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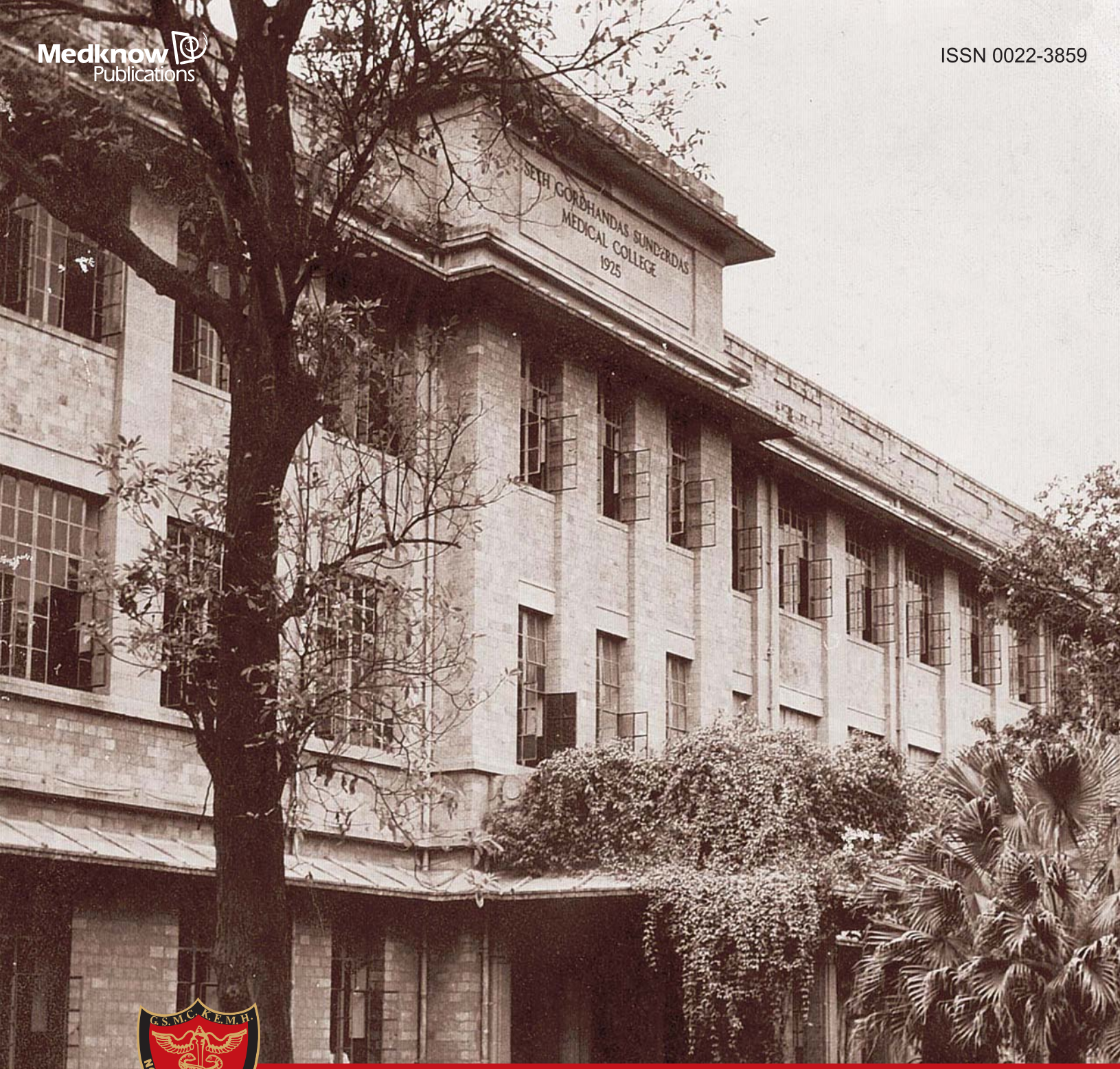

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MTHFR A1298C polymorphism and idiopathic male infertility

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ABSTRACT

Background: DNA methylation is an important epigenetic feature of DNA that plays a pivotal role in gene expression regulation during spermatogenesis. The enzyme methylenetetrahydrofolate reductase (MTHFR) catalyses the formation of folate intermediates that are vital for DNA synthesis and methylation reactions. C677T and A1298C variants of MTHFR result in reduced plasma folate and increase the susceptibility to various multifactorial disorders. We have already shown that homozygosity for 677 (C →T) mutation in the MTHFR gene, is a risk factor for idiopathic male infertility in an Indian population. Aim: Recently, we showed that homozygosity for the 677(C→T) mutation in the MTHFR gene is a risk factor for idiopathic male infertility and now we aim to assess whether the A1298C mutation in the same gene is an additional risk factor for idiopathic male infertility in an Indian population. **Setting and Designs:** In a case-control study 151 idiopathic male infertile patients and 140 healthy fertile control individuals were recruited from the University hospital and infertility clinics in Varanasi city, India. **Materials and Methods**: Genotyping for A1298C change of the MTHFR gene was done by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. **Statistical Analysis:** Allele frequencies were calculated using Fisher's exact test. Odds ratio was calculated as the measure of the association between the MTHFR genotype and idiopathic male infertility. **Results:** The homozygous (C/C) A1298C polymorphism of the MTHFR gene was present at a statistically high significance in idiopathic azoospermic infertile men (OR=3.4494, CI: 1.0092 to 11.7899, $P<0.05$). **Conclusion:** The MTHFR 1298CC genotype is an additional genetic risk factor for idiopathic male infertility in an Indian population.

KEY WORDS: Azoospermia, male infertility, methylene tetrahydrofolate reductase

Introduction

Male infertility is a multifactorial disorder and the spectrum of factors affecting male infertility is diverse ranging from genetic to environmental. Spermatogenesis is governed by a tightly controlled series of gene expression events. Both Y-chromosomal as well as autosomal genes regulate the spermatogenesis at different steps and the aberrant expression of these genes may lead to infertility.[1] One of the mechanisms regulating their expression is DNA methylation. Folate is indispensable for DNA synthesis and methylation of DNA and histones. Methylenetetrahydrofolate reductase (MTHFR) is a candidate gene of the folate and homocysteine metabolic pathway and catalyses methylation of 5, 10 methylenetetrahydrofolate, which contributes to the methyl group in the conversion of homocysteine to methionine. Methionine gets converted to 5-adenosylmethionine, the lone donor of $-CH_3$ to cytosine and lysine residues, respectively in DNA and histones.[2]

DNA and histone methylation regulates the expression of candidate genes of spermatogenesis pathway. Hypomethylation leading to aberrant expression of these genes may result from MTHFR deficiency. Inactivation of MTHFR results in absence of germinal cells and spermatogenesis arrest.[3] The above reports suggest the role of MTHFR in spermatogenesis and indicate that its deregulation could be involved in male infertility.

Materials and Methods

This case-control study included a total of 151 patients classified as idiopathic azoospermic visiting the Out Patient Door facility of the Department of Endocrinology and Metabolism, Institute of Medical Sciences, Banaras Hindu University, Varanasi and infertility clinics in Varanasi city. 140 fertile control individuals of a comparable age group (30, SD ±3), belonging to the same geographical region were included in the study. At least three seminal fluid examinations, carried out after three to four days of sexual abstinence, were performed to ascertain their infertility status. Each patient's family history, habits (smoking, alcoholism etc.), and disease were recorded through a questionnaire. Those who suffered from varicocele, diabetes, mumps and orchitis etc. were excluded from the study. Informed consent was obtained from all the subjects to carry out molecular analyses. Institutional

ethical committee approval was obtained.

Genotyping for A1298C change in the MTHFR gene was done by PCR-RFLP method. An A→C change at base pair 1298 abolishes an M*bo*II restriction site. PCR product of 163bp was digested with M*bo*II following which wild type 1298AA and mutant 1298CC alleles would yield, respectively 5 (56, 31, 30, 28, 18bp) and 4 fragments (84, 31, 30, 18bp). The identification bands for each were of 56 and 84bp,[4] shown in Figure 1.

Statistical analysis

Allele frequencies were calculated for each genotype and differences between the case and control were determined using Fisher's exact test crude. Odds ratio for the heterozygous and homozygous mutant between the MTHFR genotypes were calculated as the measure of the association between the MTHFR genotype and idiopathic male infertility, and used as an estimate of relative risk. Throughout, a two-tailed *P* value of < 0.05 was interpreted as statistically significant.

Results

PCR-RFLP analysis was done for DNA extracted from both infertile patients and healthy fertile controls. PCR amplified product was digested with MboII and resolved in 4% agarose gel. Figure 1 illustrates the three genotypes (AA, AC and CC). 151 idiopathic infertile subjects and 140 fertile control individuals were genotyped for A1298C single nucleotide polymorphism in the MTHFR gene. The genotype frequency for the MTHFR A1298C polymorphism is shown in Table 1. The population was in the Hardy-Weinberg Equilibrium (HWE) for C677T genotypes;[5] however, for A1298C genotypes only patient subpopulation was in HWE, the control subpopulation was not. The 1298CC genotype was higher in patients compared to controls and was associated with increased risk for male infertility (OR=3.4494, CI: 1.0092 to 11.7899, P<0.05). The cross-tabulation of genotype combinations of MTHFR C677T and A1298C revealed none of the subjects to be homozygous for both the mutations. Combined heterozygosity AC/TT frequency was quite comparable in both the groups (10.6% in cases and 9.3% in controls).

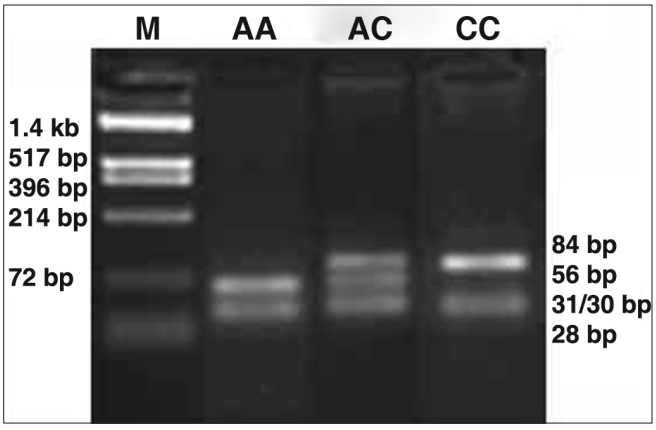

Figure 1: RFLP analysis for the 1298(A→C) mutation on a 163-bp PCR fragment with MboII. The 1298(A→C) abolishes an MboII restriction site. Digestion of the 163-bp fragment of the 1298AA genotype gives five fragments, of 56, 31, 30, 28 and 18 bp, whereas the 1298CC genotype results in four fragments, namely, 84, 31, 30, and 18 bp. The 18 bp fragment has been run off the gel

Discussion

Our study on MTHFR SNP A1298C arises out of the consideration that DNA methylation plays an important role in the regulation of spermatogenesis and that SAM, the lone donor of methyl group is the product of homocysteine (Hcy) metabolism.[6] It is also known that certain SNPs in one of the genes in this pathway, MTHFR , lead to impaired enzyme activity, elevation of Hcy levels and serve as risk factor for various multifactorial disorders. Animal model studies suggest that MTHFR plays a critical role in spermatogenesis.[3] Previously, we have reported that MTHFR, C677T, is a risk factor for infertility in an Indian population.[5] Since A1298C is associated with the lowering of MTHFR enzyme activity, and elevated Hcy level in Indian populations,[7] it is logical to see its association with infertility, particularly in the individuals already analyzed for C677T polymorphism. 1298CC genotype showed significant difference in its frequency between the cases and controls.

Heterogeneous distribution of C677T and A1298C is recorded globally and may be attributable to different ethnic, environmental and genetic backgrounds. The present study has certain limitations. For A1298C polymorphism only patient group was in HWE not the control group. Similar results has previously been reported from North India where patient subpopulation was only in HWE for A1298C polymorphism.[8,9] The present study was done in the Indian context thus generalizability to the other ethnic groups remains uncertain.

The identification of genetic risk is an important step for the management of idiopathic male infertility. The findings from the present study support the hypothesis that 1298CC homozygosity is an additional risk for idiopathic male infertility in an Indian population.

Table 1: Genotype frequency of A1298C, MTHFR in case as well as controls

	Cases	Controls	Odds ratio	95% CI	*P*
Total	151	140			
Genotypes					
AA	66 (43.7)	64 (45.7)	1.0		0
AC	76 (50.3)	74 (52.9)	1.004	0.6231 to 1.5918	
CC	09 (6.0)	02 (1.40)	3.4494	1.0092 to 11.7899	<0.05
AC +CC	85 (56.3)	76 (54.3)	1.0842	0.6833 to 1.7205	

Figures in parentheses are in percentage

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