CHAPTER V

DISCUSSION

Based on evidences from both human and animal models, two way hypotheses suspecting a single gene mutation or a mutation in a regulatory region were generated to determine the mechanism causing Mesomelic Dysplasia, Kantaputra type. A linkage result showed that a locus of MDK was 2q24-q32 which spanned about 22.7 cM consisting about 230 genes. The number of gene in within the most sharing haplotype markers; D2S2284 and D2S2314 is about 43 genes from *MYO3B* gene to *LNP* gene.

Using candidate gene approach, some genes in *HOXD* cluster and nearby region were selected consisting *HOXD10*, *HOXD11*, *EVX-2* and *LNP*. The *HOXD* cluster contains 9 genes; *HOXD1*, *HOXD3*, *HOXD4*, *HOXD8*, *HOXD9*, *HOXD10*, *HOXD11*, *HOXD12* and *HOXD13*. The *HOXD11* was selected as a candidate gene instead of choosing whole genes within *HOXD* cluster based on the evidence of involvement of *HOXD11* gene in the ulna and radius development.

However, none of mutation was found in MDK patient. Mutation screening of *LNP*, *EVX-2* and *HOXD10* also showed none of mutation. However, TA repeats in MDK patients and normal controls were found and seemed to be polymorphic. The data from NCBI confirmed that this TA repeats have been used as a part of a STS, so it was not implied anything related to MDK pathogenesis. Although none of mutation was found in any candidate genes, it is too fast to conclude that these genes are not involve in MDK pathogenesis because of mutation screening in coding regions, splice sites and untranslated regions are not cover all of possibilities of mutation. Mutation in an intron or a promoter can also result in pathogenesis.

Mutation screening in cDNA could shed the light on this limitation. Not only mutation detection in coding region or untranslated regions can be done, but also introns. However, spatio-temporal expression information is required before working with cDNA.

It is notably that only double homozygous mutation (*Hoxa11* -/-, *Hoxd11* -/-) caused shortening of radius and ulna, whereas heterozygous mutation of *Hoxa11* did not. Since MDK is an autosomal dominant disease, it informs that mutation in a single allele of disease-causing gene can lead to MDK due to haploinsufficiency. It is unlikely that mutation in coding region or untranslated region of *HOXD11* is the cause of MDK.

It is possible that mutation of other gene within 2q24-q32 region could also involve in MDK pathogenesis. Nevertheless, narrow down of MDK locus could be a good choice before further analyses.

Based on evidences of existence of GCR found in mouse models, it informed that mutation in a regulatory region could lead to a phenotype similar to MDK. The studies in *Ulnaless* mice showed that balanced inversion caused changes in expression pattern of *Evx-2* and *Hoxd13* which implied an existence of global control region.

Although there was an evidence of the presence of GCR, the balanced paracentric inversion can lead to another clue other than GCR. The breakpoints found in *Ulnaless* mice could lead to other hypothesis. Since the centromeric breakpoint was within *LNP* gene, so at least one mutated allele of *LNP* gene could be considered as a cause of *Ulnaless* phenotype. While, the telomeric breakpoint did not break any gene located downstream of *Mtx2* gene.

Studies of mesomelic dysplasia in Italian patients also indicated the clue of mutation in a regulatory region because of reciprocal translocation breakpoint in chromosome 2, located 56 kb away from *HOXD1* and upstream of *MTX2* gene, did not break any gene. This finding pointed to another regulatory region located at opposite site of GCR.

This could lead to another regulatory called "Early Limb control region" (ELCR), located at telomeric side of *HOXD* cluster. The telomeric *Ulnaless* breakpoint and the breakpoint from the Italian mesomelic dysplasia patient could possibly be the evidence of ELCR.

However, it is preferable that the mutation in the regulatory region is the cause of *Ulnaless* phenotype, based on the evidences of serial deletion and duplication of genes in the *HOXD* cluster. However, further studies must be done to elucidate the real mechanism.

An overexpression of *LNP* in a female patient with MDK observed in this study implied that *LNP* gene might involve in MDK pathogenesis. This could due to mutation in the *LNP* gene in another region of gene other than the coding regions or due to mutation in the regulatory region. It is possible that human GCR may exist and mutation in GCR or ELCR could lead to overexpression of *LNP* gene. Nevertheless, further expression study of other cell type in case, related control, and unrelated control is necessary. However, this finding raises many questions;

1. Why is *LNP* gene overexpressed in lymphoblastoid cell line at this time?

2. Is there any DNA rearrangement which effects the expression of *LNP*?

3. Are there any changes in gene expression of the nearby genes?

4. Is *LNP* gene upregulated in another cell type?