CHAPTER II

REVIEW OF RELATED LITERATURE

1. Frontoethmoidal encephalomeningocele (FEEM)

The Frontoethmoidal encephalomeningocele (FEEM) is a endemic neural tube defect (NTD) which is frequently found in Southeast Asia and rarely seen in Western Europe, Japan, Australia, and North America. [1,2,4,6] In Thailand, the estimated incidence was found to be 1 in 6,000. [1,2,31] The reason for this particular geographic distribution is unknown, because the etiology has not yet been clarified.

Generally, NTD was classified as open, if neural tissue is exposed or covered only by membrane, or closed, if the defect is covered by normal skin[32]. In the West, it is generally accepted 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[33]. Consistent with reports[34,35] proposed the embryologic basis that in human, as in mice, closure of the neural tube occurs at several sites and that the clinical types of NTDs depending on the site at which the closure failed (figure 1). 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[5] 1) nasofrontal 2) nasoethmoidal and 3) naso-orbital encephalocele (figure 2). 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)[5] 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.[36]

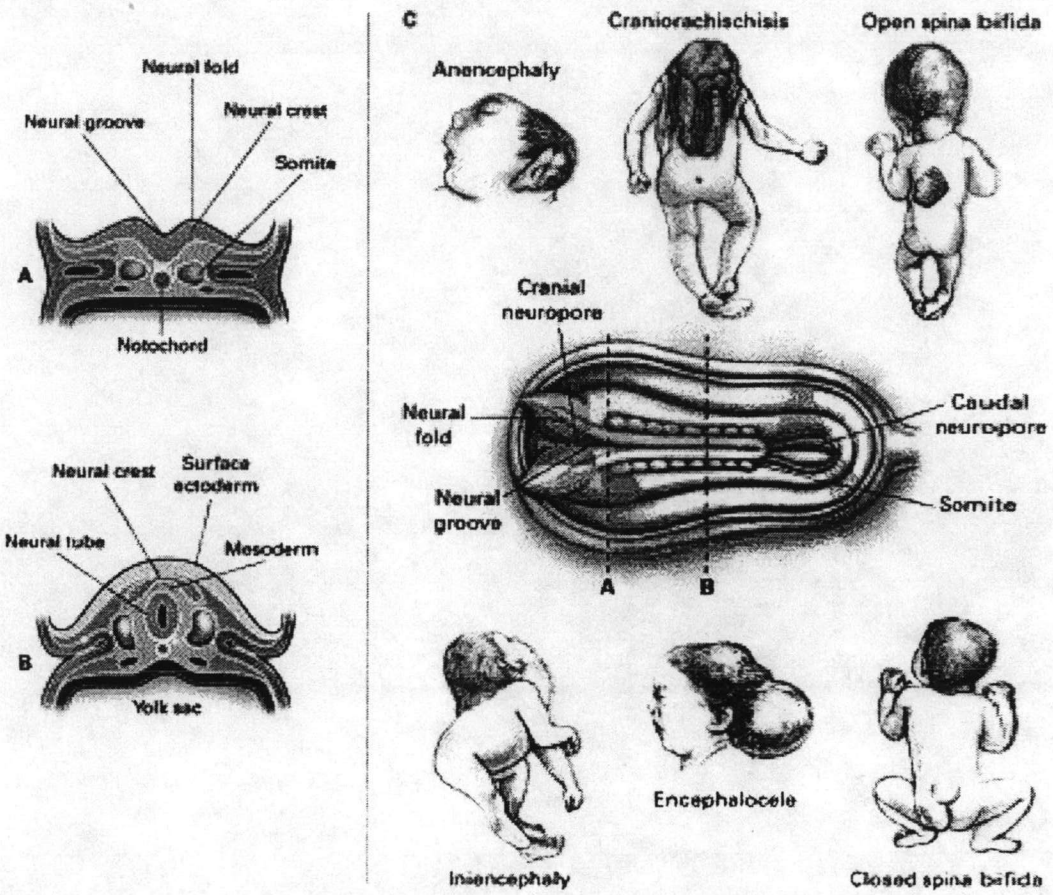

Figure 1[37] Features of Neural-Tube Development and Neural-Tube Defects.

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.

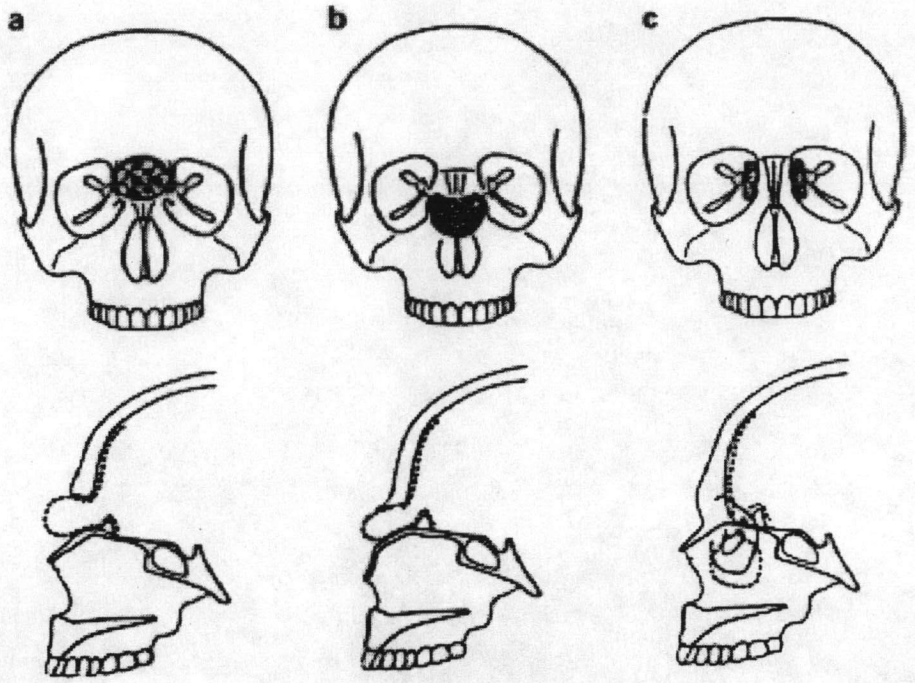

Figure 2[36] Frontoethmoidal encephalomeningoceles are characterized by an internal skull defect at or near the site of foramen caecum. The subdivision into nasofrontal (a), nasoethmoidal (b) and naso orbital (c) encephaloceles is based on the location of external defect in the facial skeleton

Etiologies

About the etiology many theories have been proposed, and a multifactorial genesis seem to be most propable.[9,37] Similar to other NTDs, environmental and genetic factors may play a joint role in the causation of FEEM.

Genetic factors

In the previous report by Suwanwela [4,6] 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[38] 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.[3] 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.

Of the candidate gene for FEEM, many genes suggested in the recent reports seem to be involved with FEEM development.[17] Because of similarity between FEEM and many craniofacial malformations to their origins in specific embryological process, including abnormalities of brain patterning of the migration and fusion of tissues in the face, and of bone differentiation in the skull vault, a number of genes such as *MSX* gene family, *FGFR* gene family, *SHH* gene and etc., may be one of candidate genes for FEEM development. However, further studies in the gene function is might additionally be documented to find whether they can be caused FEEM related phenotypes.

Environmental factors

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 [39,40] and maternal obesity [41,42] have been proposed to giving birth of affected baby. Other suggestion such as maternal diabetes,[43,44] maternal used of some antiepileptic drug,[45] and even the higher incidence of NTD in groups with lower socioeconomic status[46], 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.[47-49] 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.[50] Interestingly, the recent study 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.[51,52] 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, particularly folic aid, lead to cause NTD, is widely accepted.

In view of FEEM, due to the similarity between FEEM and NTD according to their pathogenesis, FEEM development seem to be invulved with environmental factor as reported in NTD. It was suggested to be related with time of birth that higher proportion than expected was born in rainy season, and also suggested the possible of maternal economic status which lead to malnutrition before conception or during pregnancy. Especially, the suggestion of nutritional involvement is the most favorable to foster FEEM development. Since the nutrition insufficiency are widespread in Thailand.[53] However, unlike NTDs that the studies of effective prevention by folic acid supplementations and multivitamin used have widely been documented, those in FEEM have not been yet clarified to date.

Genes and environments

Concidering NTD, the 1970s epidermiologic evidence suggested that broadly defined environmental factors interacting with genetic factors had an important role in causing NTD. Because of marked changes in incidence in many area of the world, it was clear that some important factor must have affected large segments of the population. The increased risk of NTD among people in lower socioeconomic groups has offered a clue to the factor that make poor family different from rich families. These may lead to poor nutrition. Poor nutrition, as the one factor, depend on other factors such as cultures, social class, countries and importantly effected by person's genetic makeup.[37]

Several studies suggest that complex interactions may occur among genes, between alleles of the same gene, and between certain genotypes and environment factors. For example, the risk of NTD might vary if mutations *MTHFR* occurred with mutations in other folate-related genes[54-56] or if two different mutations in the *MTHFR* gene occurred together.[28,57] Some studies also suggested that the risk related with maternal vitamin B12[58] or folate level.[59] All suggestion of the role of gene and environmental factors in NTDs are still needed to confirm and warrant further investigation. However, these interaction between genetic and environment factors suggested in NTDs, tend to favor to development of FEEM as considered to their similarity in pathogenesis.

2. Clef lip with or without clef palate (CL/P)

Nonsyndromic clef lip with or without clef palate (CL/P:MIM119530) is among the most congenital anomalies occurs in approximately 1/700-1/1,000 in Caucasians [12,60] and 1/600 in Thais.[12] The frequency of CL/P is highest in American Indians (over 3.6 per 1,000 births)[12,61] Clinically, there is great variability in the extent of clefting. Microforms include bifid uvula, submucous clefting of thesoft palate or linear lip indentation. Cleft Lip (CL) is a developmental defect that occurs in the womb in the fourth to six weeks of life. Basically, it means that portions of the upper lip do not fuse together, which creates an opening in the lip between the mouth and nose on the right, left site, or on both sites.

Cleft palate (CP) is a birth defect that occurs in 8^{th} to 12^{th} weeks after conception. It is an opening in the roof of the mouth that exists because structures between the mouth and the nose did not fuse together properly. The roof of the mouth is divided in two parts, the hard and soft palate. The hard palate does not move and provides bony separation between the front portions of the mouth and nose. The soft palate consists of muscles , and typically elevates during speech and swallowing to separate the nose and the mouth. In mild forms of CP, there is just a slight notching of the soft palate. Most clefts of the palate involve both the soft and hard portions of the roof of the mouth. A child can have a cleft lip, a cleft palate or both.

Etiologies

The etiologies of CL/P is suggested to caused by the multifactorial factors in which environmental and genetic factor also play a joint role.[15,16]

Genetic factors

There are certain generalities about the genetics of CL/P. Given this consideration, the risk of normal parents to have child affected with cleft lip will be approximately 1 per 1,000. However, in family having the affected first born tend to have affected second born with approximately 4 percent.[62] Additionally, the risk for third affected child becomes approximately 10 percent. Calculation of recurrent risk for CL/P must be individualized and will dependent on multiple factors, including associated

syndromes. Segregation analysis and epidemiological studies have shown that 25-35 percent of CL/P and 10-20% of Cleft palate only (CPO) patients have a family history of clefting and that simple Mendalian-inheritance in families segregating clefting.[63,64] Molecularly, approximately 3 percent of orofacial clefting disorders are believed to be related etiologically to a single gene aberration that is associated with a syndrome.[65] In addition, CL/P are heterogeneous traits with an estimated 2-20 genes interacting multiplicatively to cause clefts, including possible major gene that may account for 10 to 50 percent of incidence of this birth defect.[16,66,67] To identify gene(s) involved in Cl/P, association and linkage studies were evaluate candidate genes. Association with *TGFA, RARA,D4S191* and *BCL3* have been found for CL/P.[64,68]

Environmental factors

Recent studies revealed effects of a variety of physical and chemical agents on lip and palate development. However, most of these studies documented on animal models and it is recognized that teratogens are the major specific in their effects. 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.[69,70] Ericson et al [71]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. 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.[47-49] There is some evidences for this in humans,[49] and that administration of vitamins, in particular those of the B group, can prevent the occurrence of CL/P.[72,73] In 1982, Tolarova[74] reported that periconceptional vitamin supplementation plus 10 mg of folic acid, one of vitamin B, reduced the recurrent risk of cleft lip. To date, the possible preventive of folic acid has not been rule out.

Genes and environments

As other complex diseases, disorder with pure genetic etiology without environmental influence is difficult to characterized. Etiologies of general complex

disorders including CL/P are also explained by multifactorial model. In CL/P, one of the most famous research on gene-environment is considered to gene and nutrition status. It has been suggested that maternal folic acid supplementation in early pregnancy decreases the recurrence risk of CL/P.[23] Although folate's protective effect is recognized, the mechanism by which some people develop low folate level is still matter debate on birth defects including CL/P. Recent studies focus on interaction between CL/P and *MTHFR* polymorphism and lead to a number of further studies[75,76] documented on the environmental factor such as nutrient status of individuals and *MTHFR* gene.

3. *MTHFR* Gene and Gene Product

The *MTHFR* gene is located on chromosome 1 at 1p36.3. The cDNA sequence is 2.2 kilobases long and appears to consist of 11 exons[77]. Alternative splicing of the gene has been observed both in humans and in mice[77]. The major product of the MTHFR gene in humans is a catalytically active 77-kDa protein, although a smaller isoform of approximately 70kDa has been observed in some tissues[78]. MTHFR catalyzes the conversion of 5,10 methylenetetrahydrofolate into 5-methyltetrahydrofolate (5-MTHF) which is the major circulating form of folate (Figure 3).

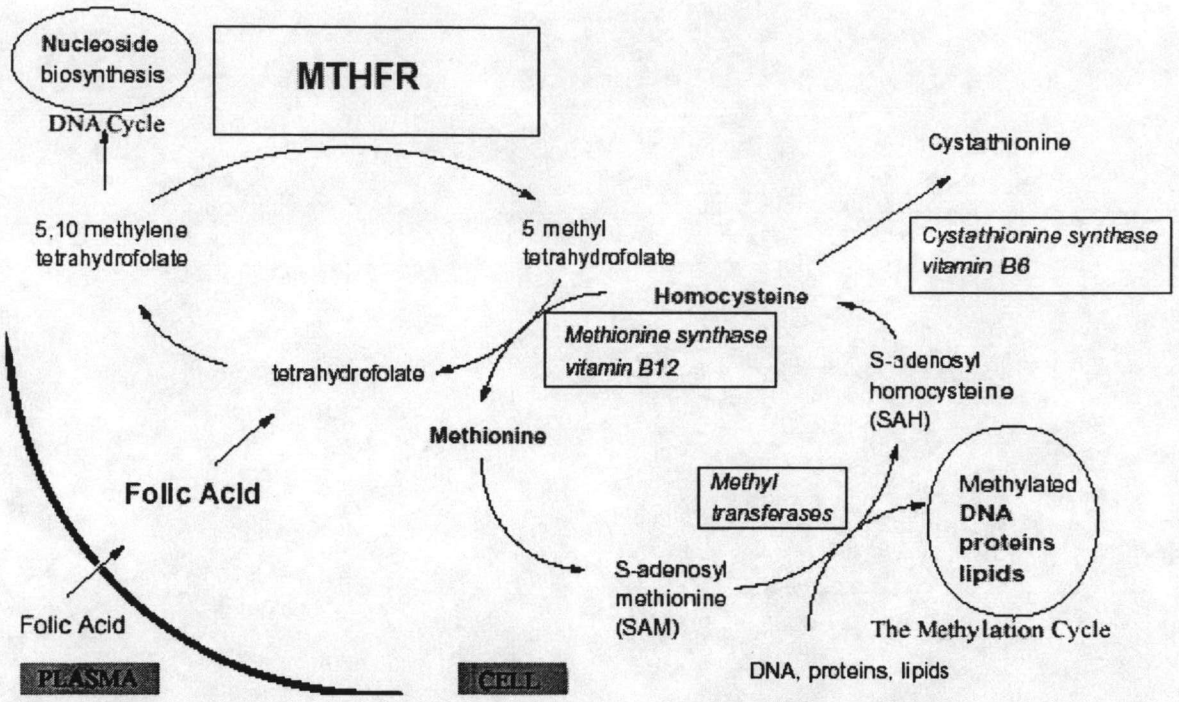

Figure 3 simplified metabolic pathways involving *MTHFR*

The biochemical pathways involving folic acid and MTHFR are complex and have been reviewed in detail[79]. Briefly, 5-MTHF, the methylated form of folate, provides the carbon moiety that is used to convert for example homocysteine into methionine, a reaction catalyzed by methionine synthase. The remethylation of homocysteine to methionine is an important step in the metabolic network that regulates the biosynthesis of nucleosides, the methylation of DNA, proteins and lipids, and the levels of homocysteine and methionine (Figure 3). The metabolic network is complex and relies on multiple activators and inhibitors. For instance, a derivate of methionine, S-adenosyl methionine (SAM) (Figure 3), is an allosteric inhibitor of MTHFR and an activator of cystathionine ß-synthase and regulates two main outflow paths of homocysteine. Although the complete effects of normal and abnormal folate metabolism are still incompletely understood, there is growing evidence that normal MTHFR activity may contribute to maintaining the pool of available circulating folate and methionine and prevent a buildup of homocysteine; conversely, abnormally low MTFHR activity may lead to lower levels of circulating folate, lower availability of methionine, and higher levels of homocysteine.

How these biochemical effects translate into derangements of developing embryonic structures is still unknown. On a basic level, methionine is used in the formation of SAM; SAM in turn plays a major role in many methylation processes, including methylation of DNA, proteins, neurotransmitters, and phospholipids. It has also been suggested that homocysteine or its derivatives may be toxic in high doses to developing tissues[80]. To add to the system's complexity, many enzymes in addition to MTHFR are involved in folate-related metabolic pathways (Figure 3).[79]

MTHFR Gene Variants

Of the gene variants reported to date, most were identified in patients with homocystinuria, a rare, severe, autosomal recessive metabolic disorder. These 14 mutations were associated with severe MTHFR deficiency[81-83] and are individually very rare, each having been found in only one or two families. Because these variants have not been linked to common birth defects so far, they will not be discussed further.

Two MTHFR variants, however, the 677C→T and the 1298A→C polymorphisms, are common in many populations and have been studied in relation to birth defects, mainly spina bifida and anencephaly. One study reported a third common polymorphism (1059T→C), that was described as a silent polymorphism apparently cotrasmitted within the same gene that harbored the A1298C polymorphism in that particular study group. The 677C→T and the 1298A→C polymorphisms will be the focus of this review. Although these two variants also have been associated with an increased risk for diseases other than congenital malformations, in particular adult cardiovascular disease, stroke, and coagulation abnormalities, these associations will not be discussed.

677C→T Allele

The 677C→T allele is a single base pair polymorphism in which a cytosine is converted to a thymine at basepair 677, resulting in an amino acid substitution (alanine to valine)in the enzyme [27,78]. The molecular genetics of the polymorphism have been recently reviewed[78]. Functionally, the encoded protein has a reduced enzymatic activity at 37 degrees C[84] and higher, so that the 677C→T polymorphism is often termed

"thermolabile". For instance, compared to similarly treated controls, samples from 677C→T homozygotes have 50 to 60 percent lower MTHFR activity at 37 degrees C, and about 65 percent lower activity at 46 degrees C[78]. Heterozygotes are in the intermediate range.

The variations in MTHFR activity associated with the 677C→T polymorphism appear to correlate with biochemical abnormalities. For instance, compared to controls, 677C→T homozygous persons have higher plasmatic levels of homocysteine[78] ; the homocysteine levels, however, depend in part on folate levels in that homocysteine is increased among those in whom plasma folate is at the lower end of the normal range, but not when folate levels are normal. Erythrocyte folate, on the other hand, is not decreased, since 5-MTHF is the major circulating form of folate and not a significant storage form[78].

1298A→C Allele

The 1298A→C variant of MTHFR was first identified in 1995 during a study of ovarian cancer[85]. In the 1298→C allele, a point polymorphism in exon 7 results in the coding of a glutamate instead of an alanine residue [28,85,86]. This allele has also been termed 1289C→A polymorphism by other authors.[57]

Functionally, the polymorphism results in mildly decreased MTHFR activity [28,86]. Unlike the case with the 677C→T polymorphism, neither homozygous nor heterozygous individuals had significantly higher plasma homocysteine or lower plasma folate compared to controls[28,86]; however, persons who were compound heterozygotes for the 1298A→C and 677C→T polymorphisms presented a biochemical profile that included increased homocysteine and decreased plasma folate levels similar to that seen in persons homozygous for the 677C→T polymorphism[86].

Folic acid

Folic acid (folinic acid, folacin, pteroylglutamic acid) was first isolated from spinach leaves (its name derives from the Latin *folium* meaning "leaf") in 1964 and early on was found to be essential in the prevention of anemia in animals. Later it was

discovered that ensuring that mothers had adequate folic acid levels could prevent neural tube defects in human babies.

Folic acid is essential for the synthesis of adenine and thymine, two of the four nucleic acids that make up our genes, DNA and chromosomes. It is now also clear that folic acid is required for the proper metabolism of the essential amino acid methionine that is found primarily in animal proteins. Methionine is converted to homocysteine in the body. Homocysteine, in turn, may be converted back to methionine in a process requiring folic acid (tetrahydrofolate) and vitamin B12 (cobalamin) as a catalyst or it may be metabolized into cysteine in a process catalyzed by vitamin B6 (pyridoxine). Cysteine is a vital link in the synthesis of glutathione, one of our most important antioxidants. A high blood level of homocysteine has been found to be highly detrimental to health and is invariably accompanied by a low level of folic acid.[87,88]

Folic acid protects the brain

A low or deficient blood level of folate (folic acid) has been detected in 15 to 38 per cent of adults suffering from depression. There is now increasing evidence that supplementation with therapeutic amounts of folate can significantly improve the condition of depressed patients. In a recent trial involving 20 elderly patients with depressive disorders, treatment with 50 mg/day of methylfolate was associated with an 81 per cent response rate within six weeks. Folate supplementation (15 mg/day of methylfolate) has also been found to markedly improve the effect of treatment with standard antidepressants. Researchers at the Harvard Medical School point out that chronic diseases (e.g. rheumatoid arthritis), certain cancer treatments, alcoholism, and a poor diet can all lead to a folate deficiency and the potential for depression.[99,90]

Research has also shown that many drugs such as methotrexate, levopoda, niacin, phenytoin (Dilantin), carbamazepine, and theophylline can markedly reduce folate levels.[88,91,92]Researchers at Oxford University recently reported that Alzheimer's patients have substantially lower levels of folic acid and vitamin B12 than do normal people of the same age. They also found that a high homocysteine level is a potent risk factor for Alzheimer's disease (AD); study participants with a level above 14 micromol/L had an almost five times higher risk than participants with levels below 11 micromol/L.

Participants with low folate and vitamin B12 levels had a three to four times higher risk of AD than did people with normal levels.[93]

Folic acid is especially important for women

It is now firmly established that women can reduce their risk of giving birth to a baby with neural tube defects (e.g. spina bifida) by supplementing with folic acid prior to conception and during pregnancy.[91,92,94] Perhaps less well known is the finding that women can also markedly reduce their risk of giving birth to a child with a cleft lip or palate by supplementing daily with a multivitamin containing 0.4 to 0.8 mg of folic acid.[23] These findings are particularly important in view of the fact that oral contraceptives reduce folate levels significantly. Women who have been "on the pill" need to boost their folate status if they are planning a pregnancy.[95-97]

Low folate levels are also heavily implicated in the development of cervical cancer. Cervical dysplasia is the precursor of cervical cancer and is usually first detected through a routine Pap smear. Fortunately, folate supplementation (0.8-3.0 mg/day) is very effective in reversing cervical dysplasia and preventing the cancer.[98-100] Researchers at the Harvard Medical School recently reported that women who supplemented with folic acid (0.4 mg/day or more for at least 15 years) had a four times lower risk of developing colon cancer than did women with a daily intake of 0.2 mg/day or less (the daily contribution of a typical North American diet)[101-103] Many postmenopausal women have increased homocysteine levels that are believed to contribute to the risk of osteoporosis; folate supplementation can reverse these high levels.[98]

There is no question that folic acid is extremely important to health and wellbeing. Not only is it important for heart health, mental health and women's health, but it is now also clear that it affects many other facets of health and disease. Researchers at the Cleveland Clinic Foundation have found that patients with end-stage renal disease have extremely high homocysteine levels and can be protected from cardiovascular events by supplementing with folic acid, vitamins B6 and B12.[104] Diabetes patients tend to have high homocysteine levels and folate is especially important for them.[105] Recent

research has also shown that low folate levels (high homocysteine levels) are implicated in age-related hearing loss, psoriasis, and restless leg syndrome.[106-108]

It is indeed astounding that one single vitamin, folic acid, can have such a profound effect on our health and yet perhaps it is not so surprising when one considers its vital role in DNA synthesis and folate-homocysteine metabolism (figure3).

Strategies of identifying genes

To identify human disease genes, there have been 4 approaches overlapping together which are generally suggested:

1) Functional cloning : this approach will be employed to identify gene if information of gene functions are available. Base on the biochemical knowledge, several methods were used to test function or biochemical product of those gene.
2) Positional cloning : The data sets of chromosome locations, imply to genetic markers, are available for identifying gene by using linkage analysis. This approach is suggested to identify disease gene if no biochemical data is presented.
3) Candidate gene approach : This approach requires sufficient information of molecular basis of pathogenesis or the knowledge of animal experiment in which gene function is identified. This approach seem to call the reasonable guess.
4) Positional candidate gene approach : This strategy combines the positional and candidate gene approach together. To date, it's powerful method to identify human disease gene.

Mapping genes for complex disease.

The term of complex disease refer to trait phenotype with unknown mode of inheritance. Since it was suggested to be dealt with a number of gene, it is difficult to map it's related genes, However, the availability of high-quality, dense genetic markers, have led to effective way to identify genes of complex traits. No single method is optimal to identify gene of complex disease. A multiapproach was suggested.[109]

1) Linkage analysis is widely used and appear to be useful since the increasing availability of more markers across genome are available. Linkage analysis is employed to test linkage within family by using multipoint analysis to test linkage between closely linked markers and a disease-predisposing gene.[110,111]

2) Association study is the most common approach widely used to mapping gene. There are two type of association study.

2.1 Case-control study which is used to compare allele frequencies in set of unrelated affected individuals to those in set of matched controls. The control populations should be matched with respect ethnicity as well as other factors such as age. However, spurious associations may result from population stratification, for example, the existence of multiple population subtypes in what is assumed to be relatively homogeneous population. A strategy, applicable to both simple and compiex disease, is allelic association (often referred to as linkage disequilibrium). This method only works when most (or all) of the individuals with the disease in the study population are descended from the individual in whom the disease mutation originally occurred. In such cases, there will be a difference in allele frequencies between individuals with the disease and the general population, for markers very close to the disease gene. This method is widely used when candidate genes are being tested, by studying alleles of the candidate gene for differences in frequency between patients and controls.

2.2 Family-based study is designed to avoid problem of population stratification by using family members such as parents to be internal control. Two family-based approach widely used including.

2.2.1 The affected-family-based control (AFBAC) method[112] which uses parental and proband genotypes to compare all transmitted allele with untransmitted allele.

2.2.2 The transmission disequilibrium test (TDT)[113] which is also used to test difference between transmitted and untransmitted allele derived from parents.

AFBAC has greater power to defect association than TDT has when no population structure exists, which can be proved by Hardy-Weinberg equilibrium for two generation.[114] In the presence of population structure TDT is more powerful, since AFBAC loses power by a larger denominator.

Overview, regardless statistic artifact or population stratification, the presence of association between a disease D and allele A is due to the following[115]

1) Natural selection ; people who have disease D 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 D. A allele is neither necessary nor sufficient for individuals to develop disease D, but it increase likelihood. Also, A allele is expected to associate with disease D 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 D. The particular allele at A locus (A_1,A_2,...) that is associated with disease D may be differed in different population.

Polymorphism in human DNA[115]

Genetic polymorphism is defined as the occurrence of multiple alleles at a locus, where at least two alleles appear with frequencies greater than 1 percent. There are millions of sites in human DNA that are different between individuals. These include:

1. Restriction fragment length polymorphisms (RFLPs) result from a mutation in a restriction enzyme site. Detected by Southern blotting/hybridisation or by PCR/restriction enzyme analysis.

2. Tandem repeat sequences (also known as microsatellies), such as dinucleotides $(CA)_n$, tri- and tetra-nucleotides, that are variable for the number of repeats. They are detected by PCR across the repeat and gel electrophoresis.

3. Single nucleotide polymorphisms (SNPs) in genes or in non-coding DNA, that may or may not affect phenotype.

Most of the first 2 types of polymorphism are in non-coding DNA. They have been used to construct complete linkage maps of all the human chromosomes. The third type (SNPs) are currently the focus of much interest, since it is believed that SNPs in many genes are responsible for susceptibility to complex disease.