# CHAPTER I
# INTRODUCTION

## Background and rationale

Frontoethmoidal encephalomeningocele (FEEM) and cleft lip with or without cleft palate (CL/P) are among the most severe congenital abnormalities. FEEM is an endemic neural tube defect (NTD) affecting babies born in Southern and Southeast Asia and is rarely found in Westen Europe, Japan, Australia, and North America.[1,2] In Thailand, the incidence of FEEM is relatively high with approximately 1/6,000.[3] In contrast to NTDs in most parts of the world that failure of closure of neuropore are usually located in the lumbosacral and occipital regions, NTDs in Southeast Asia including Thailand are usaully situated at the root of the nose resulting in FEEM.[4] 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[5] and may result in the neurological and ocular problem in addition to the presence of facial dysmorphology[2,6-8]. 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.[9]

Cleft lip with or without cleft palate (CL/P MIM 119530) is the birth defect characteristics of genetically complex trait.[10] It results from the defect of the facial bone in which the failure of fusion of the medial nasal, maxillary, and frontonasal process, are found. The incidence of CL/P is about 1/700 -1/1,000 in Caucacians[11,12] compared to the incidence of 1/600 which is relatively high in Thailand.[13] Regarding etiology of CL/P, several studies suggested that environmental and genetic factor have a joint role in the causation of CL/P[14], and the multifactorial model was advanced to explain the causes of CL/P.[15,16]

Since etiologies of FEEM and CL/P are considered 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. Although the information of interaction of genes and FEEM development is as yet

uninvestigated, the data sets of craniofacial development related genes tend to favor of the major gene contributing for FEEM.[17] Also in CL/P that the effects of predisposing genes are considered, the previous studies revealed heterogeneity of CL/P with estimated 2-20 genes interacting to cause cleft, including possible major gene that may account for 10-50 per cent of the incidence of these defects.[16,18] Because of the similarity between FEEM and CL/P to their pathogenesis of causing craniofacial bone defect and their relationship of the same group of gene controlling developmental process, they may particularly be effected by the same group of genes.

Of more immediate practical interest than genes depend on development process, the involvement of genetic and micronutrient was considered in predisposition to birth defect. Previous evidences show that reductions in the risks of NTD,[19-21] CL/P[22-24] and other congenital abnormalities[25,26] are associated with folic supplementation in early pregnancy. These suggested the genetic susceptibility of folate metabolism. 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*). Previously, two common point polymorphisms have been found in *MTHFR* gene. The first identified polymorphism in exon 4 of the *MTHFR* gene, is a cytosine (C) to thymine (T) substitution at nucleotide 667 (677C→T)[27], which convert alanine to a valine (A222V). Recently, a second common *MTHFR* polymorphism,1298A→C, has been reported with the alteration of an adenine (A) to cytosine (C) in exon 11 that results in a glutamate-to-alanine conversion (E429A)[28]. The previous study showed that the 677C→T were found to be associated with CL/P[29] whereas the presence of the 677C→T and the compound heterozygosity for 677C→T and 1298A→C were found to be associated with NTDs.[28,30] Although there have been several reports on the NTD associated *MTHFR* polymorphism, mostly documented on other type of NTD such as anencephaly, spinabifida, and other encephaloceles. No report on the relationship between the *MTHFR* polymorphisms and FEEM has been found so far. Moreover, the previous reports on the correlation between *MTHFR* polymorphisms and NTD or CL/P, are documented among the Western population which may not explain relationship between these two *MTHFR* polymorphisms and

different ethnic groups such as Thai population. In order to locate possible genetic risk factor of FEEM and CL/P in the Thai population due to *MTHFR* polymorphisms, this study aims to determine the prevalences of *MTHFR* 677C→T and 1298A→C polymorphisms among Thai population and to find out the association between the *MTHFR* polymorphisms and risks of FEEM and CL/P.

## Research Questions

1. What are the prevalences of *MTHFR* 677C→T and 1298A→C among Thai population ?

2. Do the *MTHFR* 677C→T and 1298A→C polymorphisms confer risks of FEEM and CL/P among Thai population ?

## Objectives

1. To determine the prevalences of *MTHFR* 677C→T and 1298A→C polymorphisms among Thai population

2. To find association between the *MTHFR* 677C→T and 1298A→C polymorphisms, and FEEM and CL/P

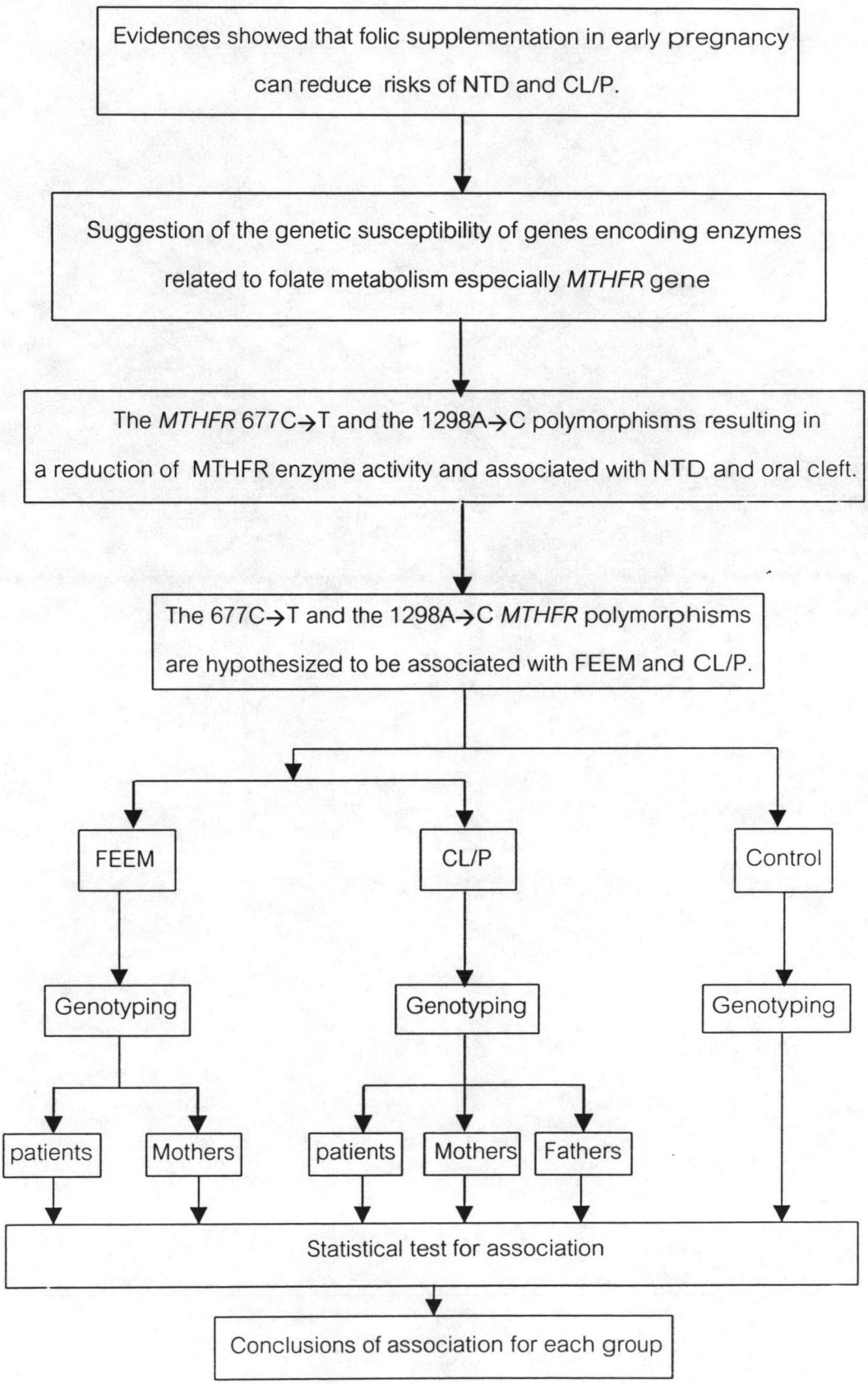
## Hypothesis

The *MTHFR* 677C→T and 1298A→C polymorphisms is the possible genetic risk factors for FEEM and CL/P in Thai population.

## Assumption

Individuals enrolled to this study from several parts of Thailand including Bangkok, Maehongsorn, Nan, Uthaithani, Nakornratchasima, Srakaew, and Trang, are Thai in ethnicity.

**Conceptual framework**



**Limitation**

Because of unavailable of sample from FEEM fathers, Transmission Disequilibrium test can not be performed for FEEM group.

**Operational Definition**

Because this study considers both genotypes and haplotypes, to avoid the misunderstand to their abbreviations, genotypes for the 677C→T polymorphism are represented by 677CC, 677CT, and 677TT whereas the 1298A→C genotypes are represented by 1298AA, 1298AC and 1298CC, respectively.

In case of genotype combination for 677C→T and 1298A→C, a "/" is used; for example 677CT/1298AC represents individual who is 677CT accompanying with 1298AC, whereas 677CC/1298CC implies individual who is 677CC with 1298CC.

Regarding haplotypes, a "-" is used; for example C-A/T-C means that one allele presents C and A at nucleotides 677 and 1298 respectively, and the other allele contains T at nucleotide 677 and C at nulcleotide 1298. In addition, if we mention the presence of only one allele, we still use C-A to represent an allele which presents C at nucleotide 677 and A at nucleotide 1298.

**Expected Benefit**

Given that there is an association between a genotype and a congenital anomaly, folate supplementation in early pregnancies should be encouraged for all Thai women planning tohave children in an attempt to decreased the incidence of birth defect. In addition, the result from this study will produce the basis for future studies, including searching for other causative genes.

**Research Methodology**

1. Sample Collection

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

1.1.1 FEEM patients : patients recruited from the Genetic Clinic of the King Chulalongkorn Memorial Hospital, Bangkok and from Uthaithani Province.

1.1.2 CL/P patients : Patients with CL/P and their parents were enrolled to this study from several parts of Thailand including from Maehongsorn, Nan, Uthaithani, Nakornratchasima, Srakaew, and Trang

1.2 Controls : Control group is the group of unrelated healthy blood donors from the Thai Red Cross of Bangkok and Nakorn Ratchasima.

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 electrophpresis

3. Data collection and analysis