CHAPTER V

DISCUSSION

The concept of multifactorial inheritance has been proposed to account for the common congenital malformations, such as oral cleft and FEEM. This concept seems to be a type of non-Mendelian familial aggregation. These disorders are thought to result from the interaction of genetic and environmental factors. According to the liability/threshold model for multifactorial inheritance, the population's genetic and environmental susceptibility, which is known as liability, is normally distributed. Individuals are affected if their liability exceeds a threshold superimposed on the liability curve (figure 10). Regarding to genetic factors, loci that contribute to susceptibility for multifactorial disorders can be identified by linkage analysis and search for disease associations.

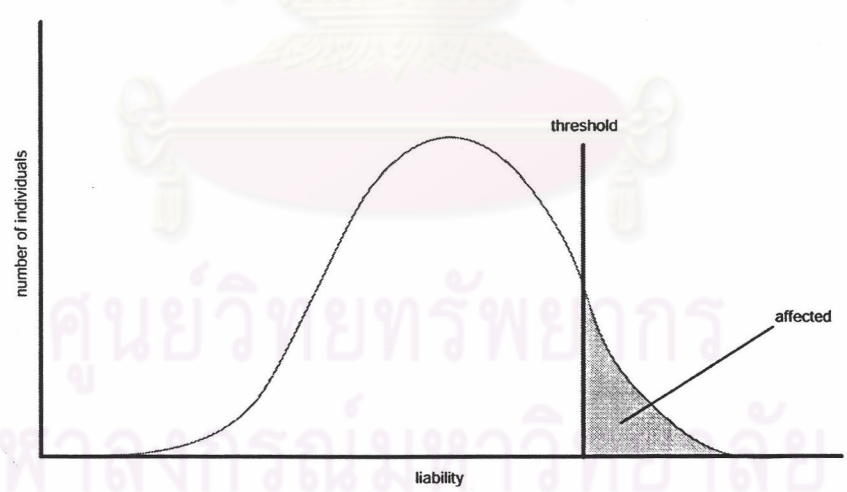

Figure 10 Threshold theory

Although linkage method has been remarkably successful in identifying linkage regions whereas it has not been successful in identifying genes the are involved in the complex forms of disease. Because multifactorial disorders have a genetically heterogeneous, in many regions have been mapped by using linkage analysis, so other approaches such as association study base on population or family were applied to

identify genes that are involved in complex diseases. In our study aim to identify genes that associated with oral cleft and FEEM by using candidate-gene approach base on population scale and transmission disequilibrium test (TDT), family base study. Association study by using candidate-gene approach has more powerful than linkage analysis. Because it can be defined as the study of the genetic influences on a complex trait by generating hypotheses about, and identifying candidate genes that might have a role in, the etiology of the disease and their identifying variants that might either cause a change in the protein or its expression with functional change.[94] Additionally to reduce the effect of population stratification on our association study, TDT have been developed that use controts selected from the families of affected probands. TDT focuses on only heterozygous parental genotypes, thereby providing a joint test of linkage and association that eliminates the effects of stratification when applied to single probands and parents.[95]

In this study, genes in transforming growth factor β signal pathway are selected, consist of *transforming growth factor β-3* (*TGFβ-3*), *Interferon regulatory factor 6* (*IFR6*), and *SKI*. Additionally, interesting gene in folate pathway, *trifunctional enzyme methylenetetrahydrofolate dehydrogenes/ methylenetetrahydrofolate cyclohydrolase/formyltetrahydrofolate synthese* (*MTHFD1*) is included for a candidate gene. The effect of all variants dose not proposes so we hypothesize that our candidate gene variants might have a role in functional modulation. The the two variants in *transforming growth factor β-3* (*TGFβ-3*), the changing of threonine to methionine by *TGFβ-3* 179C→T (Thr60Met) and the substitution of arginine for lysine by *TGFβ-3* 383A→G (Lys128Arg), are the data from online Single Nucleotide Polymorphism (dbSNP) Database (http://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?rs=4252315), and the rare variants in Iowa population reported by Lidal et al, respectively.[45] As for transcription factor, *interferon regulatory factor 6* (*IFR6*), *IRF6* 820G→A (Val274Ile) is selected. This variant is the substitution of an isoleucine for an evolutionarily conversed valine residue at codon 274 in a protein binding domain (SMIR domain).[26] For *SKI* proto-oncogene, which act as a repressor for transforming growth factor β signal,[111] two variants are selected. First is *SKI* 185C→G (Ala62Gly) [AY331180] that change an alanine to glycine, this variant locate on exon1 which crucial role for SKI function.[117] The

second one, located on exon3, is *SKI* 1163C→T (Ala388Val) [AH013034], which an alnnine is substituted by valine. These two variants are reported in submitted nucleotide sequences on NCBI homepage. The report about *MTHFD1* 1958G→A (Arg653Gln) that substitution which causes the replacement of the arginine residue at position 653 by a glutamine in the deduced protein, this position is located on C-terminal of MTHFD1 which corresponding to synthethase activity residual.[113]

In case of *TGFβ-3*, the previous data showed interesting evidences of an association between 2 copies allele of CA variant and oral cleft, whereas this marker may be located on 3' untranslated region of unknown gene that near the *TGFβ-3* locus. Thus, mutation or causative SNPs that contribute liability of oral cleft may be located on *TGFβ-3* locus or other. The non-synonymous SNPs within TGFβ-3 are determined, from the online data, *TGFβ-3* 179C→T shows average allele frequency 0.98 for C allele and 0.02 for T allele while homozygotes are detected in individual. Reported from Lidral et al suggested *TGFβ-3* 383A→G variant may be a rare, functionally neutral, but this amino is conserved in mammals.[118] Interestingly, patient who was submucous cleft palate, heterozygous that transmitted from mother was identified. While mother and her relatives had scarring of the lip, which apparently was due to spontaneous in utero repair of cleft lip. However, it had not been identified in almost 350 controls.[22] Whereas no polymorphics are observed at *TGFβ-3* 179C→T and *TGFβ-3* 383A→G in our approximately 100 subjects. These results suggest that the polymorphism is not frequent in Thai population. As for *SKI* 1163C→T, has no additional detail for describable it's property, while no polymorphic was observed in our samples. It is again suggests that this polymorphism is not exist in Thai population.

Regarding to polymorphic SNPs in our population, allele frequencies are described. For *IRF6* 820G→A, high frequency (0.38) was found in A allele while in European-descended and Asian population were 0.03 and 0.22, respectively.[26] Genotyping of *SKI* 185C→G, very low frequency were observed in G allele (0.03). As for *MTHFD1* 1958G→A, 0.18 was determined with A allele whereas a high frequency was observed in Dutch (0.44)[113] and Dublin (0.45).[44] Overall genotypic distribution for these three SNPs were consistent with Hardy Weinberg equilibrium (HWE). As described, data

of allele frequencies observed across population suggested that allele frequencies may likely depend upon population ethnic.

Analysis of *IRF6* 820G→A, genotype and allele frequencies differ significantly between patient with CL/P group and control group (Table 9) and genotype frequencies showed a significantly excess of the GA genotype in CL/Ps, compared with control individuals (Table 18). There were GA group in co-dominance mode, GA vs. GG, (OR = 0.57 [0.37 <OR< 0.86]), dominance mode, GA/AA vs. GG, (0.60 [0.40 <OR< 0.88]) and heterozygous genotype that compared with the other, GA vs. GG/AA, (0.62 [0.42 <OR< 0.91]). This results show that GA is a protective status in normal individual. Comparable with previous report by Zucchero et al, transmission disequilibrium (TDT) analysis revealed high significant overtransmission of G allele from parents to CL/P patients ($p<10^{-5}$).[119] By change, our informative 34 trios of CL/P, no significant was found, it may be effect of calculated by only few particular of CL/P samples (34 samples from totally 192 sample). From these results, we hypothesize that an individual who is GG which is normal function of IRF6 but it susceptible for CL/P whereas an individual who has GA that still haplosufficient for normal function with single G allele and generated advantage status for protect CL/P with A allele. Question that, why AA not more protective than GA, it possible way that AA may contribute to other disease.

Alternatively, observation of *SKI* 185C→G, only two homozygous GG were found in two patients with cleft lip with cleft palate, we hypothesize that GG for *SKI* 185C→G which located on exon1 that is critical region for SKI function[117] may loss their function and contribute to clefting.

Moreover, association between *MTHFD1* 1958G→A and both patients with CPO and their maternal A allele were determined. Referring to table 14, genotype and allele frequencies of patients (n=43) showed significantly ($p<0.05$) difference from controls while mother group (n=36) was almost significance ($p=0.07$ for genotype frequency and $p=0.06$ for allele frequency). Furthermore, we observed that A allele in patient group exhibited OR = 4.42 (1.05 <OR< 16.52) for co-dominance mode (GA vs. GG). In addition to mother group, A allele effected to OR = 2.25 [1.05 <OR< 4.86] for AA group of co-dominance mode, OR = 2.17 [1.02 <OR< 4.62] for GA/AA in dominance mode and also OR = 2.23 [1.04 <OR< 4.75] for GA vs. GG/AA (Table 23). Under the

assumption that children who was AA increasing risk for CPO and A allele in mother also contribute to increasing risk for CPO in their children. For describe effect of the MTHFD1, the enzyme may play a key role in cell division, it is an important provider of carbon-1 units for *de novo* purine and pyrimidine synthesis. The 1958G→A variant may be less efficient purine and/or pyrimidine synthesis, which is critical during early development.

Despite, there is no evidence of association between our SNPs and risk of FEEM, we can not entirely conclude that no interaction of these variants development of FEEM among the Thai population. Mutation or causative variants still possible to locate on our candidate genes and we still need to clarify for these candidate genes. Especially *SKI*, that present evidence of knockout mice that exhibited failed closure of the cranial neural tube during neurulation, such as vasicularized brain mass and frontonasal clefting[30] and evidence of difference genetic background effected to variable of midline defect such as NTD and midline facial clefting.

In conclusion, we found an evidence that *IRF6* 820G→A in the patients may be a protective factor for CL/P, while AA at *MTHFD1* 1958G→A in children and heterozygous GA in mother may be a risk factor for CPO. While on association for FEEM and the polymorphism in our candidate genes.