Original Article

Prevalence of methylenetetrahydrofolate reductase 677 C-T polymorphism among mothers of Down syndrome children

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INTRODUCTION: The relationship between chromosomal non-disjunction leading to aneuploidy and folate metabolism has drawn attention in the recent years. In this study, we examined the polymorphism in the gene encoding the folate metabolizing enzyme methylenetetrahydrofolate reductase (MTHFR), namely, 677 C-T in women having Down syndrome (DS) children.
MATERIALS AND METHODS: The prevalence of these variant genotypes (MTHFR 677 C-T polymorphism) in women having DS children (case mothers) ($n = 110$) was compared with controls ($n = 111$) from Punjab. Genotyping was done using the polymerase chain reaction method followed by restriction fragment length polymorphism.
RESULTS: In the present study, 1.8% of case mothers had TT genotype while none of the control mothers showed this genotype. T allele frequency among cases was 0.13 and 0.11 in controls. The Chi-square value showed a non-significant difference between cases and controls.
CONCLUSION: No association has been observed between 677 C-T polymorphism and risk of non-disjunction in case mothers. Detection of polymorphisms in more genes of folate pathway is required to find out the exact cause of non-disjunction.

Key words: Down syndrome, methylenetetrahydrofolate reductase, polymorphism

Introduction

The higher birth frequency of Down syndrome (DS) has been a subject of interest to clinicians and researchers due to its phenotypic expression. In addition to the advanced maternal age, studies have linked the increased frequency of polymorphism of methylenetetrahydrofolate reductase (MTHFR), 677 C-T in mothers with DS child. Abnormal folate and methyl metabolism can lead to deoxyribonucleic acid (DNA) hypomethylation and abnormal chromosomal segregation; researchers have observed that mothers with mutations in MTHFR and other genes in this pathway have elevated levels of plasma homocysteine. An increase in plasma homocysteine was found to be a risk factor for DS in several of the studies. There seems to be a geographic variation in MTHFR gene polymorphism. It appears that DS is attributable not only to meiotic non-disjunction in mothers, but also to the gene, nutritional and environmental factors.

The gene for MTHFR is located on chromosome 1 at the position 1p36.3 having 11 exons. The most common polymorphism associated with the risk of DS, neural tube defect, mental retardation, congenital malformations is 677 C-T in the exon 4. This missense mutation leads to the substitution of valine instead of alanine residue thus creating a new restriction site for Hinf I resulting in 677 C-T polymorphism. In individuals with CT genotype, enzyme activity is reduced to 35% while with TT genotype it is reduced to 70%. Reduced MTHFR activity results in increased requirement for folic acid to maintain homocysteine remethylation, which has been considered as the risk factor for non-disjunction.[1,2] There is only one report on MTHFR polymorphism from North India,[3] whereas no data is available from Punjab. Thus, non-availability of data has prompted this first study

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on Punjabi population to establish the role of MTHFR polymorphism in women with DS children.

Materials and Methods

Blood samples of 110 mothers having DS children and 111 mothers having normal children were collected. Mothers of DS children were selected after confirming trisomy in children by cytogenetic analysis. The control mothers had normal children and did not have any abortion. Informed consent and approval of institutional ethical committee was obtained before all the investigation. Detailed family history and pedigree analysis was done. Total genomic DNA was isolated from 2 ml peripheral blood lymphocyte by phenol extraction method,[4] with modifications. Polymerase chain reaction (PCR) was carried out for 677 C-T polymorphism using specific primers[5] and conditions as follows:

F: 5'-TGAAGGAGAAGGTGTCTGCGGGA-3'
R: 5'-AGGACGGTGCGGTGAGAGTG-3'

For 35 cycles at 95°C for 45 sec, 62°C for 30 s and 72°C for 45 s. The 198 bp fragment was obtained as PCR product and was subjected to the restriction digestion by the enzyme Hinf I (4U/reaction) at 37°C overnight. Restriction digestion products were then electrophoresed in 2.5% agarose gel followed by ethidium bromide staining. Heterozygotes (CT) produced 198 bp, 175 bp and 23 bp fragments, homozygous mutant (TT) produced 175 bp and 23 bp fragments, while homozygous (CC) wild produced only 198 bp fragment.

Genotypic and allelic frequencies were calculated under the assumption of Hardy's Weinberg Equilibrium. To compare cases and controls mothers, Pearson's Chi-square test was employed and to estimate relative risk for DS, odds ratio at 95% confidence interval was calculated. Analyses were performed using software (Statistical Package for the Social Sciences Inc. 10, Chicago, IL, USA).

Results

A total of 110 cases and 111 controls mothers for 677 C-T genotyping were enrolled in the present study. The mean maternal age at the time of birth of DS child was 27.5 years, while it was 29.8 years in control mothers. In our study, 78.2% of DS mothers had CC genotype, 20% CT and 1.8% TT while 80.2% and 19.8% of control mothers had CC and CT genotype, respectively. None of the control mothers had TT genotype. The T allele frequencies among cases and controls were 0.13 and 0.11, respectively. The Chi-square (χ^2 - 2.064) showed a non-significant difference between cases and controls [Table 1]. No relative risk was observed between genotypes [Table 2].

Discussion

The aim of the present study was to estimate T allele frequency among mothers of DS children from Punjab and to establish a link between the presence of T allele and risk of having DS children. The data for T allele frequency revealed a non-significant difference between cases and controls. The frequency of homozygous wild genotype (CC) was much higher in both cases (78.2%) and (χ^2 - 2.064) showed that 677 C-T polymorphism was not associated with DS. The results from our study were consistent with various studies.[2,6-13] Several reports have suggested that higher frequency of T allele is associated with risk of non-disjunction.[1,2,14-15] Meguid *et al.*[15] reported higher genotypic frequencies (CT and TT) among cases as compared to controls with odds ratio of 2.34 and 2.75, respectively and similarly, Ruxandra *et al.*[16] observed a higher heterozygous and homozygous mutant genotypic frequencies among control mothers (45.7% and 15.2%) than cases (38.5% and 7.7%). According to van der Put *et al.*[17] single polymorphism (677 C-T) is not sufficient

Table 1: Allele and genotype distributions of 677 C-T polymorphism

Study	Number	Genotype				Allele		HWE tests	
		CC	CT	TT	CT+TT	C-allele	T-allele	X2	*P* value
Cases	110	86 (78.2)	22 (20.0)	02 (1.8)	24 (21.9)	0.97	0.13	2.064	0.3593
Controls	111	89 (80.2)	22 (19.8)	00	22 (19.8)	1	0.11		

Percentages in parenthesis

Table 2: MTHFR 677 C-T odds ratio among cases and controls

Genotype	Cases	Controls	Odds ratio 95% (CI)	*P* value
CC	86	89	0.88	0.71
CT+TT	24	22	(0.46-1.7)	
CT	22	22	1.01	0.97
CC+TT	88	89	(0.52-1.9)	

Percentages in parenthesis

to increase the risk of DS but its association with other polymorphisms in folate metabolizing genes such as 1298 A-C may lead to increased risk of non-disjunction. It is further indicated by Devi *et al*.[18] and van der Put *et al*.[17] that individuals with genotype 677 TT always had 1298 AA genotype or vice versa, thus two alleles are always in trans-configuration. Non-significant association in the present study may be due to single polymorphism study. Thus, to establish association, study with other polymorphisms in folate metabolizing genes and large sample size is required. Since very limited data has been reported from North Indian population, such studies will help in generating a base line data and to develop tools aimed at evaluating the risk for younger women having DS child.

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