CHAPTER I

INTRODUCTION

Background and rationale

Oral cleft and frontoethmoidal encephalomeningocele (FEEM) are among the most severe congenital craniofacial malformations. The patients require surgical, nutritional, dental, speech, medical and behavioral interventions and impose a substantial economic burden.[1] Oral cleft consists of two major group, cleft lip with or without cleft palate (CL/P OMIM 119530) and cleft palate only (CPO OMIM 119540). Cleft of lip results from the defect of the facial bone in which the failure of fusion of the medial nasal, maxillary, and frontonasal process, while cleft of palate is caused by the palatal shelves failing to close. The variability incidence of cleft lip and/or palate is related to geographic origin, 1/500 or higher for Asian and Amerindian, 1/700 -1/1,000 in Caucasians, and 1/2,500 in African-derived[2-4], while Thailand is 1/600.[5] According to their etiology, several studies suggested that environmental and genetics factors have a joint role in the causation[6], and the multifactorial model was advanced to explain the causes of CL/P and CPO.[7-8]

FEEM is an endemic neural tube defect (NTD) affecting children in Southern and Southeast Asia and which is rarely found in Westen Europe, Japan, Australia, and North America.[9-10]In Thailand, the incidence of FEEM is relatively high with approximately 1/6,000.[11] In contrast to NTDs, in most parts of the world that failure of neuropore closure are usually located in the lumbosacral and occipital regions, whereas NTDs in Southeast Asia including Thailand are usaully situated at the root of the nose resulting in FEEM.[12] As for the ratio of the lesions are founded in the front to that of back of the head is 9.5:1 in Thailand, whereas Western Europe, North America, Australia, and Japan, the ratio varied from 1:5 to 1:28.[13] The defect of skull base at the junction of frontal and ethmoidal bones arisen in FEEM patients lead to herniation of mininges or brain through the hole[13] and may result in the neurological and ocular problem in addition to the presence of facial dysmorphology.[14-16] Although the etiologies of FEEM

and other NTDs have not yet been clarified, many theories have been proposed and a multifactorial model seem to be most probable.[17]

Because etiologies of oral cleft and NTD are determined as multifactorial model resulting from the interaction of environmental factors and multiple genes, some of which might have a major disease effect but many of which have a relatively minor effect. According to previous reports, by using mouse models for functional study and genetics models for association and linkage studies, revealed heterogeneity for oral cleft.[4] As for FEEM, have no investigated interaction of genes and FEEM development, so the data sets of craniofacial development related genes tend to favor of the major gene contributing for FEEM.[18] For the reason that oral and FEEM cleft have similarity for pathogenesis of causing craniofacial bone defect thus they may have overlaying genes that controlling developmental process, and may effected by the same gene.

In our study focusing the genetics factors, the interesting genes in development processes are described. First is *transforming growth factor-β3* (*TGF-β3*) which is the member of transforming growth factor-β, that control cell proliferation, migration and differentiation, regulation of extracellular matrix deposition and epithelial-mesenchymal transformation.[19-20] Mice lacking TGF-β3 exhibited an incompletely penetrant failure of the palatal shelves to fuse leaded to cleft palate and no craniofacial abnormalities were observed.[21] Thus *TGF-β3* is the candidate gene for genetics study in nonsydromic clefting in humans. In addition significant linkage disequilibrium (LD) were found between CL/P and marker X5.1 of TGF-β3(T->C, at position –24 relative to intron 4/exon 5 junction) and UTR.1 (AGAGGG repeat in 5' UTR)-X5.1 haplotype[22] in Iowa population. In Japanese population, significant TDT was observed in CA repeats[23], while in Danish population one copy of CA repeats contributed risk for CL/P.[24]

The second one is *interferon regulatory factor 6* (*IFR6*); the one of nine transcription factors that shares a highly conserved helix-turn-helix DNA-binding domain and a less conserved protein-binding domain. Most of IRFs regulate the expressing of interferon-α and -β after viral infection[25], whereas the function of IRF6 is unknown. In the recent report, mutations in Smad-Interferon Regulatory Factor binding domain (SMIR domain) of *IRF6* caused Van der Woude syndrome (VWS; OMIM 119300,), oral cleft with lip pit inherited by autosomal dominance.[26] According to orofacial anomalities in VWS,

perhaps the variant *IRF6* 820G->A (Val274Ile) in *IRF6* is the substitution of an isoleucine for an evolutionarily conversed valine residue at codon 274 in a protein binding domain (SMIR domain) was associated gene with non-syndromic clefting.

The third one is *SKI* proto-oncogene, which encodes a nuclear protein that binds to DNA in association with other cellular factors and modulates transcription.[27-28] Several lines of evidence suggest that SKI may function to regulate critical decisions leading to a choice between continued proliferation or terminal difference, enhanced cell proliferation and viability, and skeletal muscle differentiation.[29] The data from *SKI* knockout mice, perinatal lethality resulting from exencephaly and defects caused by failed closure of the cranial neural tube during neurulation, such as vasicularized brain mass and frontonasal clefting, were exhibited.[30] Furthermore, null mutant mice that change background by backcrossed with other background showed a switch from a neural tube defect to midline facial clefting. The data in human, *SKI* is located at distal 1p36.3 and deleted in the entire individuals affected 1p36 deletion syndrome (syndrome that included oral cleft). Thus, *SKI* may contribute to some of the phenotypes common in 1p36 deletion syndrome, and particularly to facial clefting. In the overall, these three genes are function in the TGF-β pathway, and may have an interaction. Activation signal pathway is initiated by TGF-β3 assembles a receptor complex that phosphorylates Smads, then Smads translocate to nuclease and assemble with a transcriptional complex that regulates target genes. As for that cascade, IRF6 act as the transcriptional factor, which has Smad binding domain and SKI is the co-regulated of transcription factors.[31]

Moreover, the involvement of genetics and micronutrient was considered in predisposition to oral cleft and FEEM. Maternal folic acid supplementation during early pregnancy may reduce the risk for both oral clefts[32-34] and NTD[35-37], and other congenital abnormalities.[38-39] Therefore, researchers have recently clarified the molecular basis of several genes related with the folate mechanism including gene which encode for 5,10–Methylenetetrahydrofolate reductase (*MTHFR*). Two polymorphisms, *MTHFR* 677C->T (A222V) and *MTHFR* 1298A->C (E429A)[40-41] have been shown to have reduced MTHFR activity and associated with oral cleft and NTD risk.[41-43] Whereas the prevalence of the risk genotype explain only a small portion of the

protective effect of folic acid. In recent report the other folate-metabolism enzymes were analyzed, only *trifunctional enzyme methylenetetrahydrofolate dehydrogenes/ methylenetetrahydrofolate cyclohydrolase/formyltetrahydrofolate synthese* (*MTHFD1*) was associated with NTD risk. There was *MTHFD1* 1958G->A (Arg653Gln) that contributed risk in the mothers of NTD.[44]

According to the described data, this study aim to determine the prevalences of the non-synonymous single nucleotide polymorphisms (SNPs) of entire genes (Table 1) among Thai population and to find out the association between the polymorphisms and risks of CL/P, CPO and FEEM.

Table 1 Candidate Genes, their structures and SNPs

Gene	Locus	Gene structure			SNPs	Location
		No. of exon	g-DNA (kb)	c-DNA (kp)		
TGFβ-3	14q24.3	7	23	1.2	179C->T[a] Thr60Met	Exon1
					383A->G [22] Lys128Arg	Exon2
IRF6	1q32-q41 (critical region)	9	18	1.4	820G->A [26] Val274Ile	Exon6 SMIR domain
SKI	1p36	7	79	2.1	185C->G[b] Ala62Gly	Exon1
					1163C->T[c] Ala388Val	Exon3
MTHFD1	14q24	27	70	2.8	1958G->A [44] Arg653Gln	Exon20

a = code rs4252315 form Single Nucleotide Polymorphism (dbSNP) Database (http://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?rs=4252315)

b = data form NCBI, genomic sequences of SKI exon 1 (AY331180)

c = data from NCBI, genomic sequences of SKI exon 2 – 3 (AH013034)

Research Questions

1. What are the prevalences of studied SNPs among Thai population?
2. Are the studied SNPs associated with oral cleft and FEEM among Thai population?

Objectives

1. To ascertain the prevalences of studied SNPs among Thai population
2. To determine association between the studeed SNPs and oral cleft and FEEM

Hypothesis

Selected SNPs of the candidate genes are associated with oral cleft and FEEM in Thai population.

Assumption

Samples from the subject and control groups are al collected in the same region of Thailand. Thus, this study is assumed to have no population stratification.

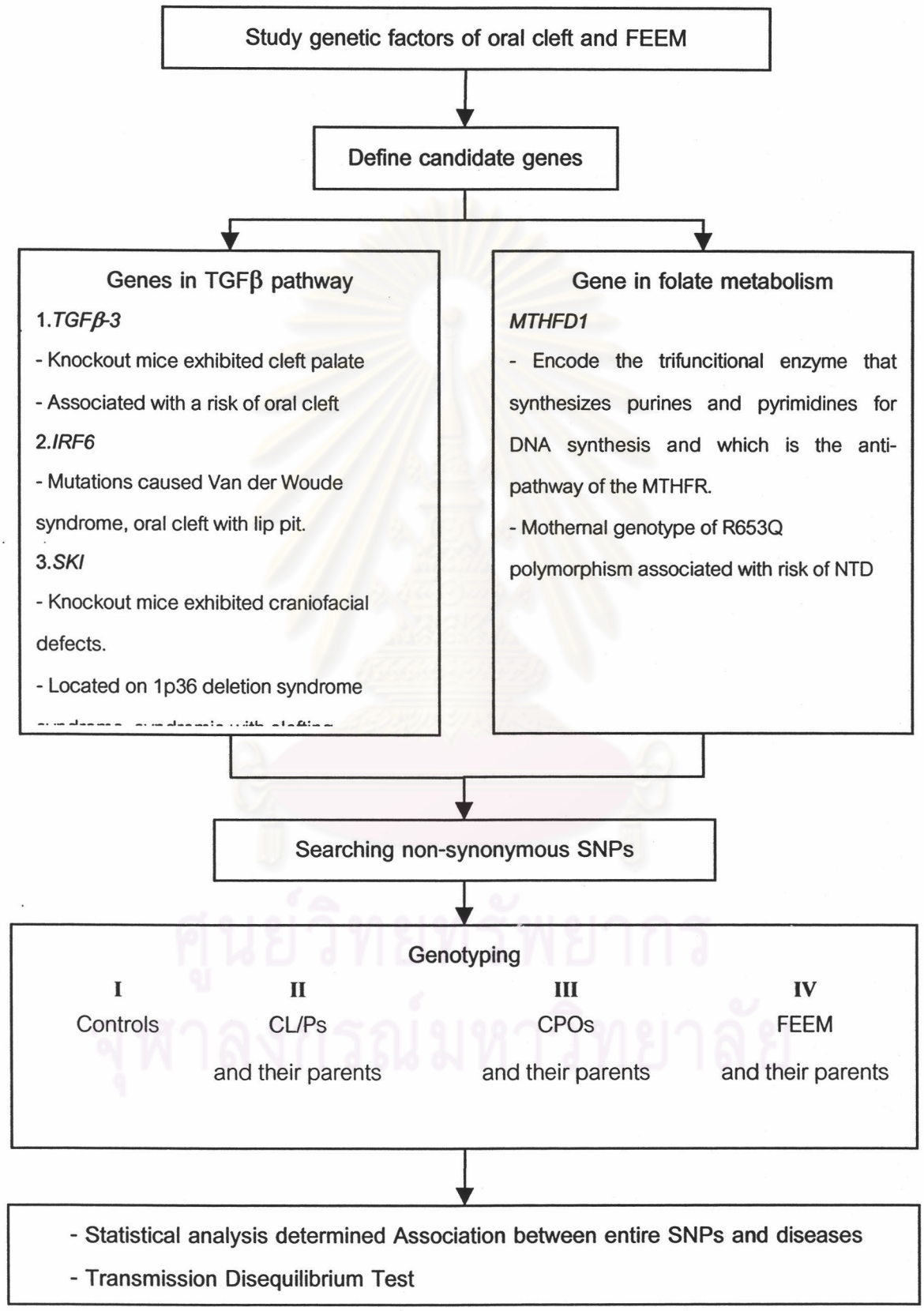
Operational Definition

1. The subjects in this study consist of 4 groups: 1) unaffected individuals or control group, 2) CL/P patients and their parents, 3) CPO patients and their parents, 4) FEEM patients and their parents.
2. Genotype is determined by RFLP patterns.

Limitation

Numbers of patients may be limited are not enough for TDT calculation.

Conceptual framework



Research Methodology

1.Sample Collection

1.1 Patients: Patients with FEEM and CL/P were characterised and clinically diagnosed by the clinicians due to their typical phenotypes.

1.1.1. Oral cleft patients: The patients with CL/P and CPO, plus their parents (trio, non-trio) recruited from Mae Hongson, Nan, Uthai Thani, Prachin Buri, Nakorn Ratchasima, Sa Kaeo, Kalsin, Nongkhia , Maha Saracham, and Trang provinces.

1.1.2. FEEM patients: The children with FEEM and their parents were collected from Uthai Thain and King Chulalongkorn Memoreal Hospital, Bangkok.

1.2 Controls: The control populations were 310 healthy individuals collected from the Thai Red Cross of Nakorn Ratchasima, Kalasin, Nong Khia and Bangkok.

2.Process of study

2.1 Blood collection

2.2 DNA extraction

2.3 DNA amplification

2.4 Restriction enzyme analysis

2.5 Agarose gel electrophoresis

3. Data collection and analysis

Expected Benefit

The result from this study may produce the basis for describe the mechanism as contribute to human clefting and FEEM.