# CHAPTER I
# INTRODUCTION

## 1. Background and rationale

The mucopolysaccharidoses are a group of rare genetic disorders of polysaccharide metabolism caused by a deficiency of either one of the seven specific lysosomal enzymes. The accumulation of undegraded mucopolysaccharides in cells of various tissues and organs results in a number of physical abnormalities. Mucopolysaccharidosis type I (MPS I) is the most common MPS disorders in Caucasians and has an incidence of 1 per 120,000 live births[1].

Mucopolysaccharidosis type I (MPS I; MIM# 252800) is an autosomal recessive disorder that results from a deficiency of the lysosomal enzyme alpha-L-iduronidase (IDUA; EC 3.2.1.76). The activity of alpha-L-iduronidase is required for the removal of non-reducing terminal iduronic acid residues, during the step-wise lysosomal degradation of the glycosaminoglycans (GAGs), dermatan sulfate and heparan sulfate in mammalian cells. An absence of IDUA activity leads to the lysosomal accumulation of undegraded substrates and the onset of pathology in specific cells, organs, and tissues[2].

Patients with MPS I show variable clinical phenotypes. The clinical manifestations of MPS I range from severe mental retardation combined with hepatosplenomegaly, dysostosis (dysosteogenesis) multiplex, corneal clouding, cardiac involvement, and death in early childhood (Hurler syndrome; MPS IH; MIM# 607014), to milder symptoms such as corneal clouding and hearing loss combined with normal intelligence and life span (Scheie syndrome; MPS IS; MIM# 607016). Following clinical presentation, diagnosis of MPS I is based on elevated levels of urinary dermatan sulfate and heparan sulfate as well as the absence or significant reduction of IDUA activity in patients' leukocytes or skin fibroblasts[3, 4].

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]. Previous studies found a frequency of Hurler syndrome being 1 per 76,000 in Northern Ireland[6] and 1 per 320,000 in Western Australia[7].

The gene encoding alpha-L-iduronidase (*IDUA*) has been fully characterized in 1991 by Scott and others[8]. It is located on chromosome 4p16.3 and extends approximately 19 kb. It contains 14 exons and produces an mRNA transcript of 2.3 kb that encodes a precursor protein of 653 amino acids. The first 2 exons are separated by an intron of 566 bp, a large intron of approximately 13 kb, and the last 12 exons are clustered within 4.5 kb[9, 10]. Alternative splicing of exons 2 and 4 to produce peptide products of 606 and 617 amino acids, respectively, has been shown to occur[9], but there is no evidence that these alternatively spliced products are functionally significant. The 81 kDa precursor is catalytically processed via 76 kDa and 70 kDa intermediates to the 69 kDa polypeptide (69 kDa form is stable in culture)[11]. The protein sequence contains six potential N-glycosylation sites (Asn residues 110, 190, 336, 372, 415, and 451)[9].

MPS I is diagnosed biochemically by the presence of dermatan sulphate in the urine, and the absence of IDUA activity in leucocyte[12, 13]. Measurement of IDUA activity can be determined using the substrate 4-methylumbelliferyl-alpha-L-iduronide[14, 15]. It is however unable to distinguish enzymatically between the three different phenotypes of MPS I. To identify which types of MPS I an individual has, it is based on the clinical features, rather than on test results. Mutation analysis of a large number of MPS I patients has shown some evidence of a genotype/phenotype correlation. However, previous studies have also suggested that non-pathogenic polymorphisms affect mutant allele expression and environmental factors may also influence gene expression[16].

In this study, mutational analysis by PCR-sequencing was performed on 2 unrelated Thai patients diagnosed clinically and biochemically as MPS I. An *in vitro* expression study of identified mutations was also carried out to investigate their pathogenicity.

## 2. Research Questions

1. What are the mutation spectrums of Thai patients with clinically diagnosed MPS I?

2. Does the novel mutation, if any, affect the alpha-L-iduronidase activity?

## 3. Objectives

1. To identify mutations in the *IDUA* gene in Thai patients with clinically diagnosed MPS I.

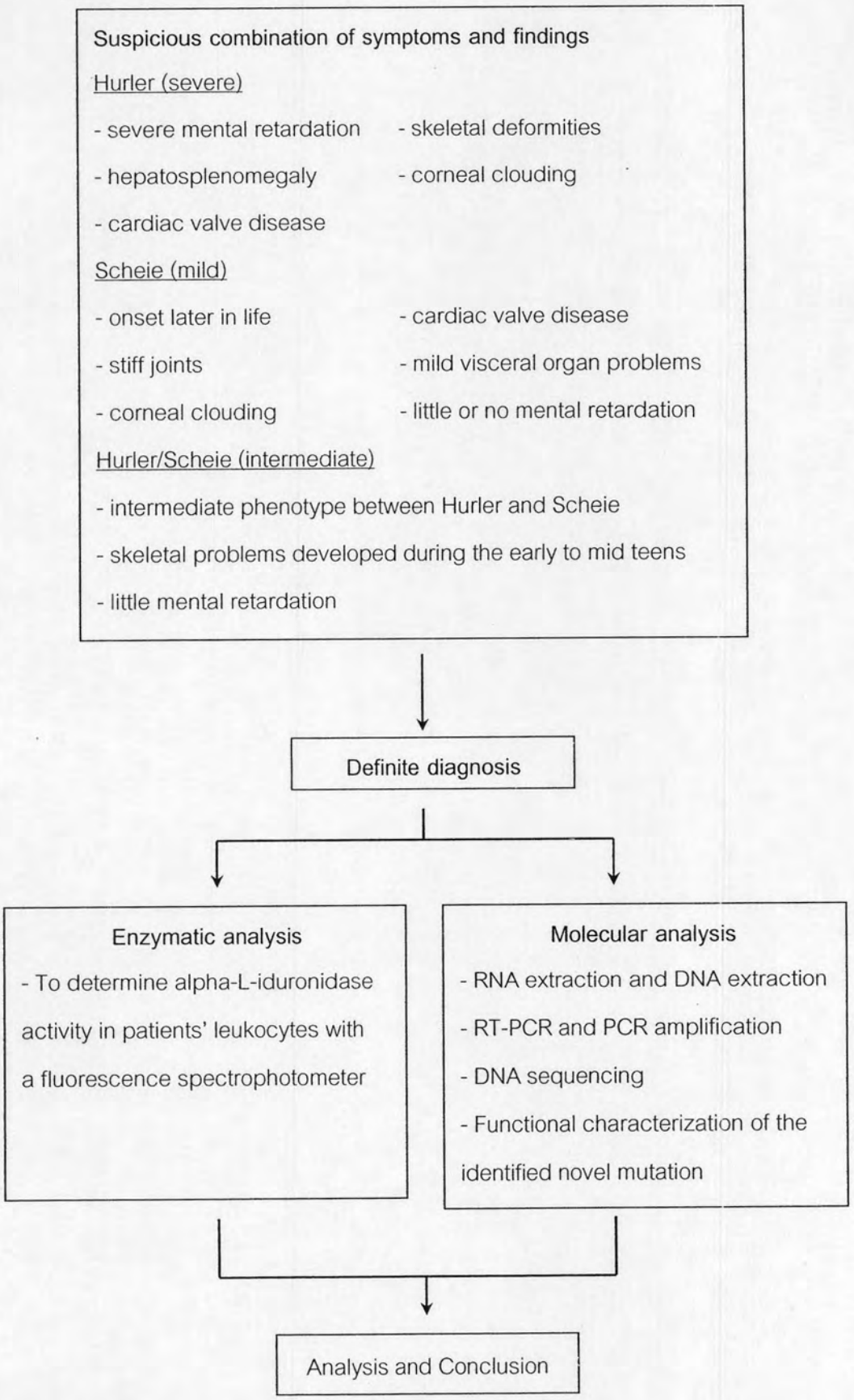
2. To determine the activity of alpha-L-iduronidase in leukocytes of patients with MPS I compared to that in Thai unaffected controls.

3. To study an effect of the novel mutation on alpha-L-iduronidase activity.

## 4. Hypothesis

Thai patients with clinically diagnosed MPS I have a mutation in the *IDUA* gene with no or reduced alpha-L-iduronidase activity.

5. Conceptual Framework



6. Assumption

Cases are Thai patients with clinically diagnosed MPS I.

Controls are healthy volunteers who are unaffected with MPS I and have no family history of MPS I.

7. Key words

Mucopolysaccharidosis type I, Hurler, Scheie, alpha-L-iduronidase, *IDUA*, mutation

8. Operational Definition

Controls are the healthy volunteers who are unaffected with MPS I and have no family history of MPS I.

Cases are the patients with clinically diagnosed MPS I.

DNA sequencing is the process of determining the nucleotide order within DNA.

Transfection is the process of delivering DNA into mammalian host cells.

Enzyme assay is the laboratory method for measuring enzymatic activity. They are vital for the study of enzyme kinetics and enzyme inhibition, the terms of activity described in enzyme units.

9. Research Design

Descriptive and *in vitro* studies

10. Ethical Considerations

The researchers collected 5 ml. of peripheral blood from the patients for RNA and DNA extraction. This study was approved by the local Ethics Committee. Written informed consent was obtained from all patients or their parents who participated in the study.

- Respect for person

The researchers have explained all the details written in the information sheet to all individuals participating in the study before giving a signed consent.

- Risk and advantage

There is a minimal risk associated with venipuncture, for example, a painful sensation at the site of venipucture or a minor bleeding which can be stopped easily.

**11. Limitation**

Small sample size

**12. Expected Benefit and Application**

This study will identify the molecular defect underlying MPS I in Thai patients as well as to expand the mutation spectrum of the *IDUA* gene. In addition, testing for *IDUA* mutations will help physicians to correctly diagnose Thai patients with MPS I. This will lead to appropriate genetic counseling including advice, therapy and ultimately prevention.

**13. Research Methodology**

1. Sample collection

1.1 Thai patients with MPS I who were examined and diagnosed by the clinical geneticists at King Chulalongkorn Memorial Hospital.

1.2 Controls were unrelated healthy blood donors who were unaffected with MPS I and had no family history of MPS I, aged over 18 years.

2. Study process

2.1 Blood collection

2.2 WBC collection

2.3 Mutation analysis

- DNA extraction
- DNA amplification
- DNA sequencing

2.4 Restriction enzyme analysis

2.5 Enzyme assay

- Determining the protein concentrations by Bradford protein assay
- Determining alpha-L-iduronidase activity by fluorimetric assay

2.6 Functional analysis

- Construction of expression vectors
- *in vitro* site directed mutagenesis

3. Data collection and analysis