CHAPTER I

INTRODUCTION

Background and rationale

X-linked agammaglobulinemia (XLA) is the first immunodeficiency disorder to be described in humans [1].XLA is a congenital disease affecting mostly in males. It is characterized by a lack of ability to produce immunoglobulin (gamma globulin). Affected individuals with the disorder are prone to develop recurrent bacterial infections, particularly in the respiratory tract due to absent or reduced levels of antibodies [1].

Agammaglobulinemia is also known as Bruton's disease after the American physician Ogden Bruton, who described a boy with immunoglobulin deficiency and recurrent infections in a 1952 case study [1].

Symptoms are usually evident within the first year of life, although there are instances of late onset of the disease. XLA originates from a block in the process of B-cell differentiation resulting in severely decreased numbers in B lymphocytes and an almost complete lack of plasma cells, as well as negligible, or very low, immunoglobulin levels of all isotypes [2]. XLA patients have increased susceptibility to mainly bacterial infections due to virtually absent humoral immune responses. The most common symptoms include sinusitis, bronchitis, otitis, and pneumonia. Patients are treated with both antibiotics and immunoglobulin replacement therapy. The frequency of XLA has been estimated to be 1:10,000 to 200,000 live births. The disease is considered to have full penetrance. Female carriers are healthy but display nonrandom X-chromosome inactivation in their B cells.

Genetic studies in humans led to the identification of the defective gene, *Bruton's tyrosine kinase* (*BTK*). It encodes a cytoplasmic tyrosine kinase that plays an essential role in regulating B-cell development. The 37.5-kb *BTK* gene, which is located at Xq21.3-Xq22 (Fig. 1), contains 19 exons and codes for a protein of 77 kDa. *BTK* is expressed in all hematopoietic lineages except for T lymphocytes and plasma cells [2].

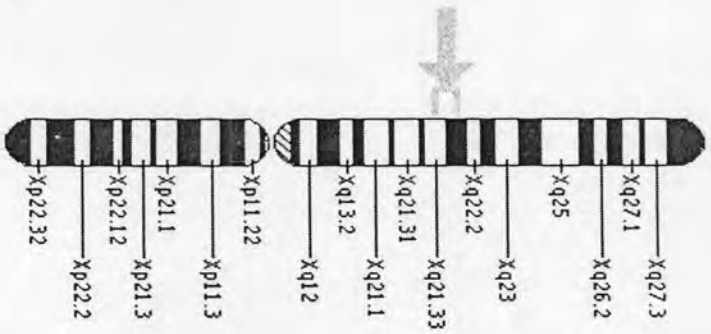


Figure 1: The *BTK* is located at the long (q) arm of the X chromosome between positions 21.33 and 22. More precisely, the *BTK* gene is located from base pair 100,491,097 to base pair 100,527,837 on the X chromosome. (ghr.nlm.nih.gov/gene=btk)

In the absence of protective immunoglobulins, affected individuals develop repeated infections. People with this disorder are particularly susceptible to bacterial infections caused by *Haemophilus influenzae,* pneumococcus *(Streptococcus pneumoniae*), and staphylococci as well as repeated viral infections. The upper respiratory tract, lungs, and skin are common sites of infection.

The goal of treatment is to reduce the number and severity of infections as well as provide genetic counseling to affected families. Intravenous infusions of immunoglobulins (gamma globulin, IVIG) help boost the immune system by providing the body with the antibodies that are decreased or missing. Routine treatment with IVIG is central to the treatment of this disorder.

Research Questions

1. Are Thai patients with clinically diagnosed XLA caused by mutations in the *BTK* gene?
2. Can the Antisense Morpholino Oligonucleotides (AMOs) correct the abnormal splicing detected in peripheral blood mononuclear cells of the XLA patient with a splice site mutation in the *BTK* gene?

Objectives

1. To identify the *BTK* gene mutations in Thai patients with clinically diagnosed XLA.

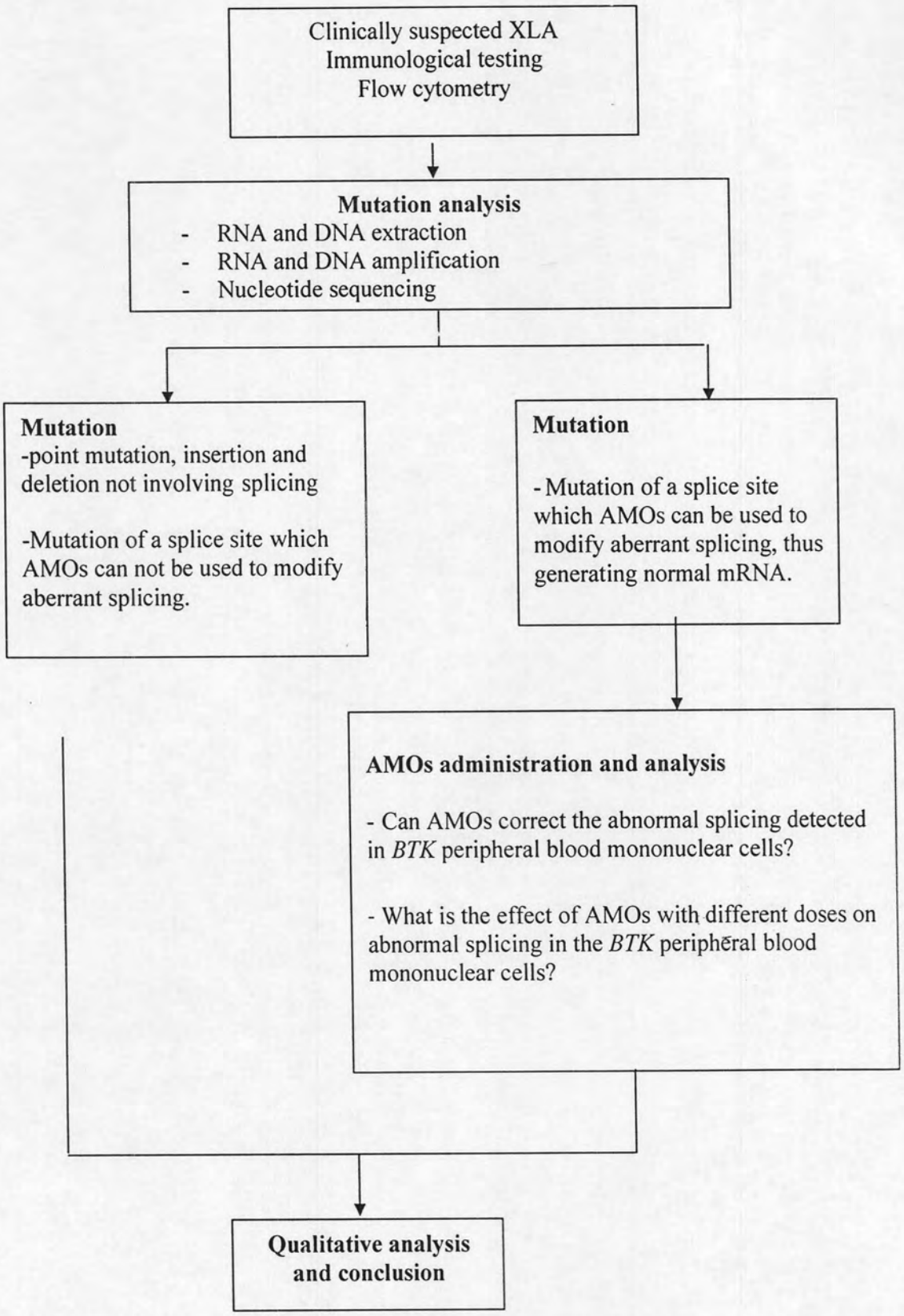
2. To investigate an effect of the AMOs on the splicing of the *BTK* gene in peripheral blood mononuclear cells of the XLA patient with a splice site mutation.

Hypothesis

Thai patients with clinically-diagnosed XLA carry disease-causing mutations in the *BTK* gene.

AMOs can target at the desired mutation which causes aberrant splicing, resulting in normal mRNA production, achieving therapeutic correction of the defect.

Conceptual framework



Assumption

Cases are the patients with clinically-diagnosed XLA and the immunological studies consistent with agammaglobulinemia

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

Key words

XLA, X-linked agammaglobulinemia, Bruton's agammaglobulinemia; *BTK*, Bruton agammaglobulinemia Tyrosine Kinase; AMOs, Antisense Morpholino Oligonucleotides; PBMC, Peripheral blood mononuclear cells

Operational Definition

Controls: Blood samples from the healthy volunteers who are unaffected with XLA and have no family history of XLA.

Cases: Blood samples from the patients who are diagnosed with XLA.

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

Cell isolation: Purification of lymphocytes from peripheral blood.

AMOs administration: The 25-mer AMOs is designed to target a splicing mutation in the pre-mRNA in accordance with the manufacturer's criteria. Endo-Porter is used as the delivery mechanism.

In Vitro splicing analysis: RT-PCR, with successful splice-modification appearing as band shifts of RT-PCR products on electrophoretic gels and Real-Time TaqMan PCR

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 25-mer AMOs and Endo-Porter which are used in these tests are unavailable in Thailand.

Expected Benefit and Application

Identification of mutations in the *BTK* gene causing XLA provides more accurate diagnosis and appropriate genetic counseling. In addition, it paves the way for a potential therapeutic strategy aimed at correcting the genetic errors at the RNA level.

Current available treatments for XLA include intravenous immunoglobulins (IVIG), stem cell transplantation and gene therapy. However, each treatment approach has its drawbacks. In this study, we highlight mutation-targeted therapies with chemicals called antisense morpholino oligonucleotides (AMOs) that mitigate mutational pathology at nuclear level. This finding provides a new therapeutic strategy in XLA and other single gene disorders, potentially applicable to a large number of cases with splicing mutations resulting in pseudoexon inclusion or partial exon deletion.

Research Methodology

1. Sample collection

1.1 Cases including five affected subjects from five unrelated families were enrolled at King Chulalongkorn Memorial Hospital and children Hospital. Initial diagnosis of XLA was based on the clinical findings and immunological testing.

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

2. Study process

2.1 Blood collection

2.2 Mutation analysis

- RNA and DNA extraction
- RNA and DNA amplification
- Nucleotide sequencing

2.3 Cell culture and AMOs administration

- Purification of lymphocytes from peripheral blood
- Restoration of corrected splicing in *BTK*-mutated peripheral blood mononuclear cells (PBMCs)
- Evaluation of *in vitro* splicing

3. Data collection and analysis