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Original Article

Polymorphism in Folate Metabolic Pathway Gene as Maternal Risk Factor for Down Syndrome

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ABSTRACT

Down syndrome (DS) with the prevalence of 1/700 to 800 live births is the most common cause of mental retardation in human. During last two decades, it was repeatedly reported that besides advanced maternal age at conception impairment in maternal folate metabolism and elevated homocysteine are also risk factors for having a DS child. Methionine synthase reductase (MTRR) is the vital enzyme of folate/methionine metabolism cycle and dysfunctional MTRR enzyme lead to higher level of homocysteine and abnormal DNA methylation. Normal DNA methylation at centromeric and pericentromeric regions is responsible for normal segregation of chromosomes. Abnormal methylation of centromeric DNA due to A66G mutation is the main reason behind the trisomy 21. A number of association studies on MTRR, have focused on possible links between the maternal MTRR A66G polymorphism and birth of DS child but no consistent results have been obtained with regard to DS birth hence the aim of present meta-analysis was to examined association between birth of DS child and maternal MTRR A66G polymorphism in published case control reports. The present meta-analysis included total six studies with 623 case samples and 936 control samples. The genotype percentages of AA, AG and GG in cases were 17.17%, 51.2% and 31.62% respectively. Meta-analysis with random effects showed that there was 73.1% heterogeneity between the six studies. The fixed effect pooled OR was 1.34 (95% CI; 1.17 to 1.54) and Cochran Q was 18.57 (df = 5; p=0.0023). The random effect pooled OR was 1.42 (95% CI; 1.05 to 1.92) and Cochran Q was 18.57 (df = 5; p=0.0023). The random effect pooled OR was significant and showed strong association between MTRR A66G maternal genotype and Down syndrome. Meta-analysis was carried out by Meta-disc (version 1.4). The pooled OR of the present meta-analysis reported with 95% certainty that carriers of the 66G allele would have more than 1.42 folds increased risk of having DS child than the women without this allele. MTRR A66G polymorphism needs to be evaluated as a risk factor for Down syndrome in larger sample size.

1. Introduction

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.

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Methionine synthase reductase (MTRR) is a key enzyme in folate dependent homocystein 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 (III)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)alanine-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 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 a 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) ×100%, 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 and AG genotypes were highest in both cases and controls (Table 2). Allelic frequencies of A and G alleles were also calculated and presented in table 3. In six 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

Author	Population /Race	No. of Controls	No. of Cases	Year	Journal
Wang et al.	Chinese	70	64	2008	J Zhejiang University ScienceB,9(2):93-99.
Scala et al	Italian	257	93	2006	Genet Med, 8:409-416.
De Silva et al	Brazilian	158	154	2005	Am J Med genet, 135:263-267.
Chango et al	French	120	119	2005	Br J Nutr,94:166-169.
O'Leary et al.	Irish	192	48	2002	Am J Med Genet 107:151–155
Hobbs et al.	White	139	145	2000	Am J Hum Genet 67:623–630

Table2: Distribution of the A66G genotypes in controls and cases

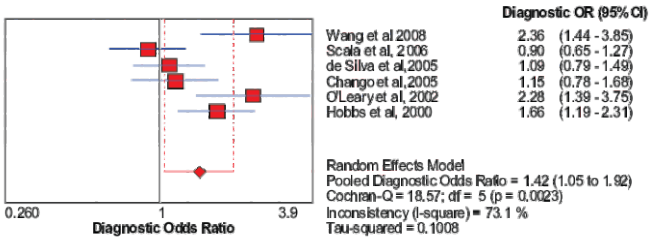
Studies	Genotypes						Allele numbers			
	AA		AG		GG		A		G	
	Cases	Control	Cases	Control	Cases	Control	Cases	Control	Cases	Control
Wang et al.,2008	10	24	28	34	26	12	48	82	80	58
Scala et al,2006	28	69	46	131	19	57	102	269	84	245
De Silva et al,2006	37	45	92	87	25	26	166	177	142	139
Chango et al,2005	5	6	66	72	48	42	76	84	162	156
O'Leary et al,2002	1	35	23	101	24	56	25	171	71	213
Hobbs et al,2000	26	39	64	68	55	32	116	146	174	132
Total	107	218	319	493	197	225	533	929	713	943
	17.17%	23.29%	51.20%	52.67%	31.62%	24.04%				

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

Studies	Allelic frequency in Cases				Allelic frequency in Control			
	A		G		A		G	
	No.	Frequency	No.	Frequency	No.	Frequency	No.	Frequency
Wang et al.,	48	0.375	80	0.625	82	0.586	58	0.414
Scala et al,2006	102	0.58	84	0.452	269	0.514	245	0.486
De Silva et l,2005	166	0.539	142	0.461	177	0.560	139	0.439
Chango et al,2005	76	0.319	162	0.681	84	0.35	156	0.65
O'Leary et al,2002	25	0.260	71	0.739	171	0.445	213	0.555
Hobbs et al,2000	116	0.24	174	0.6	146	0.525	132	0.475

Figure 1. Forest plot: Random effects (RE) odds ratio (OR) estimates with the corresponding 95% confidence interval (CI) for the allele contrast of MTRR A versus G.



The pooled Odd Ratios were estimated by both fixed effects (Mantel and Haenszel, 1959) [31]and random effects [17] models. The meta-analysis with fixed effects showed that there was 73.1% heterogeneity between the six studies. When there is large heterogeneity between studies the pooled OR is preferably estimated using the RE models. The fixed effect pooled OR was 1.34 (95% CI; 1.17 to 1.54) and Cochran Q was 18.57 (df = 5; p=0.0023). The random effect pooled OR was 1.42 (95% CI; 1.05 to 1.92) and Cochran Q was 18.57 (df = 5; p=0.0023). The random effect pooled OR was significant and shows association between maternal MTRR A66G genotype and Down syndrome (Fig. 1). The role of MTRR gene polymorphism as risk factor for Down syndrome was first evaluated by Hobbs and colleague[9].The frequency of MTRR, A66G mutation was evaluated in DNA samples of 145 mothers of children with Down syndrome and 139 control mothers. The MTRR A66G polymorphism was found to be more prevalent in mothers of children with Down syndrome with odd ratio of 1.66(95% CI: 1.19-2.31).O'Leary et al [18] evaluated the frequency of MTRR A66G polymorphism in 48 mothers of Down syndrome children and 192 control mothers. Mothers with A66G polymorphism had a 2.28 folds higher risk of having a child with Down syndrome than did mothers without this polymorphism (95% CI:1.39-3.75). Chango et al [19] reported A66G frequency in 119 DS mother and 120 control mothers from French population. Mothers with GG genotype had 1.15 times more risk having DS child. De Silva [20] and Scala et al [21] studies on DS and control population in Brazilian and Italian population respectively ,did not report any significant association. Wang et al [22] evaluated A66G polymorphism in 64 DS and 70 control mothers from Chinese population. Mothers with A66G polymorphism had a 2.36 fold higher risk of having a child with Down syndrome than did mothers without this polymorphism (95% CI: 1.44-3.85).

Maternal impairments in folate metabolism and elevated homocysteine are known risk factors for having a child with Down syndrome (DS) at a young age. A deficiency in cellular folates and methyl-donors may be associated with abnormal DNA methylation, DNA strand breaks, defective chromosome recombination, and abnormal chromosome segregation [23-26]. Both methylenetetrahydrofolate reductase (MTHFR) and methionine synthase reductase (MTRR) genes code for the key enzymes involved in homocysteine–methionine metabolism. In this cycle,homocysteine is converted into methionine and 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]. Missegregation 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 synthesis 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-azacytidien, apotent demethylating agent results in pericentromeric decondensation and profound chromatid missegregation in anaphase [37,42,7].(ii) chromosomal deletions, translocations and instability were reported in murine embryonic stem cells nullizygous for the major DNA methyltransferase (Dnmt1) gene (Chen et al.,1998). (iii) chromosomal instability 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 ICF (immunodeficiency centromeric instability and facial anomalies) disorder exhibit several chromosomal abnormalities like, undermethylation of pericentromeric satellite DNA, chromosomal decondensation and complex multiradiate chromosomes [44]. ICF 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 relation ship 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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