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Mucopolysaccharidosis Type II (Hunter Syndrome): Mutation "Hot Spots" in the Iduronate-2-Sulfatase Gene

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Summary

Mucopolysaccharidosis type II (MPS II, Hunter syndrome) is an X-chromosomal storage disorder due to deficiency of the lysosomal enzyme iduronate-2-sulfatase (IDS). We have identified IDS mutations in a total of 31 families/patients with MPS II, of which 20 are novel and unique and a further 1 is novel but has been found in 3 unrelated patients. One of the mutations detected is of special interest as an A→G substitution in an intron, far apart from the coding region, is deleterious by creating a new 5′-splice-donor site that results in the inclusion of a 78-bp intronic sequence. While the distribution of gene rearrangements (deletions, insertions, and duplications) of <20 bp seems to be random over the IDS gene, the analysis of a total of 101 point mutations lying within the coding region shows that they tend to be more frequent in exons III, VIII, and IX. Forty-seven percent of the point mutations are at CpG dinucleotides, of which G:C-to-A:T transitions constitute nearly 80%. Almost all recurrent point mutations involve CpG sites. Analysis of a collective of 50 families studied in our laboratory, to date, revealed that mutations occur more frequently in male meioses (estimated male-to-female ratio between 3.76 and 6.3).

Introduction

Hunter syndrome (mucopolysaccharidosis type II [MPS II]) is an X-chromosomal form of mucopolysaccharidosis, a lysosomal storage disorder due to deficiency of iduronate-2-sulfatase (IDS). The accumulation of partially degraded heparan and dermatan sulfates leads to progressive destruction of various tissues and organs and results in death of patients usually in 1st–2d decade (for a recent review, see Hopwood et al. 1993 and references therein). Since the cloning of the IDS cDNA (Wilson et al. 1990) and after the exon-intron structure of the gene was established (Flomen et al. 1993; Wilson et al. 1993), several reports have been published on mutation analysis on small collections of patients. The first mutation update, compiled by Hopwood et al. (1993), revealed excessive genetic heterogeneity, but the body of information was too small to recognize specific patterns, if they exist, within the mutation spectrum available at that time. Here we present our results on the identification of 21 novel IDS mutations that were collected by mutation analysis on 31 not-yet-reported families/patients with MPS II. These data, together with those reported by us and others previously, are analyzed with respect to the nature, regional distribution, and parental origin of mutations, as well as to the possible relationship between genotype and phenotype.

Material and Methods

A total of 31 families, from different European countries, with at least one patient with biochemically confirmed IDS deficiency were investigated. Genomic DNA was prepared from peripheral blood leukocytes or from cultured skin fibroblast cells by the high-salt extraction protocol (Miller et al. 1988). Total cellular RNA was isolated from cultured skin fibroblasts by the guanidinium isothiocyanate procedure (Chomczynski and Sacchi 1987). An aliquot of 4 μg of total RNA was used to synthesize cDNA as described by Bunge et al. (1992). Skin fibroblast cells were grown in Ham's F10 medium supplemented with 15% FCS (Gibco BRL).

PCR amplification on cDNA or genomic DNA of patients and unaffected controls was carried out by using oligonucleotide primers reported by Bunge et al. (1993) or shown in table 1. The coding region of the IDS cDNA was amplified in four overlapping fragments (Bunge et al. 1992). Allele-specific oligonucleotide primers were used to demonstrate that the mutations found in exon III are located in the functional IDS gene and not in the pseudogene (Rathmann et al. 1995). SSCP analysis and direct sequencing of PCR products were performed according to the method of Bunge et al. (1996).

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Table 1

Oligonucleotide Primers

Primer	Position (first nucleotide/exon-intron)	Sequence (5′–3′)
1	63 exon 1	TAACTGCGCCACCTGCTGCA
23	227+63 intron 1	AGGAAGGAGGAAGGAAGGGAGG
24	228–54 intron 1	GAGGGTTCAGTGTCAGTGCAGG
25	364+47 intron 2	AGTGCACGAAGCAGCACACACC
26	833–73 intron 5	AGACAGTGATAGAGCCACAAGC
27	1003+51 intron 6	CGACACTATGTCATCAGTGTCC
32	1131–38 intron 7	AATCATCATACCACAGCTTG
35	1131–138 intron 7	TTGGAATTACCACAGAAAGGG
40	1131–347 intron 7	GAATGAGCATGTCTTAGCAG

Southern blot analysis was carried out with 5 μg of genomic DNA digested by *Hin*dIII, *Taq*I, or *Stu*I, and alkaline transferred onto nylon membrane. Radioactively labeled probe hybridization was done by using the IDS cDNA clone (pc2S15) reported by Wilson et al. (1990). Details on specially designed primers to test the presence of normally spliced transcripts in patients with splice-site mutations can be obtained from the authors.

For the analysis of mutation origin, carrier status of mothers and other female relatives was confirmed by mutation detection. Identification of the parental origin of the X chromosome bearing the mutation was done by using intragenic IDS polymorphisms detected by *Taq*I or *Stu*I (Suthers et al. 1991) and/or the polymorphism at DXS1113 (Weber et al. 1993), which is ~10 kb distal to the IDS gene (Timms et al. 1995). Estimation of the male-to-female ratio of mutations was done according to Haldane's mutation-selection equilibrium model as applied by Oldenburg et al. (1993).

Results

We have identified IDS gene mutations in a total of 31 patients/families with MPS II. Results are summarized in table 2. Of the 31 mutations reported here, 20 are novel and unique. R88C, which has not been reported, to date, has been found in three patients of this collection, whereas family analyses suggest that the three mutations occurred independently, since the mother of patient 4 does not carry the mutation, and patients 5 and 6 originate from Turkey and Spain, respectively.

Mutations in table 2 are grouped according to their nature and the biochemical/biological consequences in which they are expected to result. Among the 31 mutant alleles, there are 26 point mutations (17 missense, 5 nonsense, and 4 affecting correct splicing), four structural rearrangements affecting <20 bp (3 deletions and 1 insertion), and 1 large inversion. The latter rearrangement, carried by patient 31, was described in several unrelated MPS II patients and is due to a homologous nonallelic recombination between the IDS gene and a recently identified IDS pseudogene in Xq27.3-q28 (see Bondeson et al. 1995 and references therein; Rathmann et al. 1995).

Of the 14 different missense mutations, 4 have already been described in other (and most likely unrelated) patients (table 2) and are therefore considered pathogenic. Five mutations are de novo, which strongly suggest that the respective gene alteration is the primary genetic cause of the disease. None of the novel missense mutations has been found on 100 X chromosomes of unaffected and unrelated controls studied in our laboratory, suggesting that these changes do not represent frequent polymorphisms. In this context, it is worth mentioning that no sequence polymorphism predicting an amino acid exchange has been reported, to date, in the IDS gene.

The four point mutations affecting splicing were studied in further detail. A third-position silent G→A transition at nt 832 (numbering according to Wilson et al. 1990) at the end of exon V of patient 24 alters the consensus donor splice sequence. Molecular analysis of the mRNA isolated from fibroblasts revealed a transcript lacking 37 nt, suggesting that an upstream cryptic splice site (GGGT) in exon V, located at nt 794–797, was used in the patient. The mutation leads to a frameshift and premature termination of protein translation due to a novel in-frame stop codon encountered after incorporation of 42 amino acids unrelated to IDS.

A further splice-site mutation was detected in a 6-year-old boy (patient 25) presenting with a mild course of the disease. Screening for mutations by amplification of single exons and SSCP has not revealed any aberration. However, analysis of the 3′ half of the IDS mRNA by agarose gel electrophoresis detected bands of three different sizes. As shown in figure 1, the most abundant transcript was ~80–100 bp larger than the mRNA of the unaffected control. In addition, there was an appar-

Table 2

Mutations of the IDS Gene in 31 Patients with MPS II

Patient (No. and Initials)	Phenotype[a]	Mutation type[b]	Codon[c] (Nucleotide)	Sequence	Protein[d]
			A. Point Mutation (missense)		
1: YA	Intermediate	Rec	63	AAT→GAT	Asn→Asp; c+
2: MP	Intermediate	Novel	85	GCC→ACC	Ala→Thr; c+
3: SI	nd	Novel, de novo	86	CCG→CAG	Pro→Gln; c++
4: AKR	Severe	Novel, de novo	88	CGC→TGC	Arg→Cys; c++
5: SK	Severe	Rec	88	CGC→TGC	Arg→Cys; c++
6: ASS	Severe	Rec	88	CGC→TGC	Arg→Cys; c++
7: TK	Severe	Novel	88	CGC→CAC	Arg→His; c++
8: FK	Mild	Novel	108	TAC→TGC	Tyr→Cys; nc
9: RH	Very mild	Novel	125	GAG→GTG	Glu→Val; nc
10: RW	Severe	Novel	134	GGA→CGA	Gly→Arg; c++
11: SJ	Mild to intermediate	Novel, de novo	184	TGC→TTC	Cys→Phe; nc
12: AS	nd	Novel, de novo	252	GAT→AAT	Asp→Asn; nc
13: JK	nd	Rec	333	TCG→TTG	Ser→Leu; c+
14: MI	nd	Novel, de novo	347	AAA→ATA	Lys→Ileu; c++
15: RM	Intermediate	Novel	403	CTT→CGT	Leu→Arg; nc
16: TG	nd	Rec	468	CGG→CAG	Arg→Gln; nc
17: GW	Severe	Rec	468	CGG→CAG	Arg→Gln; nc
			B. Point Mutation (nonsense)		
18: JBS	Severe	Rec	172	CGA→TGA	Arg→term
19: MN	Severe	Novel	298	CAG→TAG	Gln→term
20: ID	Severe	Novel	365	GGA→TGA	Gly→term
21: SW	Intermediate	Rec	443	CGA→TGA	Arg→term
22: ICG	Severe	Novel	474	CAG→TAG	Gln→term
			C. Point Mutation (splice site)		
23: HK	Mild	Novel	214	ACG→AGG	Thr→Arg; new donor splice site in ex V, mRNA: 67 nt del; fs; 43 altered aa; term
24: SS	Intermediate	Novel	236 (832G→A)	AAG→AAA	Cryptic splice site in ex V used; mRNA: 37 nt del; fs; 42 altered aa; term
25: AU	Mild	Novel	Intron 7 (1131-133A→G)		Donor splice in intron 7 activated; term
26: WK	Mild	Rec	374 (1246C→T)	GGC→GGT	New donor splice site in ex VIII; 20 aa del
			D. Deletion		
27: TK	Severe	Novel	117 (ΔS117)	TCC→---	1 aa del
28: UJ	Severe	Novel	289 (991delT)	CCT→CC–	fs; 25 altered aa; term
29: AÖ	Severe	Novel	346 (1160delG)	GCC→–CC	fs; 13 altered aa; term
			E. Insertion		
30: FF	Mild	Novel	523 (1690/1691insT)	TAT→TTAT	fs; 5 altered aa; term
			F. Inversion		
31: PF	nd	Rec	Inversion beyond nt 221		≥221 normal aa; Southern blot Hind III: 2 bands lost; 2 novel bands; all exons amplifiable

[a] Criteria of classification: mild = normal intelligence, typical but not too severe somatic changes; intermediate = medium-degree mental retardation, typical symptoms, cognitive abilities decline gradually with age; severe = severe mental retardation, never learned to speak, marked degree of somatic symptoms, diagnosis usually completed at ≤2 years of age; and nd = not determined (for further details, see Bunge et al. 1993).

[b] Novel = mutation not yet reported before; rec = recurrent mutation already reported in the literature in other (most likely unrelated) patients; and de novo = mutation occurred spontaneously in one of the family members studied.

[c] The numbers of codons and nucleotides are according to Wilson et al. (1990).

[d] fs = frame shift; aa = amino acid; nt = nucleotide; ins = insertion; term = termination; ex = exon; c = conserved amino acid in at least seven of the nine different sulfatases (Franco et al. 1995); nc = nonconserved amino acid in nonconserved region; c+ = nonconserved amino acid in conserved region; and c++ = conserved amino acid in conserved region.

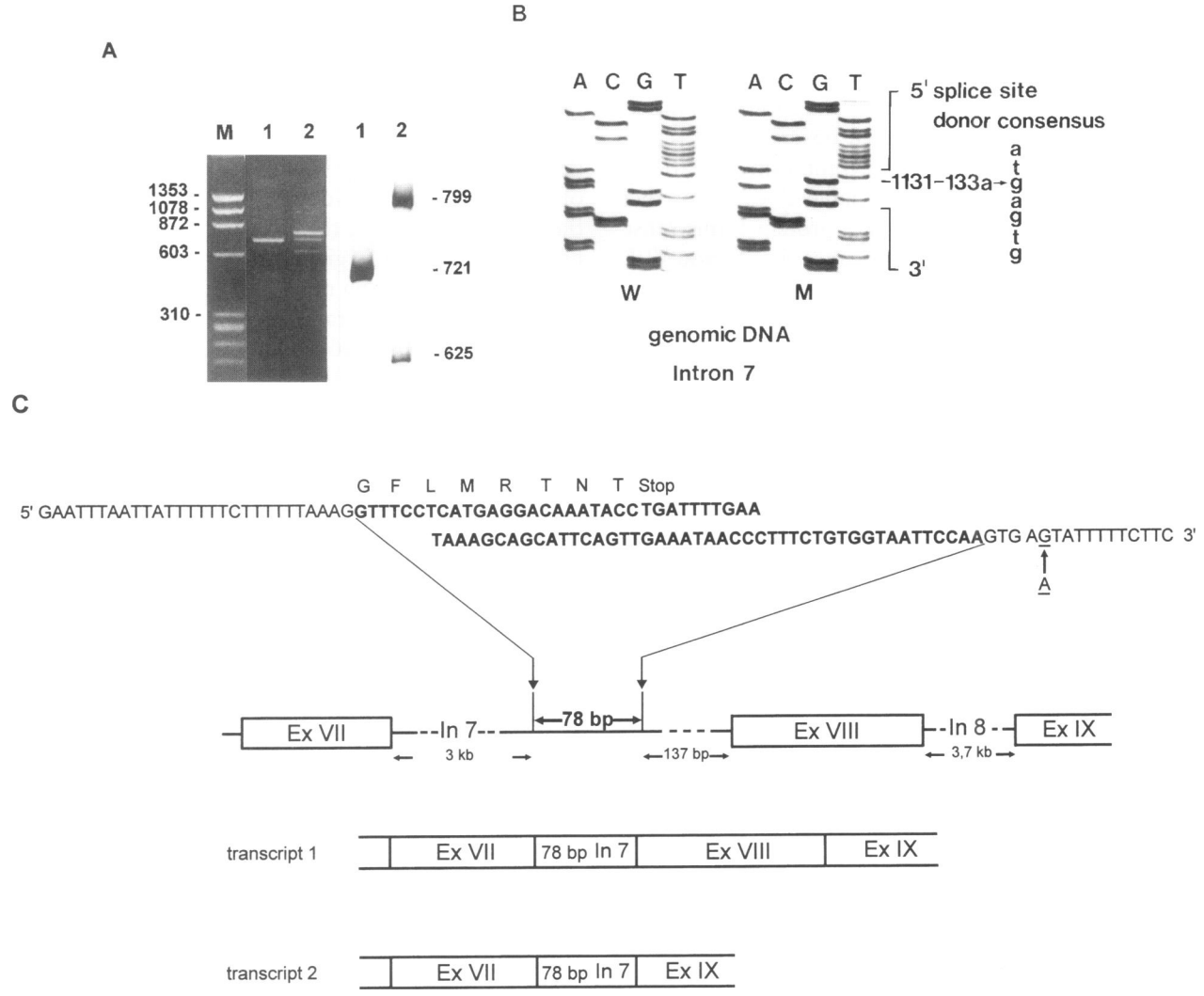

Figure 1 Molecular characterization of the various IDS transcripts detected in patient 25. *A*, Analysis of IDS-specific messages. Total mRNA was isolated from cultured fibroblasts of an unaffected control (lane 1) and patient 25 (lane 2), reverse transcribed, PCR amplified, and separated by gel electrophoresis on 1.5% agarose (*left*) and 6% polyacrylamide (*right*). M = molecular weight marker PhiX174 digested by *Hae*III. Numbers indicate fragment sizes (in base pairs). *B*, Partial DNA sequence of intron 7 of the IDS gene from an unaffected control (*W*) and patient 25 (*M*), showing the A→G transition, which creates a novel splice site. *C*, Simplified presentation of the partial structure of the human wild-type IDS gene and transcripts 1 and 2 (from *top* to *bottom*) in patient 25. Ex = exon; In = intron.

ently normal sized fragment and another one being smaller by ~90–110 bp. Direct sequencing of the corresponding PCR-amplified cDNA fragments revealed that the larger message contained a 78-bp insertion between exons VII and VIII. The same 78-bp sequence was found in the smaller transcript, although at this time it was flanked by exons VII and IX of the IDS gene (i.e., exon VIII was skipped during splicing). The DNA fragment running approximately equivalent to the normal sized message in the agarose gel electrophoresis is most likely a heteroduplex formed by the two above mRNA/cDNA species, because only the larger and smaller fragments were seen on polyacrylamide gel (fig. 1).

A search in the data bank has uncovered that the 78-bp sequence identified in the patient's mRNA is part of intron 7 of the IDS gene. Therefore, together, all findings suggested that the patient carried a mutation affecting splicing and/or activating alternative splice mechanisms. The presumed 3′-acceptor splice-consensus sequence (aggt) at the beginning of the 78-bp sequence was found to be unaltered, whereas an A→G transition of the 5th nt following the last base of the included sequence was identified (fig. 1). This substitution creates a new 5′-splice-donor site (aagtgaa→AAgtgag) and results in the inclusion of the 78-bp intronic sequence. The translation products defined by either of the two alternatively spliced transcripts (with or without exon VIII) in the patient have

probably no IDS activity, since they encode a largely truncated IDS protein due to an early termination signal in the 78-bp inclusion.

The C→T transition of the 3d nt in codon 374, found in patient 26, has repeatedly been observed and represents a frequent recurrent splice mutation in patients with MPS II. Although the base change does not predict amino acid substitution in the IDS protein (same-sense mutation), it creates a new donor splice site in exon VIII, which is preferentially used and results in skipping of 60 bp in the IDS mRNA. A very similar molecular mechanism can be postulated in patient 23, in which a missense mutation (T214R) produces a new intraexonic donor splice site and leads to "deletion" of 67 nt from the mRNA and thereby to a frame shift and premature termination of protein translation.

Three of the four splice-site mutations create an additional splice site, leaving the original one unaltered (patients 23, 25, and 26), while in the fourth case (patient 24) the original splice site remains functional, although its consensus value is reduced from 77.7 to 65.3. Because all four patients' phenotypes were intermediate or mild, the presence of (a low amount of) normally spliced transcript was suspected. RT-PCR was performed for patients 23 and 24 with a forward primer annealing in exon IV and a reverse primer located in that region of exon V, which is deleted in the abnormally spliced transcript in both. Expected PCR products span the exon IV/V-boundary, and thus accidental amplification of genomic DNA present in the RNA preparation is unlikely at the experimental conditions used, because of the large size of intron 4. For both patients, PCR products corresponding to the wild-type cDNA could be obtained (data not shown). A direct estimation of the amount of normal mRNA is difficult, in this way. It should be low, since wild-type mRNA cannot be detected at all with the "commonly" used primers annealing outside the deleted region. For patient 25, a reverse primer was constructed that consists of the first 12 nt of exon VIII and the last 11 nt of exon VII and thus can anneal only on cDNAs that have a correct junction of the corresponding exons (i.e., lack the 78-nt inclusion). In this patient, too, the wild-type message could be detected (not shown). A reverse PCR primer consisting of 12 scrambled nt and the last 11 nt of exon VII did not produce any PCR product in the patient and in control samples, indicating that partial binding of the primer to the abnormal transcript is not sufficient to direct the reaction.

Parental Origin of Mutations

During the past several years, a total of 64 patients were analyzed for mutations in our laboratory. For 50 of them (33 isolated cases, 4 patients with affected brothers, and 13 families with affected males in at least two generations), DNA of the mothers was available. In six (12%) cases, the mother did not carry the mutation in her peripheral blood leukocytes, and therefore, on the assumption of no mosaicism, a new mutation in the mothers' meioses was postulated. Because the fitness of the patients is considered to be zero (hardly any of them can reproduce), the probability (M) of a mother not being a carrier is given as $M = \mu/(2\mu + \nu)$, with μ as the female mutation rate, ν as the male mutation rate. k (male-to-female sex ratio) can be estimated by $k = (1/M) - 2$. If the observed value of noncarriers among the son/mother pairs (6/50) is applied to the formula, a k-value of 6.3 is obtained. However, ascertainment bias (familial vs. sporadic cases) cannot be completely ruled out. Therefore, another estimation for k was done for a well-defined subgroup of patients in which the mother was a carrier, and DNA from both grandparents was available (21 cases). Of the 21 grandmothers, 10 were carriers, while 11 (52%) did not carry the mutation. Of the latter cases, the IDS mutation must have occurred during spermatogenesis in maternal grandfather in all but one case, in which the parental origin of the X chromosome could not be determined, because of noninformativity of the markers used. Since no new mutation was found in grandmaternal meioses, the μ/ν ratio k would go toward infinity (10/0). The lower limit of k compatible with the observed data would be 3.76 ($P = .0016$), suggesting that the mutation frequency is significantly higher in male meiosis.

Discussion

In this communication, we report 31 mutations of the IDS gene, of which 20 are novel and unique, and another 1 is novel but has been found in three unrelated patients, while the remaining 8 have already been described.

Compiled data on a total of 50 families show that mutations (mainly, point mutations) occur more frequently in male meiosis. This observation is in accordance with findings made for other X-linked diseases such as Duchenne muscular dystrophy (Grimm et al. 1994) and hemophilia A (Knobloch et al. 1993) and B (Oldenburg et al. 1993; Becker et al. 1996). If this finding can be confirmed on a larger collection of families, it may have important implication for genetic counseling of at-risk female relatives of patients with MPS II, because the frequency of carriers among mothers of "isolated cases" seems to be much higher than the statistical value one would expect for a genetically lethal X-chromosomal disorder.

Genotype-Phenotype Correlation

In addition to genetic data, table 2 provides some information on the phenotype of the 31 patients de-

scribed here. The fact that nonsense mutations, as well as major structural rearrangements (deletions, insertions, inversions, and duplications), are in general associated with intermediate or severe phenotype is not unexpected, because most of the above changes should result in largely truncated protein (completely) lacking enzyme activity. Patients with missense mutations show largely variable phenotypes from very mild (9) to severe (e.g., 4–7). Most of the mutations affecting residues conserved among sulfatases (Tomatsu et al. 1991; Franco et al. 1995) result in intermediate or severe phenotype. Yet, R468 is an evolutionarily nonconserved amino acid in a poorly conserved region of IDS, and it is associated with a severe phenotype. It is interesting to note that the four mutations affecting splicing result in mild to intermediate phenotype. The regular splice site remains unaltered in three of the four cases, and it seems that a proportion of the primary transcript is correctly spliced in these cases, which can produce some fully active enzyme molecules assuring a limited degradation capacity sufficient to prevent a severe phenotype.

An alternative transcript of the IDS gene has recently been identified (Malmgren et al. 1995). This latter message contains 180 bp from intron 7, including the 78 bp detected in patient 25. It has been suggested that part of intron 7, which has a total length of 3,215 bp, represents an additional IDS gene exon (VIIb) coding for seven amino acids before a first stop codon appears. The corresponding alternative transcript is 1.4 kb long, i.e., 0.7 kb smaller than the mRNA coding for the wild-type enzyme, for it contains only exons I–VIIb and lacks the last two exons, VIII and IX, of the IDS gene. However, IDS messages containing the 78-bp intronic sequence together with exon VIII and/or IX, as detected in patient 25, have not been seen as normal variants (data not shown).

The functional consequence of the intronic point mutation on mRNA splicing in patient 25 is in line with the hypothesis of exon definition (Robberson et al. 1990). For the cryptic 3′-splice-acceptor site in intron 7 a consensus value of 86.4 can be calculated by using the formulas defined by Shapiro and Senapathy (1987). Nonetheless, it seems that this site is used only if an alternative IDS transcript is formed and the primary message is polyadenylated after exon VIIb (Malmgren et al. 1995). The cryptic 5′-splice-donor site in intron 7 has a score of 69.9, which is obviously insufficient to be recognized effectively by the splice machinery. However, the 1131−133A→G point mutation results in a dramatic increase of the score to 84.3. The fact that this novel 5′-splice-donor site is considerably stronger than that at the end of exon VIII (68.6) may provide a possible explanation why exon VIII is skipped in a proportion of transcripts in the patient. The size of the "newly generated" exon (78 bp) and intron (137 bp) does not differ substantially from the average size of vertebrate internal exons (having a broad peak between 100 bp and 170 bp) and introns (with the largest single size range of 80–99 bp) (Hawkins 1988). The splice mutation in patient 25 is one of the rare examples in which an intronic sequence change, far apart from the coding region, may have a deleterious effect. Because the corresponding portion of the IDS gene is not examined on mutation screening performed at the genomic DNA level and by PCR amplification of single exons, such mutations can be detected only by analyzing the mRNA/cDNA.

Distribution of Mutations over the Coding Region of the IDS Gene

Figure 2 shows the approximate location of the 30 small rearrangements and point mutations reported in this communication, together with that of 105 similar IDS mutations described so far (Hopwood et al. 1993 and references therein; Hogervorst et al. 1994; Schröder et al. 1994; Jonsson et al. 1995 and references therein; Li et al. 1994, 1995; Popowska et al. 1995; Sukegawa et al. 1995; Goldenfum et al. 1996; Olsen et al. 1996). While the distribution of small rearrangements (deletions, insertions, and duplications of <20 bp) seems to be random, point mutations tend to be more frequent in exons III, VIII, and IX. The nonrandom distribution of point mutations over the exons is highly significant (χ^2 test of goodness-of-fit = 35.28; $P < 2.5 \times 10^{-5}$). Of the 109 point mutations, 101 lie within the coding region and 8 in introns. Assuming a random distribution of the 101 point mutations over the entire IDS coding region of 1,653 bp, a relative frequency of one mutation for every 16.4 nt can be calculated. As shown in table 3, the relative frequency of point mutations in exon III is 1/8, i.e., twice as high as the average. Exon VIII also harbors a much higher number of point mutations than expected according to its size. Nevertheless, there is a fundamental difference in the specificity of mutations found in exons III and VIII. While there are several mutations affecting the same codon but different nucleotides, so far only P86R and R88C have been identified in more than one (unrelated) patient in exon III. In contrast, the 1246C→T mutation in exon VIII was reported in nine (most likely) unrelated patients and thus accounts for 45% of the point mutations identified so far in this exon. Of the 31 point mutations reported, to date, in exon IX, 20 affect two apparently highly mutable codons, 443 and 468. In contrast to exons VIII and IX, point mutations (as other types of mutations) are distributed randomly over exon III, suggesting that the high mutation rate is due to a feature specific for this part of the gene. In this context, it is worth remembering that an IDS pseudogene has recently been identified in the close proximity of the functional gene (Rathmann et al. 1995) and that the sequence of exon III is practically

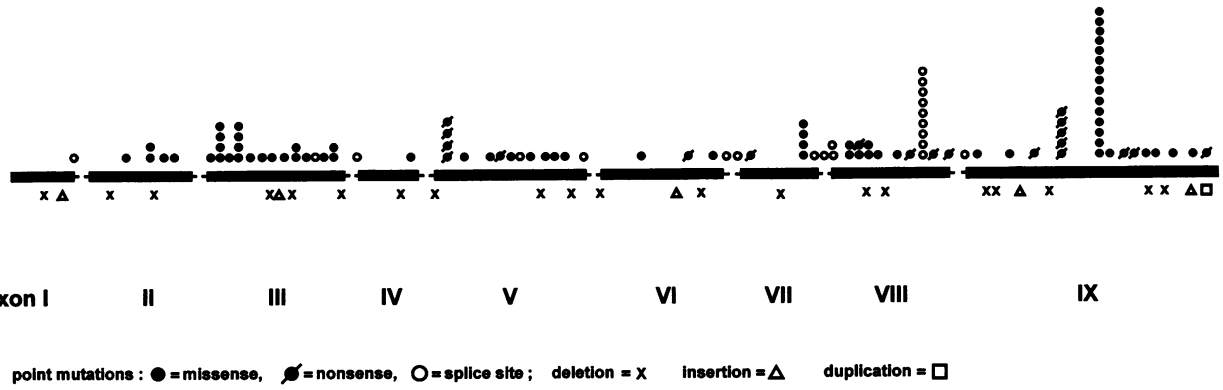

Figure 2 Distribution of 135 mutations affecting <20 bp over the human IDS gene. In addition to the 30 mutations reported in this communication, 105 further mutations were taken from the literature (see text for references).

unchanged in the pseudogene, whereas the remaining part has only a low overall homology to the functional IDS gene (Bondeson et al. 1995). However, because exon III contains two "sulfatase sequence motifs" that are evolutionarily conserved from prokaryotic sulfatases to human sulfatases (Prosite protein signal database, release 13), the high number of mutations identified in this exon may also be due to the fact that alterations in this functionally important part of the enzyme molecule always lead to a disease phenotype. Consequently, such mutations may have a higher chance of detection, while persons with alterations in less important regions of the protein may remain phenotypically normal (i.e., they are more likely to escape detection).

In addition to the location of mutations, their type is also an important factor in defining the mutation spectrum of the IDS gene. Of the 109 point mutations discussed above, 51 (47%) are at CpG dinucleotides, of which G:C-to-A:T transitions constitute ~80%. Forty-five percent of the exon VIII mutations affect the same CpG dinucleotide (1246C→T), whereas the remaining 11 are distributed randomly. In case of exon IX, ~64% of all mutations (20 of 31) are recurrent and affect the same codons (443 and 468) and occur at CpG, with the great majority (90%) being a G:C-to-A:T transition. Remarkably, point mutations recurrent in more than two unrelated patients (R88C, R172X, S333L, 1246C→T, R443X, R468Q, and R468W) involve CpG sites. All these values are very similar to those found in other genes (for review, see Cooper and Krawczak 1994).

Table 3

Relative Frequency of Point Mutations in the 9 IDS Gene Exons (n = 101)

Exon	Size (bp)	No. of Mutations	Relative Frequency
I	103	0	...
II	137	5	1/27
III	177	21	1/8
IV	90	1	1/90
V	201	14	1/14
VI	171	3	1/57
VII	127	6	1/21
VIII	174	20	1/9
IX	473	31	1/15

NOTE.—The χ^2-test of goodness-of-fit performed for the deviation of the observed number of mutations in each exon compared to the expected values (assuming a random distribution of one mutation/16.36 nt) proved high statistical significance ($\chi^2 = 35.28$; $P > 2.5 \times 10^{-5}$).

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