Disease Association Studies

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Critical review

Advances in the genetic aspects linking folate metabolism to the maternal risk of birth of a child with Down syndrome

F Coppedè[1]*

Abstract

Introduction

In 1999, it was first hypothesised that maternal polymorphisms of genes involved in folate metabolism might represent maternal risk factors for the birth of a child with Down syndrome. Several research articles have been produced worldwide to address that question, and recent meta-analyses of the literature suggest that at least two polymorphisms, namely *MTHFR* c.677C>T and *MTRR* c.66A>G, are associated with increased maternal risk for trisomy 21. Moreover, there is indication for an additive contribution of variants in folate pathway genes to the maternal risk for having a birth with Down syndrome. In addition, lack of folate supplementation at periconception combined with genetic polymorphisms of folate pathway genes, might represent maternal risk factors for congenital heart defects in the child with Down syndrome. The aim of this critical review was to discuss advances in genetic aspects linking folate metabolism to the maternal risk of giving birth to a child with Down syndrome.

Conclusion

Despite encouraging results, several factors such as ethnicity, age, dietary habits, and many others, could modulate those interactions and we are still far away from a complete understanding of the relationship between folate metabolism

and chromosome 21 non-disjunction.

Introduction

Down syndrome (DS) is a genetic disease resulting from the presence of an extra copy of the genetic material of chromosome 21, either in whole (trisomy 21) or part (due to Robertsonian translocation). The disease was named after the British physician John Langdon Down who first described it, and since 1959 the phenotype of DS has been associated with trisomy for chromosome 21(ref. 1). For the majority of DS cases (92%), the extra chromosome stems from the failure of a normal chromosome segregation during meiosis (meiotic non-disjunction) producing a gamete containing two copies of chromosome 21 that, when combined with a normal gamete from the other parent, produces a zygote with three copies of chromosome 21 (trisomy 21). In such cases, the extra chromosome originates during the development of either the egg or the sperm. However, it has been observed that the non-disjunction event is of maternal origin in most cases, occurring primarily during meiosis I in the maturing oocyte. As a result, only less than 10% of primary trisomy 21 is due to errors occurring during paternal meiosis[2].

In human females, meiosis I starts at embryonic age, but halts in diplotene of prophase I until puberty. This suspended state is referred to as the dictyotene stage and remains so until puberty. In late foetal life, all oocytes, still primary oocytes, have taken this halt in development. First, after menarche only a few of them continue to develop in every menstrual cycle. For those primary oocytes continuing to develop in each menstrual cycle,

meiosis I is completed and the primary oocyte becomes the secondary oocyte and the first polar body. Immediately after meiosis I, the secondary oocyte initiates meiosis II, which is halted in metaphase II until fertilisation, if it occurs. In summary, oocyte development takes decades to complete with the first crossover process taking place during foetal development and the final cell division occurring only 15–50 years later following fertilisation[3].

Advancing maternal age at conception and the location of genetic recombination represent the two most important risk factors for chromosome 21 non-disjunction identified so far[4,5]. However, the cellular and molecular mechanisms that underlie meiotic non-disjunction in mothers of DS individuals are still largely unknown.

In 1999, James et al.[6] were the first to hypothesise that maternal polymorphisms of genes involved in folate metabolism, also known as one-carbon metabolism (Figure 1), might act as maternal risk factors for the birth of a child with DS. Their assumption was that impairments in one-carbon metabolism could result in altered DNA methylation, probably at centromeric or pericentromeric regions, thus favouring chromosome 21 non-disjunction and malsegregation, and the hypothesis was supported by the experimental evidence of increased homocysteine levels and increased frequency of the methylenetetrahydrofolate (*MTHFR*) 677T allele in mothers of children with DS (MDS) compared with control mothers[6]. I reviewed the paper in 2009 and it stimulated considerable research in the field[7]. At the time of my review, 26 independent genetic association studies had been performed to address the maternal

* Corresponding author
Email: f.coppede@geog.unipi.it

Department of Translational Research and New Technologies in Medicine and Surgery, Division of Medical Genetics, University of Pisa, Pisa, Italy
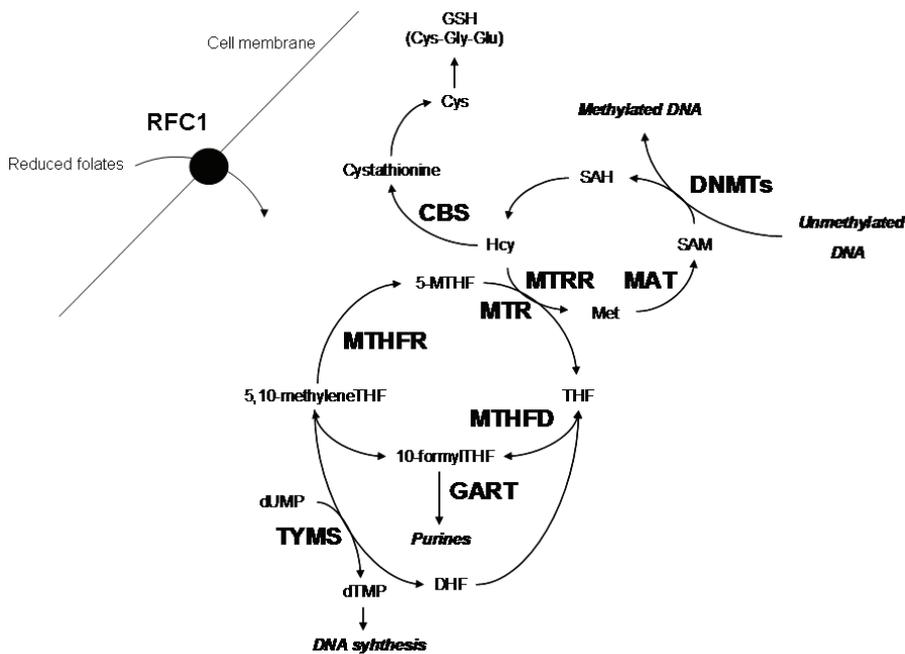
Figure 1: Overview of the folate metabolic pathway adapted from Coppedè et al[19]. Folates require several transport systems to enter the cells, the best characterised being the reduced folate carrier (RFC1). Methylenetetrahydrofolate reductase (MTHFR) reduces 5,10-methylenetetrahydrofolate (5,10-MTHF) to 5-methyltetrahydrofolate (5-MTHF). Subsequently, methionine synthase (MTR) transfers a methyl group from 5-MTHF to homocysteine (Hcy) forming methionine (Met) and tetrahydrofolate (THF). Methionine is then converted to *S*-adenosylmethionine (SAM) in a reaction catalysed by methionine adenosyltransferase (MAT). Most of the SAM generated is used in transmethylation reactions, whereby SAM is converted to *S*-adenosylhomocysteine (SAH) by DNA methyltransferases (DNMTs) that transfer the methyl group to the DNA. Vitamin B12 (or cobalamin) is a cofactor of MTR, and methionine synthase reductase (MTRR) is required for MTR maintenance in its active state. If not converted into methionine, Hcy can be condensed with serine to form cystathionine in a reaction catalysed by cystathionine b-synthase (CBS), which requires vitamin B6 as a cofactor. Cystathionine can be then utilised to form the antioxidant compound glutathione (GSH). Another important function of tetrahydrofolate derivatives is in the *de novo* synthesis of DNA and RNA precursors, where they are used by thymidylate synthase (TYMS) and methylenetetrahydrofolate dehydrogenase (MTHFD) for the synthesis of nucleic acid precursors. MTHFD is a trifunctional enzyme that interconverts tetrahydrofolate derivatives for purine, methionine and thymidylate synthesis. TYMS requires 5,10-MTHF and dUMP for the production of dTMP and dihydrofolate (DHF) in the de novo synthesis of pyrimidines. Other enzymes participate in folate metabolism such as phosphoribosylglycinamide transformylase (GART), which is a protein required for purine synthesis.

contribution of folate metabolism to the risk of birth of a child with DS[7]. Results were often conflicting; only one meta-analysis was available, and

no single gene could be consistently associated with the maternal risk. On the contrary, there was increasing evidence suggesting

an additive effect with variants in multiple genes of the folate metabolic pathway, in interaction with dietary availability of one-carbon nutrients[7].

The aim of this critical review article is to discuss the most recent advances in this field. Several articles produced from 2009 to date, and the recent meta-analyses of the literature, have provided additional information on the contribution of this pathway to the maternal risk of birth of a baby with DS, highlighting the contribution of folate pathway genes to the risk of congenital heart defects among individuals with DS[7], suggesting the more probable link of those genes to the maternal risk for a DS birth[9–11], and providing novel insight into the role of pre-conceptional folic acid supplementation and risk for chromosome 21 non-disjunction[12].

Discussion

The author has referenced some of its own studies in this review. These referenced studies have been conducted in accordance with the Declaration of Helsinki (1964) and the protocols of these studies have been approved by the relevant ethics committees related to the institution in which they were performed. All human subjects, in these referenced studies, gave informed consent to participate in these studies.

Polymorphisms of folate pathway genes and maternal risk for a DS birth: recent evidence from literature meta-analyses

In 1999, on the basis of evidence that abnormal folate and methyl metabolism can lead to DNA hypomethylation and abnormal chromosomal segregation, James et al.[6] hypothesised that the *MTHFR* c.677C>T polymorphism might be a risk factor for maternal meiotic chromosome 21 non-disjunction and DS risk in the offspring. The MTHFR enzyme is constituted by dimers in humans,

each of which has the catalytic domain-harbouring position 222 and the regulatory domain-harbouring position 429, and two common poly-morphisms changing the amino acids at those regulatory positions are known to reduce MTHFR activity: MTHFR c.677C>T (Ala222Val) and MTHFR c.1298A>C (Glu429Ala). Numerous studies have shown that the MTHFR 677T allele is associated with increased total plasma homo-cysteine (tHcy) and decreased serum folate, mainly in 677TT homozy-gous subjects. The impact of the *MTHFR* c.1298A>C polymorphism on enzyme activity is less than that of the c.677C>T one, and most studies have not detected significant differ-ences in tHcy or serum folate levels between different *MTHFR* c.1298A>C genotypes[7,13].

The *MTHFR* c.1298A>C polymor-hism showed no association with maternal risk for trisomy 21[11]. Similar results were observed by Costa-Lima et al.[10] in their meta-analysis of 20 articles from English, Spanish and Portuguese databases addressing the association of the *MTHFR* c.677C>T polymorphism with maternal risk for having a birth with DS, for a total of 2101 DS mothers and 2702 control mothers[10]. They observed a 50% increase in the association of maternal homozygous TT genotype and DS risk in both fixed-effects (OR = 1.51; 95% CI: 1.22–1.87) and random-effects models (OR = 1.54; 95% CI: 1.15–2.05). Similarly, a significant pooled OR was found for the heterozygote CT, in both fixed-effects (OR = 1.26; 95% CI: 1.10–1.43) and random-effects model (OR = 1.28; 95% CI = 1.08–1.51)[10], and stratification of data by latitude, showed association mainly in Sub-Tropical regions[10]. Also, Medica et al.[14] performed a meta-analysis of PubMed articles for a total of 1545 DS mothers and 2052 control

mothers observing the association of the *MTHFR* c.677C>T polymorphism with maternal risk for DS in both dominant (OR = 1.40; 95% CI: 1.16–1.70) and recessive models (OR = 1.35; 95% CI: 1.02–1.78). Their meta-analysis of 1007 case mothers and 1318 control mothers revealed no association of the *MTHFR* c.1298A>C polymorphism with maternal risk for having a baby with DS[14]. Taken overall, these data suggest a modest contribution of the *MTHFR* c.677C>T polymorphism to the maternal risk for having a child with DS[10,11]. The effect of this polymorphism might be more pronounced in those countries with a reduced folate bioavailability resulting from dietary habits or other environmental factors since there is an increased folate demand for proper MTHFR stability and activity in individuals harbouring the *MTHFR* 677TT genotype[7,10,13]. MTHFR plays a pivotal role in regulating DNA methylation through the reduction of 5,10-methylenetetrahydrofolate (5,10-MTHF) to 5-methyltetrahydro-folate (5-methylTHF). Subsequently, methionine synthase (MTR) transfers a methyl group from 5-methylTHF to homocysteine forming methionine and tetrahydrofolate (THF). Vitamin B12 (cobalamin) is a cofactor in this reaction and methionine synthase reductase (MTRR) is required for the maintenance of MTR in its active state. Methionine is then converted to *S*-adenosylmethionine (SAM) and DNA methyltransferases (DNMTs) transfer the methyl group from SAM to the DNA (Figure 1).

Another polymorphism that has been consistently associated with maternal risk for trisomy 21 by three independent meta-analyses[9,14,15] is the *MTRR* c.66A>G one. Human MTRR, a NADPH-dependent diflavin enzyme, is required for the reduc-tive activation of the cobalamin-dependent MTR and the c.66A>G polymorphisms might impair the binding of MTRR to MTR thus leading to reduced MTR activity and increased homocysteine levels[16]. The

first report of an association between the *MTRR* c.66A>G polymorphism and maternal risk for trisomy 21 was published in 2000 by Hobbs et al[16]. Two meta-analyses of six PubMed records for a total of 623 case mothers and 936 control mothers revealed association of the *MTRR* 66GG genotype (recessive model: OR 1.57; 95% CI: 1.06–2.31)[14] and of the *MTRR* 66G allele (OR = 1.45; 95% CI: 1.05–1.92)[15] with maternal DS risk, respectively. A more recent meta-analysis of 11 articles for a total of 1226 DS mothers and 1533 control mothers confirmed the association of G allele with DS risk (OR = 1.23; 95% CI: 1.02–1.49)[9].

Several other polymorphisms of folate pathway genes, such as *reduced folate carrier* (*RFC1* or *SLC19A1*), *cystathionine β-synthase* (*CBS*) and others, have been investigated as potential maternal risk factors for having a birth with DS[7], but results are still conflicting for most of them and the only available meta-analysis failed to detect significant individual associations[14]. Table 1 summarises the most recent findings from meta-analyses.

The additive contribution of variants in folate pathway genes to the mater-nal risk for having a birth with DS

In my previous review article[7], I summarised in detail several studies suggesting that combinations of different polymorphisms in genes of the folate metabolic pathway, rather than a single variant alone, might increase the maternal risk for gener-ating a child with DS[7]. Of particular interest are some observations[16,17] listed in Table 2, and corroborated by recent findings[18,19], indicating an additive value of variants in folate pathway genes to the maternal risk for trisomy 21 when three or more polymorphisms are considered simultaneously. For example, Bran-dalize et al.[18] observed that combined genotypes among *MTRR* c.66A>G, *MTR* c.2756A>G, *CBS* c.844ins68, and *RFC1* c.80A>G polymorphisms

Table 1. Main results of the recent meta-analyses of polymorphisms in genes involved in folate metabolism as maternal risk factors for DS.						
Genes	**Polymorphism**	**Case mothers**	**Control mothers**	**Model**	**OR (95% CI)**	**References**
MTHFR	c.677C>T	1545	2052	Dominant	1.40 (1.16–1.70)	Medica et al.[14]
MTHFR	c.677C>T	1545	2052	Recessive	1.35 (1.02–1.78)	Medica et al.[14]
MTHFR	c.677C>T	2101	2702	TT vs. CC	1.51 (1.22–1.87)	Costa-Lima et al.[10]
MTHFR	c.677C>T	2101	2702	CT vs. CC	1.26 (1.10–1.43)	Costa-Lima et al.[10]
MTHFR	c.677C>T	2806	4597	Dominant	1.30 (1.12–1.51)	Wu et al.[10]
MTHFR	c.1298A>C	2806	4597	All	Not significant	Wu et al.[10]
MTHFR	c.1298A>C	1545	2052	All	Not significant	Medica et al.[14]
MTRR	c.66A>G	623	936	Recessive	1.57 (1.06–2.31)	Medica et al.[14]
MTRR	c.66A>G	623	936	G vs. A	1.45 (1.05–1.92)	Rai [15]
MTRR	c.66A>G	1226	1533	G vs. A	1.23 (1.02–1.49)	Amorim et al.[9]
MTR	c.2756A>G	439	731	All	Not significant	Medica et al.[14]
RFC1	c.80A>G	354	644	All	Not significant	Medica et al.[14]
CBS	c.844ins68	367	542	All	Not significant	Medica et al.[14]

increase maternal DS risk, with ORs ranging from 5.8 to 6.9 depending on the number of risk alleles considered[18]. We applied a completely novel statistical approach, artificial neural networks, to understand the links among different folate pathway gene polymorphisms, chromosome damage and maternal risk for having a child with DS[19], observing complex interactions among studied variables and identifying six variables that allowed discriminating between MDS and control mothers with 90% accuracy[19].

Folic acid supplementation and risk for chromosome 21 non-disjunction
Despite several *in vitro* studies revealing that folate deficiency induces chromosome 21 aneuploidy[20,21], one of the major detractors from accepting the contribution of folate metabolism to the maternal risk for having a child with DS was the lack of consistent evidence of a reduced incidence of DS cases in countries, such as the United States, with mandatory folic acid fortification[7]. However, most of the maternal meiotic errors leading to chromosome 21 non-disjunction occur at maternal meiosis I during maternal embryogenesis in the maternal grandmother body, and are likely to be affected by the maternal grandmother diet[7]. By contrast, the

maternal nutrition status at periconception might affect some of the chromosome 21 malsegregation events occurring at maternal meiosis II[7]. A very recent study from the National Down Syndrome Project, a population-based case–control study of live born infants with trisomy 21 that was conducted at six recruitment sites in the United States from 2000 to 2004 to test the hypothesis that the lack of maternal folic acid supplementation before conception increases the odds of chromosome 21 non-disjunction, revealed an association among older mothers (≥35 years of age) experiencing meiosis II non-disjunction errors (OR = 2.00; 95% CI: 1.08–3.71), suggesting that lack of folic acid supplementation may be associated specifically with meiosis II errors in the aging oocyte[12]. Interestingly, meiosis II errors are associated with unusual peri-centromeric recombination events that are more prevalent among older than younger mothers[22–24]. If confirmed in subsequent studies, the data could provide a direct evidence of the link between folate bio-availability and chromosome 21 non-disjunction and the need to take into account maternal age and the type of meiotic error when assessing those associations, as it was suggested few years ago[7].

Maternal folate metabolism and risk of congenital heart defects in the DS child
One of the most attractive findings of recent years was the association of folate metabolism with risk of congenital heart defects (CHDs) in DS individuals[12]. CHDs occur in approximately 40% of DS cases and range from small atrial or ventricular septal defects to complete atrioventricular septal defects and other serious heart defects[25]. A recent article of the National Down Syndrome Project, including 1011 mothers of infants with DS that reported their use of supplements containing folic acid, revealed that lack of maternal folic acid supplementation was more frequent among infants with DS and atrioventricular septal defects (OR = 1.69; 95% CI: 1.08–2.63) or atrial septal defects (OR = 1.69; 95% CI: 1.11–2.58) than among infants with DS and no heart defect[25]. In 2009, Brandalize et al.[26] studied 239 mothers of DS individuals and 197 control mothers observing that the presence of the *MTHFR* 677T allele in case mothers resulted in a 2.07-fold higher odds of CHD in the offspring (*P* < 0.01). Moreover, among the 57 mothers of CHD-affected children with DS who carried the *MTHFR* 677CT or TT genotypes and did not have peri-conceptional folic acid

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Table 2. Some examples of the additive contribution of variants in folate pathway genes to the maternal risk for having a birth with DS.		
Genes	**Main results**	**Reference**
MTHFR, MTR, MTRR, CBS	The presence of increasing numbers of 5 polymorphic alleles (MTHFR 677T, MTHFR 1298C, MTR 2756G, MTRR 66G and CBS 844ins68) increases maternal risk for DS (P = 0.04)	da Silva et al.[16]
MTHFR, MTR, MTRR, RFC1	The presence of increasing numbers of 3 or more polymorphic alleles among MTHFR 677T, MTHFR 1298C, MTR 2756G and RFC1 80G, increases maternal risk (OR = 1.7; 95% CI: 1.0–3.0)	Biselli et al.[17]
MTR, MTRR, CBS, RFC1	Combined genotypes among MTR 2756A>G, MTRR 66A>G, CBS 844ins68 and RFC1 80A>G polymorphisms increase maternal DS risk. ORs ranging from 5.8 to 6.9 depending on the number of risk alleles considered	Brandalize et al.[18]
MTHFR, MTR, RFC1, TYMS	Artificial Neural Networks selected 6 variables (Micronucleus frequency, MTHFR 677TT, RFC1 80AA, TYMS 1494 6bp +/+, TYMS 28bp 3R/3R and MTR 2756AA genotypes) that allowed to discriminate between mothers of DS individuals and control mothers with 90% accuracy	Coppedè et al.[19]

Table 3. Maternal folic acid supplementation, folate pathway gene variants and risk of congenital heart defects (CHD) in the DS child.		
Folic acid supplement/genetic polymorphisms	**Main results**	**References**
Preconception folic acid supplementation	Lack of maternal folic acid supplementation was associated with DS and atrioventricular septal defects (OR = 1.69; 95% CI: 1.08–2.63) and with DS and atrial septal defects (OR = 1.69; 95% CI: 1.11–2.58)	Bean et al.[25]
Peri-conception folic acid supplementation/MTHFR c.677C>T	Lack of maternal peri-conceptional folic acid intake in carriers of the MTHFR 677CT or TT genotypes was associated with risk of CHD in the child with DS (OR = 2.26; 95% CI 1.25-4.09)	Brandalize et al.[26]
RFC1 and MTHFR genetic variants	Association of several RFC1 gene polymorphisms (SNP) and of the MTHFR c.1298A>C polymorphism with CHD in the DS offspring	Locke et al.[8]
Peri-conception folic acid supplementation/MTHFR c.677C>T and c.1298A>C polymorphisms	No association of lack of folic acid supplementation and maternal MTHFR polymorphisms with CHD in the DS offspring	Božović et al.[27]

intake, they observed a 2.26-fold increased odds (95% CI 1.25–4.09) of having any CHD-affected child with DS[26]. Locke et al.[8] screened several polymorphisms in folate pathway genes for a role in the DS-associated CHD atrioventricular septal defect. Their analysis of a group of 121 case families (mother, father and proband with DS and atrioventricular septal defect) and 122 control families (mother, father and proband with DS and no CHDs), revealed that several *RFC1* gene polymorphisms [single-nucleotide polymorphism (SNP)] showed nominally significant associations with ORs between 1.34 and 3.78, depending on the SNP and genetic model[8]. Also the functional *MTHFR* c.1298A polymorphism was over-transmitted to cases with atrioventricular septal defects (*P* = 0.05) and under-transmitted to controls (*P* = 0.02)[8]. By contrast, others have failed to observe the association of maternal *MTHFR* polymorphisms with CHDs in the DS offspring[27]. The study population included 112 DS subjects and CHDs were present in 48% of the DS subjects. The mothers of 107 DS individuals were available for the study and none were peri-conceptional folic acid users[27]. However, despite some conflicting results, the vast majority of recent studies suggest that lack of folic acid supplementation at peri-conception coupled with genetic variants of the folate metabolic pathway might represent maternal risk factors for CHDs in the DS child (Table 3).

Conclusion

This brief survey of the most recent literature concerning the link between folate metabolism and maternal risk of birth of a child with DS summarises the progresses of the

scientific research in this field. At least two common polymorphisms, namely *MTHFR* c.677C>T and *MTRR* c.66A>G, are associated with the maternal risk for having a child with DS in several independent meta-analyses of the literature performed between 2009 and 2013, and there is increasing evidence of an additive contribution of polymorphisms of folate pathway genes to the maternal risk for trisomy 21. Recent epidemiological reports, however, highlight the complex relationship between folate metabolism and chromosome 21 non-disjunction, suggesting the need to take into account other factors such as maternal age at conception and the type of meiotic error along with geographical and dietary factors. In addition, increasing evidence points to a link between maternal folic acid metabolism at peri-conception and risk of CHD in the DS offspring. Overall, those findings are extremely encouraging, but they are also revealing the need of additional research for a deeper understanding of the molecular mechanisms behind those associations. Several factors such as maternal age and ethnicity, dietary habits, food availability, alcohol consumption, latitude, and many more could interfere with folate metabolism to modulate the maternal risk for the birth of a child with DS and that of CHD or other diseases in the DS child. Since we are still far away from a complete understanding of the overall interactions among those variables, none of the polymorphisms identified so far can be used in the genetic counselling to "predict" a woman's risk for trisomy 21.

Abbreviations list

CBS, cystathionine β-synthase; CHD, congenital heart defect; CI, confidence interval; DNMTs, DNA methyltransferases; DS, Down syndrome; MDS, mothers of children with DS; MTR, methionine synthase; MTRR, methionine synthase reductase; 5-methylTHF; 5-methyltetrahydro-

folate; 5,10-MTHF, 5,10-methylene-tetrahydrofolate; OR, odds ratio; tHcy, total plasma homocysteine; THF, tetrahydrofolate; RFC1 or SLC19A1, reduced folate carrier; SAM, *S*-adenosylmethionine; SNP, single-nucleotide polymorphism.

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