CHAPTER I

INTRODUCTION

Background and rationale

Genetic disorders are caused by abnormalities in an individual's genetic material (genome). The abnormality can range from a discrete mutation in a single base in the DNA of a single gene to a gross chromosome abnormality involving the addition or subtraction of an entire chromosome or set of chromosomes.

Mutations are changes to the nucleotide sequence of the genetic material of an organism. It can be caused by copying errors in the genetic material during cell division. Mutations can affect the function of genes leading to genetic disorders. Some examples include X-linked adrenoleukodystrophy, Glycogen storage disease, Hyper-IgE syndrome, Holt-Oram syndrome, systemic lupus erythematosus.

X-linked adrenoleukodystrophy (X-ALD) is a neurodegenerative disorder, with a minimum incidence of 1:21,000 males. X-ALD is cause by alterations in the *ABCD1* (ATP-binding cassette, sub-family D [ALD]) gene. Mutations in this gene cause a defect in peroxisomal β-oxidation leading to an accumulation of saturated very long chain fatty acids (VLCFA) in all tissues of patients, which results in demyelination. Patients with X-ALD can be diagnosed by an increase in long chain fatty acid levels in plasma and confirmed by mutation analysis in the *ABCD1* gene.

Glycogen storage disease type II (Pompe disease) is an autosomal recessive progressive muscular disorder caused by mutations in the acid α-glucosidase (*GAA*) gene. Patients with Pompe disease have impaired lysosomal degradation causing an accumulation of glycogen in multiple tissues, which results in severe muscle weakness. The diagnosis of Pompe disease can be confirmed by mutation analysis in the *GAA* gene.

Hyper-IgE syndrome (HIES), a rare primary immune deficiency, is caused by mutations in the *STAT3* (SIGNAL TRANSDUCER AND ACTIVATOR OF TRANSCRIPTION 3) gene. The clinical features of HIES patients include dermatitis, and

recurrent skin and lung infections. HIES can be diagnosed by a finding of increased IgE levels in plasma or mutation analysis of the *STAT3* gene

Holt-Oram syndrome (HOS) is an autosomal dominant disorder, which has an estimated frequency of 1 in 100,000 live births. The HOS patients were found to harbor mutations in the *TBX5* gene. The clinical features of HOS patients are a variety of upper limb skeleton malformations and congenital heart defects. HOS can be diagnosed by clinical features showing upper limb skeleton malformations with congenital heart defects or mutation analysis by PCR-sequencing of the *TBX5* gene.

Systemic lupus erythematosus (SLE) is an autoimmune disease inflicting damage to multiple organs. The disease prevalence is 0.05% in general population, with 80-90% of patients being female. The exact etiology of SLE has not been clear, but genetics, gender, and environment are involved in its pathogenesis. A previous report in 2007 showed that overexpression of DcR3 in mice resulted in a SLE–like syndrome. In addition, another report in 2008 revealed that SLE patients had elevated serum DcR3 levels when compared with DcR3 levels of normal controls. DcR3 might be a new diagnostic parameter and risk factor for SLE. The *DcR3* gene becomes an interesting new candidate for SLE.

Development in genetic testing has led to significant benefits in improving patient management including more accurate diagnosis, genetic counseling as well as prenatal diagnosis.

In this study, we demonstrated various approaches for mutation analysis in several genetic disorders. These methods were developed to help improve diagnosis of particular genetic disorders.

Research questions

1. Are Thai patients with clinically diagnosed ALD, Pompe disease, HIE, HOS, and SLE caused by mutations in the *ABCD1*, *GAA*, *STAT3*, *TBX5*, *SALL4*, *and DcR3* respectively?
2. Are these techniques practical to detect mutations in *ABCD1*, *GAA*, *STAT3*, *TBX5*, *SALL4*, *and DcR3*?

Objective

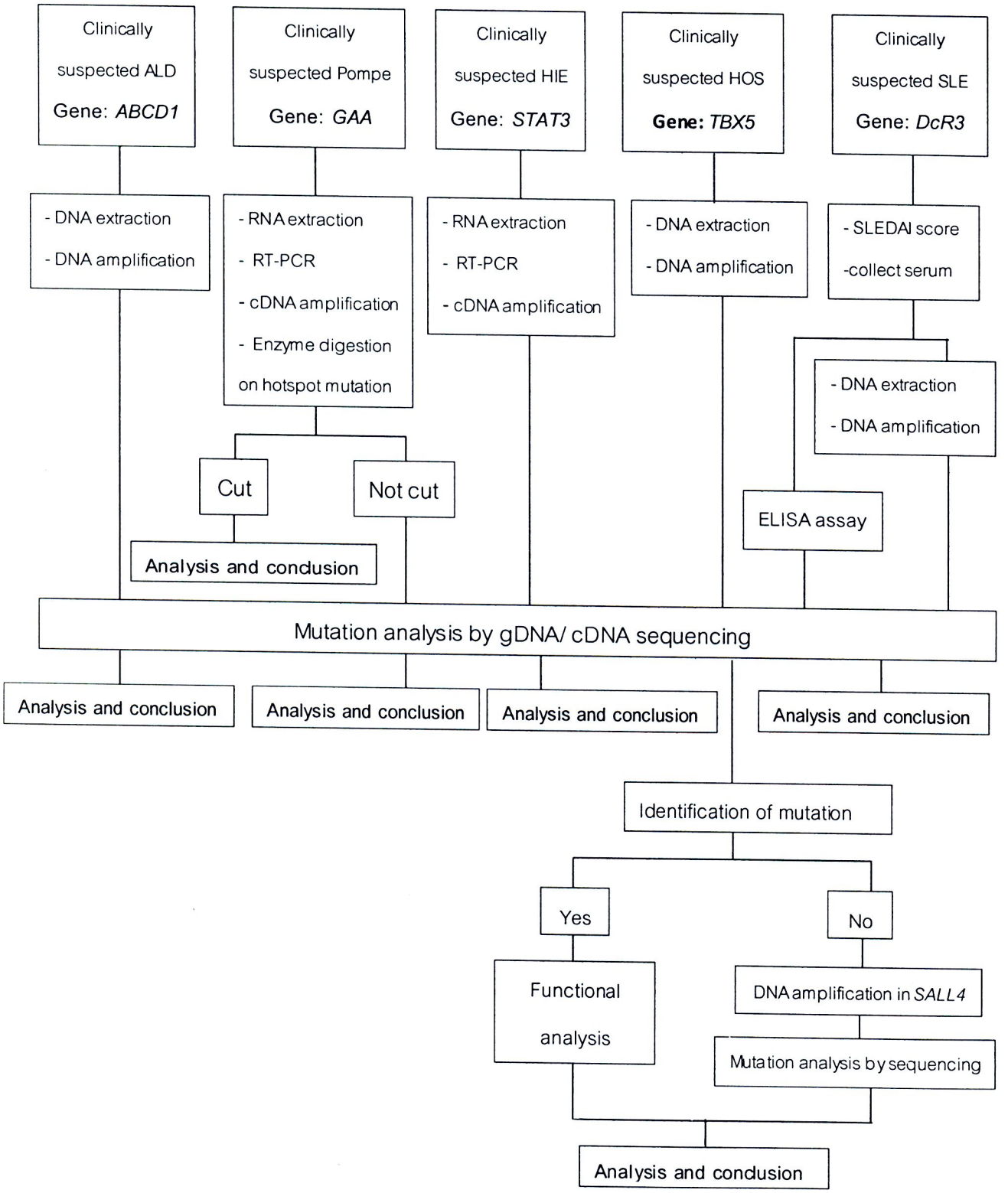
1. To develop techniques of mutation detection in the *ABCD1*, *GAA*, *STAT3*, *TBX5*, *SALL4*, *and DcR3* genes in patients with clinical features consistent with ALD, Pompe disease, HIE, HOS, and SLE, respectively.
2. To investigate efficiency of techniques for detection mutation in the *ABCD1*, *GAA*, *STAT3*, *TBX5*, *SALL4*, *and DcR3* genes.

Hypothesis

Thai patients with clinically-diagnosed ALD, Pompe disease, HIE, HOS, and SLE carry mutations in the *ABCD1*, *GAA*, *STAT3*, *TBX5*, *SALL4*, *and DcR3* genes, respectively.

Techniques of mutation detection have efficiency to detect mutations in Thai patients with these particular disorders.

Conceptual framework



Assumption

Cases are the patients with clinically-diagnosed ALD, Pompe, HIE, HOS, or SLE in which selection criteria were based on clinical presentation.

Controls are healthy volunteers who are unaffected with ALD, Pompe, HIE, HOS, SLE and have no family history of ALD, Pompe, HIE, HOS, SLE.

Key words

Mutation analysis, X-linked adrenoleukodystrophy, X-ALD, *ABCD1*, Glycogen Storage Disease Type II, Pompe disease, *GAA*, Hyper-IgE Syndrome, HIES, *STAT3*, Holt-Oram Syndrome, HOS, *TBX5*, *SALL4*, Systemic Lupus Erythematosus, SLE, *DcR3*

Operational Definition

Controls: Blood samples from the healthy volunteers who are unaffected with ALD, Pompe, HIE, HOS and SLE and have no family history of ALD, Pompe, HIE, HOS, and SLE.

Cases: Blood samples from the patients who are diagnosed with ALD, Pompe, HIE, HOS or SLE.

Sequencing is the process of determining the nucleotide order within DNA and RNA.

Enzyme-linked immunosorbent assay (ELISA) is a biochemical technique used mainly in immunology to detect the presence of an antibody or an antigen in a sample.

Research Design

Descriptive and *in vitro* studies

Ethical Consideration

This study has been approved by the local Ethics Committee. Written informed consent was obtained from all patients or their parents who participated in the study.

Limitation

Some diseases have small sample size. Some genes do not express in leukocytes.

Expected Benefit and Application

This study will help identify Thai patients with ALD, Pompe, HIE, HOS and SLE as well as to expand the mutation spectrum of the *ABCD1*, *GAA*, *STAT3*, *TBX5*, *SALL4* and *DcR3* genes. In addition, testing for mutations will help physicians to correctly diagnose Thai patients leading to appropriate genetic counseling, therapy and prenatal diagnosis.

Research Methodology

1. Sample collection

1.1 Cases wereThai patients with ALD, Pompe, HIE, HOS or SLE who were diagnosed by clinical geneticists at King Chulalongkorn Memorial Hospital. Initial diagnosis was based on the clinical presentations.

1.2 Controls were unrelated healthy blood donors who were unaffected with ALD, Pompe, HIE, HOS and SLE and had no family history of ALD, Pompe, HIE, HOS and SLE.

2. Study process

2.1 Blood collection

2.2 Mutation analysis

- RNA and DNA extraction
- RNA and DNA amplification
- PCR-RFLP on particular regions
- Nucleotide sequencing

2.3 Agarose gel electrophoresis

2.4 Enzyme-linked immunosorbent assay

3. Data collection and analysis