



Frequency of 677C → T and 1298A → C polymorphisms in the 5,10-methylenetetrahydrofolate reductase (MTHFR) gene in Turner syndrome individuals

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Abstract

Turner syndrome (TS) is an interesting model for investigating the association between methylenetetrahydrofolate reductase (MTHFR) gene polymorphisms and non-disjunction because of the high frequency of chromosomal mosaicism among patients with this syndrome. We determined the frequencies of MTHFR 677C → T and 1298A → C polymorphic mutations in 49 patients with TS and 200 control individuals. The frequency of the 677C → T allele was 0.39 for patients and 0.29 for controls while that of the 1298A → C allele was 0.28 for patients and 0.25 for controls. Genotype frequencies were shown to be different in patients and controls ($\chi^2 = 12.143$; $p = 0.033$), and this was attributable to the higher frequency of the C677C → T/677C → T genotype among TS patients. In homozygotes, this mutation might have an effect on somatic chromosome disjunction by decreasing MTHFR activity.

Key words: chromosomal non-disjunction, MTHFR gene, Turner syndrome.

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Defects in folic acid metabolism because of mutations in the 5,10-methylenetetrahydrofolate reductase (MTHFR) gene have been considered as a possible cause of chromosomal non-disjunction by hypomethylation (James *et al.*, 1999; Hobbs *et al.*, 2000). The *MTHFR* gene is located on the short arm of chromosome 1 (1p36.3) and has 11 exons (Goyette *et al.*, 1994) which code for a cytosolic flavoprotein that catalyzes the reduction of 5,10-methylenetetrahydrofolate (5,10-methyleneTHF) to 5-methyltetrahydrofolate (5-methylTHF), the predominant circulating form of folic acid (Weisberg *et al.*, 1998). One of the metabolic products, S-adenosylmethionine (SAM), is a methyl donor in various methylation reactions, including DNA and protein methylation and phospholipid synthesis (Chiang *et al.*, 1996).

Frosst *et al.* (1995) identified a C to T substitution at nucleotide 677 in the coding region of the *MTHFR* gene

converting an alanine to a valine residue in the gene product and creating a new *HinfI* restriction site resulting in the *MTHFR* 677C → T polymorphism and a reduction in enzyme activity which may lead to a decrease in the levels of SAM, inhibition of methyltransferase and subsequent DNA hypomethylation. The importance of the stable methylation of pericentromeric DNA for chromosomal stabilization and segregation has been supported both by clinical and experimental data (reviewed by Hobbs *et al.*, 2000). Therefore, in homozygosis, the 677C → T mutation may be a contributing risk factor to somatic chromosomal non-disjunction.

James *et al.* (1999) studied the mothers of Down syndrome children and found a high frequency of mothers who were heterozygous for the *MTHFR* 677C → T polymorphism, suggesting that this mutation might be a risk factor for chromosome 21 non-disjunction. Hobbs *et al.* (2000) further investigated this association and in addition studied the methionine synthase reductase (*MTRR*) gene, also involved in folate metabolism, and found not only that the *MTRR* 66A → G polymorphism was independently associated with a 2.6 fold increase in the estimated risk for Down

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syndrome when mothers were homozygous for this gene but that the presence of both the polymorphisms was associated with a greater risk than was the presence of either alone.

However, the association between the *MTHFR* 677C → T and *MTRR* 66A → G polymorphisms is still controversial. In an extensive study, Hassold *et al.* (2001) determined the frequencies of the *MTHFR* 677C → T polymorphism in mothers of 93 cases of sex chromosome trisomy, 158 cases of chromosome 2, 7, 10, 13, 14, 15, 16 and 22 trisomy and 44 cases of trisomy 18 but detected significant increase in the 677C → T polymorphism only in mothers of trisomy 18 concepti. Even so, Chadeaux-Vekeman *et al.* (2002) did not detect any significant differences in 677C → T genotype frequencies among 85 mothers of fetuses with DS and 107 controls. A similar finding was reported by Petersen *et al.* (2001), who analyzed 177 mothers of DS patients resulting from maternal non-disjunction in meiosis I or II.

A possible explanation for these contradictory findings is the influence of maternal age, and it may be that *MTHFR* deficiency is more prevalent as a cause of non-disjunction among younger mothers of Down syndrome children while in older mothers maternal age may be the risk factor. In the study by Chadeaux-Vekemans *et al.* (2002) the mean age of the mothers of Down syndrome children was 34.9 years (range: 20 to 47 years), whereas in the study by James *et al.* (1999) the mean age was 29.7 years and all the mothers were less than 40 years old.

The A to C substitution at base pair 1298 of the *MTHFR* gene (van der Put *et al.*, 1998) causes a glutamate to alanine substitution (the *MTHFR* 1298A → C polymorphism) which abolishes the *Mbo*II restriction site created by the *MTHFR* 677C → T polymorphism.

Turner syndrome individuals frequently show mosaicism (Hook and Warbuton, 1983; Robinson, 1990) and the presence of a normal cell lineage along with the 45,X lineage is considered to increase the probability of survival of those with 45,X Turner syndrome (Held *et al.*, 1992, Kelly *et al.*, 1992).

Our study was carried out because Turner syndrome can be used as a model for investigating the contribution of the *MTHFR* gene polymorphisms to somatic chromosomal non-disjunction and help to shed light on the role of this genetic mechanism in both Down and Turner syndrome.

The study group consisted of 49 Turner syndrome individuals (mean age 15.6 ± 6.4 ; range 3 to 33 years) of predominantly European descent. For each individual we assessed metaphases from a minimum of 50 and a maximum of 113 blood lymphocytes, 50 cells providing a probability of $\approx 99.9\%$ of detecting a mosaicism in which the minority cell line occurs with a frequency of at least 10% or a probability of $\approx 96.2\%$ of detecting a mosaicism including a minority cell line with a frequency of at least 5% (Bei-

guelman, 1982). In our group 24 individuals had a 45,X karyotype and 25 had at least one additional cell line (*i.e.* 22 individuals with 45,X/46,XX, one with 45,X/46,XX/47,XXX, another with 45,X/46,XY and a third with 45,X/46,XX/47,XX + 13). The control group was randomly selected from a group of blood donors in apparently good health and consisted of 106 females plus 94 males ($n = 200$ with a mean age = 27.3 ± 6.1 years; range 18 to 43 years), 110 being of predominantly European descent and 90 of predominantly African descent. This study was approved by the ethics committee of our institution and informed consent was obtained from each individual or their legal guardians.

Genomic DNA of members of the study and control groups was extracted from lymphocytes and purified (Woodhead *et al.*, 1986) and the 677 region of the *MTHFR* gene PCR amplified using forward (5'-TGAAGGAGAAGGTGTCTGCGGGA-3') and reverse (5'-AGGCGGTGCGGTGAGAGTG-3') primers to generate a 198-bp fragment which was digested with *Hinf*I to yield a 23 and 175 bp fragment that were separated by polyacrylamide gel electrophoresis (Figure 1).

We also conducted PCR to amplify the 1298 region of the *MTHFR* gene using forward (5'-CTTTGCGGAGCTGAAGGACTACTAC-3') and reverse 5'-CACTTTGTGACCATTCCGGTTTG-3') primers to generate a 163-bp fragment which was digested with *Mbo*II to yield 56, 31, 30, 28 and 18 bp fragments from the normal gene and 84, 31, 30 and 18 bp fragments from the *MTHFR* 1298A → C polymorphism which were separated by polyacrylamide gel electrophoresis (Figure 2).

In the 49 Turners syndrome individuals the frequency of the two alleles was 677C → T = 0.39 and 1298A → C = 0.28 while that in the 200 control individuals was 677C → T = 0.29 and 1298A → C = 0.25 (Table 1). Although the differences between the two groups were not significant by the chi-squared test ($\chi^2 = 5.5$; for 2 degrees of freedom (df) at $p = 0.064$) analysis of the adjusted residuals using Haberman's test suggested that the frequency of the normal allele was somewhat decreased in the Turner syn-

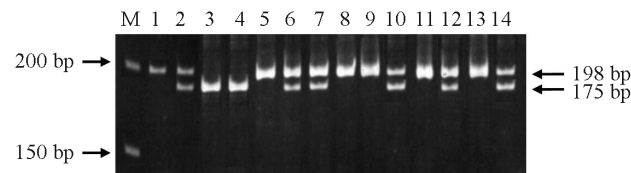


Figure 1 - Polymorphism analysis of the methylenetetrahydrofolate reductase (*MTHFR*) 677C → T allele: *Hinf*I-digested PCR fragments from the 677C → T polymorphism (175 bp) and from the normal allele (198 bp). M = molecular weight markers; 1 = undigested control fragment; 2 = 677C → T heterozygous control; 3 and 4 = 677C → T homozygous Turner syndrome (TS) individuals; 5, 8, 9, 11 and 13 = 677C → T null TS individuals without the polymorphism; 6, 7, 10, 12 and 14 = 677C → T heterozygous TS individuals.

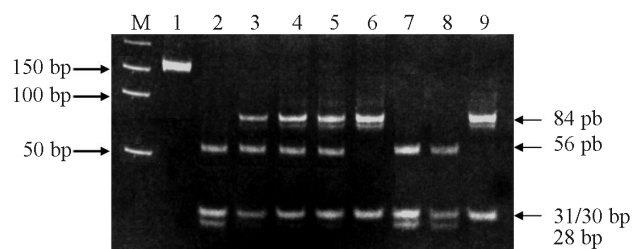


Figure 2 - Polymorphism analysis of the methylenetetrahydrofolate reductase (MTHFR) of the 1298A → C allele: *Mbo*I-digested PCR fragments from the 1298A → C mutation (84 and 31/30 bp) and from the normal allele (56, 31/30 and 28 bp). M = molecular weight marker; 1 = undigested control fragment; 2 = control without the polymorphism; 3, 4 and 5 = 1298A → C heterozygous Turner syndrome (TS) individuals; 6 and 9 = → C homozygous TS individual; 7 and 8 = 1298A → C null TS individuals.

Table 1 - Frequencies of the methylenetetrahydrofolate reductase (MTHFR) 677C → T and 1298A → C alleles in 49 individuals with Turner syndrome and 200 controls.

Alleles	Turner syndrome		Controls	
	Number	%	Number	%
677C → T	117	29	38	39
1298A → C	102	25	28	28
Wild-type allele	181	46	32	33
Total	400	100	98	100

drome group. When the Turner syndrome individuals were grouped genotypically according to polymorphism a significant difference was observed for the 677C → T polymorphism ($\chi^2 = 8.822$; $df = 2$; $p = 0.012$) but not for the 1298A → C polymorphism ($\chi^2 = 0.448$; $df = 2$; $p = 0.799$). Subsequent analysis of all possible genotypes using both alleles (Table 2) revealed a significant difference between Turner syndrome individuals and controls ($\chi^2 = 12.143$; $df = 5$; $p = 0.033$), the 677C → T/677C → T homozygote genotype being more frequent among Turner syndrome individuals as shown by an analysis of adjusted residuals using Haberman's test. When individuals of African descent were excluded from the control group, the differences persisted ($\chi^2 = 12.46$; $df = 5$; $p = 0.029$).

Table 2 - Genotypic distribution of the methylenetetrahydrofolate reductase (MTHFR) 677C → T and 1298A → C alleles in 49 individuals with Turner syndrome and 200 controls.

Genotypes	Turner syndrome	Controls
677C → T homozygote	9	11
677C → T heterozygote	13	62
1298A → C homozygote	5	15
1298A → C heterozygote	11	39
Compound heterozygote	7	33
Wild-type homozygotes	4	40

Our results show an increase in the frequency of the MTHFR 677C → T homozygote genotype in Turner syndrome individuals. Mosaicism among these patients is frequent and it is believed that the presence of a normal cell lineage along with the 45,X lineage confers viability to a conceptus (Kelly *et al.*, 1992). Indeed, in our study about half of the Turner syndrome individuals tested were mosaics, implying post-zygotic non-disjunction events. As mentioned above, the 677C → T polymorphism causes a reduction in enzyme activity (Frosst *et al.*, 1995) which may lead to inhibition of methyltransferase through decreased levels of SAM and result in DNA hypomethylation. Both clinical and experimental data support the importance of pericentromeric DNA methylation for chromosomal stabilization and segregation (Hobbs *et al.*, 2000) and it appears that in homozygosis the 677C → T mutation may be a contributing risk factor to somatic chromosomal non-disjunction.

References

- Beiguelman B (1982) As cromossomopatias autossômicas. In: Citogenética Humana. Guanabara Koogan, Rio de Janeiro, pp 179-218.
- Chadefaux-Vekemans B, Coude M, Muller F, Oury JF, Chabli A, Jais J and Kamoun P (2002) Methylenetetrahydrofolate reductase polymorphism in the etiology of Down syndrome. *Pediatr Res* 51:766-767.
- Chiang PK, Gordon RK, Tal J, Zeng GC, Doctor BP, Pardhasaradhi K and MacAnn PP (1996) S-adenosylmethionine and methylation. *FASEB J* 10:471-480.
- Frosst P, Blom HJ, Milos R, Goyette P, Sheppard CA, Matthews RG, Boers GJ, Den Heijer M, Kluijtmans LA, Van Den Heuvel LP and Rozen R (1995) A candidate genetic risk factor for vascular disease: A common mutation in methylenetetrahydrofolate reductase. *Nat Genet* 10:111-113.
- Goyette P, Summer JS, Milos R, Duncan AMV, Rosenblatt DS, Matthews RG and Rozen R (1994) Human methylenetetrahydrofolate reductase: Isolation of cDNA, mapping and mutation identification. *Nat Genet* 7:195-200.
- Hassold T (1986) Chromosome abnormalities in human reproductive wastage. *Trends Genet* 2:105-110.
- Hassold JT, Burrage LC, Chan E, Judis LM, Chwartz S, James SJ, Jacobs PA and Thomas NS (2001) Maternal folate polymorphisms and the etiology of human nondisjunction. *Am J Hum Genet* 69:434-439.
- Held KR, Kerber S, Kaminsky E, Singh S, Goetz P, Seemanova E and Goedde HW (1992) Mosaicism in 45, X Turner syndrome: Does survival in early pregnancy depend on the presence of two sex chromosomes? *Hum Genet* 29:94-97.
- Hobbs CA, Sherman SL, Yi P, Hopkins SE, Torfs CP, Ne RJ, Pogribna M, Rozen R and Mes SJ (2000) Polymorphisms in genes involved in folate metabolism as maternal risk factors for Down syndrome. *Am J Hum Genet* 67:623-630.
- Hook EB and Warbuton D (1983) The distribution of chromosomal genotypes associated with Turner's syndrome, live birth prevalence rates and evidence for diminished fetal mortality and severity in genotypes associated with structural X abnormalities or mosaicism. *Hum Genet* 64:24-27.

- James SJ, Pogribna M, Pogubny IP, Melnyk S, Hine RJ, Gibson JB, Yi P, Tafoya DL, Swenson DH, Wilson VL and Gaylor DW (1999) Abnormal folate metabolism and mutation in the methylenetetrahydrofolate reductase gene may be maternal risk factors for Down syndrome. *Am J Clin Nutr* 70:495-501.
- Kelly TE, Ferguson JE and Golden W (1992) Survival of fetuses with 45, X: An instructive case and an hypothesis. *Am J Med Genet* 42:825-826.
- Petersen MB, Grigoriadou M and Mikkelsen M (2001) Polymorphisms in genes involved in folate metabolism are not maternal risk factors for Down syndrome. *Am J Hum Genet* 69(suppl) 2:141.
- Robinson A (1990) Demography and prevalence of Turner syndrome. In: Rosenfeld RG and Grumbach MM (eds) *Turner Syndrome*. Basel & Dekker, New York, pp 93-99.
- van der Put NMJ, Gabreëls F, Stevens EM, Smeitink JA, Trijbels FJ, Eskes TK, Van Den Heuvel LP and Blom HJ (1998) A second common mutation in the methylene-tetrahydrofolate reductase gene: An additional risk for neural-tube defects? *Am J Hum Genet* 62:1044-1051.
- Weisberg GI, Tran P, Christensen B, Sibani S and Rozen R (1998) A second genetic polymorphism in methylenetetrahydrofolate reductase (MTHFR) associated with decreased enzyme activity. *Mol Genet Metab* 64:169-172.
- Woodhead JL, Fallon R, Figuered H, Longdale J and Malcon ADB (1986) Alternative methodology of gene diagnosis. In: Daves KE (ed) *Human Genetic Disease - A Practical Approach*. IRL Press Limited, Oxford, pp 88- 124.

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