CHAPTER II

BACKGROUND AND LITERATURE REVIEWS

1. The mucopolysacchridoses (MPS)

The mucopolysacchridoses (MPS) are a group of inherited lysosomal storage disorders, each with a distinctive phenotype and a progressive course due to a deficiency of an enzyme which usually catalyses a step in the degradation of glycosaminoglycans (GAG). Mucopolysaccharidosis type I (MPS I) is considered the prototypic lysosomal storage disease of MPS groups. Individuals of alpha-L-iduronidase deficiency have been classified into three clinical phenotypes: Hurler syndrome, Hurler-Scheie syndrome, and Scheie syndrome[2, 5].

2. Discovery of mucopolysaccharidosis type I (MPS I)

Dr. Gertrud Hurler first described two patients with the most severe form[17]. Brante later introduced the term mucopolysaccharidosis and classified the group of disorders based on chemical analysis and staining of inclusion bodies. Dorfman and Lorincz detected mucopolysaccharides in urine from Hurler patients[18]. In 1965, Danes and Bearn revealed the storage of mucopolysaccharide in cultured fibroblasts from patients with Hurler syndrome[19]. Fratantoni and others demonstrated that the cultured fibroblasts from patients with Hurler and Hunter syndromes incorporated radiosulphate (^{35}S-sulphate) into GAG more rapidly than those from normal individuals because of defective GAG degradation rather than accelerated synthesis. Correction of this defect by the products of cells with other genotype was also demonstrated[20]. Scheie syndrome was recognized in 1962[21]. In 1971, Barton and Neufeld demonstrated that abnormal GAG degradation of Hurler cells could be corrected by growing them in medium which had been used to culture Hunter cells. In addition, these cells were shown to be defective in the same "corrective factor" as Hurler syndrome identified as alpha-L-iduronidase deficiency and could not correct the defect in Scheie cells, indicating that the two diseases were probably allelic[22, 23].

3. Clinical classification

Mucopolysaccharidosis type I (MPS I; MIM# 252800) is a lysosomal storage disorder caused by a deficiency in the lysosomal acid hydrolase, alpha-L-iduronidase (EC 3.2.1.76). The alpha-L-iduronidase specifically cleaves iduronic acid residues from the nonreducing terminus of the long-chain polysaccharides derived from the proteoglycans, dermatan and heparan sulfates (Figure 1). Deficiency of alpha-L-iduronidase causes the blockage of further sequential degradation of these glycosaminoglycans resulting in the lysosomal accumulation and urinary excretion of the partially degraded substrates. MPS I patients show a wide spectrum of clinical presentations ranging from the severe archetypical Hurler syndrome to an attenuated form called Scheie syndrome. Patients at the severe end of the clinical spectrum present with hydrocephalus, coarse facial features, corneal clouding, enlarged tongue, hepatosplenomegaly, hernia, cardiac diseases, short stature, dysostosis multiplex, joint stiffness, clawed hands, fatigue and delayed development. In this severe form, the disease onset is rapid and progressive, with patients often suffering an early death before the teenage years. Scheie syndrome patients typically display variable clinical symptoms that can include coarse facial features, corneal clouding, cardiac valve diseases, joint stiffness, clawed hands, malaise/fatigue and other somatic features. The disease onset is usually delayed compared to that in Hurler syndrome patients and the disease progression is less rapid. In some patients with an attenuated form of the disorder, intelligence can be normal and the patient can have a normal lifespan[3, 4].

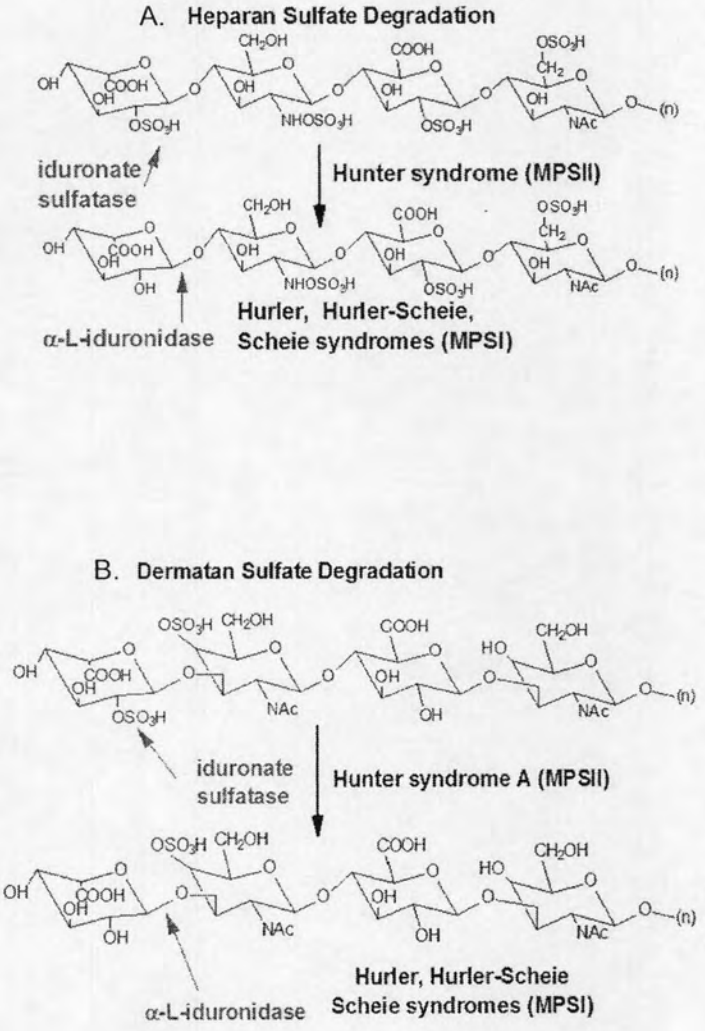

Figure 1 Schematic representation of the structures of heparan sulfates (A) and dermatan sulfates (B) as are degraded by the indicated enzymes. Enzyme names are shown in green. Defective enzyme activity leads to the disorders indicated in blue. The chains of heparan sulfates have alternative uronic acid and glucosamine residues, the reaction catalyzed by a sulfatase. Dermatan sulfates are composed of alternating uronic acid and sulfated *N*-acetylgalactosamine residues.

4. Incidence and inheritance

In the United States, the overall incidence of Hurler syndrome, Scheie syndrome and Hurler/Scheie syndrome is 1 per 100,000, 1 per 500,000 and 1 per 115,000, respectively[5]. Nelson and others conducted a comprehensive study, which yielded a frequency of Hurler syndrome being 1 per 76,000 in Northern Ireland[6] and 1 per 320,000 in Western Australia[7].

5. Etiology and molecular genetics of MPS I

5.1 Alpha-L-iduronidase structure

Alpha-L-iduronidase is synthesized in the endoplasmic reticulum (ER) as 653 amino acid polypeptide (following signal peptide cleavage) and is glycosylated with six N-linked oligosaccharides to produce a 74-kDa precursor molecule. The N-linked oligosaccharides on alpha-L-iduronidase are modified to produce mainly "complex type" oligosaccharides and at least two of these N-linked oligosaccharides have been shown to be mannose-6-phosphorylated[24]. Alpha-L-iduronidase has been found to undergo extensive proteolytic processing to produce at least 10 polypeptides (Mr 74, 69, 65, 60, 49, 44, 25, 16, 9 and 5 kDa)[11]. This extensive proteolysis is thought to occur intracellularly, as a result of normal residence in the endosome-lysosome compartment.

O-Glycosyl hydrolases are a widespread group of enzymes that hydrolyse the glycosidic bond between two or more carbohydrates, or between a carbohydrate and a non-carbohydrate moiety. A glycoside hydrolase family 39 (CAZY:GH39) comprises enzymes with several known activities; *alpha-L-iduronidase* (EC:3.2.1.76); *beta-xylosidase* (EC:3.2.1.37).

The most highly conserved regions in these enzymes are located in their N-terminal sections. These contain a glutamic acid residue which, probably acts as the proton donor in their catalytic mechanism.

Crystal structure of the catalytic domain of a homology model for the IDUA enzyme was constructed based on the recently solved crystal structure of the β-xylosidase from *Thermoanaerobacterium saccharolyticum* (XyTS, EC 3.2.1.37), both of which belong to the same sequence-related family (CAZY family 39)[25] (Figure 2).

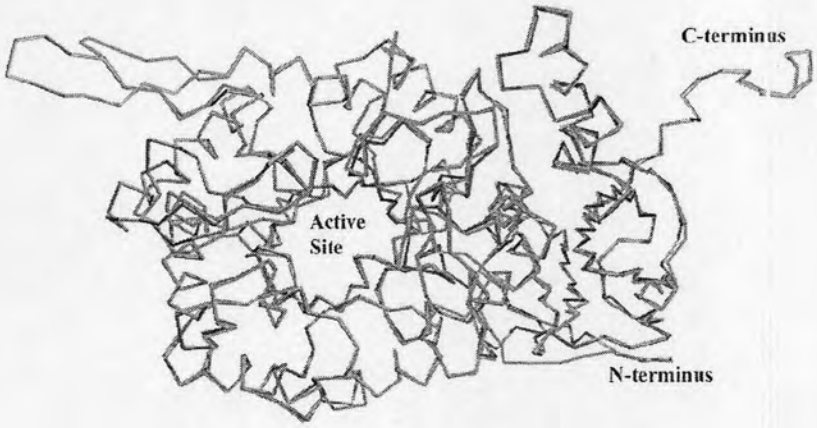

Figure 2 C-α traces overlaid for the IDUA homology model (green) and the crystal structure of the XyTS (blue) used as the basis for the homology model[25].

As to the active site of IDUA, Brooks and others[26] predicted the putative acid/base catalyst and nucleophile residues to be E182 and E299 (Figure 3), respectively. Then, they expressed site-directed mutants p.E182A and p.E299A in Chinese hamster ovary (CHO)-K1 cells and showed that the mutant proteins were catalytically inactive. These results suggest that these residues are important for the catalytic mechanism of IDUA.

5.1.1 Catalytic mechanism

Enzymatic hydrolysis of the glycosidic bond takes place via general acid catalysis that requires two critical residues, a proton donor and a nucleophile/base. This hydrolysis occurs via two major mechanisms giving rise to either an overall retention, or an inversion, of anomeric configuration.

IDUA consists of two domains: an $(\alpha/\beta)_8$-barrel domain, and an antiparallel β sheet domain (Figure 3).

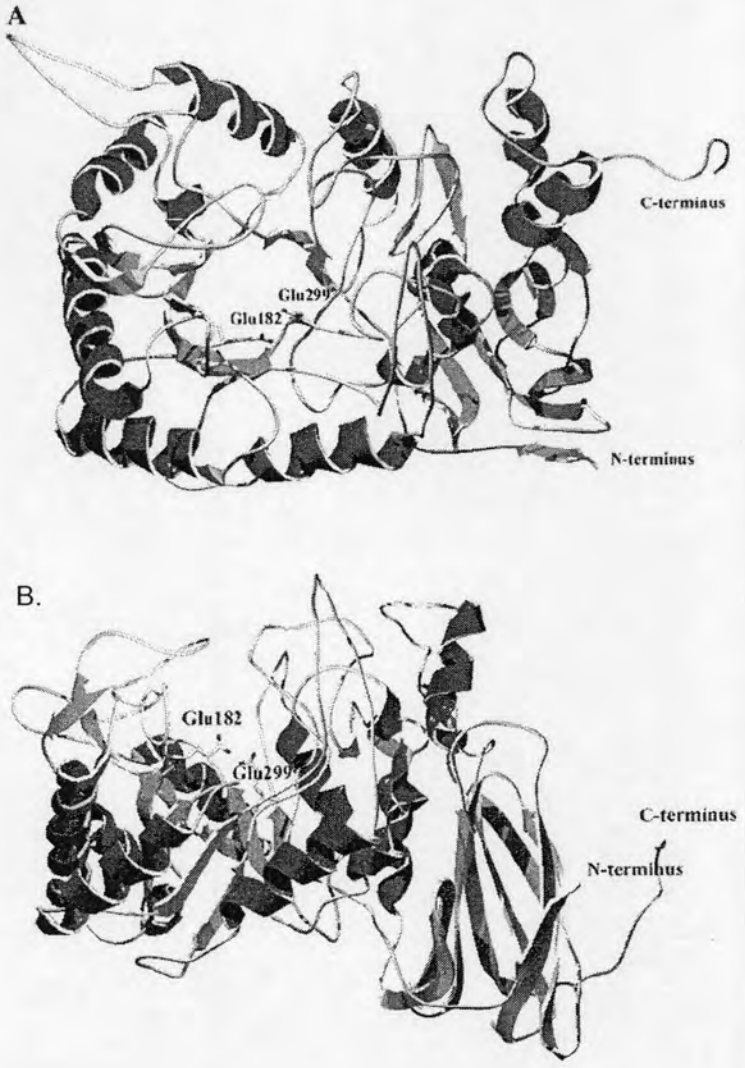

Figure 3 Ribbon representation of the IDUA model. (A) View along the $(\alpha/\beta)_8$ barrel axis. (B) View perpendicular to the $(\alpha/\beta)_8$ barrel axis. Note that α helices are colored red and β sheets green[25].

5.2 Enzymatic and molecular diagnosis

In the 1970s, a range of substrates were developed for alpha-L-iduronidase, allowing the characterization of the enzymatic defect associated with MPS I. The methods to purify alpha-L-iduronidase were developed and the catalytic properties of alpha-L-iduronidase in both crude cell/tissue extracts were defined[13, 27]. Theoretically, an alpha-L-iduronidase deficiency could be resulted from the production of an inactive protein, a low level of mutant protein or a residual amount of normal protein. A previous study revealed that the residual alpha-L-iduronidase activity detected in MPS I patient

fibroblast cell extracts was associated with a low level of mutant alpha-L-iduronidase in most cases[28]. This low level of the mutant protein was later shown to be caused by incorrect folding by the endoplasmic reticulum, causing subsequent degradation as part of the cell's quality control process[29, 30]. In MPS I patients where the mutation does not abrogate enzyme activity, this phenomenon is a major contributing factor to the onset of disease, due to the removal of the mutant but partially functional protein.

The concept of catalytic capacity (a measure of the effective amount of enzyme activity in cells) was originally developed for a lysosomal protein called 4-sulfatase[31] and represented a combination of the mutant protein detection and enzymology technologies. Catalytic capacity was calculated as the product of catalytic efficiency and the amount of specific normal/mutant protein per milligram of total cell protein in a cell extract. The measure of alpha-L-iduronidase catalytic capacity n patient cell extracts provided a good correlation with the clinical severity of patients, by taking into account the level of the mutant protein and its residual catalytic activity[28].

Table 1 Alpha-L-iduronidase activity values for control and patients[32].

Tissue	Control values* (nmol/h/mg)	Patient Values Range (nmol/h/mg)
Leukocytes	39.7±13.8	0-1.0
Cultured skin fibroblasts	83.4±62.1	0-0.5
Aminotic fluid cells	45.4±36.9	-
Chorionic villi	8.52±4.47	-

* Values are given as mean±SD

At least 109 different disease-causing mutations in the *IDUA* gene have been described with the majority being missense/nonsense mutations. The splice-junction alterations and nucleotide insertions/deletions have also been reported (Human Gene Mutation Database, http://www.hgmd.cf.ac.uk, accessed March, 2009). The two mutations, p.W402X and p.Q70X, are most commonly found in Caucasians, responsible for as much as 70% of the disease alleles in some European countries. The p.W402X and p.Q70X mutations have been shown to produce an undetectable level of alpha-L-

iduronidase in MPS I patient fibroblasts leading to a very severe clinical presentation in the homozygous condition[33-35]. The p.P533R mutation has been reported frequently in Italy and is the most common mutation in Moroccan and Sicilian Italian MPS I patients . MPS I patients homozygous for the p.P533R present the similar phenotype compared to that of classical Hurler syndrome patients. In Japanese MPS I patients, the p.R89Q mutation is one of the most common mutant alleles and is associated with an attenuated clinical phenotype[36, 37]. In addition, the 704ins5 has been found in both Japanese and Korean patients[38, 39]. Even though certain mutations such as p.W402X and p.Q70X show a higher prevalence in certain geographic locations, there is a high degree of molecular heterogeneity leading to the wide clinical variability observed in MPS I patients. Prediction of a patient's clinical phenotype through genetic analysis of the *IDUA* gene is complicated by the high number of disease-causing mutations and polymorphisms present in the *IDUA* gene. It has been hypothesized that a combination of mutations, polymorphisms, and genetic background contribute to the clinical phenotype.

6. Genotype-phenotype correlations

Enzyme assay alone has been unable to predict the severity of MPS I disorder. Over 70% of mutations were repeated and thus may be helpful in phenotype prediction. However, because many non-recurrent alleles have been identified, the ability to accurately predict phenotype based on genotype may be limited.

Genotype-phenotype correlations in individuals with MPS I are complex and further research is required before they can be useful clinically. A comprehensive review of genotype-phenotype correlations illustrates the limitations related to the frequency of private mutations at this locus[40]. Rempel et al. proposed a crystal structure of human alpha-L-iduronidase protein and suggested that mapping mutations onto this proposed crystal structure may be helpful in predicting possible effects of missense mutations on alpha –L-iduronidase processing and function[41].

In general, the three phenotypes appear to be caused by differences in the combinations of mutant alleles at the *IDUA* gene locus. Hurler patients are predicted to have two severe alleles preventing production of any functional enzyme while Scheie

patients are predicted to have at least one mild allele that allows some residual enzyme activity. The intermediate Hurler/Scheie patients are predicted to have one severe and one mild allele. Mutational analysis of a large number of MPS I patients has shown that although there is some evidence of a genotype/phenotype correlation, there are suggestions that non-pathogenic polymorphisms affect mutant allele expression, and environmental factors may also influence gene expression[42]. However, genotype-phenotype correlation based on a single observation should be considered with caution.

Earlier predictions suggested that a series of mutations, present in the homozygous state, would confer either a severe (Hurler syndrome) or a mild (Scheie syndrome) phenotype, whereas compound state would confer an intermediate phenotype. This has been proven to be highly unlikely[43].

Although recurrent mutations have been described, many patients have at least one "private mutation" which limits the usefulness of the genotype in predicting the phenotype. Conceptually the following pattern of genotype–phenotype correlation is emerging for MPS I.

1. There does not appear to be strong evidence for the involvement of "other" genes in the modulation of the phenotype. Therefore it appears that it is the different IDUA sequence in individual patients which predicts the disease severity. It is important to note that although the mutations predict the overall severity of the disease, prediction of the individual's exact disease complications is impossible.

2. Most patients are compound heterozygotes for two separate mutations at the *IDUA* locus. Therefore both mutations need to be recurrent mutations before phenotype can be predicted. Large deletions or insertions in the *IDUA* gene are not common.

3. Individual mutation frequencies vary considerably in each population.

4. Severe patients are most often compound heterozygous or homozygous for one of the following recurrent mutations: W402X, Q70X, 474-2A>G, P533R, A327P, A75T, and L218P.

5. Intermediate severity patients most often have one allele containing a mutation that has been associated with severe disease and the other allele represents a private mutation that is invariably a missense mutation.

- The plethora of private missense mutations in the intermediate patients

is what underlies the vast clinical heterogeneity of this group. Interestingly these observations point out the fine modulation of disease phenotype that is conferred by very small modulations of residual activity. Using conventional IDUA activity assays the differences between intermediate and severe patients' IDUA activity cannot be detected.

6. Patients with attenuated disease can be homozygous for a mutation that confers attenuated disease but may also be compound heterozygous for a common severe allele and an allele which modulates the phenotype. Although two common mutations have been found to underlie the attenuated phenotype, 678-7A>G and R89Q, these only represent 30% of attenuated alleles[44].

The large number of mutations underlying MPS I and the large number of protein polymorphisms make genotype–phenotype correlations difficult to interpret[42, 45].

7. Genetic counseling

7.1 Carrier detection

The measurement of alpha-L-iduronidase enzyme activity in leukocytes is not a reliable method of carrier determination. Molecular genetic testing of *IDUA* is clinically available and can be used to identify carriers among at-risk family members when both mutations have been identified in an affected family member.

Molecular genetic testing of *IDUA* to determine carrier status can be offered to both parents of an affected child with known mutations. If both parents are found to be carriers, the diagnosis of MPS I in the proband is confirmed and carrier testing can be offered to other family members. If only one parent has an identifiable *IDUA* mutation, carrier testing using molecular genetic techniques would be available to that parent's family members.

7.2 Prenatal diagnosis

In cases in which the molecular basis of MPS I is known, prenatal diagnosis should be performed by molecular genetic testing as enzyme activity measurements, particularly those performed by laboratories with limited experience, have potential inherent difficulties.

7.2.1 **Molecular genetic testing**: Prenatal diagnosis for pregnancies with an increased risk is possible by analysis of DNA extracted from fetal cells obtained by amniocentesis usually performed at about 15-18 weeks' gestation or chorionic villus sampling (CVS) at about ten to 12 weeks' gestation. Both disease-causing alleles in the family must be identified before prenatal testing can be performed.

7.2.2 **Biochemical genetic testing**: Prenatal testing is available for pregnancies with an increased risk for MPS I by measuring alpha-L-iduronidase enzyme activity in cultured cells obtained by amniocentesis usually performed at about 15-18 weeks' gestation or CVS at about ten to 12 weeks' gestation.

Difficulty with prenatal diagnosis for MPS I may result from the reduce alpha-L-iduronidase enzyme activity in normal chorionic villi[46]. However, difficulties in interpreting borderline reduction of alpha-L-iduronidase enzyme activity can be overcome by performing enzyme activity assay in cultured rather than uncultured CVS cells[47], provided analysis for possible maternal contamination is also performed.

7.2.3 **Preimplantation genetic diagnosis (PGD)**: by polar body analysis for MPS I has been reported and may be available for families in which the disease-causing mutations have been identified[48].