship of genotype to phenotype and to study the population distribution of the mutant gene. Two mutations, Trp_{402}→Ter and Gln_{70}→Ter, have been recently found to account for half of the *IDUA* alleles in MPS IH patients of predominantly Caucasian, Anglo-Saxon origin (Scott et al. 1992*b*, 1992*c*).

MPS I is a rare disorder; a survey conducted in British Columbia reported the incidence of 1 case of MPS IH/

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100,000 births and a much lower frequency for MPS IS (Lowry and Renwick 1971). It is thought not to be enriched in any particular ethnic group. However, 13 Arab patients but only 1 Jewish patient with Hurler syndrome have been diagnosed in Israel, where ascertainment of the disorder has been complete for 15 years (Schaap and Bach 1980). The mutation in the one Jewish patient is described elsewhere (Moskowitz et al. 1993). The Arab patients came from eight families, five of which were Druze and three Muslim. The Druze and Muslim Arab populations have been separated by religion since the inception of the Ismailia or Druze religion in Egypt in the 11th century A.D., although there has been some contact in various periods of history. At present the Druze live in a defined geographic area of southern Syria, southern Lebanon, and northern Israel; they maintain an isolated social structure with a high rate of consanguineous marriages. The Druze population in Israel numbers about 60,000. We anticipated that, in this closed society, MPS I would be caused by one founder mutation, which might or might not be shared with the Muslim patients residing in the surrounding area.

We now describe the surprising finding of four mutant alleles of the *IDUA* gene in the Arab MPS IH patients in Israel, three of these among the Druze. A preliminary account of this work has been reported in abstract form (Moskowitz et al. 1992*b*).

Subjects, Material, and Methods

Patient Material

We studied the mutations in eight families, five Druze (D_1–D_5) and three Muslim (M_1–M_3). Family M_3, which has been described elsewhere (Kohn et al. 1978), lives in Gaza. The other seven families live in different villages in the lower Galilee in an area of about 20×20 km; there are no known family ties between them, though it is possible that the two families who live in the same village, D_4 and D_5, might be related.

Five MPS IH patients (D_1, D_2, D_3, M_1, and M_2) had been diagnosed, and fibroblast cultures had been initiated, at Hadassah Medical Center. These patients all had the severe manifestations of MPS IH as described above. Leukocyte DNA was provided by Dr. Joel Zlotogora for the parents and relatives of these patients, as well as for parents in families D_4 and D_5; no cells were available from their affected children, now deceased. Fibroblasts from M_3 and from his mother and father were obtained from the Human Genetic Mutant Cell Repository (Coriell Institute for Medical Research, Camden, NJ), where they are listed as GM 01898, GM 02016, and GM 02017, respectively. Fibroblasts from other MPS IH patients (GM 00034B, GM 00415, GM 00798, GM 00887, GM 01391, and GM 06214) were likewise obtained from the repository.

The cells used as control were IMR 90 fibroblasts and GM 03441 lymphoblasts, also from the Coriell Institute. Some DNA samples used as controls were from a previous study of G_{M2} gangliosidosis (Paw et al. 1990).

Cell Culture and Isolation of Nucleic Acids

Fibroblasts and Cos-1 cells were cultured as described elsewhere (Paw et al. 1991). Genomic DNA was isolated from leukocytes or cultured cells by standard methods (Sambrook et al. 1989). Total RNA was isolated from cultured fibroblasts by acidic phenol-chloroform extraction in guanidinium thiocyanate (Chomczynski and Sacchi 1987); the isolated RNA was treated with RNase-free DNase I (Stratagene), extracted with phenol-chloroform, and reprecipitated. Integrity of the RNA preparations was determined after electrophoresis in 1% agarose NA (Pharmacia).

Cloning and Characterization of cDNA and Gene Encoding α-L-Iduronidase

Canine α-L-iduronidase cDNA (Stoltzfus et al. 1992) and fragments thereof were used to isolate the human α-L-iduronidase cDNA and gene. A human testis cDNA library (Clontech) was the source of the cDNA; the human cDNA was sequenced on both strands by the dideoxy termination method (Sanger et al. 1977). The sequence, 81% identical to that of the canine cDNA (Moskowitz et al. 1992*a*), was identical to that reported by Scott et al. (1991), except for a base substitution attributed to the library construction. To isolate the gene, size-selected libraries of *Eco*RI-digested genomic DNA were prepared from the lymphoblast line GM 03441; because of the presence of internal *Eco*RI sites, the gene was isolated from both a 9-kb fragment which contained the first 2 exons and a 17-kb fragment which contained the downstream 12 exons (S. M. Moskowitz and E. F. Neufeld, unpublished results). Our results were in agreement with the structure and sequence of the human *IDUA* gene reported by Scott et al. (1992*a*).

Reverse Transcription and PCR Amplification

Oligonucleotide primers were synthesized on an Applied Biosystems 391 DNA Synthesizer. The coding sequence of α-L-iduronidase was amplified in seven overlapping segments, using the primers listed in table 1, group 1 (in some experiments, only six segments were

Table I

Oligonucleotide Primers

Segment[a]	Oligonucleotide
Group I—primers for RT-PCR amplification of RNA/cDNA:	
1. Exon 1-3:	
Sense, −42→−19	5′ CAGTGCAGCCCGAAGCCCCGCAGT 3′
Antisense, 379→356	5′ GGAGCTGGTTCTCCCTGAGAAGGT 3′
2. Exon 3-7:	
Sense, 321→344	5′ GAGCTACAACTTCACCCACCTGGA 3′
Antisense, 825→802	5′ CTGCTCCAGGATGGAGATGGAGCT 3′
3. Exon 6-8:	
Sense, 752→775	5′ AGGCGGGCGTGCGGCTGGACTACA 3′
Antisense, 1083→1063	5′ CGCGAAGGGGTGCGGGTGGTA 3′
4. Exon 8-9:	
Sense, 1037→1057	5′ TGAGCAACGACAATGCCTTCC 3′
Antisense, 1341→1318	5′ GCGGGTGTCGTCGCTCGCGTAGAT 3′
5. Exon 9-10:	
Sense, 1293→1313	5′ CGACGCCTGGCGCGCCGCGGT 3′
Antisense, 1524→1501	5′ CTCAGCCGCGCGCATGCGCCGGAA 3′
6. Exon 10-12:	
Sense, 1409→1432	5′ TCTACGTCACGCGCTACCTGGACA 3′
Antisense, 1727→1704	5′ TTGGAGCCCACGTGTTCATCCGAC 3′
7. Exon 12-14:	
Sense, 1651→1671	5′ GTCACGCGGCTCCGCGCCCTG 3′
Antisense, 2008→1985	5′ CGCTGACTGCCGGTGGAGGTGCAA 3′
Group II—primers for PCR amplification of genomic DNA:	
8. Intron 1-exon 2:	
Sense, −45→−23[b]	5′ GCCATGCTGAGGCTCGGGACTGA 3′
Antisense, 276→254	5′ CCAGTGGGTCCGGACCTGCTTGA 3′
9. Exon 6-exon 7:	
Sense, 752→775	5′ AGGCGGGCGTGCGGCTGGACTACA 3′
Antisense, 972→952	5′ CTTCACCACCATGGCCGCGTA 3′
10. Exon 8:	
Sense, 1037→1057	5′ TGAGCAACGACAATGCCTTCC 3′
Antisense, 1124→1104	5′ CGGGTGTTGTTGACCTGGAAG 3′
11. Exon 8-exon 9:	
Sense, 1150→1170	5′ AAGCCGGTGCTCACGGCCATG 3′
Antisense, 1341→1318	5′ GCGGGTGTCGTCGCTCGCGTAGAT 3′
12. Exon 14:	
Sense, 1858→1878	5′ GTTCGAGCCCTGGACTACTGG 3′
Antisense, 2054→2034	5′ AAAGGGGGTGATGGGAGGGCA 3′
Group III—mutagenic oligonucleotides:	
13. Thr_{366}→Pro, 1107→1084	5′ GAAGCGCGCGGGGAGCGTGCGCTG 3′
14. Gly_{409}→Arg, 1236→1213	5′ CAGGACGGTCCGGGCCTGCGACAC 3′
15. Ter_{654}→Cys, 1971→1948	5′ AGCACAGGCACATGGATTGCCCGG 3′

[a] Except for intronic oligonucleotide 8 (see footnote b below), the numbers following the sense and antisense designations refer to the nucleotide positions in cDNA, where 1 is the adenine of the initiator methionine codon. To convert to the numbering of the published sequence (Scott et al. 1991), it is necessary to add 88 nucleotides. The abnormal base in the mutagenic oligonucleotides is underlined.

[b] Numbered from the intron 1/exon 2 junction.

amplified, using the sense primer of the third pair and the antisense primer of the fourth pair). Total RNA (1–12 μg) was reverse transcribed with 20 units of avian myeloblastosis-virus reverse transcriptase (Promega) in the presence of 50 pmol of antisense primer, 1 mM each dNTP, 1 mg BSA/ml, 50 mM KCl, 2.5 mM $MgCl_2$, and 16–20 units of RNase inhibitor (RNAguard; Pharmacia) in 20 mM Tris buffer pH 8.3; the

reaction was incubated at 23°C for 10 min and then at 42°C for 1 h. After the reverse transcription reaction was terminated by heating at 95°C for 3 min, 50 pmol of sense primer was added, and the reaction mixture was adjusted to 0.2 mM each dNTP, 0.2 mg BSA/ml, and 1.3 mM $MgCl_2$. After preincubation at 72°C for 10 min, 2.5 units AmpliTaq DNA polymerase (Perkin Elmer) was added, and the reaction mixture was subjected to 30 cycles of amplification, using the following profile: 1.5 min at 97°C, 1 min at annealing temperature, and 1 min at 72°C. The annealing temperature was determined, according to the primers used, to be 4–5°C lower than T_m, up to a maximum of 72°C. The high temperature of denaturation was used because of the high GC content of α-L-iduronidase DNA. Additional *Taq* polymerase, 2.5 units, was added after 15 cycles. Reaction products were examined by electrophoresis on 5% polyacrylamide gel.

PCR amplification of segments of genomic DNA was performed essentially as above, using primers designed to detect specific mutations (table 1, group II). Genomic DNA (1.5 μg) was mixed with 50 pmol of sense and antisense primers in 50 mM KCl, 0.2 mM dNTP, 20 mM Tris pH 8.3, and 1–4 mM $MgCl_2$ (optimized for each primer pair). Reaction conditions were as described above for amplification of cDNA, except for extension at 72°C for 1.5 min.

Cycle Sequencing

The PCR products were passed through a Bio-Spin 6 column (Bio-Rad Laboratories) to remove salts and unincorporated nucleotides. Amplification primers or internal primers were radiolabeled at the 5′ terminal with [γ-^{32}P]ATP by T4 polynucleotide kinase (Sambrook et al. 1989). Cycle sequencing (Smith et al. 1990) was carried out using Δ*Taq* polymerase (version 2 sequencing kit; United States Biochemicals) and the thermal profile: 95°C for 1 min, annealing temperature for 1 min, and 72°C for 3 min. The annealing temperature was determined as described above for PCR amplification. The reaction was carried out for 30 cycles and was terminated by the addition of sequencing dye.

Northern Analysis

Total RNA (15 μg) was loaded onto each lane of a 1.2% agarose gel containing 2 M formaldehyde and was subjected to electrophoresis for 300 V-h. Nucleic acids were transferred to a nylon membrane (Nytran; Schleicher and Schuell) as recommended by the supplier. A 505-bp segment was PCR amplified from cloned human α-L-iduronidase cDNA, using primer set 2 (table 1), and was gel purified; it was selected for use as a probe in preference to full-length cDNA in order to minimize nonspecific hybridization caused by GC-rich regions in the cDNA. A cDNA probe encoding the 3′ untranslated region of human β-actin was provided by Dr. Laurence Kedes. Both probes were labeled with [α-^{32}P]dCTP by random priming (Multiprime Kit; Amersham). Membranes were prehybridized and hybridized as described elsewhere (Church and Gilbert 1984), washed once at 55°C (β-actin) or 60°C (α-L-iduronidase) for 30 min in 0.2 × SSC and 2% SDS, and subjected to autoradiography.

Expression of Mutagenized cDNA

Site-specific mutagenesis of codons 366, 409, and 654 was performed by the method of Kunkel et al. (1987), using the Muta-Gene M13 In Vitro Mutagenesis Kit, version 2.0, as recommended by the supplier (Bio-Rad Laboratories). The mutagenic oligonucleotides are specified in group III of table 1. The normal and mutagenized α-L-iduronidase cDNAs were subcloned into pSVL (Pharmacia LKB Biotechnology). The doubly mutagenized cDNA was prepared by ligation at a *Bst*EII site of an upstream 1.4-kb segment of the Gly_{409}→Arg clone to a 750-bp segment of the Ter→Cys clone prior to insertion into the pSVL vector. All mutations and ligations were verified by standard dideoxy sequence analysis (Sanger et al. 1977).

Transfection by means of Lipofectin™ (Bethesda Research Laboratories) and measurement of α-L-iduronidase expression were performed as described elsewhere (Stoltzfus et al. 1992), except that no NH_4Cl was added to the growth medium. Cotransfection with pXCG5 (Nichols Institute) followed by radioimmunoassay for human growth hormone allowed measurement of transfection efficiency.

Results

The mutations were first identified by reverse transcription PCR (RT-PCR) of the α-L-iduronidase coding sequence of fibroblast RNA, followed by cycle sequencing; they were then confirmed by sequencing of the corresponding amplified regions of genomic DNA. As there was no discrepancy between the two sets of results, only the genomic sequences are shown in figure 1. The entire coding region was analyzed by cycle sequencing for all the patients except M_1. Additional information was obtained by restriction analysis of the amplified genomic segments of DNA from the probands and their parents.

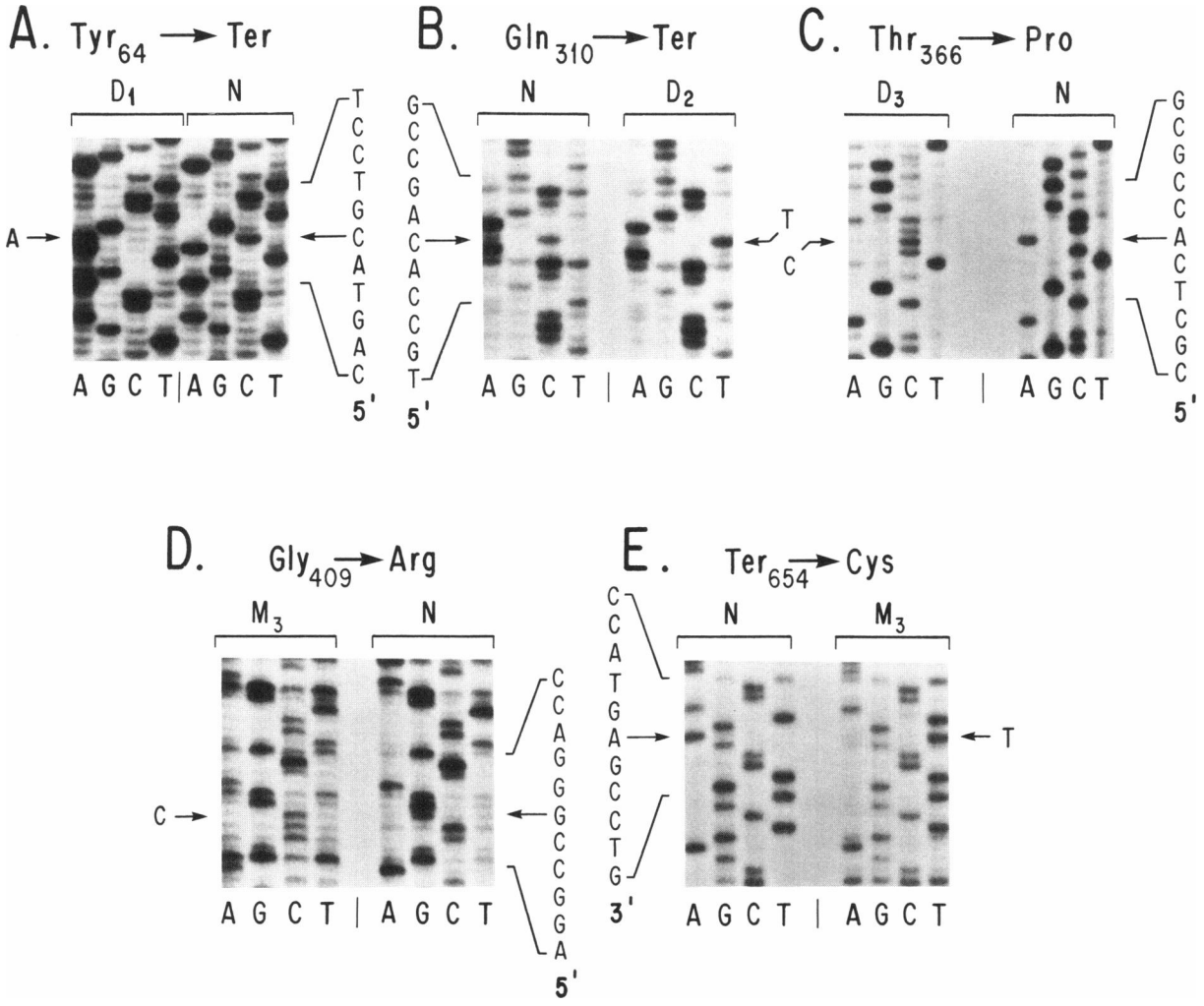

Figure 1 Identification of mutations by sequence analysis of genomic DNA of the Hurler patients. The nucleotide ladder in the region of the mutation is shown for DNA of the patients (D_1, D_2, D_3, and M_3) indicated and for normal control DNA (N). In each case, the mutant sequence was identical to that of the control, except for the nucleotide indicated by the arrow. Primer sets used for PCR amplification (table 1) were set 8 for D_1, set 9 for D_2, set 10 for D_3, and sets 11 and 12 for the exon 9 and exon 14 mutations, respectively, in M_3. The sequence in panel *E* is reported as the sense strand, though it was determined in the antisense direction.

A C→A transversion in exon 2, converting Tyr_{64} (TAC) to a stop codon (TAA), was identified in patients D_1 (fig. 1*A*) and M_1 (not shown). The mutation destroys the site at which *Rsa*I cleaves a 163-bp segment to 86 bp and 77 bp (fig. 2*A*). Both parents of D_1, as well as an aunt and uncle whose own affected child was deceased, were heterozygous for this mutation as determined by restriction analysis (not shown). On a northern blot, the mutation was associated with a very low level of mRNA, less than 5% of that of the normal control after normalization for β-actin (fig. 3*A*, lane M_1). In addition, the RT-PCR using primer set 1 resulted in two products, the smaller product predominating (fig. 3*B*, lane D_1); sequence analysis showed that the smaller product was a fusion of exons 1 and 3, exon 2 having been skipped.

A C→T transition in exon 7, converting Gln_{310} (CAG) to a stop codon (TAG), was identified in patient D_2 (fig. 1*B*). There is no restriction site in the area of the mutation. Both parents were found heterozygous for the mutation, by sequence analysis (not shown). The level of mRNA in fibroblasts of the patient was apparently half that of normal, after normalization for β-actin (fig. 3*A*, lane D_2). The product of RT-PCR amplifi-

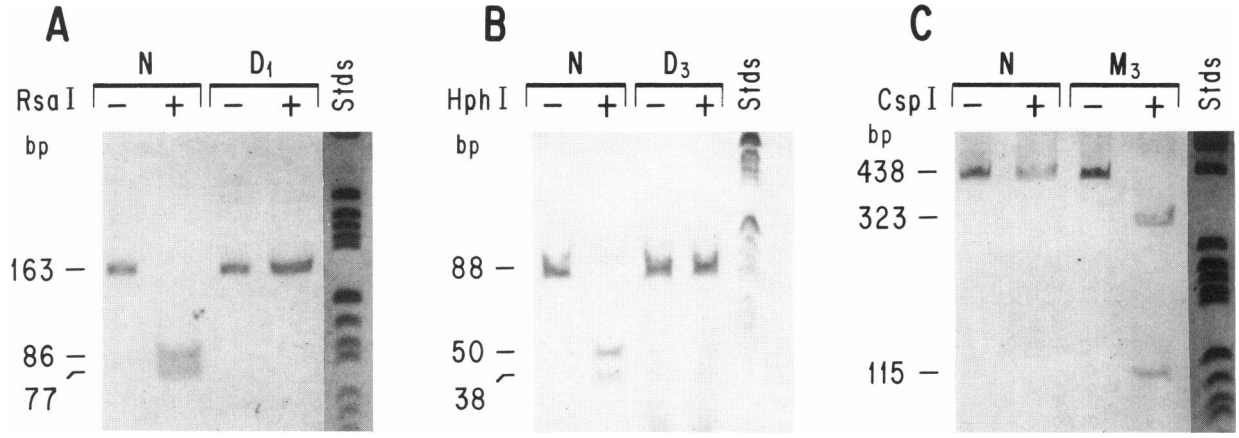

Figure 2 Restriction-enzyme analysis of the mutations. PCR-amplified genomic DNA segments were incubated with or without the restriction enzymes, as indicated, and the products were analyzed on a 5% or 7% polyacrylamide gel. Standard lanes (Stds) from noncontiguous areas of the gel are juxtaposed. Negative prints of ethidium bromide–stained gels are presented.

cation with primer set 2 migrated electrophoretically as a doublet, with the upper band slightly larger than the normal band of 505 bp (fig. 3*C*). Sequence analysis (not shown) demonstrated an admixture of a species with 28 nt of intronic sequence, indicating the utilization of a cryptic splice site in intron 5.

An A→C transversion in exon 8 was identified in patients D_3 (fig. 1*C*) and M_2 (not shown), converting Thr_{366} (ACC) to Pro (CCC). The mutation destroys the site at which *Hph*I cleaves an 88-bp segment to 50 bp and 38 bp (fig. 2*B*). The parents of D_3 and M_2 were found heterozygous for the mutation, by restriction analysis (not shown). In addition, two sets of parents from families D_4 and D_5, whose children were not available for study, were also found heterozygous for this mutation, by restriction analysis and cycle sequencing of the amplified DNA segment (not shown). The level of mRNA in D_3 and M_2 was three times higher than that of the normal control (IMR 90) tested concurrently (fig. 3*A*). Transfection of cDNA containing the Thr_{366}→Pro mutation into Cos-1 cells resulted in expression of less than 1% of the α-L-iduronidase activity obtained on transfection with normal cDNA (table 2).

Six cell strains derived from Caucasian MPS IH patients of unrelated origin (GM 00034B, GM 00415, GM 00798, GM 00887, GM 01391, and GM 06214) were found not to have the above mutations, as determined by restriction analysis for Tyr_{64}→Ter and Thr_{366}→Pro and by sequence analysis for Gln_{310}→Ter. These cell lines were subsequently found to have the common Caucasian mutations, Gln_{70}→Ter and Trp_{402}→Ter (Scott et al. 1992*b*, 1992*c*), on 11 of the 12 chromosomes (P. T. Tieu, R. Chan, and E. F. Neufeld, unpublished results).

Two base substitutions were identified in patient M_3 (fig. 1*D* and *E*). A G→C transversion in exon 9 converts Gly_{409} (GGG) to Arg (CGG), and an A→T transversion in the termination codon (TGA) converts the termination codon to a Cys (TGT) residue (fig. 1*E*). The two mutations were present in homozygous form, indicating that they occur on the same allele. The cDNA sequence predicts an extension of 38 amino acids before the next termination codon is reached. The exon 9 mutation creates a *Csp*I restriction site, at which a 438-bp segment is cleaved to 323 bp and 115 bp (fig. 2*C*), whereas the mutation at the termination codon does not alter any restriction site. Both mutations were found in heterozygous form in the DNA of each parent of M_3, confirming that they were present on the same allele. Expression of cDNA mutagenized at one or both positions showed that Gly_{409}→Arg caused a reduction of less than half the α-L-iduronidase activity, whereas Ter→Gly reduced activity by 98% compared with expression of normal cDNA (table 2). Figure 4 summarizes the *IDUA* alleles found in the Druze and Muslim Arab MPS IH families.

Discussion

Of the four mutant alleles characterized in this study, two contained nonsense mutations and manifested some abnormalities of RNA metabolism. The cells of D_1 and M_1, Tyr_{64}→Ter homozygotes (mutation in exon 2), showed a predominance of exon 2 skipping as

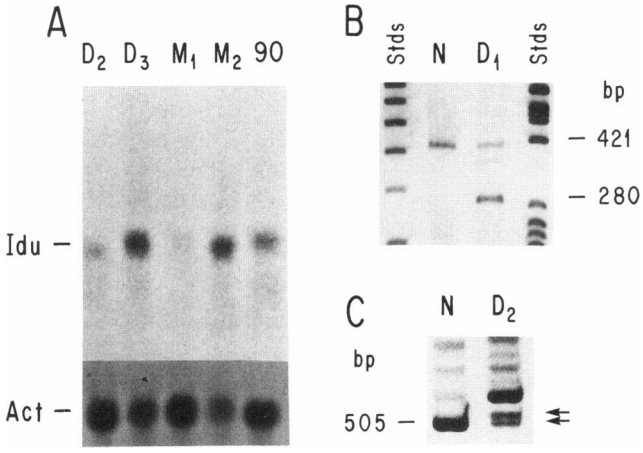

Figure 3 RNA abnormalities in MPS I cells. *A*, Northern analysis of fibroblast RNA from patients indicated and from the IMR 90 control cell strain; "Idu" and "Act" represent α-L-iduronidase and β-actin mRNA, respectively. The densitometric ratios of α-L-iduronidase mRNA/β-actin mRNA, expressed as a fraction of that found for the IMR 90 control, were (from left to right) 0.4 for D_2, 3 for D_3, 0.02 for M_1, and 4 for M_2. *B*, Presence of exon 2–less RNA in cells of D_1, demonstrated by RT-PCR amplification using primer set 1; the normal 421-bp product is present in the patient's cells, but the smaller, 280-bp product, without exon 2, predominates. *C*, RT-PCR amplification using primer set 2, showing a doublet (arrows) in the cells of D_2, in contrast to the single band of 505 bp seen in the normal control. The bands above the doublet may represent a combination of heteroduplex and nonspecific PCR products.

seen in RT-PCR analysis. Such skipping may be present in trace amount in normal cells (Scott et al. 1991). The cells of M_1 were also shown to have a very low level of mRNA as seen on northern analysis. Premature translation termination has frequently been found associated with a low steady-state level of mRNA (e.g., see Baserga and Benz 1988; Myerowitz and Costigan 1988; Urlaub et al. 1989; Cheng et al. 1990; Kadowaki et al. 1990). It may be that the exon 2–less mRNA is the predominant species in cells of D_1 and M_1 because it is more stable than the full-length mRNA with a premature termination codon. An alternative hypothesis—that a premature termination codon can actually induce exon skipping by altering splice-site selection—has been recently proposed (Dietz et al. 1993).

The cells of D_2 (Gln_{310}→Ter, in exon 7) had nearly half the mRNA of the normal control after normalization for β-actin mRNA; however, part of this mRNA was spliced at a cryptic site (nt −28) in intron 5. This alternative splicing creates a frameshift and an open reading frame until exon 9. Alternative splicing at the same position of intron 5 had been found previously in another mutant *IDUA* allele with premature translation termination in exon 6 (Moskowitz et al. 1993).

The cells of D_3 and M_2, homozygous for the Thr_{366}→Pro missense mutation, had qualitatively normal mRNA present at a level several times higher than that of mRNA derived from the control IMR 90 cells. Whether this represents a genuine increase or merely variability of the normal range is not yet known.

The nonconservative replacement of threonine by proline, a helix breaker often found at turns (Chou and Fasman 1978), alters the structure of the enzyme sufficiently to completely prevent expression of α-L-iduronidase activity, as shown by transfection of cDNA mutagenized at that position. Whether the loss of activity is caused by interference with transport, stability, or catalytic function has not yet been determined. Of the two

Table 2

Expression of α-L-Iduronidase Activity by Transfected Cos-1 Cells

Transfection Vector	α-L-Iduronidase Activity (units/mg cell protein)		Total (% of normal)
	Intracellular	Secreted	
pSVL	1.9	<.1	0
pSVLIdu	19.5	18.4	100
pSVLIdu Thr_{366} → Pro	1.9	.1	<.1
pSVLIdu Gly_{409} → Arg	14.1	9.7	61
pSVLIdu Ter_{654} → Cys	2.6	.2	2
pSVLIdu Gly_{409} → Arg, Ter_{654} → Cys	2.4	.2	2

[a] The activity of endogenous α-L-iduronidase was subtracted before the percentage of normal was calculated. No correction was applied for transfection efficiency, which was similar in all plates.

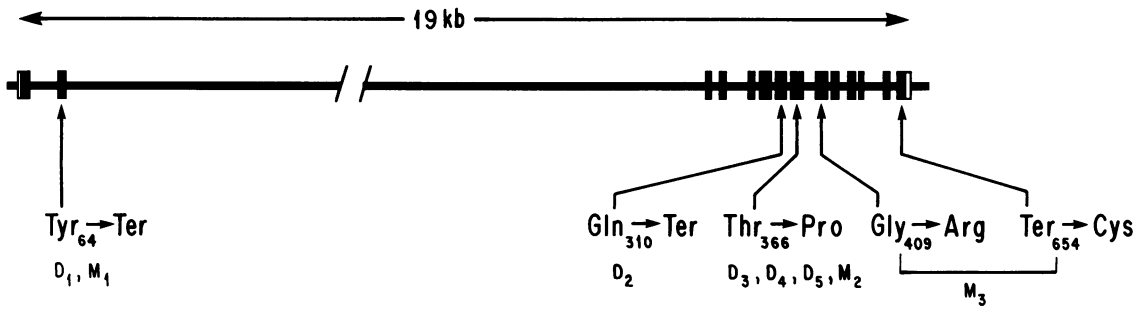

Figure 4 Position of the Druze (D) and Muslim (M) Israeli Arab mutations on the 14-exon *IDUA* gene

substitutions in M_3, only Ter→Cys, which predicts an extension of 38 amino acids at the carboxyl-terminus, interferes with expression of α-L-iduronidase activity and may therefore be considered the cause of the disease; on the other hand, the Gly_{409}→Arg substitution appears to be benign, since it permits the expression of half as much activity as does the normal cDNA. Because of its proximity to Asp 415, a functional glycosylation site (A. Matinya and E. F. Neufeld, unpublished results), and because of its ability to accommodate the substitution of Gly by the much bulkier Arg, codon 409 may be presumed to lie on the outside of the protein.

The cells of patient M_3 (with the double mutation) are listed in the catalog of the Human Genetic Mutant Cell Repository as "Hurler/Scheie." However, the patient at age 6 years had been described as having a typical MPS IH phenotype except for apparently normal intelligence (measured across a language barrier), and his similarly affected brother had died at age 9 years; a diagnosis of MPS IH/S had been excluded, and yet another form of α-L-iduronidase-deficiency disease had been proposed, by Kohn et al. (1978). It seems to us that the classification of MPS IH is as appropriate for this patient as is that of MPS IH/S, if not more so. This instance illustrates the occasional difficulties created by subdivision of MPS I into three groups which are not as much rigidly demarcated entities as they are a continuum of clinical phenotypes.

The presence of seven MPS IH families residing in a very small geographic area would be considered a priori a classic case of founder effect, with frequent appearance of the mutant allele in homozygous form because of the frequency of consanguineous marriages. Therefore the finding of three mutant *IDUA* alleles in this population was completely unexpected and is not readily explained. The three alleles are not common among Caucasians, as they were not found in fibroblasts derived from six Caucasian patients, obtained from the Human Genetic Mutant Cell Repository. Whether they are common among Druze and Muslim Arabs residing in other countries of the Middle East cannot be determined at this time, for lack of contact. Regardless of where the alleles might have originated, their clustering in a small geographic area remains puzzling. Selective pressures which would favor mutant *IDUA* genes cannot be ruled out, including social pressures which might have driven families with affected children (whose appearance is quite distinctive) to migrate together and to settle in close proximity to each other. But, in spite of such proximity, marriages had not occurred between MPS IH families in neighboring villages, as is indicated by the absence of compound heterozygosity among the patients.

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