CHAPTER V

DISCUSSION

Down syndrome, or trisomy 21, is a complex genetic disease resulting from the presence of three copies of chromosome 21. It is the leading genetic cause of mental retardation and is estimated to occur 1 of every 150 conceptions and ~1/600 – 1,000 live births. The origin of the extra chromosome is maternal in 95% of cases and is due to the failure of normal chromosomal segregation during meiosis (non disjunction). Despite its prevalence and consequence, the biochemical and molecular mechanisms the predispose to maternal non disjunction are not understood. Until recently, the only well established risk factor for Down syndrome is advanced maternal age and the associated risk increase exponential at age >30 years.[27]

A dietary folate and methyl deficiencies in vivo result in DNA hypomethylation[32-33], DNA strand breaks [34], abnormal gene expression [35-36], defective chromosome recombination [34,37,106,107] and abnormal chromosome segregation.[31,108,109] It has therefore been suggested that gene-nutrient interactions affecting the one-carbon metabolism may increase the risk of chromosome nondisjunction.

On the basis of the evidence that abnormal folate and methyl metabolism can lead to DNA hypomethylation and abnormal chromosome segregation. In this study, we aim to find out the association between *MTHFR, MTRR* and *MTR* polymorphisms in Thai mothers who had children with Down Syndrome and to determine the prevalence of *MTRR* 66A->G and *MTR* 2756A->G in Thai population.

We have established *MTHFR, MTRR* and *MTR* genotypes in 109 Thai mothers having children with Down syndrome and 186 controls. There were no significant differences between groups in term of mean age at conception (χ^2=0.034, P = 0.85).

Our results do not support the presence of an increased risk of Down syndrome in mother carriers of the *MTHFR* 677T allele in Thai women population. These results were consistent with previous four case-control studies as shown in table 2.

In this study, 186 control individuals were found to have the 677T allele frequency of 0.15. Which is consistent that of 0.15 [101] and 0.142 [116] reported in the

previous report in Thai population and 0.164 in the Indonesian population.[117] However, the frequency in Southeast Asia is relatively low when compared with the 677T allele frequencies of 0.41-0.48 among Hispanic [27,118,119], 0.4 in Sicily, 0.24-0.43 [40] in Europe, 0.34 among European American, 0.352 in Japan and 0.38 in China.[77,114] However, the T allele frequency of 0.06 among African is lower than ours.

We further analyzed the *MTHFR* 1298A->C polymorphism among the same samples. The frequency of variant C allele in Thais was found to be 0.28 which consistent with that of 0.25 reported among Thai population in previous study[101] and 0.20 reported among Chinese population in Taiwan[121] In Europe, it is responsible for 0.35 in Netherlands, 0.33 in USA[74] and 0.31 in Austria.[122] However, we did not find any significant differences in case mother compare to age-match control mothers.

Similar to the variant of 677T allele frequency, 1298C allele frequency among Thais was found to be lower than those found in Caucasians. These suggest that frequencies of variant allele of these two *MTHFR* polymorphisms are depended on population ethnic. The reason for the variation among many population still unclear, the possible explanation of the low frequencies of 677Tallele and 1298C allele in Thai population are cause founder effect. However, additional studies should be conducted to evaluate the reason of variation in distribution of a common *MTHFR* polymorphism in different ethnic groups.

This may explain by MTHFR genotype distribution, the previous reports on 677C->T genotype distribution appears in high variance among each ethnic group and geographical area.

Moreover, the present study examines the genotype distribution of each locus (nucleotide677 and nucleotide1298) separately, combined and also examines the haplotype distribution. In addition, this study evaluates the association between genotypes of each polymorphisms and risks of mother for having children with Down syndrome.

In case of *MTRR* polymorphism, 186 control individuals Thais were found to have the 66 G allele frequency of 0.28. In North American, it is respondsible for 0.47 [27],0.56 [69] in Irish and 0.53 in Sicily.[40] The frequency of the *MTRR* 66G allele were not significant higher among case mothers than among control mothers in our study

(χ^2 =0.086, P = 0.769). However, there is no published data on allele frequencies or genotype distribution in Thais before that we had more difficulty evaluating potential bias in the association between *MTRR* and Down syndrome.

Furthermore, we analyzed the *MTR* 2756A->G polymorphism among the same samples. The frequency of variant G allele in Thais was found to be 0.15 which consistent with that of 0.10 reported among Sicily population. [40] However, The frequency of the *MTR* 2756G allele were not significant higher among case mothers than among control mothers in our study (χ^2 =0.315, P = 0.575). As same as *MTRR* 66A->G, there is no ppublished data on allele frequencies or genotype distribution in Thais before.

The allele frequencies of each polymorphism vary among populations in different geographical areas and may explain the discrepancies observed among studies performed in North America, Northern Europe, and Southern Europe. [69]

We hypothesized that one or combined polymorphisms of *MTHFR*, *MTRR* and *MTR* are associated with the risk factor for having children with Down syndrome similar to many studies previously considered. We first analyzed each locus separately and, then combined loci. We found no significant difference in genetic polymorphisms distribution of *MTHFR*, *MTRR* and *MTR* between case mothers and control mothers. Single locus analysis examine each locus separately did not show association between all genotypes and risk for having children with Down syndrome in Thai woman population. After combined genotype was analyzed, no association between all combined genotypes was found, eithers.

Moreover, the present study examines the haplotype distribution and also examines the association between *MTHFR* haplotypes and risk of mother for having children with Down syndrome. We failed to find any difference in the frequency of this genotype between case mothers and control mothers.

In addition, a previous study indicated that the 677C->T mutation in the *MTHFR* gene was a risk factor in mothers aged 30 years old or less to have children with Down syndromes[27]. Of our 109 case mothers, 32 were $\leq$ 30 years old when their Down syndrome children were born. 64 out of 186 control mothers were $\leq$ 30 years olds.

There were no significant differences between groups in term of mean age at conception (χ^2=0, *P* = 1). We found no significant difference in genetic polymorphisms distribution of *MTHFR*, *MTRR* and *MTR* between these two groups. However, Hassold et al[123] found no evidence of age-related alterations in genotype frequencies at either MTHFR or MTRR of mothers having children with Down syndrome. Thus, our results do not support that SNPs in folate metabolism is a age-related risk factor in mothers having children with Down syndrome.

Cautiously, our case mothers are not homogeneous. Because of Trisomy 21 can be resulted from non disjunction in maternal (75%) and paternal (25%) meiosis but we did not determine this. In addition, it could occur in meiosis I or meiosis II. A further study should determine the parental origin and the origin of meiotic stage of the extra chromosome. Although, the extra chromosome 21 was know to be maternally derived. Hassold et al [123]found no evidence of an association between genotype and the stage of origin of trisomy, since the genotypic distributions were similar for MI- and MII-derived cases. Additionally, not only impairment of *MTHFR, MTRR* and *MTR* enzymes which are responsible for elevated homocysteine levels, the other enzymes in folate-homocysteine metabolism such as cystathionine β–synthase (CβS), betaine methyltransferase, trifunctional enzyme methylenetetrahydrofolate dehydrogenes/ methylenetetrahydrofolate cyclohydrolase/formyltetrahydrofolate synthese (*MTHFD1*) and s-adenosyl homocysteine hydrolase should be documented to their susceptibilities for having children with Down syndrome. Finally, it could be that the previous results on trisomy 21 were fortuitous and that maternal folate polymorphism have little, if any, independent effect on meiotic non disjunction in humans. Moreover, a previous study failed to observe a decline in the occurrence of trisomy 21 following folic acid food fortification.[124] This idea suggested that other susceptible genes may be responsible for nondisjunction in addition to the effects from genes in folate-homocysteine metabolism.