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Original Article

Maternal MTHFR gene polymorphisms and the risk of Down syndrome offspring

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ABSTRACT

Background: Methylenetetrahydrofolate reductase has been associated with Down's syndrome. **Aim**: To study the frequency of methylenetetrahydrofolate reductase (MTHFR) 677C⟶T mutation in Egyptian mothers having a child with Down syndrome (DS) and matched control mothers. **Materials and methods:** Eighty mothers, age < 40 years, with a history of previous delivery of a DS with karyotypically confirmed trisomy 21 and 30 mothers with healthy children as matched control mothers. A case-control study to examine the association of genetic polymorphism in the MTHFR gene implicated in folate metabolism that known to let down the activity of this enzyme. Estimation of maternal plasma homocysteine (*Hyc*): methionine (*Met*) ratio and lymphocyte methotrexate (MTX) cytotoxicity to assess the occurrence of MTHFR 677C⟶ T mutation. **Results:** The MTHFR 677C⟶T polymorphism is more prevalent among mothers of infant with DS compared with the controls, with an odd ratio of 1.9. In addition, mothers of infant with DS have significantly increased plasma levels as well as lymphocyte MTX cytotoxicity relative to the control**. Conclusion:** Aberrant folic acid metabolism secondary to MTHFR polymorphism leads to a significant reduction in plasma methionine and increasing the materials risk for having DS infant.

Key words: Down syndrome, polymorphism, methylenetetrahydrofolate reductase, gene

INTRODUCTION

Folates are members of B9 vitamins. They are basically derived from diets such as fruits, green vegetables, cereals, beans and liver. After intestinal absorption, folate metabolism includes the conversion of 5,10-Methylenetetrahydrofolate (by reduction and methylation) to 5-methlytetrahydrofolate (5-methylTHF) by means of the enzyme MTHFR. The release of 5-methylTHF into the blood and the subsequent cellular uptake is used for the synthesis of DNA and RNA precursors or for the conversion of *Hcy* to *Met*, which is then used to form the main DNA methylating agent *S*-adenosyl methionine (SAM).[1-3] Folic acid is converted to a natural biological form of the vitamins, 5-methylTHF as it passes through the intestinal wall.[1-3]

MTHFR gene is situated on the short arm (*p*) of chromosome 1 at position 36.3. 5-methylTHF is the methyl donor for vitamin B12-dependent re-methylation of *Hcy* to *Met*. *Met* is the precursor for the synthesis of SAM, the major cellular methyl donor for DNA and RNA.[4] Severe MTHFR deficiency is though

rare but caused by mutations resulting in 0-21% residual enzyme activity. Patients with MTHFR deficiency have hyperhomocysteinemia as well as low to normal plasma *Met* levels.[1-5]

Down syndrome (trisomy 21) may result from failure of chromosome 21 segregation during meiosis. Chromosome 21 contains between 300 and 400 genes.[5] in 95% of the cases the non-disjunction event is of maternal origin, occurring primarily during meiosis I in the maturing oocyte.[6] There is an increased risk of having a DS infant as a result of genetic polymorphism consequent to impairments of folate metabolism. It has been reported that the variant 677T allele of the MTHFR gene might be a maternal risk factor for having a child with DS, and increased plasma *Hcy* levels in mothers of DS children was also observed when compared with mothers of healthy children.[7]

The impetus for undertaking this research was to study the possible role of maternal MTHFR gene mutation in prediction of the risk of having a DS child.

MATERIALS AND METHODS

Subjects

The study incorporated 110 pregnant women classified into two groups. The case group consists of 80 mothers <40 years old with previous history of giving birth to a DS with karyotypically confirmed full trisomy 21. The inclusion criteria were women < 40 years at the time of conception and delivery of a DS with karyotypically confirmed full trisomy 21. The control group consisted of 30 healthy mothers having the same age, similar social class, no history of miscarriages, no abnormal pregnancies, and has given birth to normal children.

Patients were recruited from the Paediatrics and Obstetrics division of University Hospital during the period of January 2008 to December 2001. Informed consent obtained from all patients according to local procedures in accordance to the declaration of Helsinki. The ethical committees of the department approved the protocol

Methodology

A 10 ml of fasting blood sample were withdrawn from the antecubital vein of each patient and transferred to the laboratory in a heparinized sample tube. Lymphocytes were isolated immediately from the whole blood by centrifugation. Some lymphocytes were stored at -20^{o}C until analyzed for genotype. The presence of polymorphisms in MTHFR 677C⟶T was detected by polymerase chain reaction/restriction fragment length polymorphism method.[9] Measurement of the amount of chromosomal damage in peripheral blood lymphocytes was done by lymphocytes sensitivity to MTX cytotoxicity.[9] MTX inhibits dihydrofolate reductase thus limits the synthesis of reduced folates, which are necessary co-factors in *de novo* purine and pyrimidine synthesis. The distribution of MTHFR genotype in the control mother was found to be in Hardy-Weinberg equilibrium as shown below:

Hardy-Weinberg equation: ($p^2 + 2pq + q^2 = 1$)

Where p is defined as the frequency of the dominant allele and q as the frequency of the recessive allele for a trait controlled by a pair of alleles (A and a).[10]

The separated plasma, obtained within 30 minutes of blood collection, frozen at -70 ^{o}C for high-performance liquid chromatography (HPLC) was analysed for homocysteine and methionine.[11]

Statistical analysis

Statistical analyses were done using SPSS software package. Statistical differences were determined using the chi-square with Pearson statistics.

RESULT

As regards mean age at conception, mean number of pregnancy and maternal family history of cancer, table 1 shows that there were no significant differences between the control and case group. However, mothers of DS children having MTHFR polymorphism showed a higher positive history of maternal cardiovascular disease and twinning than their counterparts with DS children with a normal C/C genotype in addition to control mothers.

The distribution of MTHRF genotypes in the control mother, according to Hardy-Weinberg equilibrium (HWE) is shown in table 2. The frequency of MTHFR 677C⟶T allele was significantly higher among mothers having an infant with DS compared with the controls.

Table 1: The characteristics of age-matched control and case group

Variable	Control N=30		MDS N = 80	
	C/C (N = 13)	C/T (N = 18)	C/C (N = 18)	C/T (N = 62)
Mean age at conception	22.2	22.5	23.1	23.5
Mean no live birth	2.1	2.35	2.8	2.9
Percentage of Miscarriage	0	0	13.5	16.2
At time of conception • Taking 400U folic Acid	23%	29%	22%	26%
• Following a weight reduction	0%	0%	11%	16%
Maternal family history				
• Twins • Heart disease • Cancer	8 % 31% 17%	12% 29% 17%	22% 21% 45%	23% 52% 64%

MDS = Mother of Down syndrome child

Table 2: The association between material MTHFR genotype and Down syndrome affected pregnancies and control mother

Variable	Control (N = 13)		MDS (N= 80)			
	No	%	No	%	Odd ratio (95%CI)	*P*
Homozygous (C/C)	13	43.33	18	22.5	---	---
Heterozygous (C/T)	12	40	48	60	2.4	<0.05
Homozygous (T/T)	5	16.67	14	17.5	3.1	>0.05
Combined maternal (C/t + T/	17	56.67	62	77.5	2.5	<0.01
** Mutant allele frequency	0.28	---	0.41	----	----	

** Both population were in Hardy Weinberg Equilibrium p > 0.1
MDS = Mothers of Down syndrome children, *P*= *P*-value of X^2 test

Table 3: Fasting plasma *Hcy* and *Met* concentrations in mothers having Down syndrome babies and controls

Variable	Control mothers (N=30)		MDS (N=80)	
	C/C	C/T+T/T	C/C	C/T+T/T
	No=13	N=17	N=18	N=62
Plasma *Hcy* (umol/L)	7.5±0.7	8.1±0.5	11.0±0.4*	13.1±3.0*
Plasma *Met* (umol/L)	34.3±2.5	31.6±3.7	21.2±3.6*	27.4±1.5*
Homocysteine: Methionine	0.22±0.02	0.26±0.02	0.52±0.02*	0.48±0.03*

NB: There are eight possible DNA mismatches of varying stability and structure, namely A/A, A/C, C/T, G/G, G/A, G/T and T/T. *Hcy* =homocysteine, *Met* =Methione, MDS = mothers of Down syndrome children Significant difference between both groups (*P*<0.001).

Table 4: Lymphocytes sensitivity to MTX cytotoxicity in mothers of Down syndrome babies and controls

	All genotypes		C/T genotype	
	Control (N=10)[%1]	MDS (N=20)[%1]	Control (N=3)[%1]	MDS (N=22)[%1]
0.22 (umol/L)	8.3±1.3	70.1±25.1*	86.1±2.0	73.1±2.2[2]
0.44 (umol/L)	65.6±1.5	52.0±0.3*	72.1±2.7	54.5±2.6[3]

NB. MTX-induced cytotoxicity was significantly greater in lymphocytes of mothers having an infant with DS than among the controls.1=percentage of viable lymphocytes after 24 hours exposure; *=Chi-square; 2, 3=(p<0.05), MTX = methotrexate; MDS =Mothers of down syndrome child

The mean fasting plasma homocysteine levels in the mother of DS children with one or both 677T alleles was higher in lymphocytes of mothers having an infant with DS than among the controls (tables 3). Methotrexate-induced cytotoxicity was significantly greater in lymphocytes of mothers having an infant with DS than among the controls (table 4)

DISCUSSION

Around 90% of cases with Down syndrome are caused by non-disjunction of chromosome 21 during maternal meiosis. Advanced maternal age (>35 years) is a known risk factor for DS.[3] Nevertheless, a high number of DS children are also born to young mothers (1/1,250 births); one of the major challenges in DS aetiology is to identify possible risk factors

responsible for predisposing young mothers to DS-affected pregnancy.[13]

James *et al.*[7] reported the association of a polymorphism (C677T), in the MTHFR gene with case mothers with DS children. Thereafter, several investigators have carried out numerous studies to clarify the possible role of folate metabolism in the risk of having a DS child, yet the problem is still unsolved because conflicting results have often emerged.[14-21]

Shashi *et al*. associated low folate status with MTHFR 677C⟶T polymorphism as maternal risk factors, and folate deficiency implicated as a risk factor for human aneuplidy.[22]

In 2001, Al-Gazali *et al.* described a case of a mother and her child, in which the child had DS and cervical meningomyelocele.[23] In both the mother and her child, they found the homozygous 677T allele of the MTHFR gene 677C⟶T polymorphism. They further investigated the folate metabolism in the mother and her child, and found a secondarily altered folate status in the mother with an increased homocysteine level. The mother had not supplemented her intake of folic acid during her periconceptional period. Based on this study, Al-Gazali *et al.* suggested that the homozygous TT mutation in the MTHRF gene in both mother and her child had contributed to the presentation of DS and the neural tube defected in child.[23]

The existing study revealed that the risk of having a child with DS is strongly associated with 677C⟶T mutation.[23] The marginal significance (P<0.10) in risk mother with T/T homozygous mutant genotype was most likely due to low number in this group.[23] Nevertheless, there was a significant increase in the frequency of heterozygous genotype among the mother of children with DS than in control mothers.[23]

The result is in contrast with the previous study that reported distribution of the 677C T mutation in parents of children with neural tube defects (NTDs), in whom the increased risk is more strongly associated with the homozygous T/T genotype.[24]

The likely cause of the predominance of the heterozygous genotype in mother of DS children is that foetal viability may be lower in mother with homozygous T/T genotype, so these pregnancies are spontaneously lost before diagnosed.

The results of the existing study indicate that MTHFR 677C⟶T polymorphism conferred about 2.5 fold rise in the maternal risk of having a DS child. The most accepted explanation for this predominance of the heterozygous genotype in the mothers of DS children is that error in meiotic division is embryonic-lethal and results in foetal loss in homozygous mother with T/T genotype.
Interestingly, rare cases of DS are paternal in origin. Young and colleagues observed that men with high folate intake had lower frequencies of sperm with disomy 21 compared with men with lower intake, providing additional evidence for the importance of folates in human non-disjunction events.[27]

This study depicted that *Hcy* concentrations is significantly higher in mothers of infants with DS with the normal C/C genotype than in their control counterparts with identical C/T genotype. Similarly, there is a high MTX sensitivity in the mothers of DS children than in their control counterparts with identical C/T genotype. These findings suggest that MTHFR polymorphism compromises folate homeostasis.

The view on the association between the metabolism of folate/*Hcy* and human trisomy 21 is recently revised. The current opinion is that maternal and embryonic combinations for variants in folate metabolizing genes, coupled with the maternal nutritional and life style status during pregnancy, may strongly influence the probability that some embryos with trisomy 21 survive to birth.[26] Moreover, since most of chromosome 21 non-disjunctions occur during maternal embryogenesis in the grandmother body, also the maternal grandmother genotype and nutritional status during pregnancy might strongly affect the probability that some maternal eggs will carry two copies of chromosome 21.[21,27]

Lastly, we concluded that aberrant folic acid metabolism secondary to MTHFR polymorphism leads to a significant reduction in plasma methionine and increase in homocysteine concentrations. These metabolic dysfunctions seem to be implicated in increasing the maternal risk for having DS infant.

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