THE MOLECULAR GENETICS OF MUCOPOLYSACCHARIDOSIS TYPE I

by

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THESIS SUMMARY

Mucopolysaccharidosis type I (MPS-I, eponyms: Hurler, Hurler/Scheie and Scheie syndromes) is an autosomal recessive genetic disease caused by a deficiency of the glycosidase α-L-iduronidase (IDUA) which is required for the lysosomal degradation of the glycosaminoglycans heparan sulphate and dermatan sulphate. Patients with MPS-I store these partially degraded glycosaminoglycans in their lysosomes. MPS-I patients have a wide range of clinical presentations (Hurler (severe clinical phenotype), Hurler/Scheie (intermediate) and Scheie (mild) syndromes), which makes it difficult to predict patient phenotype that is necessary for genetic counselling and also impedes the selection and evaluation of patients undergoing therapy such as bone marrow transplantation. This work was performed with the aim of cloning the gene for IDUA, to enable molecular genetic diagnosis of mutations causing MPS-I for more accurate disease prognosis, and make possible new therapy protocols such as enzyme replacement therapy and gene replacement therapy in the near future. An understanding of the enzymology of IDUA and lysosomal biogenesis may also be obtained. The following results were obtained.

1.) Peptide data obtained from purified IDUA was used to design oligonucleotide probes which were used to screen cDNA and genomic libraries. An EMBL3 genomic clone positive to the oligonucleotide probe ID47 (λID475) was confirmed to be IDUA by showing that it contained nucleotide sequence colinear to the peptide sequence of purified IDUA.

2.) *IDUA* had previously been localized to chromosome 22. To further confirm the identity of the genomic clone, chromosomal localization studies were performed by *in situ* hybridization to human metaphase chromosomes, Southern blot analysis of human-mouse cell hybrids, and immunocapture of human IDUA from human-mouse cell hybrids. These studies revised the localization of *IDUA* from chromosome 22 to chromosome 4p16.3.

3.) Screening of a random-primed colon cDNA library with a 1.6-kb *Pst* I subclone of λID475 produced a single positive with a 729-bp insert which contained nucleotide sequence colinear to 6 peptide sequences of purified IDUA. Screening of seven other cDNA libraries produced a further 58 positives, the longest of which (λE8A) was 1765-bp long. Of these clones, 45 ended at the same 5' base as λE8A.

4.) Regional mapping of the 1.6-kb *Pst* I genomic fragment had further localized the gene for IDUA to 1100-kb from the telomere of chromosome 4p and 1000-kb from either of the two most likely regions for the Huntington disease gene and within a cosmid clone A157.1. Using a revised peptide model for IDUA, it was hypothesized that the 74/13-kDa N-terminal peptide sequence was 5' to the sequence that had thus far been obtained. An oligonucleotide probe to this peptide sequence was used to screen the cosmid A157.1 and a 2.8-kb *Bam*HI fragment positive to this probe was subcloned and partially sequenced. An exon encoding an initiating methionine, a signal peptide, the 74/13-kDa N-terminal sequence and part of a tryptic peptide from IDUA was identified.

5.) Oligonucleotide primers made to exon I of IDUA and the 5' end of the cDNA clone λE8A were used to PCR amplify IDUA mRNA sequence from cDNA, and two PCR products were obtained. The major PCR product contained exon II of IDUA including sequence that encoded the last unlocated tryptic peptide for IDUA and the minor product was shown to be the product of alternative splicing of exon II of the IDUA gene. Alternative splicing was also found to occur for exon IV of IDUA.

6.) A full-length cDNA construct for IDUA was constructed using PCR, cloned into the expression vector pRSVN.07 and transfected into Chinese hamster ovary cells. A 160-fold increase in IDUA activity was observed and monoclonal antibodies were used to prove that the transfected cell line was producing human IDUA of a normal specific activity. This was the final proof that the sequence obtained was indeed the sequence that encoded human IDUA. Thus, IDUA is translated as a 653 amino acid precursor with a 26 amino acid signal peptide that is cleaved immediately prior to the 74/13-kDa

N-terminus. The protein sequence contains six potential N-glycosylation sites. Northern blot analysis revealed only a single 2.3-kb mRNA.

7.) Mapping and sequencing of the EMBL3 genomic clone λID475 and the cosmid clone A157.1 showed that the gene for IDUA was split into 14 exons spread over 20-kb. The potential promoter for IDUA has only GC box type consensus sequences consistent with a housekeeping promoter and is bounded by an Alu-type repeat sequence. The first 2 exons are separated by an intron of 566-bp followed by a large intron of approximately 13-kb (intron 2). The last 12 exons are clustered in a 4.5-kb segment. No consensus polyadenylation signals were found although two variant polyadenylation signals are proposed.

8.) Two polymorphisms were found to exist in the IDUA gene, a *Kpn* I polymorphism in exon III and a variable number of tandem repeats (VNTR) polymorphism with three common alleles in intron 2. This VNTR polymorphism is the well characterized *D4S111* polymorphic locus used in Huntington disease diagnosis. The analysis of allele and haplotype frequencies for these two polymorphisms in the normal population and in MPS-I patients revealed the presence of linkage disequilibrium. The frequency of the 2,2 (VNTR, *Kpn* I) allele in MPS-I patients was 57% compared to only 37% in the normal population. This implied the presence of a major MPS-I allele. There was also slight disequilibrium between the 2,1 haplotype and MPS-I implying a second common mutation.

9.) On the basis of this haplotyping, a group of patients with the 2,2 haplotype and some with the 2,1 haplotype were selected for mutation analysis by chemical cleavage and direct PCR sequencing. Several patients with mild phenotypes and unusual biochemistry were also selected for mutation analysis. A G to A substitution was detected in exon IX of IDUA that altered a tryptophan codon at position 402 (TGG) to a stop codon (TAG), thus resulting in premature termination of translation approximately two thirds of the way through the 653 amino acid IDUA protein ($W_{402}X$). $W_{402}X$ was found associated with the 2,2 haplotype and accounted for 33% of MPS-I alleles in a group of 76 patients studied. Patients homozygous for $W_{402}X$ have an extremely

severe Hurler-like clinical phenotype. Patients heterozygous for $W_{402}X$ have the full range of clinical phenotypes found in MPS-I.

10.) A C to T substitution in exon II which altered a glutamine codon at position 70 (CAG) to a stop codon (TAG) was found associated with the 2,1 haplotype and accounted for 15% of MPS-I alleles in the patient group ($Q_{70}X$). $Q_{70}X$ is also associated with a very severe Hurler-like clinical phenotype as can be seen in patients with the $W_{402}X$, $Q_{70}X$ genotype. Thus two mutations accounting for 48% of MPS-I allele and therefore 23% of MPS-I genotypes have been defined. The present clinical phenotype of patients who have had both alleles defined as either $W_{402}X$ or $Q_{70}X$, and have undergone bone marrow transplantation provides encouraging evidence that MPS-I is treatable by bone marrow transplantation and therefore also possibly treatable by enzyme replacement and gene replacement therapy in the future.

11.) Three amino acid substitutions, one single base deletion and two splicing mutations have also been found. One splicing mutation, $g_{1487}a$, was observed in three mildly affected patients. It is hypothesized that a small amount of normal mRNA is produced from this mutant allele leading to the extremely mild phenotype seen in these patients, two of whom are heterozygous for $W_{402}X$. Neither the biochemical or clinical outcome of $t_{1978}c$ splicing mutation is known. One of the amino acid substitution mutations, $R_{89}Q$, is also associated with a mild phenotype. These are the first observations to explain the vast difference in the extremes of the clinical spectrum for MPS-I. An amino acid mutation, $D_{349}N$, may be involved in active site catalysis and/or processing of IDUA.