CHAPTER II

REVIEW OF RELATED LITRATURE

1. Down syndrome

Down syndrome (OMIM 190685) is the commonest chromosomal anomaly with an incidence of ~1/600 – 1,000 live births[1]. Most Down syndrome cases result from total trisomy 21 (95%), translocations involving chromosome 21 (4%) and with trisomy 21 mosaicism (1%).[3-9]

Clinical features

Down syndrome was first described by JL Down in 1866 [2], includes a phenotype with mental retardation caused by a microscopically demonstrable chromosomal aberration; characteristic facies with oblique eye fissure, epicanthus, flat nasal bridge, protruding tongue; short broad hands and wide space between first and second toes, hypotonia and other associated congenital anomalies and development disorders.

Individual with Down syndrome often have specific major congenital malformation such as those of the heart (30-40% in some studies), particularly the atrioventricular canal, and of the gastrointestinal tract, such as duodenal stenosis or atresia, imperforate anus, and Hirschsprung disease.[43-45] In addition, 90% of all Down syndrome patients have a significant hearing loss, usually of the conductive type.[46]

Etiologies

Involving total trisomy 21 results from nondisjunction, usually in formation of the eggs or sperm, where a gamete ends up with an extra chromosome 21. Non disjunction may occur in the first meiotic stage (MI) or second meiotic stage (MII).[16-19]

The extra chromosome 21 is of maternal origin in 80-93% of the cases and paternal origin in 7-20 percent of the cases.[11-15] Plausible biological explanation for the predominance of maternal non disjunction is in the human male, meiosis begins with puberty and the important events are sequential; in adult testis, cells progress from prophase to metaphase I and on to metaphase II without an interventing delays. By

contrast, the meiotic process in the human female: all oocytes enter meiosis I during fetal development, undergo DNA replication and homologous recombination and then remain arrested in prophase I (diplotene stage) for several decades until initiation of oocyte maturation and ovulation in the adult female.[20]

Among trisomy 21 cases of maternal origin, approximately 75 percent result from nondisjunction in MI and 25 percent in MII while 40 percent of trisomy 21 cases of paternal origin occur from nondisjunction in MI and 60 percent from nondisjunction in MII.[16-19]

Demographic factor

The only well established risk factor for Down syndrome is advanced maternal age. The association between increasing maternal age and Down syndrome was recognized as early as 1933 [47] more than 25 years before it was determined that Down syndrome was caused by trisomy 21. Age-specific rates have been well documented. One study found that women who had a reduced ovarian complement (congenital absence or removal of an ovary) were at increased risk of having an infant with Down syndrome. [23-24] This may suggest that the increased risk of Down syndrome with increased maternal age may be related to the physiological status of the ovaries or the eggs. Other potential explanations for the association between Down syndrome risk and advanced maternal age include delayed fertilization, changing hormone levels, and "relaxed selection". [23]

In a few studies, advanced paternal age (>49 years) has been associated with increased risk of Down syndrome births. [48-51] The risk for advanced paternal age has not been large, and is considerably diminish with the appropriate adjustment for maternal age. A large number of studies have failed to find evidence of the effect. [8, 52-53] Moreover, some studies found an association between risk of Down syndrome and age of the maternal grandmother at the mother's birth. [54-55] Female meiosis start in fetal life and nondisjunction in the first meiotic division of female might be induced during the fetal period, especially if her mother is order.

Prevalence of Down syndrome is known to vary by race/ethnicity. Hispanic infants exhibit higher rates of Down syndrome than other infants, even after differences in maternal age was considered. [56] Another studies also found Down syndrome rates to be highest in Hispanics, followed by Asians, white, Native American, and African-America.[57]

Environmental factors

A recent investigation reported that risk of a recognized Down syndrome conceptus was reduced with high alcohol consumption[31] and high coffee consumption. Smoking did not appear to affect risk of having a recognized Down syndrome conceptus.[58-59] The authors suggested that high coffee consumption may reduce the viability of a conceptus with Down syndrome so that the conceptus may be lost in early pregnancy. Other environmental have been suggest, including parental irradiation [60], oral contraceptives and fertility drug [61], thyroid antibodies [62], seasonally [63], parity [64], maternal diabetes[65], consanguinity [66], and the presence of certain types of chromosome polymorphism.[67] However, none of these or any other association have been proven.

Genetic factors

Many studies have reported that women who had infants or fetuses with Down syndrome were more likely to have abnormal folate metabolism and mutations in methylenetetrahydrofolate reductase (*MTHFR*), methionine synthase reductase (*MTRR)* gene and methionine synthase (*MTR*) gene [27,40,68-69] as shown in table 1.

Table 1. Results of previous studies that found the association between SNPs of *MTHFR*, *MTRR* and *MTR* and the risk for having children with Down syndrome.

Reference	Number of Case	Study population	SNPs	OR (95 %CI)
Jame et al., 1999 [27]	57	North American	*1. MTHFR* 677C->T	2.6 (1.2-5.8)
Hobbs et al., 2000 [68]	157	North American	1. *MTHFR* 677C->T 2. *MTRR* 66A->G 3.Combined *MTHFR* with MTRR (677C->T + 66A->G)	1.91 (1.19-3.05) 2.57 (1.33-4.99) 4.08 (1.94-8.56)
O'Leary et al.,2002 [69]	48	Irish	1. *MTHFR* 677C->T 2. *MTRR* 66A->G 3. Combined *MTHFR* with *MTRR* (677C->T + 66A->G)	1.13 (0.6-2.2) 10.47 (1.4-78.6) 2.98 (1.19-7.46)
Paolo et al., 2003 [40]	63	Sicily	1.*MTHFR* 677C->T 2. *MTHFR* 1298A->C 3. *MTRR* 66A->G 4. *MTR* 2756A->G 5. Combined *MTRR* with *MTR* (66A->G+2756A->G)	No significant* No significant No significant 3.5 (1.2-10.9) 5.0 (1.1-24.1)

* No significant mean no significant differences in genotype frequencies between case and control

However, Contradictory findings have been published as shown in table 2.

Table 2. Results of previous study that failed to find the association between *MTHFR*, *MTRR* and *MTR* as the risk for having children with Down syndrome.

Reference	Number of Case	Study population	SNPs	OR (95 %CI)
Chadefaux et al., 2002 [70]	85	French	*MTHFR* 677C->T	No significant*
O'Leary et al.,2002 [69]	48	Irish	*MTHFR* 677C->T	1.13 (0.6-2.2)
Stuppia et al.,2002 [71]	64	Italian	*MTHFR* 677C->T	No significant*
Paolo et al., 2003 [40]	63	Sicily	1.*MTHFR* 677C->T 2. *MTHFR* 1298A->C 3. *MTRR* 66A->G	No significant* No significant No significant

* No significant mean no significant differences in genotype frequencies between case and control

2. *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.[72] The major product of the MTHFR gene in humans is catalytically active 77-kDa protein.

MTHFR or 5,10-methylenetetrahydrofolate reductase (OMIM 236250) acts at a critical metabolic juncture in the regulation of cellular methylation reactions, catalyzing the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, the methyl donor for the remethylation of homocysteine to methionine (Figure 1).

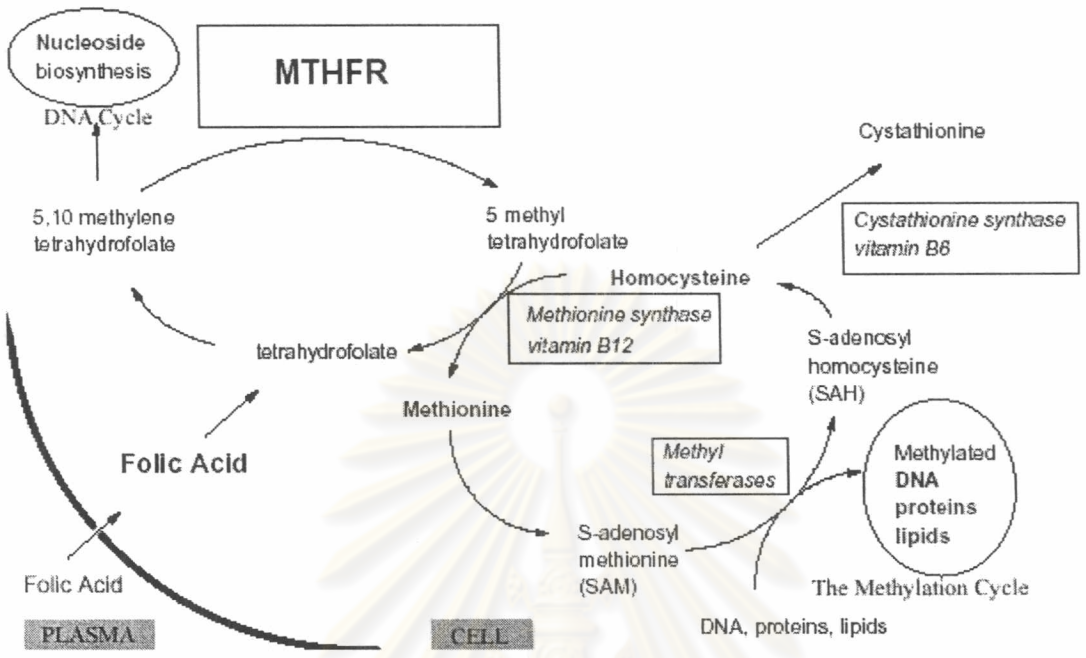

Figure 1. Simplified folic acid metabolic pathways involving *MTHFR, MTRR* and *MTR*

This reaction is important for the synthesis of S-adenosylmethionine (SAM), the major intracellular methyl donor for DNA, protein, and lipid methylation reactions. Reduced MTHFR activity results in an increased requirement for folic acid to maintain normal homocysteine remethylation to methionine. In the absence of sufficient folic acid, intracellular homocysteine accumulated, methionine resynthesis is reduced, and essential methylation reactions are compromised. An increase in homocysteine and a decrease in methionine results in a decreased ration of SAM to s-adenosylhomocysteine (SAH), which has been associated with DNA hypomethylation.[27]

MTHFR Gene Variants

677C->T Allele

The C to T transition mutation at position 677 within the *MTHFR* gene (677C->T) causes an alanine to valine substitution in the MTHFR protein and The 677 TT genotype of the MTHFR gene is associated with reduced in vitro enzyme activity. [73-74] Relative to the normal *C/C* genotype, the specific activity of the *MTHFR* is reduced approximately

35 % with the heterozygous *C/T* genotype and approximately 70% with the homozygous *T/T* genotype.[75]

Moreover, reduced MTHFR activity has been associated with mild hyperhomocysteinemia under conditions of impaired folate status [39,73] and may increase risk for neural tube defects [39,76-78], Down's Syndrome [27,68], cardiovascular disease [79], and some cancers [80-85]. However, the 677 TT genotype is also associated with decreased risk of colorectal neoplasias [86-87], acute leukemia in adults [88], and malignant lymphoma.[89] The protective effect of the 677 TT genotype in certain cancers may be related to the increased availability of 5,10-methylenetetrahydrofolate, which is required for normal DNA synthesis and repair.

1298A->C Allele

The A to C transition mutation at position 1298 within the *MTHFR* gene (1298 A-> C) causes a glutamine to an alanine substitution in the MTHFR protein and results in decreased in vitro MTHFR activity. Relative to the normal *A/A* genotype, the specific activity of the MTHFR is reduced approximately 20 % with the heterozygous *A/C* genotype and approximately 40% with the homozygous *C/C* genotype.[90]

In contrast to the C677T polymorphism, the A1298C polymorphism is not associated with increased plasma homocysteine [90-95] or lower blood folate concentrations.[91,93,96] Combined heterozygosity for the A1298C and C677T polymorphisms, however, is associated with reduced in vitro enzyme activity (~40% to 50% reduction [90], higher plasma homocysteine concentrations, and decreased plasma folate levels in some studies [91,96] but not all.[93,95] Preliminary data suggest that the A1298C polymorphism and/or combined heterozygosity for the two MTHFR variants may modulate risk for early onset coronary artery disease [97], neural tube defects [74,76,98], other obstetric complications [99,100], CL/P [101] and some cancers. [88,89,102]

3. *MTR* gene (*MS*) and Gene product

The *MTR* gene was mapped to 1q42.3-1q44.[103] The MTR cDNA probes detected 7.5 and 10-kb transcripts in a range of different human tissues and encodes 1,265 amino acids of protein.

Methionine synthase, a vitamin B12-dependent enzyme catalyzes the remethylation of homocysteine to methionine via a reaction in which methylcobalamin serves as an intermediate methyl carrier. Over time, the cob(I)alamin cofactor of methionine synthase becomes oxidized to cob(II)alamin, rendering the enzyme inactive. Regeneration of functional enzyme requires reductive methylation via a reaction in which S-adenosylmethionine is utilized as a methyl donor.

Methionine is an essential amino acid in mammals. It is required for protein synthesis and is a central player in one carbon metabolism. In its activated form, S-adenosylmethionine, it is the methyl donor in hundreds of biologic transmethylation reactions and the donor of propylamine in polyamine synthesis. The eventual product of the demethylation of methionine is homocysteine.

MTR Gene Variants

2756A->G Allele

The A to G transition mutation at position 2756 within the *MTR* gene (2756 A->G) causes a aspartic acid to glycine substitution in the MTR protein[103] but results of MTR activity from this substitution still unknown.

In 1996, Li [103]reviewed the role of *MTR* in homocysteine metabolism. They noted that loss-of-function mutations in MTR would cause increased levels of plasma homocysteine. They noted also that defects in MTR activity may play a role in tumorigenesis, since approximately 50% of tumor cells require the addition of exogenous methionine for growth and homocysteine and folate cannot replace methionine. He concluded that since methionine can only be synthesized by methylation of homocysteine, the inability of tumor cells to grow on homocysteine suggests that they have a defect in methionine synthase. In addition, patients of the cbl E complementation group of disorders of folate/cobalamin metabolism who are defective in reductive

activation of methionine synthase exhibit megaloblastic anemia, developmental delay, hyperhomocysteinemia, and hypomethioninemia.

The A2756G polymorphism is associated with decrease activity of methionine synthase (the specific activity of the MTR was not determine), increase plasma homocysteine or lower blood folate and methionine concentration.[42] In addition, Jacques et al [39] suggested that enzyme was associated with frequent polymorphism that alter the primary structure of the proteins and have been subject to extensive analysis of metabolic and disease associations such as spina bifida[38] and premature coronary disease. [104]

4. *MTRR* gene and Gene product

In 1998, Leclerc [105] cloned a cDNA corresponding to the 'methionine synthase reductase'. Northern blot analysis revealed that the gene, symbolized *MTRR*, is expressed as a predominant mRNA of 3.6 kb. The deduced protein, a novel member of the FNR family of electron transferases, contains 698 amino acids with a predicted molecular mass of 77,700. The MTRR gene was mapped to 5p15.3-p15.2 by a combination of somatic cell hybrid analysis and fluorescence in situ hybridization.

Methionine synthase reductase is reducing system required for maintenance of the methionine synthase in a functional state.

MTRR Gene Variants

66A->G Allele

The cloning of the cDNA for MTRR led to the identification of a polymorphism, 66A-G[105]

The A to G transition mutation at position 66 within the *MTRR* gene (66 A->G) causes a isoleucine to methionine substitution in the MTRR protein but results of MTR activity from this substitution still unknown as same as *MTR* 2756A->G.

In addition, Jacques et al [39] suggested that this enzyme had frequent polymorphism that alter the primary structure of the proteins and have been subject to

extensive analysis of metabolic and disease associations such as spina bifida[38] and premature coronary disease.[104]

Folic acid

Folic acid, also as folate, is a B-vitamin that can be found in some enriched foods and vitamin pills. Excellent sources of folic acid include nutritional yeast, rice or wheat germ, legumes and liver. Good sources include dark green leafy vegetables and nuts.

Moreover, folic acid is essential for the de novo synthesis of nucleotide precursors for normal DNA synthesis and is also essential for normal cellular methylation reactions. Chronic folate/methyl deficiency in vivo and in vitro has been associated with abnormal DNA methylation [32-33], DNA strand breaks [34], altered chromosome recombination [34,37,106,107], and aberrant chromosome segregation. [31,108-109] On the basic evidence, Jame et al (1999) [27] suggested the possibility that gene-nutrient interactions associated with abnormal folate metabolism and DNA hypomethylation might increase risk of chromosome nondisjunction.

5. Candidate gene approach[110]

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.

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.[111]

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.[66] 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 nucleotide polymorphisms (SNPs), repeat polymorphisms, and insertions or deletions. Most of the DNA sequence variation in the human genome is in form of SNPs. Information about the location and type of the sequence variants in a gene can be used to prioritize polymorphisms. 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.

6. The association study[112]

The association for SNPs and diseases is determined by the statistical methods. Relative risk or odds ratios 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.