CHAPTER I

INTRODUCTION

Background and rationale

Down syndrome (OMIM 190685) is one of the most common genetic diseases with an incidence of ~1/600 – 1,000 live birth. [1] It was first described by JL Down [2] in 1866 and includes a phenotype with mental retardation, 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.

Since etiologies of Down syndrome are considered, as much as 95 percent of Down syndrome is caused by trisomy 21 results from the presence of 3 copies of chromosome 21 and 5 percent is caused by translocations involving chromosome 21 and trisomy 21 mosaicism. [3-9] In the most cases of trisomy 21, the extra chromosome comes from the failure of normal chromosomal segregation during meiosis (meiotic nondisjunction).[10] The nondisjunction can occur during meiosis I (MI) when the chromosome pairs failed separate or during meiosis II (MII) when the chromatid fail to separate.

The extra chromosome 21 is of maternal origin in 80-93 percent of cases and of paternal origin in 7-20 percent of the cases [11-15]. 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] Plausible biological explanation for the predominance of maternal non disjunction is in human female primodial 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]

The only well established risk factor for Down syndrome is advanced maternal age.[21-22] Two studies 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 of the eggs.

For the birth prevalence rates of Down syndrome, women under the age of 25 years having the lowest prevalence rate (1/1,400 births). For women 35 years old, the rate is approximately 1/350 births and for women 45 years and older the rate rises to 1/25 births. [25-26] Although advanced maternal age is a major risk factor for trisomy 21, most children with Down syndrome are born to mothers less than 30 years of age.[27]

Despite the prevalence of this common genetics disease, the cellular and molecular mechanisms underlying meiotic nondisjunction and trisomy 21 are not yet understood.

Both clinical and experimental studies have shown that genomic DNA hypomethylation is associated with abnormal instability and abnormal segregation. For example, a rare autosomal disorder, ICF syndrome (immune deficiency, centromeric instability, and facies anomalies) is characterized by pericentromeric hypomethylation [28] and impaired chromosome segregation.[29] In the cultured plant and animal cells, chemically induced DNA hypomethylation with 5-azacytidine treatment induced chromosomal instability and aneuploidy.[30-31]

In 1998 Jame SJ [27] and others have shown that dietary folate and methyl deficiency in vivo results in DNA hypomethylation [32-33], DNA strand breaks [34] and abnormal gene expression. [35-36] On the basis of evidences that abnormal folate and methyl metabolism can lead to DNA hypomethylation, abnormal chromosome segregation and might increase risk of chromosome nondisjunction.

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.[37] DNA strand breaks, altered chromosome recombination, and aberrant chromosome segregation.

In pathway of folate and homocysteine metabolism, there are many metabolizing enzyme that related such as 5,10-methylene tetrahydrofolate reductase (*MTHFR*), methionine synthase reductase (*MTRR*), methionine synthase or methylene tetrahydrofolate reductase S-homocysteine methyltranferase (*MTR*). All of enzymes act at a critical metabolic juncture in the regulation of folate and homocysteine level by synthesis of S-adenosyl methionine (SAM), the major intracellular methyl donor for DNA, protein and lipid methylation reactions.

Polymorphisms in genes of folate and homocysteine metabolism that reduced enzyme activity lead to increased requirement for folic acid to maintain normal homocysteine remethylation to methionine. In the absence of sufficient folic acid, intracellular homocysteine accumulates, methionine resynthesis is reduced, and essential methylation reactions are compromised. An increased in homocysteine and a decreased in methionine results in a decreased ratio of SAM to S-adenosylhomocysteine (SAH), which has been associated with DNA hypomethylation.[27,38-42]

Although, many experimental studies have shown that polymorphisms in maternal genes of folate and homocysteine metabolism may be a risk factor for maternal meiotic nondisjunction and having a child with Down syndrome. However, the previous studies are documented in different population and their result is contradictory. Moreover, the polymorphisms in genes of folate and homocysteine metabolism among Thai women does not study before. In order to find possible maternal genetic risk factor to having child with Down syndrome in Thai women due to *MTHFR, MTRR* and *MTR* polymorphisms, this study aims to determine the prevalences of *MTRR* 66A->G and *MTR* 2756A->G in Thai population and to find out the association between *MTHFR, MTRR* and *MTR* polymorphisms in mother who having children with Down Syndrome.

Research Questions

1. What are the prevalence of *MTRR* 66A->G and *MTR* 2756A->G among Thai population?

2. Are polymorphisms in *MTHFR*, *MTRR* and *MTR* the maternal risk factors for Thai women to have children with Down syndrome?

Objectives

1. To determine the prevalence of *MTRR* 66A->G and *MTR* 2756A-G polymorphisms among Thai population.

2. To find association between polymorphisms in *MTHFR, MTRR* and *MTR* and mothers who have children with Down syndrome.

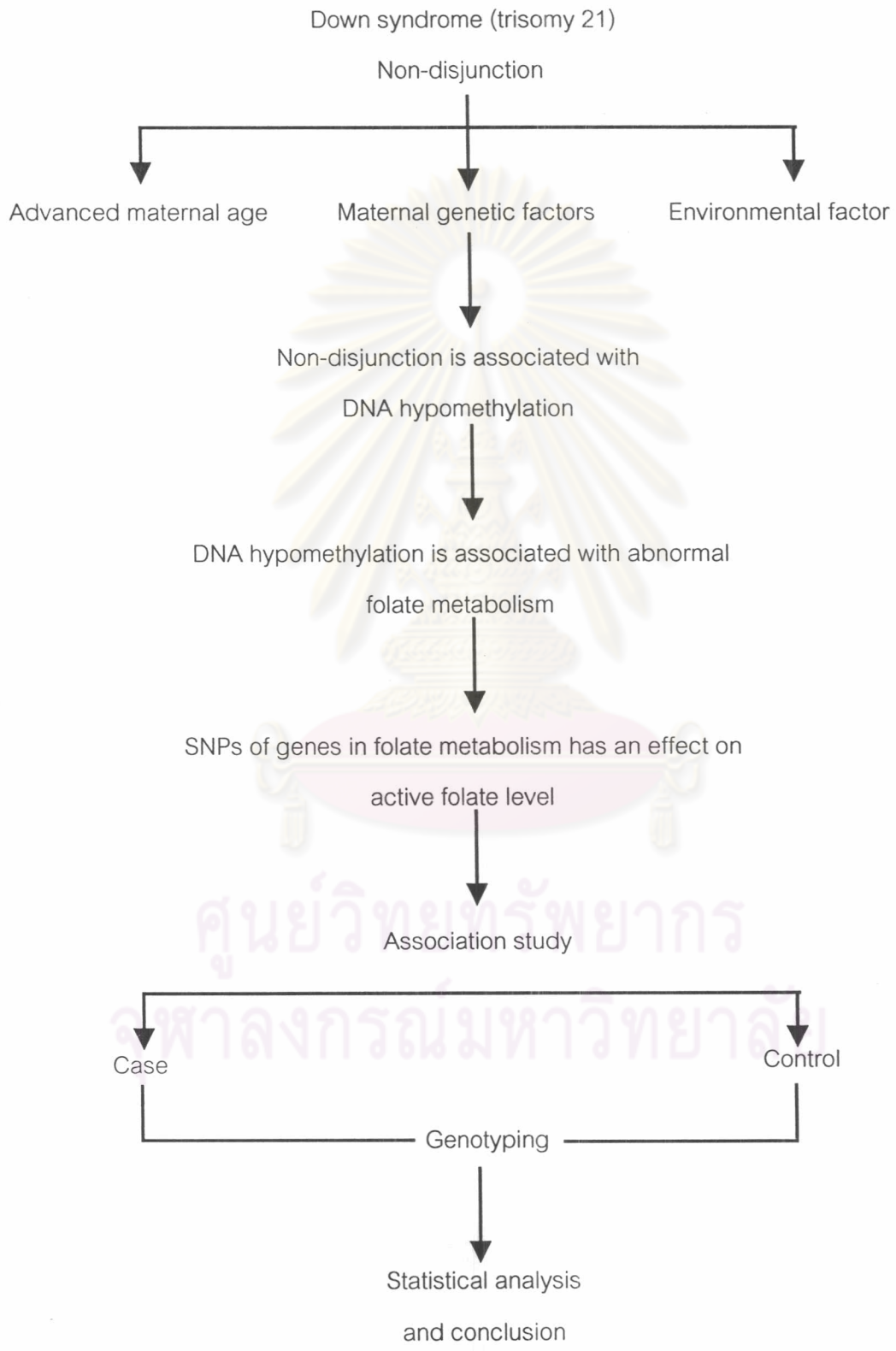
Hypothesis

Polymorphisms in *MTHFR*, *MTRR* and *MTR* gene in folate metabolism are maternal risk factors for having children with Down syndrome?

Assumption

The subjects in this study consist of 2 groups: 1) control used in this study were From the antenatal care clinic at King Chulalongkorn Memorial Hospital and were followed up to verify that their children were not dysmorphic 2) Case mothers were women who had children with Down syndrome presenting to Rachanukul hospital or King Chulalongkorn Memorial Hospital, Bangkok. Their children with Down syndrome had to have karyotypically confirmed full trisomy 21.

Conceptual framework



Limitation

Can not calculate the number of sample because *MTRR* and *MTR* gene do not have a previous study in Thai population.

Operational Definition

1. All genotypes are determined by RFLP patterns
2. This study considers many genotype, to avoid the misunderstood and their abbreviation, genotypes for the 677C->T polymorphism are represented by 677CC, 677CT, and 677TT whereas the 1298A->C genotypes are represented by 1298AA, 1298AC and 1298CC, respectively. In addition, genotypes for the 66A->G polymorphism are represented by 66AA, 66AG and 66GG while the 2756AA, 2756AG and 2756GG, respectively. In case of genotype combination for 677C->T and 1298A->C, 677C->T and 66A->G, 66A->G and 2756A->G, a "/" is used; for example 677CC/1298AA represents individual who is 677CC with 1298AA, whereas 66AG/2756AG implies individual who is 66AG accompanying with 2756AG.
3. In case of haplotypes, a '"-" is used; for example C-A/T-C means that one allele presents C and A at nucleotides 677 and 1298 respectively, and the other allele contains T at nucleotide 677 and C at nucleotide 1298. In addition, if we mention the presence of only one allele, we still use C-A to represent an allele that presents C at nucleotide 677 and A at nucleotide 1298.

Expected Benefit

The result from this study may uses to be an index for the risk to having children with Down syndrome in Thai women who have polymorphisms of *MTHFR, MTRR* and *MTR* gene in folate metabolism.

Research Methodology

1. Sample Collection

1.1 Cases are women who have children with Down syndrome coming to King Chulalongkorn Memorial hospital and Rachanukul hospital.

1.2 Controls are pregnant women coming for antenatal care at King Chulalongkorn Memorial Hospital.

For each case mother, one or two age-matched control mothers were recruited. Control mothers were Thai (They described themselves as "Thai" and could speak Thai clearly and fluently). In addition, they had to have experience no miscarriages, other abnormal pregnancies or had previous children with possible genetic factor related to *MTHFR, MTRR* and *MTR* SNPs, i.e. cleft lip with or without cleft palate (CL/P) and neural tube defect (NTD).

2. Process of study
    2.1 Blood collection
    2.2 DNA extraction
    2.3 DNA amplification
    2.4 Restriction enzyme analysis
    2.5 Agarose gel electrophoresis

3. Data collection and analysis