CHAPTER I

INTRODUCTION

Background and Rationale

Resistance to thyroid hormones (RTH) is an autosomal dominant inherited syndrome characterized by a variable degree of reduced tissue sensitivity to thyroid hormone (TH) resulting in elevated serum TH levels, normal or elevated serum thyroid stimulating hormone (TSH) levels, and goiter[1]. The clinical phenotype varies from clinically asymptomatic to thyrotoxicosis and hypothyroidsm. The variability in clinical manifestations may be due to the severity of the hormonal resistance, effectiveness of compensatory mechanisms, genetic factors, and effects of prior therapy[1, 2]. The most common clinical findings are goiter, learning disabilities with or without hyperactive behavior, developmental delay, and sinus tachycardia[3]. The clinical manifestation can be summarized in table 1.

Table 1 The clinical manifestation of RTH and its frequency [3]

Clinical manifestation	Frequency (%)
Nervous system	
Attention deficit hyperactivity disorder	40–60
Emotional disturbance	60
Hyperkinetic behavior	33–68
Learning disability	30
Mental retardation	4-16
Sensorineural hearing loss	10-22
Thyroid gland	
Goitre	66–95
Cardiovascular system	
Tachycardia	33–75
Musculoskeletal system	
Delayed bone age	29–47
Low body mass index (in children)	33
Short stature	18–25
Immune system	
Recurrent ear and throat infection	55

In 1967 Refetoff *et al.* reported the first case of RTH[1]. Linkage between RTH and the *thyroid hormone receptor (TR)-β* gene was shown in 1988[4]. It was located on chromosomes 3 encoding the receptor for thyroid hormone. More than 300 families with RTH have been found to harbor mutations in this gene. The majority of patients with RTH display an autosomal dominant inheritance pattern, with the exception in only one family showing an autosomal recessive inheritance[1, 5, 6]. The incidence of RTH is 1 in 40,000 to 50,000 live births[7, 8].

The diagnosis of RTH is based on clinical findings and measurement of free T_3 (FT_3), free T_4 (FT_4) and TSH levels. An increase in FT_3 and FT_4 with normal or elevated TSH identified in patients with RTH can overlap with findings in the TSH-secreting pituitary adenoma and interfering antibodies to thyroid hormones, which may lead to misdiagnosis[3]. Thus, genetic screening of the *TRβ* gene provides specific diagnosis of RTH. This will lead to appropriate genetic counseling including advice, therapy and ultimately prevention. In addition, functional characterization of the *TRβ* mutants gives more insight into the pathogenic mechanism leading to better therapeutic strategies.

Research Questions

1. Do Thai patients with clinically-diagnosed RTH harbor mutations in the *TRβ* gene?

2. Do mutations found in the *TRβ* gene affect the protein function and exhibit dominant negative effect?

Objectives

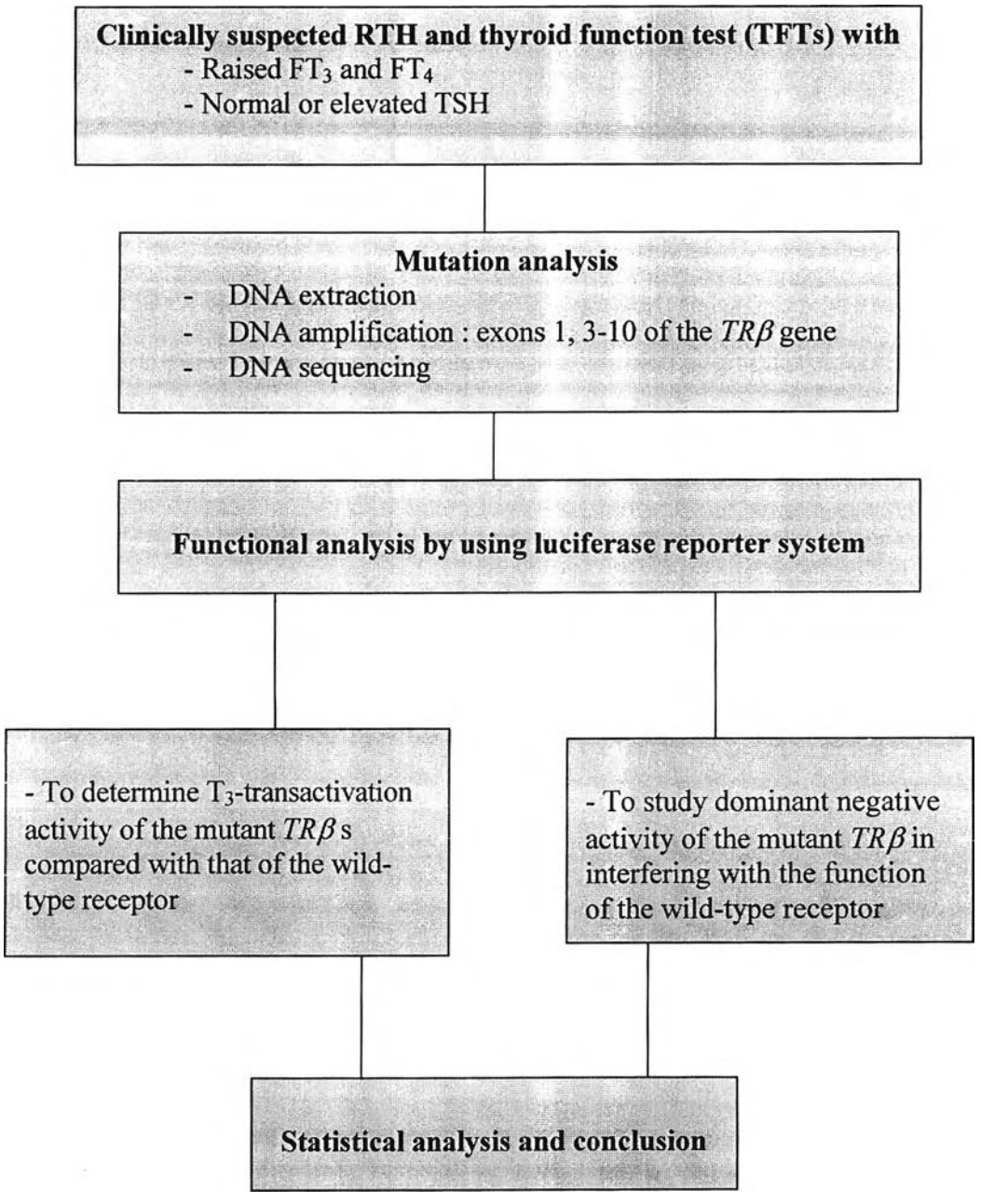
1. To identify the *TRβ* gene mutations in Thai patients with clinically diagnosed RTH.

2. To investigate an effect of the uncharacterized mutant TRβs on their transcriptional activity and on the activity of the wild type TRβ.

Hypothesis

Thai patients with clinically-diagnosed RTH carry disease-causing mutations in the *TRβ* gene. The uncharacterized mutant TRβs have reduced transcriptional activity and/or interfere with the function of the wild type TRβ, major mechanisms underlying the RTH pathogenesis.

Conceptual Framework



Assumption

Cases are the patients with clinically-diagnosed RTH.

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

Key words

RTH, resistance to thyroid hormone; TR, thyroid hormone receptor; TRE, thyroid response element; FT_4, free thyroxine; FT_3, free triiodothyronine; TSH, thyroid stimulating hormone

Operational Definition

Control: DNA from the healthy volunteers who are unaffected with RTH and have no family history of RTH.

Case: DNA from the patients who were diagnosed with RTH.

Sequencing: The process of determining the nucleotide order within DNA.

Transfection: The process of delivering DNA into mammalian host cells.

Luciferase reporter assay: The luciferase reporter containing a binding site for the thyroid hormone receptor (transcription factor) located upstream of the luciferase gene to determine the expression regulation of the target gene (luciferase gene) by the thyroid hormone receptor. Thus, the results are expressed as luciferase activity.

T_3-dependent transactivation: An effect of thyroid hormone receptor in activation of target gene expression when induced by T_3.

Dominant negative effect (DNE): The mechanism of the mutant TR in interfering with the function of the wild-type TR.

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

The sample size was small.

Besides thyroid function test, the diagnosis also requires T_3 suppression test as well as TRH stimulation test. The T_3 and TRH which are used in these tests are unavailable in Thailand.

Expected Benefit and Application

The clinical features of RTH can overlap with those of thyrotoxic, euthyroid and hypothyroid subjects from other causes and lead to misdiagnosis. Thus, testing for *TRβ* mutations will help physicians to correctly diagnose Thai patients with RTH. Genetic counseling including advice, therapy and ultimate prevention can be appropriately provided. In addition, functional characterization of the *TRβ* mutants gives more insight into the pathogenic mechanism leading to better therapeutic strategies.

Research Methodology

1. Sample collection

1.1 Cases including six affected subjects from six unrelated families were enrolled at King Chulalongkorn Memorial Hospital. Initial diagnosis of RTH was based on the clinical findings and thyroid function test.

1.2 Controls were unrelated healthy blood donors who were unaffected with RTH and had no family history of RTH.

2. Study process

2.1 Blood collection

2.2 Mutation analysis

- DNA extraction
- DNA amplification
- DNA sequencing

2.3 Functional analysis by luciferase reporter system

- Construction of expression vectors
- Construction of a luciferase reporter vector
- *in vitro* site directed mutagenesis

3. Data collection and analysis