THE MOLECULAR BASIS OF MUCOPOLYSACCHARIDOSIS TYPE I IN TWO THAI PATIENTS

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Abstract. Two Thai patients diagnosed with Hurler syndrome (mucopolysaccharidosis type 1, MPS I) were found to have no detectable α-iduronidase (E.C. 3.2.1.76) activity in leukocytes, while normal Thai children all had significant activity, with a mean of 135 ± 30 nmol/mg/18h. One patient was heterozygous for A75T (311G>A) and S633L (1986C>T) mutation, previously reported to cause MPS I, together with 9 other heterozygous polymorphisms also found in normal controls. The other patient had the previously described frameshift mutation 252insert C and a new nonsense mutation E299X (983G>T).

INTRODUCTION

Mucopolysaccharidosis type I (MPS I, MIM 252800) is caused by a deficiency of lysosomal α-L-iduronidase (E.C. 3.2.1.76) that results in accumulation of dermatan sulfate and heparan sulfate, which are normally degraded by this enzyme in the lysosome (Scott *et al*, 1995). MPS I causes a wide range of clinical disorders, from Hurler syndrome at the severe end to Scheie syndrome at the mild end.

Many mutations have been identified in the α-L-iduronidase gene, which is located on chromosome 4 at 4p16.3 and contains 14 exons spanning approximately 19 kb, encoding a 653 amino acid protein (Human Gene Mutation Database: http://www.hgmd.org; Scott *et al*, 1993,

1995; Bunge *et al*, 1995; Yamagishi *et al*, 1996; Beesley *et al*, 2001; Matte *et al*, 2003). Mutations that cause severe MPS I (Hurler syndrome) include nonsense, missense and splice-site mutations, while milder MPS I defects identified include mainly missense mutations and small deletions that leave most of the protein intact. The mutations, W402X and Q70X, are especially common in European populations (Scott *et al*, 1993, 1995; Bunge *et al*, 1996; Beesley *et al*, 2001), while the milder R89Q mutation is often found in Japan (Yamagishi *et al*, 1996). Before this work, there have been no reports on the molecular characterization of this defect in Thailand.

PATIENTS AND METHODS

Subjects

Patient 1 was first diagnosed as having Hurler syndrome at one year of age. At 4 years old, her symptoms included macrocephaly, coarse facial features, cloudy cornea, claw-hand deformity, pectus deformities, hepatosplenomegaly, dysostosis multiplex and mental retardation.

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Patient 2 was diagnosed with Hurler syndrome at two and a half years of age. Symptoms included coarse facies, cloudy cornea, claw-hand deformity, hepatosplenomegaly, hearing loss and mental retardation.

Determination of α-iduronidase enzyme activity

The urine of both patients was tested for mucopolysaccharides (dermatan sulfate and heparan sulfate) as described by Stone (1998).

Leukocytes were isolated from fresh heparinized blood of control children, aged 4 to15 years, and of the patients (ages 4 and 6). Leukocytes were suspended in 0.9% NaCl, lysed by sonication and the protein concentration of the lysate was determined by the Bradford assay (Bradford, 1976). Cell homogenates were assayed for α-L-iduronidase activity by measuring the release of phenol from 1.3 mM phenyl-α-iduronide in 0.08 M sodium formate, pH 3.5, 12.5 mM NaCl at 25°C after 18 hours (Dulaney *et al*, 1976).

Gene sequencing

RNA and genomic DNA was extracted from leukocytes using blood RNA and QIAamp blood DNA extraction kit (QIAGEN, Germany) respectively. The mRNA was reverse-transcribed using oligo dT_{15} primer, then PCR amplified with F30 (CCGGCAGTGCAGCCCGAAG) and R2000 (AGCCCCAGCTCGCTGACTG) primer to produce a cDNA fragment that included the whole coding region. This fragment was directly sequenced with internal primers to determine the sequence of the whole coding region. For the genomic DNA, exons or sets of closely spaced exons were amplified using primers from within the surrounding introns, or occasionally within the exons. These products were similarly directly sequenced. Primers used in genomic DNA amplification and sequencing were as follows: exon 1, F30 and R9 (AAGCTCTCACAGTGCGCAGTC); exon 2, F9 (TGCCAGCCATGCTGAGGCTC) and R8 (CACAGCCAGCAAGGACACGCT); exons 3 and 4, F12 (CTGGAGCATGGAGCTGTGT) and R11 (AGGCAGCAGCCTGCGTGAT); exon 5, F13 (AGTCACTGAGGCGAGATTCAC) and R5 (AGGCATCGTAGTAGTTCAGGA); exon 6, F2 (GAGCCAGACCACCACGACT) and R4 (CCGCCTCGTCGTTGTAAATG); exon 7, IDUA3 (AGGCGGGCGTGCG

GCTGGACTACAT) and R3 (AGCACCGGCTTGCGCAACA); exon 8, F3 (TGGAGCAGGAGAAGGTCGT) and R16 (CCTTCGCAGGAGCCGCTC); exon 9, F15 (AGGGGGAGCGAGTGGTG) and R2 (CAGAGCCCGTTGTCCAGGTA); exon 10, F5 (TGATCTACGCGAGCGACGA) and R14 (CCTCCGCGGCCCACCTA); exon 11, F16 (TCCGAGGCGGTGTGGGTG) and R1 (TTCATCCGACCAGACCAGAA); exon 12, F6 (CCGTCGCTTTTGCTGGTGCA) and R15 (CACCCAGTACTGCCCTGA); exon 13, F17 (ACCTTTGAGGACTGTCTTGA) and R15; exon 14, F7 (AGTTCGAGCCCTGGACTACT) and R2000. The polymorphisms were detected in control subjects and patients by restriction digests of amplified exons or by sequencing, as listed in Table 1.

RESULTS

The patients exhibited severe signs of MPS I or Hurler syndrome and excreted mucopolysaccharides (dermatan sufate and heparan sulfate) in their urine, consistent with the diagnosis of MPS I. The activity of leukocyte α-iduronidase in both patients was not detectable (< 10 nmol/mg/18 h), while normal Thai controls (n = 28) all had higher levels with a mean of 135 ± 30 nmol/mg/18 hours.

To determine the molecular basis of the defects, cDNA and genomic coding regions of the α-L-iduronidase gene were PCR amplitied and directly sequenced (Fig 1). For patient 1, 11 heterozygous nucleotide locations were identified within the coding region, while all homozygous positions matched those of the database (Genbank Accession M74715). Six of the polymorphisms caused amino acid changes in the protein: Q33H (CAG>CAT), A75T (GCC>ACC), R105Q (CGG>CAG), A361T (GCG>ACG), V454I (GTC>ATC) and S633L (TCG>TTG); the others were silent L118 (CTG>TTG), N181 (AAT>AAC), A314 (GCG>GCC), T388 (ACG>ACC), and T410 (ACC>ACG). The cDNA and genomic DNA of the patient's mother was also sequenced and found to have all of the same polymorphisms, except for Q33H and S633L.

Patient 2 had the previously reported 252-insert C polymorphism (Bunge *et al*, 1995), which results in a frameshift from residue 56 onwards,

Table 1
α-Iduronidase mutations and polymorphisms in Thai MPS I patients.

Amino acid change	Nucleotide change	Location (Exon)	Restriction site test	Percent in Thai controls	Phenotype and reference
Q33H	187G>T	1	Nal III	12%	Normal (Scott et al, 1992)
frame-shift	252 insert C	2	Sequencing	0%	Hurler (Bunge et al, 1995)
A75T	311G>A	2	AsuHP I	0%	Hurler (Clarke et al, 1994)
R105Q	402G>A	3	AlwN I	19%	Normal (Scott et al, 1991)
E299X	983G>T	7	Mnl I	0%	Hurler, (new, this work)
A361T	1169G>A	8	FnuD II	19%	Normal (Scott et al, 1993)
V454I	1448G>A	9	Sequencing	19%	Normal (Scott et al, 1995)
S633L	1986C>T	14	Sequencing	0%	Scheie, (Beesley et al, 2001)

Nucleotide positions listed are those in the cDNA. Percentages are from 100 chromosomes from normal Thai control subjects. Phenotypes listed are those in the literature, except for the new E299X mutation.

A. A75T GCC>ACC	B. S633L TCG>TTG	C. 252insert C	D. E299X GAG-> TAG (N297N) (AAC>AAT)

ACATA GGC GAGGTTG   CCCCTTC CTCG GACCCT   GGGGGGCTGGGGACAA   CCGC CTC GTC GTT GTA
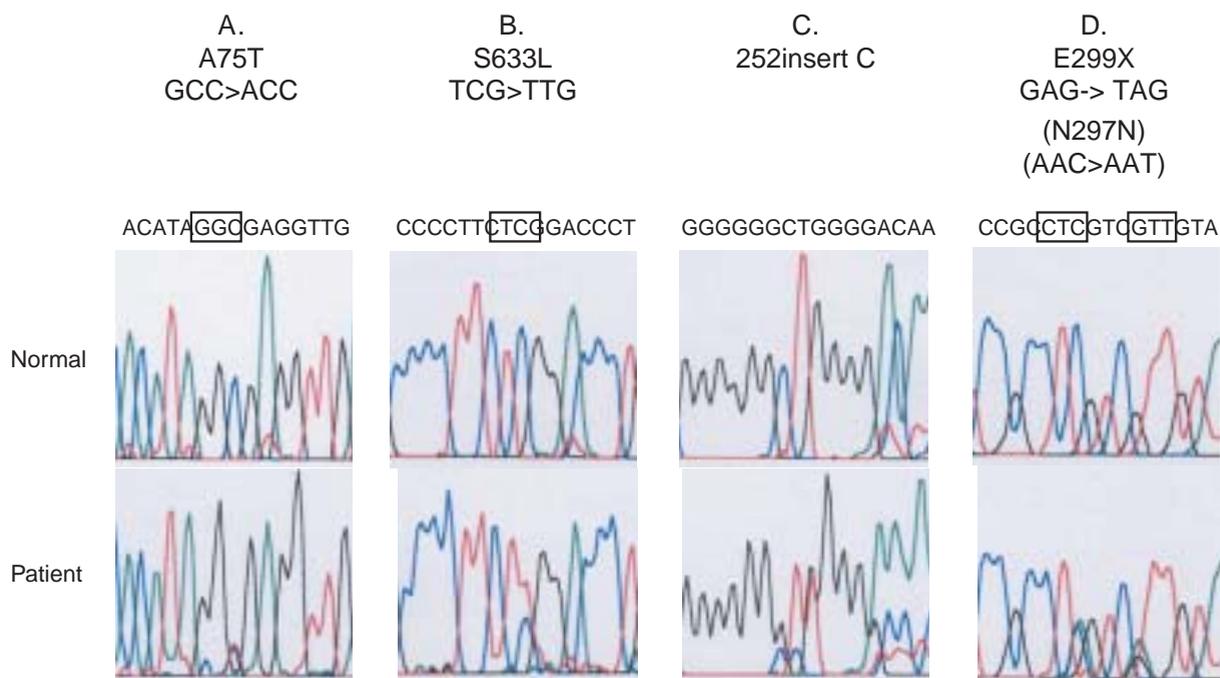
Fig 1–Sequenograms of the putative disease-causing mutations. A. normal control and patient 1 at the A75T mutation (noncoding strand, C>T); B. normal control and patient 1 at S633L mutation (coding strand, C>T); C. normal control and patient 2 at 252 insert C mutation (noncoding strand, inserted G causes heterologous signal after point of insertion); D. normal control and patient 2 at E299X (noncoding strand, C>A).

and a new nonsense mutation, E299X (GAG> TAG) (shown in Fig 1).

To verify which mutations were deleterious, 100 chromosomes from normal Thai patients were analyzed for all the polymorphisms causing amino acid changes. As shown in Table 1, the mutations, A75T, S633L, 252insert C, and E299X, were not detected in the normal controls, but Q33H was found in 12%, while the R105Q, A361T and V454I were found in 19% and always in the same subjects, together with a T>C change in IVS 9 at nucleotide 36.

DISCUSSION

Of the missense polymorphisms that may change the α-iduronidase activity of patient 1, only A75T and S633L have been identified as causing a defect (Clarke *et al*, 1994; Beesley *et al*, 2001). The others, Q33H, R105Q, A361T and V454I, have been identified as being nonpathogenic mutations, although it is possible they may modulate the affects of other mutations (Scott *et al*, 1995). This is consistent with the observation that A75T and S633L were not found in the unaffected Thai population, but the others were. Both the mutations found in patient 2 should result in a truncated protein lacking the catalytic nucleophile, which has been shown to be E299 (Nieman *et al*, 2003; Brooks *et al*, 2001). Again, the absence of these polymorphisms from the general population supports their identification as the pathogenic mutations.

Clarke *et al* (1994) identified A75T as a missense mutation that results in severe MPS I when found in combination with a nonsense mutation on the sister chromosome. Recently, S633L was found to cause Scheie syndrome when homozygous and to completely lack activity when expressed in a mammalian system (Beesley *et al*, 2001). The Hurler syndrome seen in patient 1 is likely to be primarily due to compound heterozygosity of A75T and S633L. The association with A75T, and perhaps other polymorphisms, leads to a more severe phenotype than might be expected for the S633L mutation. In patient 2, the new mutation which replaces the catalytic nucleophile residue, E299, with a stop codon, together with a frameshift mutation at residue 56, would lead to the absence of the complete protein, resulting in no activity and explaining the severe phenotype in this patient.

The Q33, Q105, T361, I454 genotype has previously been reported to be common in Taiwan and to result in high α-iduronidase activity when expressed in mammalian cells (Lee-Chen *et al*, 1998). The presence of these polymorphisms in the Thai population suggests this haplotype may be derived from the same source. Though no information of ethnicity is available for the normal controls, the abundance of Chinese population in Thailand and Taiwan suggests this may be a common haplotype found throughout the Chinese population.

Identification of the new E299X mutation and the 3 previously reported mutations and the common polymorphisms in Thailand should be helpful in characterizing new cases of MPS I in the Southeast Asian population.

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