CHAPTER I

INTRODUCTION

Background and rationale

Mesomelic dysplasia Kantaputra type (MDK) (OMIM *156232) is a new type autosomal dominant skeletal dysplasia. It was first described in 3 generation of a large Thai family and characterized by dwarfism, shortening of the forearms/lower-legs, carpal/tarsal synostosis, and dorsolateral foot deviation (*1*). To date, only two families of MDK were reported, first in Thailand and second in Dutch family (*2*).

Another type of skeletal dysplasia was reported (*3*). The disease in this Italian family was characterized by multiple skeletal abnormalities involving shortening of the forearm, bowing of the radius, Madelung deformity, cubitus valgus with limited movement, fusion between the C1 and C2 vertebrate, and the cleft L5 and S1. The cytogenetical result showed that all affected individuals in two generations had a balanced reciprocal translocation [t(2;8)(q31;p21)]. Thus, the putative disease gene may located to either of the breakpoints.

The clinical and radiological similarity of the diseases in the Thai and Italian families suggested that they were allelic, although the condition in the latter family was much less severe. A linkage analysis was performed in the Thai family using 50 CA-repeat markers mapped to the nearby regions (2q22-q34 and 8p24-p21). Haplotype analysis and LOD scores showed that MDK was most likely mapped to 2q24-q32.

Since there is, so far, no additional mapping to narrow down this region, thus candidate gene approach is the selected. *HOX* genes in a *HOXD* cluster and some nearby genes in 2q32 region were hotspots for candidate genes selection because several reports showed that genes in this cluster involved embryonic development of structure along the trunk and limb axes. For instance, mutation

HOXD13 causes synpolydactyly type II (*4*) and *HOXD10* mutation was associated with Charcot-Marie-Tooth disease (*5*). Moreover, synpolydactyly was found in patients with 117-kb microdeletion removing *HOXD9-HOXD13* and *EVX-2* genes. However, to identify the cause of MDK, more evidences and implications for this mysterious disease are needed.

Hence, a searching of clues in animal models is an answer. There are also evidences of *Hoxd* mutation causing limb defects in mice. In *Hoxa11/Hoxd11* double homozygous mutants mice, the shortening or absence of radius and ulna were found. This indicated that *Hoxd 11* gene also involve in the limb development (*6*).

Another interesting evidence involving limb patterning is the regulation of gene expression in the *Hoxd* cluster and some nearby genes by cis-acting elements. In ulnaless mice, caused by X-ray induction, severe reduction of the proximal limb and less severe reductions of the distal limb were observed, the mutation mapped on chromosome 2, tightly linked to the *Hoxd* cluster.

Previous study suggested that mutation in Ulnaless mice was a regulatory mutation that interfered with a control mechanism shared by multiple genes. A balanced paracentric inversion in chromosome 2 of ulnaless mice was found that one breakpoint was telomeric to *Hoxd* cluster and another breakpoint was within a *Lnp* (Lunapark) gene, centromeric to *Hoxd* cluster and the downregulation of *Hoxd13* and *Evx2* expression compared to normal mice were found. From studies of the presence of the regulatory region controlling the *Hoxd* cluster and neighbor genes by sequence comparison among human, mouse and puffer fish and observation of the activity of the hypothesized regulatory region by transposon-based, locus-targeted enhancer trap procedure, they found evolutionary conservation and can localized the enhancer region which called "Global control region" (GCR). It could be concluded that inversion in ulnaless mice caused change in genomic topography then many genes nearby the GCR are no longer under the control and might lead to the ulnaless phenotype.

From these evidences in both human and animal model, it could be referred that there are several possibilities of the mechanism causing MDK. To find the cause of MDK, candidate gene approach and relative mRNA quantification were selected. The importance of this study is to find the clues or evidences that can lead to identification of the disease-causing mechanism of MDK. Once, the mechanism is identified, hopefully, many of those who suffered or have a risk from MDK could be relieved in term of prevention and ,if possible, of treatment.

Research questions

1. Is the mutation in *HOXD11* the cause of MDK?

2. Is there any Global Control Region (GCR) in human?

Objectives

1. To find the mechanism(s) which cause MDK.

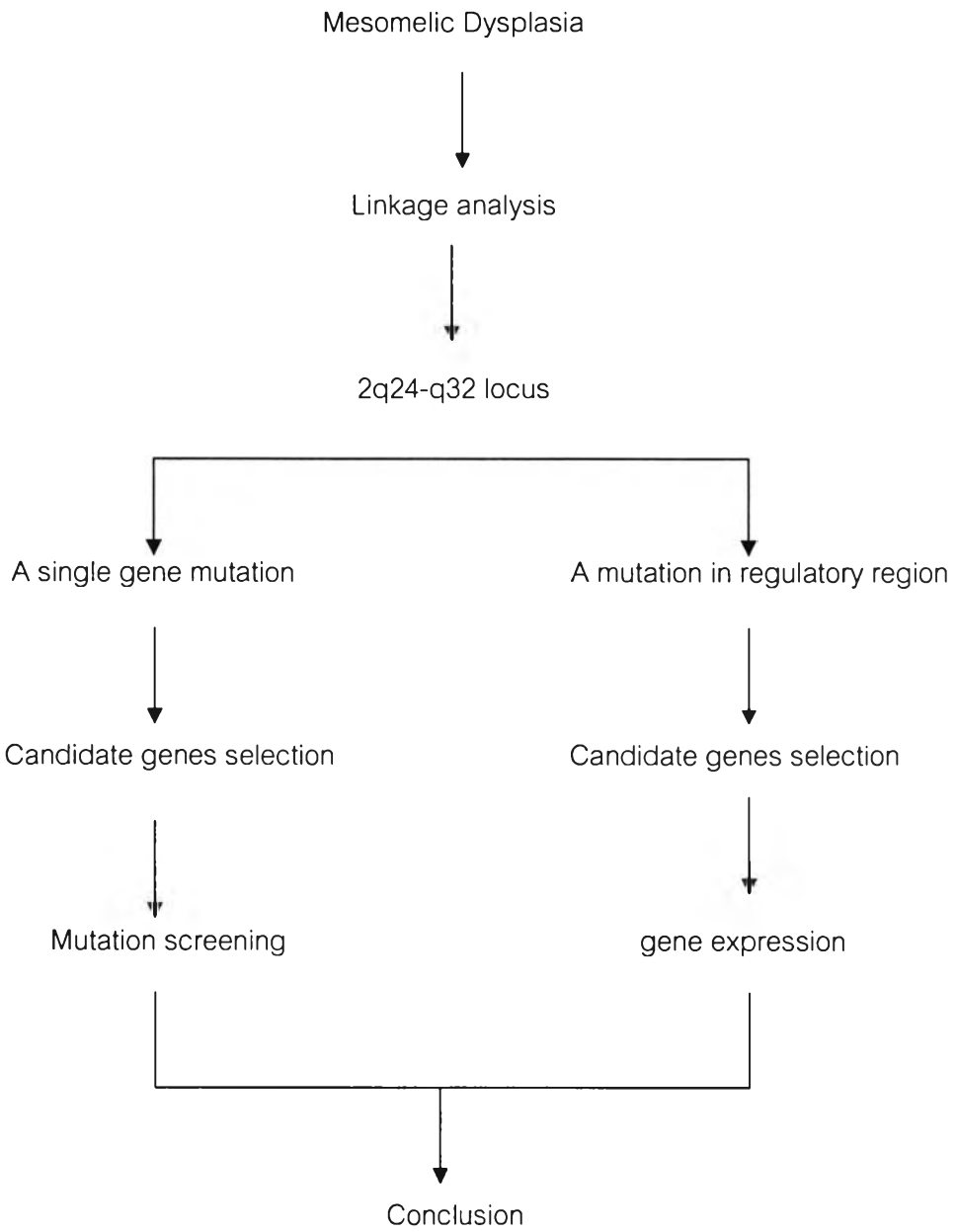
2. To provide preliminary data to prove the existence of human GCR.

Hypothesis

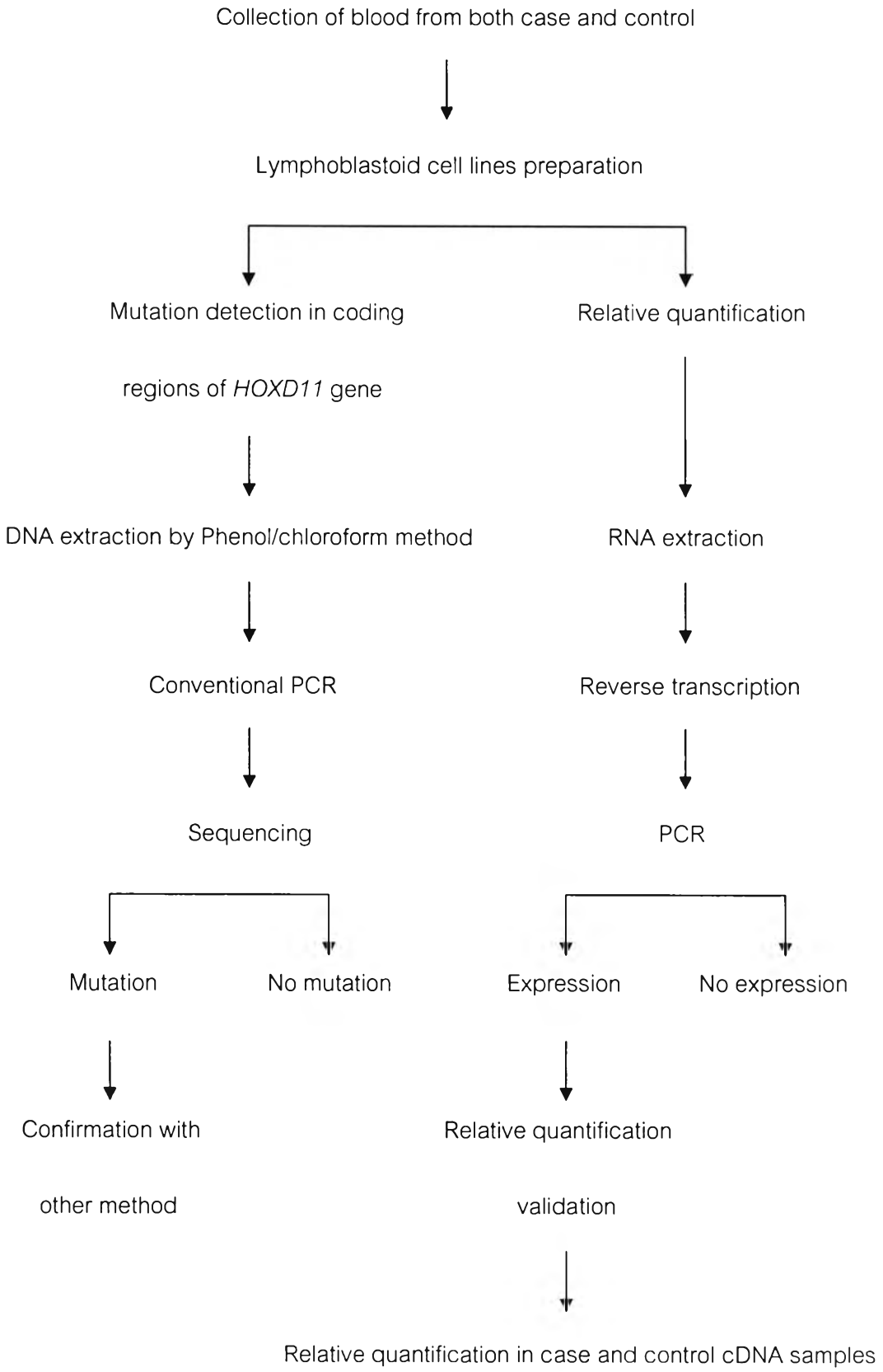
1. The mutation in *HOXD11* gene is the cause of MDK.

2. The gene expression of *LNP*, *EVX2* and *HOXD11* are controlled by GCR.

Conceptual framework



Methodologies



Limitation

Only one case of MDK is available for relative quantification in lymphoblastoid cell lines, whereas relative quantification in other tissues can not be done.

Expected Benefit

The result from this study may provide a clue for an extensive study in MDK disease.

Research Methodology

1. Collection of blood form MDK patients and controls.
2. Preparation of lymphoblastoid cell lines.
3. DNA extraction
4. DNA amplification
5. Sequencing
6. RNA extraction
7. Two-step RT-PCR
8. Agarose gel electrophoresis
9. Relative realtime RT-PCR
10. Data analysis