

## SHORT REPORT

# C677T mutation in the 5,10-MTHFR gene and risk of Down syndrome in Italy

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The C677T polymorphism of the MTHFR gene has been associated to maternal risk of Down syndrome, due to the detection of an higher prevalence of the T allele among mothers of children with trisomy 21, compared to control mothers. In order to confirm this association, we studied the presence of the C677T in 64 mothers of Down syndrome children and 112 controls from central Italy. An higher incidence of the mutant T allele in controls (48.2%) than in Down syndrome children mothers (44%) was detected. These results do not support the presence of an increased risk of Down syndrome in mothers carriers of the T allele in the Italian population.

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### Introduction

Methylenetetrahydrofolate reductase (MTHFR) has a crucial role in regulating cellular methylation, through the conversion of 5,10 methyl-THF to 5-methyl-THF, the methyl donor in the transformation of homocysteine to methionine.<sup>1</sup> The C-T common polymorphism at nucleotide 677 (C677T), which results into an alanine to valine substitution in the MTHFR protein, causes higher thermolability and reduced enzyme activity in lymphocyte extracts.<sup>2</sup> Increased folic acid intake is required to maintain homocysteine remethylation to methionine at normal levels. Reduced MTHFR activity due to the C677T change has been associated with different disorders, including vascular diseases,<sup>2</sup> neural tube defects,<sup>3–5</sup> male infertility.<sup>6</sup> However, studies performed in different populations have often shown

inconsistent results, disputing these associations.<sup>7–10</sup> Recently, the C677T polymorphism has been related also to maternal risk for Down syndrome (DS), because of an higher prevalence of the T allele among mothers of children with trisomy 21, compared to control mothers.<sup>11,12</sup> The hypothetical effect of 677T onto maternal non-disjunction was linked to an altered DNA methylation pattern on the oocyte, secondary to reduced MTHFR activity. Available results refer only to DS and control mothers from USA and Canada. Recently, Hassold *et al*<sup>13</sup> analysed maternal polymorphism at MTHFR in cases of trisomies of other chromosomes, detecting significant association only with trisomy 18. The identification of the C677T as a factor of maternal risk for DS would be of great importance in the genetic counselling of this disease, since in this case other female relatives of a carrier DS mother should be tested for the presence of this mutation. In order to support the association between the MTHFR genotype and the risk of DS, we investigated the prevalence of the C677T polymorphism in a sample of Italian mothers of DS children.

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**Table 1** Prevalence of the MTHFR genotypes in DS mothers and controls

Genotype	C/C (%)	DS mothers (n=64)			C/T+T/T (%)	C/C (%)	Controls (n=112)		
		C/T (%)	T/T (%)	C/T (%)			T/T (%)	C/T+T/T (%)	
James <i>et al</i> <sup>11</sup>	26.3	59.6	14	73.6	48	44	8	52	
Hobbs <i>et al</i> <sup>12</sup>	32	54	14	68	48	42	10	52	
Present study	31	50.7	18.3	69	24.1	55.4	20.5	75.9	

## Materials and methods

Sixty-four mothers of trisomy 21 children and 112 controls from the Abruzzo region in central Italy entered this study. Karyotypes of DS parents were normal. The mean age at conception of case mothers was 30.9 years (range 20–46) and 50 mothers were less than 35 years. Informed consent was obtained from all the participants. The 112 controls were mothers with the same mean age, who had unremarkable pregnancy.

MTHFR genotypes were analysed on DNA from peripheral blood cells of mothers by PCR amplification of a 198 bp fragment, followed by *Hinf*I restriction endonuclease digestion.<sup>2</sup> After digestion, samples were run on a 4% agarose or 8% polyacrilamide gels. Allele frequencies were calculated for each genotype, and the  $\chi^2$  test was used for evaluating the differences in allele frequencies between case and control mothers.

## Results

In the case mothers, the C/C wild homozygous genotype was found in 31%, the C/T heterozygous genotype in 50.7% and the T/T mutant homozygous genotype in 18.3%. In the mothers of DS children aged less than 35 years at conception, the C/C genotype frequency was 32.5%, C/T 46.1%, and T/T 21.4%. In the control mothers, the C/C genotype frequency was 24.1%, C/T 55.4% and T/T 20.5% (Table 1). Genotypes of control mothers were in the Hardy Weinberg equilibrium ( $\chi^2=1.16$ ,  $P>0.2$ ). Overall, the frequency of the C allele was 56% in DS mothers and 51.8% in control mothers, while the frequency of the T allele was 44% in DS mothers and 48.2% in the control mothers (Table 2).

## Discussion

Data obtained in this study does not support an increase of DS in the children of mothers with the T allele in our sample, the C/T or T/T genotypes being more common in controls than in DS children mothers. Conversely, we have detected a quite high frequency of the T allele in controls and a lower frequency in mothers of DS patients. This is a point of interest since the genotypes in the mothers of DS children were comparable to those reported by James *et al*<sup>11</sup> and Hobbs *et al*,<sup>12</sup> while controls disclosed an higher frequency of the T allele, the C/T and T/T genotypes accounting for 75.9% of the analysed controls. The difference between case and controls was not significant

**Table 2** Allele frequency of MTHFR 677C->T in DS mothers and controls

Allele	DS mothers	Controls	$\chi^2$	P
C	0.56	0.52	0.6	>0.2
T	0.44	0.48		

( $\chi^2=2.59$ ,  $P>0.05$ ), even considering only mothers aged less than 35 years ( $\chi^2=3.11$ ,  $P>0.05$ ).

These data confirm previous studies by showing an high prevalence of the T allele in the Italian population.<sup>5,14–16</sup> Present results do not rule out an effect of the 677T allele on maternal non disjunction, but suggest that in populations with adequate folate dietary intake the reduced enzymatic activity of the MTHFR in the carriers of the T allele is balanced by folates, thus reducing the risk of cellular demethylation and non disjunction. This also agrees with the observation that, despite an higher prevalence of the 677T in Italy, compared to other European countries, the prevalence of neural tube defects is not increased in the Italian population.<sup>5</sup> Thus, genotyping of the MTHFR gene at present does not appear useful to identify women with increased risk of non disjunction in Italian subjects.

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