Down syndrome (DS) is characterized by trisomy of chromosome 21 as a result of defects in chromosome segregation during maternal meiosis in the majority of cases [1]. It represents the most common cause of mental retardation in humans, and occurs with a prevalence of 1 in 700–800 live births and 1/150 conceptions [2]. The molecular mechanisms underlying meiotic nondisjunction leading to trisomy 21 are still poorly understood and the major risk factor for trisomy 21 is advanced maternal age at conception [3,4]. Risk of having a child with DS is lower among mothers under age 20 (1/1,600) than in mothers over age 35 (1/370) [5], but since younger women have a higher birth rate, 75% of the affected children are born to women under age 35 [6]. James et al [7] reported an increased maternal risk of DS in mothers carrying methylenetetrahydrofolate reductase (MTHFR) gene polymorphism associated with elevated plasma homocysteine and low folate status was published.
Methionine synthase reductase (MTRR) is a key enzyme in folate dependent homocysteine metabolism and its gene is located on chromosome 5p15.2-15.3. This enzyme is responsible for methionine synthase (MTR) regulation by reductive methylation and disturbances in catalytic activity can lead to higher levels of homocysteine. MTRR catalyzes the conversion of the inactive form of methionine synthase (MTR) into its active form, by regeneration of methyl ([II])cobalamin, the cofactor of MTR. The most common polymorphism in MTRR gene is A66G substitution [8], leading to a change of isoleucine to methionine in amino acid 22 (I22M). The I22M variant is located in the putative FMN-binding domain of the MTRR enzyme that is suggested to interact with MTR. Substitution in this part of the enzyme thus disrupted the binding of MTRR to the MTR-cob(I)alamin-complex, thereby decreasing the rate of homocysteine remethylation [8]. In the last few years, a number of studies have evaluated a possible link between polymorphism in maternal folate metabolism genes and Down syndrome [9,10]. The present meta-analysis was carried out to assess the role of maternal MTRR A66G polymorphism are available in the literature. The present meta-analysis was carried out to assess the role of maternal MTRR A66G polymorphism and Down syndrome by conducting meta-analysis [9,10].

The world-wide frequency of A66G polymorphism is ~30% [7,11,12]. However, its frequency varies in different ethnic and geographical regions as reported by Rady et al [13] the lowest frequency in the Hispanic population (28.65%) compared to 34% among African-Americans, 43.1% among Ashkenazi Jews and among Caucasians (54.4%). Several conflicting or inconclusive results regarding role of maternal MTRR A66G polymorphism are available in the literature. The present meta-analysis was carried out to assess the role of maternal MTRR A66G genotype in the risk of DS offspring.

2. Materials and Method

Author assessed the association between the maternal MTRR A66G polymorphism and Down syndrome by conducting meta-analyses of published case-control genetic association studies. All research articles that investigate the association of the maternal MTRR A66G polymorphism with the risk of DS child published before December, 2010 were extracted by computer based search of 'Pubmed' database. Only free full case-control genetic association publications were included in the present meta-analysis. The control group included individuals who have no family history of any genetic and psychiatric disorder. Genome scans, linkage studies were not included only studies using validated method for case control studies were included in the present meta-analysis.

2.1. Search strategy

Author identified six eligible studies by searching Pubmed for all publications up to December 2010. Search terms were used “MTRR”, “Methionine synthase reductase”, and “A66G” in combination with “Down syndrome”.

2.2. Data extraction

Relevant information’s were extracted from all selected studies like- author name, journal name, year of publication and number of cases and controls for each A66G genotypes (AA, AG and GG genotypes). Allelic frequencies for the cases and controls were calculated from corresponding genotypes. Allele frequency was calculated by simple gene count method. In any large, randomly mating population, in which there is a constant mutation rate, and no migration or selection against a particular genotype, the proportions of the various genotypes will remain unchanged from one generation to another. To test for population stratification, the distribution of genotypes in control subjects of each individual population was tested for departure from Hardy-Weinberg equilibrium.

2.3. Meta-analysis

The present meta-analysis tested the overall association of G allele of MTRR with the risk of Down syndrome in comparison to MTRR A allele. The association was tested as odd ratios (OR) with the 95% confidence interval (CI). On the basis of individual ORs, a combined pooled OR was estimated. Author also tested heterogeneity between studies using Cochran’s chi-square-based Q-statistic and estimated the degree of heterogeneity with I2 (I2 = ((Q-(k-1))/Q) x100%, where k indicates number of studies). I2 ranges from 0% to 100%. It indicates the proportion of between-study variability in point estimates that was due to heterogeneity rather than sampling error [14,15]. An overall OR and 95% confidence interval (CI) was estimated under the Mantel-Haenszel’s fixed-effects model [16]. if there was no evidence for heterogeneity (I2 < 50%), otherwise (I2 = 50%) under the DerSimonian-Laird random-effects model [17]. The statistical analyses were performed using the program Meta-analysis with Meta-disc (version 1.4).

3. Result and discussion

Six studies were found suitable for the inclusion in the present meta-analysis [9,18,19,20,21,22]. The studies were carried out in Brazil [20], Italy [21], France [19], China [22], Ireland [18] and America [9]. Author has assessed whether the frequencies of AA, AG and GG genotypes among controls in individual studies were consistent with the expected distribution (that is in Hardy-Weinberg equilibrium) by using the x2 test. Genotypes were in Hardy-Weinberg equilibrium in all controls. Details of six studies included in the present meta-analysis is summarised in table 1. In all six studies, total cases were 623 with AA (107), AG (319) and GG (197), and controls were 936 with AA (218), AG (493), and GG (225). In controls genotype percentage of AA, AG and GG were 23.29%, 52.67% and 24.04% respectively. In total cases genotype percentage of AA, AG and GG were 17.17%, 51.20% and 31.62% respectively. Frequencies of AA, AG and GG genotypes were highest in individual studies.
included in the present meta-analysis the frequency of G alleles in controls was in the range of 0.414 to 0.65 and in cases the G allele frequency was slightly higher, and in the range of 0.452 to 0.739.

**Table 1: Details of six studies included in the meta-analysis**

<table>
<thead>
<tr>
<th>Author</th>
<th>Population/Race</th>
<th>No. of Controls</th>
<th>No. of Cases</th>
<th>Year</th>
<th>Journal</th>
</tr>
</thead>
</table>

**Table 2: Distribution of the A66G genotypes in controls and cases**

<table>
<thead>
<tr>
<th>Studies</th>
<th>Genotypes</th>
<th>Allele numbers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AA Cases</td>
<td>A Control</td>
</tr>
<tr>
<td>Wang et al, 2008</td>
<td>10</td>
<td>24</td>
</tr>
<tr>
<td>Scala et al, 2006</td>
<td>28</td>
<td>69</td>
</tr>
<tr>
<td>De Silva et al, 2006</td>
<td>37</td>
<td>45</td>
</tr>
<tr>
<td>Chango et al, 2005</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>O’Leary et al, 2002</td>
<td>1</td>
<td>35</td>
</tr>
<tr>
<td>Hobbs et al, 2000</td>
<td>26</td>
<td>39</td>
</tr>
<tr>
<td>Total</td>
<td>107</td>
<td>218</td>
</tr>
</tbody>
</table>

Total No of Samples = 1559  
Total No of alleles = 3118  
Total No of Control samples = 936  
Total No of alleles in controls = 1872  
Total No of Case samples = 623  
Total No of alleles in cases = 1246

**Table 3: Allelic frequencies in different studies**

<table>
<thead>
<tr>
<th>Studies</th>
<th>Allelic frequency in Cases</th>
<th>Allelic frequency in Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A No.</td>
<td>Frequency</td>
</tr>
<tr>
<td>Wang et al, 2006</td>
<td>48</td>
<td>0.375</td>
</tr>
<tr>
<td>Scala et al, 2006</td>
<td>102</td>
<td>0.58</td>
</tr>
<tr>
<td>De Silva et al, 2006</td>
<td>166</td>
<td>0.539</td>
</tr>
<tr>
<td>Chango et al, 2005</td>
<td>76</td>
<td>0.319</td>
</tr>
<tr>
<td>O’Leary et al, 2002</td>
<td>25</td>
<td>0.260</td>
</tr>
<tr>
<td>Hobbs et al, 2000</td>
<td>116</td>
<td>0.24</td>
</tr>
</tbody>
</table>
methionine is converted to S-adenosylmethionine (SAM), the lone donor of a methyl group for epigenetic modification of DNA (cytosine methylation) and post-translational methylation of histones [27]. Methionine synthase and MTRR enzymes are necessary for the homocysteine to methionine conversion.

In 1999, Wilson and colleagues reported that homozygous mutant genotype (GG) when combined with low cobalamin levels, greatly increases the risk for giving birth to child with Down syndrome and congenital defects especially neural tube defects. Since then several studies reported A66G polymorphism as risk factor for neural tube defects [7,8] and coronary artery diseases [28,12]. Olteanu et al [29] have reported that the I22M variant (A66G) MTRR enzyme exhibits four-fold lower activity than the wild-type protein in the reactivation of MTR in vivo. Hence the level of active MTR is reduced and so the availability of SAM, as methyl donor is also decreased, thus leading to DNA hypomethylation and it was pointed out by several studies that the DNA hypomethylation is the main causative factor in the chromosome missegregation, micronucleus formation, and defective gene expression etc [30]. Misregulation of chromosomes and altered gene expression are the main causative factors behind the role of A66G polymorphism and as risk factor of hereditary disorders [7,31].

MTRR is an enzyme of homocysteine/methionine metabolism pathway that maintains the active state of methionine synthase for the remethylation of homocysteine to methionine. A66G polymorphism reduces enzymatic activity of MTRR enzyme and consequently homocysteine level is higher and methionine level is lower in individuals with AG and GG genotype. Chronic elevation in intracellular homocysteine can lead to a decrease in SAM to SAH that inhibits DNA methyltransferase [32,33,34,7]. Chronic folate/methyl deficiency in vivo and in vitro has been associated with abnormal DNA methylation [32,26], and aberrant chromosome segregation [35-39,7]. James et al [1999] were the first to propose the hypothesis that altered DNA methylation patterns resulting from abnormal folate metabolism may increase DNA hypomethylation in centromeric and pericentromeric regions, thus increasing the risk of chromosome nondisjunction [27,40]. Several evidences supported the importance of normal pericentromeric DNA methylation for normal segregation of chromosomes during meiosis ([i]) treatment of cultured cells with 5-azacytidine, apotent demethylating agent results in DNA hypomethylation and (ii) chromosomal instability and (iii) chromosomal deletions, pericentromeric decondensation and profound chromatid anomalies) disorder exhibit several chromosomal abnormalities and aneuploidy are present in almost all human cancers and it has been shown by Vilain et al [43] that chromosomal instability is related to DNA hypomethylation and (iv) lymphocytes from individuals with 1CF (immunodeficiency centromeric instability and facial anomalies) disorder exhibit several chromosomal abnormalities like, undermethylation of pericentromeric satellite DNA, chromosomal decondensation and complex multiradiate chromosomes [44]. 1CF disorder is caused by mutation in DNA methyltransferase 3B (DNMT3B) and strongly supported the association between DNA hypomethylation and abnormal chromosome segregation [45,39,7].
The present meta-analysis is examined the MTRR A66G gene polymorphism and its relationship to the risk of developing DS. The strength of the present analysis was based on an accumulation of published data, providing greater information to detect significant differences. Limitations of the present meta-analysis were: (i) small number of studies (only six studies), (ii) small sample size, (iii) age factor is not considered in several included studies. Controls were not age matched and young mothers were included as control in many studies. The absence of maternal DS in young mothers does not exclude the possibility of DS later; (iv) single gene polymorphism is considered. Gene polymorphism in several other genes involved in homocysteine and folate pathway and their interactions might be a major determinant of disease risk rather than the individual polymorphism[7], and (v) higher heterogeneity.

The pooled OR of the present meta-analysis reported with 95% certainty that carriers of the 66G allele would have more than 1.42 fold increased odds of maternal DS. MTRR A66G polymorphism is one of the classical cases of gene nutrient interaction as this polymorphism similar to MTHFR (C677T) polymorphism raises the dietary need of folate to maintain normal homocysteine levels. Polymorphism of several folate metabolism genes with each with moderate effect might play a role in Down syndrome etiology. A meta-analysis considering larger sample size, population diversity of included studies, age of mothers of as inclusion/exclusion criteria for DS and control mothers, serum homocysteine, folate and B12 levels need to be performed before coming to the any conclusion about the genetics of Down syndrome.

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4.References


