CHAPTER II

REVIEW OF RELATED LITERATURE

1. Oral cleft

Clinical features and incidence

Craniofacial abnormalities are among the most common features of all birth defects. The most frequent of these are the oral clefts, which consist of two major groups, cleft lip with or without cleft palate (CL/P OMIM 119530) and cleft palate only (CPO OMIM 119540). Cleft of the 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 the palate is caused by the palatal shelves failing to close. Approximately 50% of cleft lip patients also have cleft palate, which is thought to be a secondary effect resulting from the defect in facial prominence fusion that precedes palate formation. Thus cleft palate only (CPO) occurring alone is therefore considered to be etiologically distinct from cleft lip with or without cleft palate (CL/P). The majority of CL/P, account for 70%, are regarded as non-syndromic. The remaining syndromic cases have additional characteristic features that can be subdivided into categories of chromosomal abnormalities, recognizable Mendelian single gene syndromes, teratogenic effects and various unknown syndromes.[46] According to figure 1, clef palate only is shown as diagrams ***a*** and ***e*** while cleft lip and cleft lip and palate are presented by diagrams ***b c d f g*** and ***h***.

The variability incidence of cleft lip and/or palate (CL/P) 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]

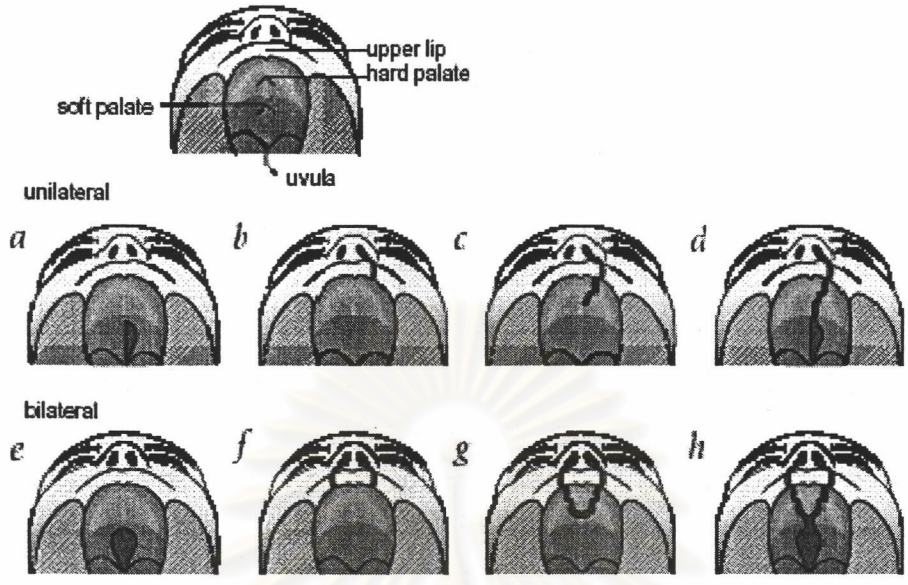

Figure 1 Diagram of various types of orofacial clefting. Normal lip and palate in top panel. ***a***,***e***, Unilateral and bilateral cleft of the soft palate only. ***b***,***c***, Cleft lip and various degrees of cleft lip and cleft palate involvingthe hard (*c*, *g*) and soft (*d*, *g*) palate (CL/P). Note that unilateral and bilateral CL/P can be easily differentiated (*b*, *c*, *f*, *g*), whereas this is not the case for unilateral or bilateral clefts of the soft palate.[47]

Etiologies

Epidemiological investigations have identified a number of factors that may be related to the occurrence of non-syndromic clefting. Many of these factors have not been implicated in the etiology of these conditions in several studies and, therefore, represent important areas of further investigation.

The evidence of genetic factors and non-genetic factors were reported. About potential risk factors included race, ethnicity, and nationality. There was substantial variation in the birth prevalence of orofacial clefts across populations, with Asian populations reported higher rates than Caucasian or African populations. There was some evidence that the prevalence of clefts among the offspring of immigrants remained similar to that in country of origin.[48] According to family analysis, increasing recurrent risk in families of patients were found, estimated that the risk of recurrence of CL/P in subsequently born children was 4% if one child had it, 4% if one parent has it, 17% if one parent and one child had it, and 9% if two children have it.[49] Additionally, a concordance in monozygotic (MZ) twin ranged between 40% and 60%, whereas only 5% was found in dizygotic twins. The lack

of 100% concordance in monozygotic twins suggests that genetic events alone are not responsible for the clefting phenotype.[4] As for inheritance analysis, Hecht et al. (1991) performed complex segregation analysis of nonsyndromic CL/P in 79 families ascertained through a proband diagnosed at the Mayo clinic. In one analysis, the dominant or codominant mendelian major locus models of inheritance provided the most parsimonious fit. In another, the multifactorial threshold model and the mixed model were also consistent with the data. However, the high heritability (0.93) in the multifactorial threshold model suggested that any random exogenous factors were unlikely to be the underlying mechanism, and the mixed model indicated that this high heritability was accounted for by a major dominant locus component. Thus, the best explanation for the findings of the study was a putative major locus associated with markedly decreased penetrance[50]

Alternatively, an environmental component to clefting was defined. Oral cleft recognized as associated maternal nutritional deficiencies with cleft palate in rats.[51] As for teratogen that cause clefts include rare exposures, such as phenytoin, valproic acid and thalidomide, and also common environmental exposures, such as maternal alcohol or cigarette use[52], herbicides such as dioxin[53], and altitude.[54] The agent that has been studied most extensively is maternal smoking which account for a two-to six fold increase in the relative risk for clefts among smokers.[55-56] Ericson et al performed a case-control study on smoking habits of woman in Sweden who gave birth to infants with CL/P during 1975. Of 66 cases, it was observed that significantly more women who had infants with CL/P smoked than did control women.[57] In addition, a considerable body of data from animal experiments suggested that vitamin deficiencies play a role in the pathogenesis of cranial soft tissue and bone defects, in particular CL/P.[58-60] There is some evidences for this in humans[60], and that administration of vitamins, in particular those of the B group, can prevent the occurrence of CL/P.[61-62] In 1982, Tolarova reported that periconceptional vitamin supplementation plus 10 mg of folic acid, one of vitamin B, reduced the recurrent risk of cleft lip.[63] To date, the possible preventive of folic acid has not been rule out.

Gene studies

Animal models have also provided tools for studying human facial development. Studies of gene expression in tissue and organ pointed to a role for their development. Insertional mutants or disruption gene functions have caused craniofacial abnormalities.[64] The function of a gene might be obscured if it has a critical role at earlier developmental stages (for example, at implatation or gastrulation), such that its loss causes an early lethal phenotype (for example, before embryonic day (E)7 in mice). For palate clefting, occurrence in the absence of other malformations in the *Jag2* (*Jagged2*), *TGFβ-3* (*transforming growth factor-β 3*) and *Lhx8*[65] knockouts. In *Jag2-/-* mice, the palatal shelves fail to elevate[66], whereas in *TGFβ-3 -/-* mice, correctly positioned palatal shelve fail to fuse.[21] The strong evidence of *Msx1* was reported, 100% of knockout mice were exhibited cleft palate and their expression was presented in developing craniofacial structures.[67-68]

According to linkage study, Isolated cleft lip with or without cleft palate (CL/P) is genetically heterogeneous. Data suggested loci for clefts on chromosome 4, 6, 17 and 19.[69-70] Linkage has been excluded at these same loci in other datasets. Only loci on 6p have consistently shown linkage to CL/P in Denmark,[71] Italy,[72-73] and the UK.[74] One genome-wide screen has been carried out using approximately 100 sib-pairs from the UK.[74] Although on highly significant loci were identified in this study, nine regions of interest were confirmed in a 5-cm scan. Three of these (1p36, 2p13 and 6p24) are near genes or loci suggested in other studies. The interestingly region, 1p36, has at least three interesting genes, which are *SKI*, *P73* and *MTHFR*. Additionally, association studies have also been used extensively to examine candidate genes in oral cleft. Associations with *SKI*, *MTHFR*, *TGFβ*, *TGFA*, *MSX1*, *PVRL1*, *GABRAB3*, *RARA*, and *BCL3* have been found for oral cleft.[4]

2. Frontoethmoidal encephalomeningocele (FEEM)

Clinical features and incidence

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] Generally, NTD was classified as open, if neural tissue is exposed or covered only

by membrane, or closed, if the defect is cover by normal skin.[75] In the West, it is generally accept that NTDs which usual lesions located in the lumbosacral and occipital regions resulting in spina bifida, anencephaly, and occipital encephalocele, are caused by the failure of the neural tube to close.[76] Consistent with reports[77-78] proposed the embryologic basis that in human, as in mice, closure of the neural tube occurred at several sites and that the clinical types of NTDs depended on the site at which the closure failed (Figure 2). According to this hypothesis, FEEM, as a type of NTD, was found to be caused by the failure of neural tube closure usually at the root of the nose and subsequently result in the neurological and problems in addition to the presence of facial dysmorphology. Classification of FEEM was recently characterized according to their internal defect of the cranium between the frontal and ethmoidal bone, and most frequently situated at the site of the foramen caecum. Base on the different locations of skull defect, FEEM can be divided into nasofrontal, nasoethmoidal, and naso-orbital encephalocele(Figure 3).[14-16] Although pathogenesis of FEEM is still unclear, various theories have also been proposed. The most popular classification of this deformity is the one proposed by Suwanwela (1972)[11] based on the site trough which the herniated cerebral tissue exist. Recently, there has been the report suggested that the defect is based on a disturbance in separation of neural and surface ectoderm at the site of final closure during the final phase of neurulation in the 4th week of gestation. The nonseparation of neural and surface ectoderm will result in a midline mesodermal defect as that in FEEM.[79] 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.[11]

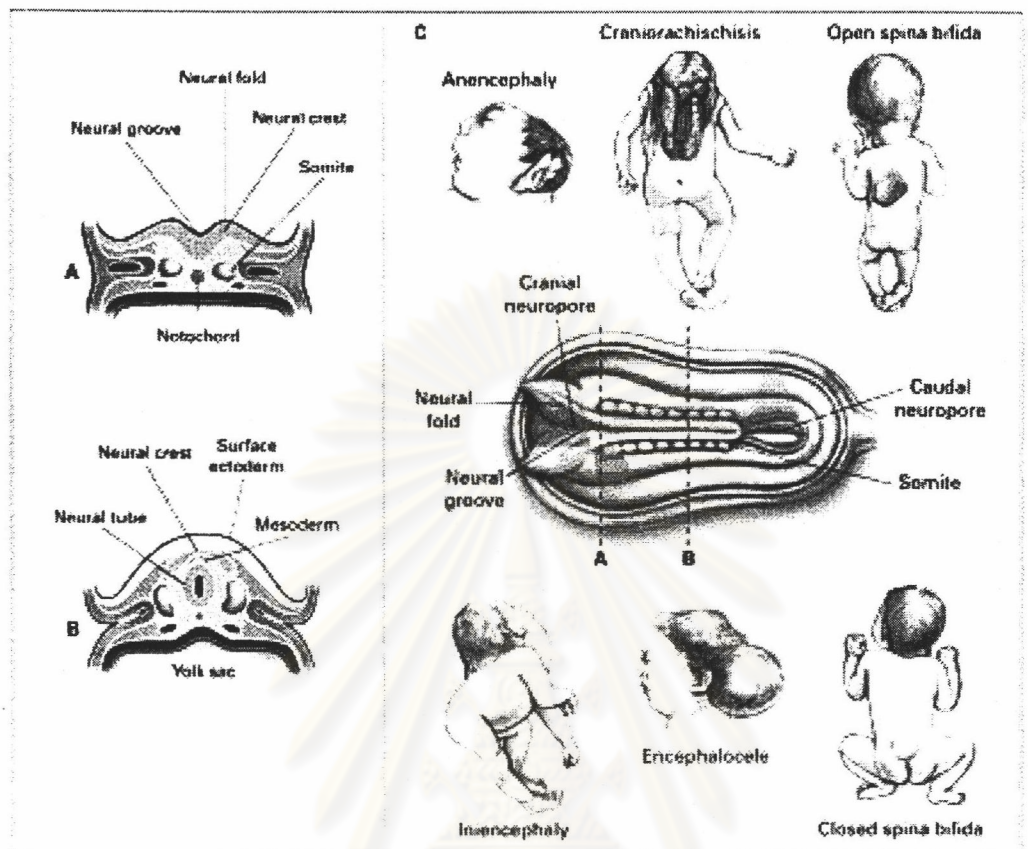

Figure 2 Features of Neural-Tube Development and Neural-Tube Defects.Panel A shows a cross section of the rostral end of the embryo at approximately three weeks after conception, showing the neural groove in the process of closing, overlying the notochord. The neural folds are the rising margins of the neural tube, topped by the neural crest, and demarcate the neural groove centrally. Panel B shows a cross section of the middle portion of the embryo after the neural tube has closed. The neural tube, which will ultimately develop into the spinal cord, is now covered by surface ectoderm (later, the skin). The intervening mesoderm will form the bony spine. The notochord is regressing. Panel C shows the developmental and clinical features of the main types of neural-tube defects. The diagram in the center is a dorsal view of a developing embryo, showing a neural tube that is closed in the center but still open at the cranial and caudal ends. The dotted lines marked A and B refer to the cross sections shown in Panels A and B. Shaded bars point to the region of the neural tube relevant to each defect.[80]

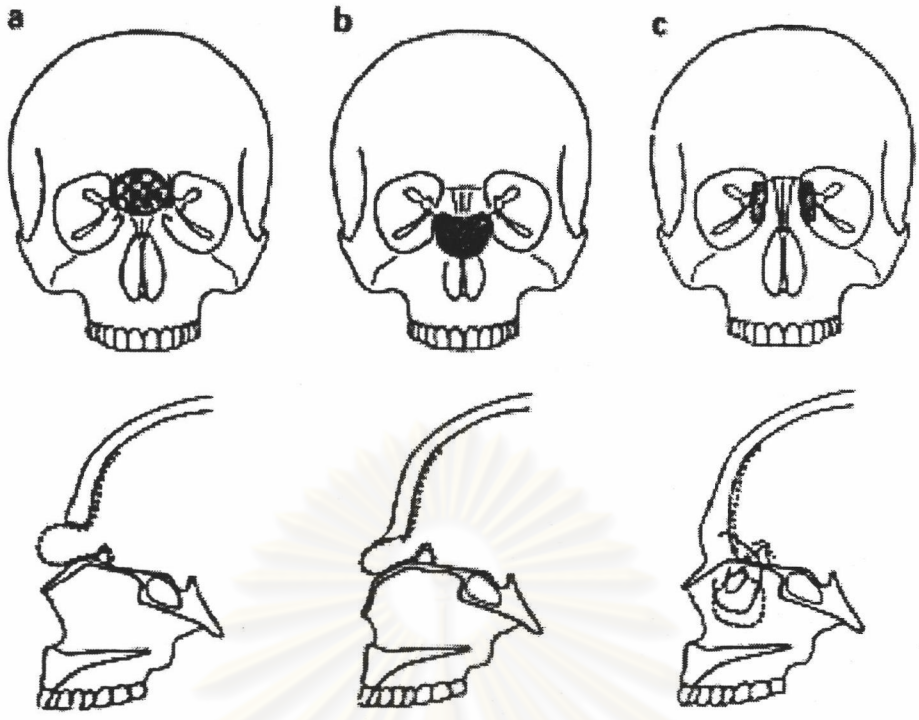

Figure 3 Frontoethmoidal encephalomeningoceles are characterized by an internal skull defect at or near the site of foramen caecum. The subdivision into nasofrontal (a), nasoethmoidal (b) and nasoorbital (c) encephaloceles is based on the location of external defect in the facial skeleton.[79]

Etiologies

About the etiology many theories have been proposed, and a multifactorial genesis seem to be most probable.[17,80] Similar to other NTDs, environmental and genetic factors may play a joint role in the causation of FEEM. In the previous report by Suwanwela[12,14] reported the lack of familial incidence and negative chromosome study and the discordant affection in a pair of identical twin. These suggested that no genetic mechanism as primary cause. Consistently, Thu Aung[81] reported that no first degree relative of a subject was effected. In view of the incidence among the various ethnic groups, significant different was found between incidence of Thai native patients and patients came from Chinese or Indian immigrants of ethnic which contrast to other study that showed non-genetic involvement that no difference between the Malay, Chinese and Indian subjects.[11] Because of unclear result of genetic involvement documented on incidence of FEEM among various populations, the studies documented on the widely larger population are preferable to clarify this role.

Regarding evidences documented on other types of NTDs, many explanations were suggest as the environmental factors which foster to development of NTDs. In some studies, fever and hyperthermia in early pregnancy[82-83] and maternal obesity[84-85] have been proposed to giving birth of affected baby. Other suggestion such as maternal diabetes,[86-87] maternal used of some antiepileptic drug,[88] and even the higher incidence of NTD in groups with lower socioeconomic status[89], have also been proposed to be NTDs causes. Moreover, several studies in animal model suggested that vitamin insufficiencies play an important role in pathogenesis of cranial soft tissue and bone defects.[58-60] Consistently, in human, there has been a study report that women who gave birth to babies with NTD had low serum level of micronutrients including some Vitamins.[90] Interestingly, the recent study[91-92] indicated that the risk of recurrent and occurrent of NTD was significant lower among women who were supplemented by folic acid daily than women who did not. These was supported by the most recent study that supplementation of 400 μg of folic acid daily can effectively prevent NTD in an area of China. To date, the theory of nutrition deficiency such as lacking of folate, lead to cause NTD, is widely accept.

Gene studies

A genetics model of human NTDs seems likely to be a non-syndromic NTD with a degree of genetic complexity. Few of the less than 60 mutations caused NTDs in mice, and many of these mutations cause other morphological defects, while numerous of nulls homozygous mutant mice had syndromes of multiple severe defects, lethal during embryogenesis that included failure to close the neural tube, in particular in the head. Their function were involved embryo development process, such as signal transduction, receptor, transcription factor, transcription coactivator, enzyme, tumour suppressor, DNA methytransferase and cytoskeleton.[93]

According genes in folic metabolic pathway, a variant form of *methylenetetravydrofolate reductase* (*MTHFR*) 677C->T is a know risk factor for NTDs, However the association of the *MTHFR* 677 C->T polymorphism with NTDs remains controversial, with several studies finding no association. Several additional folate-related genes had also been examined in relation to NTDs; however,no association had been found. 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]

3. Candidate gene approach

Association studies with candidate genes have been widely used for the study of complex diseases. The contents in the method consist of 1) a population-based case-control study, 2) candidate genes, 3) gene variants and 4) statistical methods. This approach 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 aetiology of the disease; identifying variants in or near those genes that might either cause a change in the protein or its expression, or be in linkage disequilibrium (LD) with functional changes; genotyping the variants in a population; and by using statistical methods to determine whether there is a correlation between those variants and the phenotype.[94]

Sampling strategies for case-control study, by necessary, identify ethnic and matching ethnic between cases and controls are performed. Because allele frequency differences between cases and controls can appear as disease association, even if they only reflect the results of evolutionary or migratory history, gender differences, mating practices or other independent processes.[95]

For selection candidate genes, investigators must choose, from a very large number of potential factors, those factors that are most likely to be involved in the phenotype. The first step is usually to examine published studies of the phenotype of interest for suggestions about the types and the number of risk factors, or candidate genes, that are involved. In addition, linkage studies might provide information about genomic regions that can be explored further. These studies can be evaluated from several perspectives, including the population characteristics, the phenotypic definition and the number and density of the markers used.[96] Evidence can also be evaluated for the involvement of specific genes in the phenotype. It is necessary to consider carefully the genes and variants that are selected for these studies and the reasons for their selection. Until recently, a study might have examined a gene solely because of the existence of an easily genotyped polymorphism. Therefore, it is important to determine whether any of the

variants that were examined have a functional consequence. Finally, there might be biological, aetiological and pathological models of the disease of interest. Expression studies might provide important information about the tissues and cells that are involved in the disease.

Regarding to DNA variation that has an allele frequency of at least 1% in a population. There are several types of polymorphism in the genome: single nucleotied polymorphisms (SNPs), repeat polymorphisms, and insertions or deletions. Most of the DNA sequence variation in the human genome is in form of SNPs.[97] Information about the location and type of the sequence variants in a gene can be used to prioritize polymorphisms (Table 2). For some polymorphisms, it might be obvious that a DNA variation changes the function of a protein — for instance, a non-synonymous (missense) variant that alters an amino acid in a protein, or a nonsense change that results in a premature stop codon. These types of polymorphism account for most known disease associations, and therefore they should be given the highest priority for genotyping in candidate-gene studies.

The association for SNPs and diseases is determined by the statistical methods. Relative risk or odds ratio for is used for determine SNPs is a risk factor for diseases. This association is described with:

1) Natural selection; people who have disease might be more likely to survive and have children if they also have A allele.

2) Direct causation; having allele A markers you susceptible to disease. A allele is neither necessary nor sufficient for individuals to develop disease, but it increase likelihood. Also, A allele is expected to associate with disease in any populations unless the causes of the disease vary from one population to another.

3) Linkage disequilibrium(LD); or allelic association is the association of closed allele. Base on hypothesis that most disease-bearing chromosome in the population are descended from one or a few ancestor chromosomes. If LD is the cause of association, there should be a gene near to the A locus that has mutation in people with disease. The particular allele at A locus (A_1,A_2,...) that is associated with disease may be differed in different population.

Alternatively, another approach is family-based study designs to avoid problem of population stratification by using family members such as parents to be internal control. Two family-based approach widely used including, such as the transmission disequilibrium test (TDT)[98] which is also used to test difference between transmitted and untransmitted allele derived from parents.

Table 2 Priorities for single-nucleotide-polymorphism selection

Type of variant	Location	Functional effect	Frequency in genome	Predicted relative risk of phenotype
Nonsense	Coding sequence	Premature termination of amino-acid sequence	Very low	Very high
Missense/ non-synonymous (non-conservative)	Coding sequence	Changes an amino acid in protein to one with different properties	Low	Moderate to very high, depending on location
Missense/non-synonymous (conservative)	Coding sequence	Changes an amino acid in protein to one with similar properties	Low	Low to very high, depending on location
Insertions/deletions (frameshift)	Coding sequence	Changes the frame of the protein-coding region, usually with very negative consequence of the protein	Low	Low to very high, depending on location
Insertions/deletions(in frame)	Coding sequence	Change amino acid sequence	Low	Very high, depending on location
Sense/synonymous	Coding sequence or non-coding sequence	Does not change the amino acid in the protein – but can alter splicing	Medium	Low to very high
Promoter/regulatory region	Promotor, 5'UTR, 3'UTR	Does not change the amino acid, but can affect the level, location or timing of gene expression	Low to medium	Low to high
Splice site/intron-exon boundary	With in 10 bp of the exon	Might change the splicing pattern or efficiency of intron	Low	Low to high
Intronic	Deep within introns	No know function, but might affect expression or mRNA stability	Medium	Very low
Intergenic	Non-coding regions between genes	No know function, but might affect expression through enhances or other mechanism	High	Very low

4. Candidate genes

Transforming growth factor-β3 (TGF-β3)

The three mammalian Transforming growth factor-β, consist of 3 isoforms, have been conserved in evolution and are closely related: the mature region of TGF-β3 protien has approximately 80% sequence identity to the mature regions of both TGF-β1 and TGF-β2. Each is encoded by different gene in located on a different chromosome.[99] The *Transforming growth factor-β3* (*TGF-β3*) gene is located on chromosome 14 at 14q24.3. The gDNA sequence is 23 kilobases while cDNA sequence is 1.2 kilobases and appears to consist of 7 exons, encode 412 amino acids. *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. Regarding to murine palate formation by in situ hybridization analysis, TGF-β3 expression was associated with palate formation. At 13.5 days TGF-β3 trascrips are localized to the short stretch of MEE cells delineating those regions that will eventually fuse, then at 14.5 day TGF-β3 also seen on the epithelial surface of the nasal septum which will the shelves, but only the diffuse signal is detected throughout the palatal mesenchyme. Around 15 days palatal shelves were fused, and TGF-β3 hybridization signal was still detected in the MEE cells of the midline seam.[100] 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] Additionally in organ culture, palatal shelves were dissected from embryonic day 13.5 (E13.5) mouse embryos. Shelves were placed in homologous (+/+ vs +/+, -/- vs -/-, +/- vs +/-) or heterologous (+/+ vs -/-, +/- vs -/-, +/+ vs +/-) paired combinations and examined by macroscopy and histology. Pairs of -/- and -/- shelves failed to fuse over 72 hours of culture whereas pairs of +/+ (wild-type) and +/+ or +/- (heterozygote) and +/-, as well as +/+ and -/- shelves, fused within the first 48 hour period. Histological examination of the fused +/+ and +/+ shelves showed complete disappearance of the midline epithelial seam whereas -/- and +/+ shelves still had some seam remnants. In order to investigate the ability of TGF-β family members to rescue the fusion between -/- and -/- palatal shelves in vitro, either recombinant human (rh) TGF-β1, porcine (p) TGF-β2, rh TGF-β3, rh activin, or p inhibin was added to the medium in different concentrations at specific times and for various

periods during the culture. In untreated organ culture -/- palate pairs completely failed to fuse, treatment with TGF-β3 induced complete palatal fusion, TGF-β1 or TGF-β2 near normal fusion, but activin and inhibin had no effect. Thus *TGF-β3* is the candidate gene for genetics study in nonsydromic clefting in humans.[101] 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 in Iowa population.[22] 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]

Interferon regulatory factor 6 (IFR6)

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. *Interferon regulatory factor 6* (*IFR6*) gene is located on chromosome 1 at 1q32-q41. The gDNA sequence is 18 kilobases while cDNA sequence is 1.4 kilobases and appears to consist of 9 exons, encode 467 amino acids. IRF6 has two conserved domains, a winged-helix DNA-binding domain (amino acids 13-113) and a protein-binding domain (amino acids 226-394) termed SMIR (Smad-interferom regulatory factor- binding domain).[102] Most of IRFs regulate the expressing of interferon-α and -β after viral infection, whereas the function of IRF6 is unknown. In the recent report, mutation in *IRF6* caused Van der Woude syndrome (VWS; OMIM 119300) and popliteal pterygium syndromes (PPS; OMIM 119500). The two syndromes are a single gene disease inherit by autosomal dominance, VWS consist of oral cleft with lip pit while PPS are similar facial cleft and also include skin and genital anomalies.[103-104]VWS are due to a compleat loss of function of the mutated IRF6 protein, affecting both DNA and protein binding, whereas missense mutations causing PPS affect only DNA binding domain. The ability of the mutated IRF6 to bind to other protein is unaffected, and it therefore forms inactive transcription complexes; thus, this is a dominant-negative mutation. Expression of *IRF6*, greeter expression seemed to be occur in secondary palates dissected from day 14.5-15 mouse embryos and in adult skin.[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.

SKI

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 differenciate, enhanced cell proliferation and viability, and skeletal muscle differentiation. *SKI* located on chromosome 1 region 1p36. The gDNA is 79 kilobase length, compost of 7 exons. *SKI* consists of 2 forms, *c-SKI* is a full-length form and *v-SKI* is the truncated version. *c-SKI* contain 728 amino acids encoded from 2.1 kilobases cDNA, while truncated form, *v-SKI*, missing 20 amion acids from the N-terminus and 292 amino acids from the C-terminus.[29] This truncation, which removes a carboxyl-terminal dimerization domain, plays no role in the activation of ski as an oncogene.[107-109] Over expression of either *c-SKI* or *v-SKI* induces transformation in chicken embryo fibroblasts (CEFs)[108] and their muscle differentiation or transformation in cultured quail embryo fibroblasts (QEFs), depending on conditions.[110] To understand how the two phenotypes are derived from a single gene, Zheng et al identified functionally important regions in *v-SKI* and to test whether these regions can promote one phenotype without the other. They had not found complete separation of the myogenic and transforming properties. Alternatively, Nicol and Stavnezer demonstrate that both *c-SKI* and *v-SKI* were included in nuclear complex that binds to a specific consensus sequence GTCTAGAC DNA binding site. Additionally, Xu et al were identified Smad2, Smad3, Smad4 as the protein complex that bind with SKI and proposed this complex acted as repressor of transforming growth factor type β.[111]

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. 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 (Figure 4). 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.

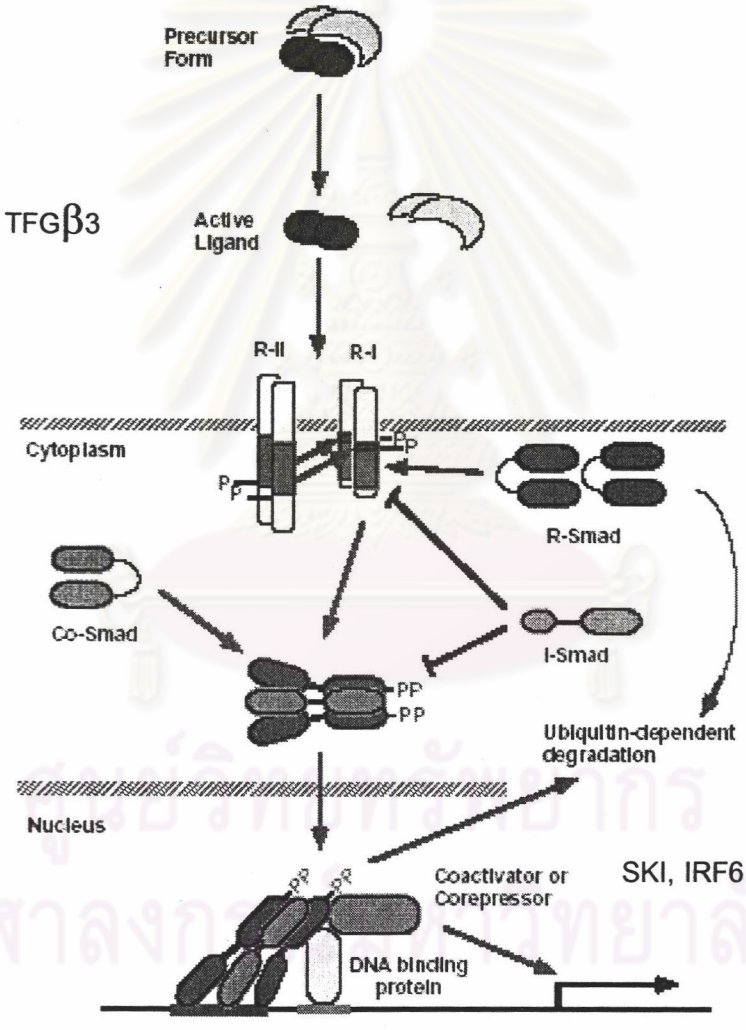

Figure 4 Signaling by TGF-β through serine/threonine kinase receptors and Smad proteins. I-Smads inhibit signaling by R-Smad-Co-Smad complexes. R-I and R-II represent type I and type II receptors, respectively.[112]

MTHFD1

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 and , and other congenital abnormalities. 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) have been shown to have reduced MTHFR activity and associated with oral cleft and NTD risk. Whereas the prevalence of the risk genotype explain only a small portion of the protective effect of folic acid. In 1998, Hol et al reported 2 polymorphisms in *trifunctional enzyme methylenetetrahydrofolate dehydrogenes/ methylenetetrahydrofolate cyclohydrolase/formyltetrahydrofolate synthese* (*MTHFD1*). The first one was nucleotide substitution 878G->A leading to the exchange of arginine for histidine at position 29 of the polypeptide (R293H) and the second one was 1958G->A substitution which causes the replacement of the arginine residue at position 653 by a glutamine in the deduced protein (R653Q). The R293H was detected in a patient with familial spina bifida and not in 300 control individuals whereas the R653Q was presented in both patients and controls with similar frequencies.[113] 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] The *MTHFD1* located on chromosome 14q24 consisted of 27 exons, 2.8 kilobase cDNA, which encode 935 amino acid length.[114] MTHFD1 possessed three enzymatic properties: 5,10-methylenetetracydrofolate dehydrogenase, 5,10-methenyltetrahydrofolate cyclohydrolase and 10-formyltetrahydrofolate synthetase, respectively, and catalyses three sequential reaction in the interconversion of one-carbon derivatives of tetrahydrofolate which are substrates for methionine, thymidilate, and de novo purine synthesis (Figure 5).[44] Proteolysis experiments with mammalian trifunctional enzymes demonstrated that the protein can be separated in two functional domains: an N-terminal domain of approximately one-third of the native polypeptide which contains the cyclohydrolase and NADP-dependent dehydrogenase activities and a C-termminal domain

corresponding to about two-third of the original protein, in which the ATP- dependent synthetase activity resides.[115]

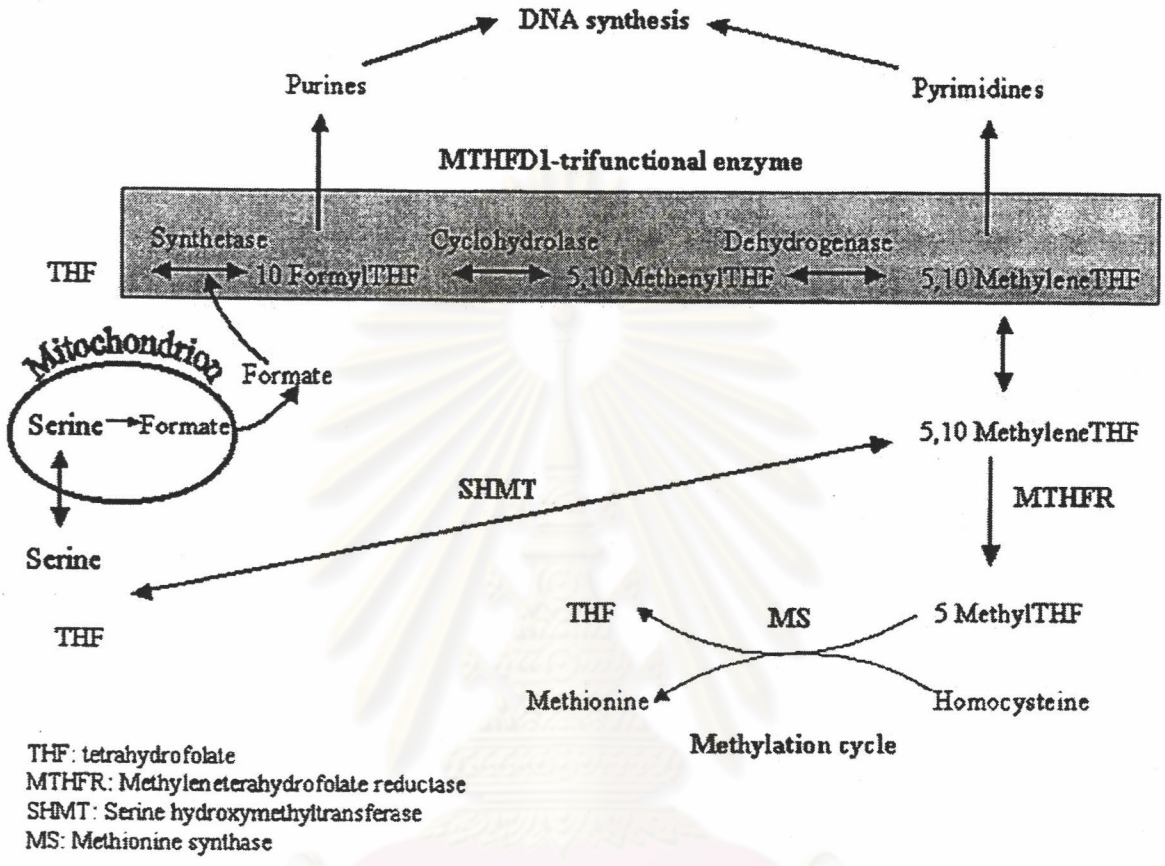

Figure 5 Role of MTHFD1 in DNA synthesis. The main role for MTHFD1 (*shaded*) is in providing 10-formyl THF and 5,10-methylene THF for purine and pyrimidine synthesis. 10-Formyl THF can be synthesized directly from formate and THF, via the synthetase activity, or from 5,10-methenyl THF, via the cyclohydrolase activity, which is channeled from 5,10-methylene THF via the dehydrogenase activity. MTHFD1 plays an indirect role in homocysteine metabolism by providing some of the 5,10-methylene THF pool, but the major source of this is SHMT. The enzymes MTHFR and MS are directly involved in homocysteine metabolism.[44]