Does MTHFR C677T polymorphism predispose asthma? A North Indian study

Research Article

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Summary

Background: Involvement of impaired folate metabolism has been hypothesized to be a potential risk factor for the predisposition of asthma. The reports are conflicting on the potential association between atopic disease and polymorphism of the methylene-tetrahydrofolate reductase (MTHFR)-gene.

Objective: We designed the study to investigate the association of C677T polymorphism of the MTHFR gene with asthma in the Indian population.

Methodology: This was a hospital based case control study in which 200 asthmatic cases and 329 controls from North India population were enrolled and the MTHFR gene C677T polymorphism was genotyped by PCR-RFLP method.

Result: We observed 92.85% of control group as wildtype homozygous, 11.24% and 1.51% as heterozygous and mutant homozygous respectively, while 98.25% and 3.75% of case group as wildtype homozygous and heterozygous respectively.

Conclusion: Our finding suggests a lack of association of MTHFR C677T polymorphism with asthma. Further association studies in worldwide populations, involving different ethnic group samples with narrowly defined phenotypes of asthma, could contribute to the understanding of genetic susceptibility to asthma.

I. Introduction:

Asthma is an inflammatory disease of small airways of the lungs- characterized by an intermittent airway narrowing and airflow obstruction that leads to symptoms of wheeze and shortness of breath (Cookson W et al., 1999). The genetic etiology of asthma is multi-factorial and still remains elusive. In developing countries, prevalence of asthma and allergy increased in the past 20–30 years and the cause remains unclear (Edwards A et al., 1991). Multiple genetic components can predispose an individual to asthma they are: ADAM33, PHF11, DPP10, GRPA, SPINK5, IL5, FceRI-β, CFTR and MTHFR (Van EP et al., 2002; Zhang Y et al., 2003; Allen M et al., 2003; Laitinen T et al., 2004; Walley AJ et al.,
while the involvement of MTHFR gene polymorphism has not been studied in asthma patients of Indian population. MTHFR gene encoded Methyl-enetetrahydrofolatereductase catalyses conversion of 5, 10 methylenetetrahydrofolate to 5-methlytetrahydrofolate in the metabolism of folate and homocysteine. The reduced efficiency of the enzyme has been linked to two functional polymorphism-C677T and A1298C-that in turn resulting in decreased serum level of folate and elevated level of homocysteine(Frosst P et al., 1995; Jacques PF et al., 1996). However, folate supplement has been shown to reverse back the enzyme activity to its optimal level. This is due to excess of vitamin B2, cofactor which gets dissociated very soon from the enzyme synthesized by the gene of C677T polymorphism (Guenther BD et al., 1999; Yamada K et al., 2001). Folate has an essential role in basic cellular functions-nucleotide synthesis, cell division, cell differentiation, DNA methylation and fetal development (Haberg SE et al., 2009). In late 20th century, it was reported that the MTHFR (C677T) genotype and inadequate folate status induces an altered th1/th2 balance attributing to the development of atopic allergy. The non-allergic th1-type immune responses requires folate- necessary for methyl group transfer reaction in proliferative reactiontherefore reduced folate level (TT genotype) favors the th-2 type immune responses over the th-1 type resulting in atopic reaction (Lucey DR et al., 1996; Hansen G et al., 1999). In addition, folate deficiency in an adult and pregnant women results in numerous complications that include neural tube defects, cardiovascular defects, atherosclerosis, congenital heart defects, Down syndrome and various cancers (Brustolin S et al., 2010). The C677T polymorphism in the MTHFR gene has been reported to be associated with asthma in the Denmark population (Thuesen BH et al., 2010). However, another report from the same population has failed to show such association with asthma (Thuesen BH et al., 2009). These observations raise the question of whether genetic variation of the MTHFR gene confers susceptibility to asthma. In the present study, we intended to investigate whether C677T polymorphism of MTHFR gene has any association with asthma in the Indian population.

II. Material and Method:
Study Design and Subjects:
This was a case-control study jointly conducted at the Department of Pediatrics, ChhatrapatiShahujiMaharaj Medical University and the Department of Genetics, SGPGIMS, Lucknow, Uttar Pradesh. Included in the study were 200 cases of bronchial asthma and a total of 329 controls. Institutional Ethical Committee approved the study, cases and controls were recruited after obtaining informed consent.

Genotyping:
The MTHFR gene C677T polymorphism was analyzed by PCR-RFLP method. The target sequence was amplified with the primer reported elsewhere (Tripathi R et al., 2010). Amplification was carried out in 25ul reaction that contains 25ng of DNA, 200mM of dNTP, 2.5ul of 10X reaction buffer and 0.5U of taq polymerase. The reaction mixture was cycled as follows: 95°C of one cycle for 2 minutes was followed by 35 cycles, at 95°C for 30 sec, 58°C for 20 seconds and 72°C for 30 seconds, and a final extension at 72°C for 2 minutes. The amplification generates 198bp product; a 10 ul of PCR product was then restriction digested with 10U of HinfI restriction enzyme. Digestion products were separated by electrophoresis on 3.5% agarose gel and visualized by ultraviolet light after staining with ethidium bromide (CC, CT and TT genotype will produce 198bp, 198bp+175bp+23bp and 175bp+23bp respectively).
Statistical analysis
The data's were fed manually in to Microsoft® office excel® 2007 V12.0. We calculated allele frequencies for each genotype by manual allele counting. Fisher's exact test was applied, the test was two tailed and \( p<0.05 \) was considered as significant, GraphpadInStat V3.10 was used for both Fisher’s exact test and OR

III. Results and discussions:
We analyzed the MTHFR gene C677T polymorphism in a total of 529 samples, 200 asthmatic cases and 329 healthy controls. Table I shows the genotype distribution; surprisingly, number of heterozygous (11.24%) and mutant homozygous (1.51%) genotypes were higher in control cohort as in comparison with the case cohort (Heterozygous - 3.5% & homozygous-0%). The odds ratio (OR (95% CI) p value) was protective for CT genotype (0.28 (0.13-0.64) 0.002) with a significant \( p \) value. Similarly, at allele level wild type allele was common in the case cohort (98.25%) as compared with control cohort (92.85%); minor allele frequency among the cases and control cohort was about 1.75% and 7.14 % respectively (Table 2).

To our knowledge, this is the first study of the association between C677T polymorphism of the MTHFR gene and asthma. Interestingly, we found variant allele (minor allele) to be common among control cohort as compared to that of cases suggesting the polymorphism of MTHFR gene is negatively associated with asthma susceptibility in the Indian population. In contrast, an association of this variant with asthma (self reported doctor-diagnosed) was reported in a study consisting of 6784 individuals from a general population of Denmark. The authors observed that low serum folate levels and the TT genotype of the MTHFR-C677T polymorphism were associated with increased prevalence of self-reported doctor-diagnosed asthma and attacks of shortness of breath. The OR for developing asthma and shortness of breath in individuals with the T/T genotype was 1.52 and 1.47 compared to individuals with the C/C genotype, respectively (Thuesen BH et al., 2010). The discrepancy between this data and ours could be explained by differences in the ethnicity of the investigated patient groups. Alternatively, it could be attributed to specific structures of the surveyed population. Studies in other populations have also failed to replicate its association with asthma (Thuesen, BH et al., 2009; Matsui EC et al., 2009), suggesting that the association of this polymorphism is population specific. However, the genotype distribution of present study deviates significantly from that expected by Hardy – Weinberg equilibrium, as estimated by Fisher’s exact test and in turn it could be attributed to difference in ethnicity among study population. In fact, studies that investigated the genetic association of C677T polymorphism in asthma patients support this hypothesis. Zou CC et al., (2003) conducted a case-control study in the Chinese population, involving 433 asthmatic and 1249 control subjects. The authors observed that both the T allele and TT genotype showed a significantly higher frequency in asthma patients. The OR for the TT genotype compared with all CC and CT genotypes was 1.61 (95% CI 1.06 approximately 2.47). Similarly, in a cross-sectional population-based study of 1,671 male and female residents of Copenhagen County, Denmark, it was suggested that TT individuals had a significantly higher risk of atopy compared with CC/CT individuals [odds ratio 1.76, 95% confidence interval (95% CI) 1.19-2.60] (Thuesen BH et al., 2009). However, the same has not been replicated in other populations, European and US (Thuesen, BH et al., 2009; Matsui EC et al., 2009). Interestingly, it has been suggested that folate
supplementation in mothers during early pregnancy increased the risk of wheezing, and respiratory disease among infants born to these mothers (Husemoen LL et al., 2006). Similarly, a folate-enriched diet also increased the severity of allergic disease in a mouse model of allergic asthma, suggesting that high folate levels induced epigenetic changes, which in turn responsible for inducing allergic diseases (Lucey DR et al., 1996; Hansen G et al., 1999).

In conclusion our finding suggests a lack of association of MTHFR C677T polymorphism with asthma. Further association studies in worldwide populations, involving different ethnic group samples with narrowly defined phenotypes of asthma, could contribute to the understanding of genetic susceptibility to asthma.

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<table>
<thead>
<tr>
<th>Genotype</th>
<th>Number</th>
<th>Frequency (%)</th>
<th>Genotype</th>
<th>Number</th>
<th>Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type</td>
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<td>87.23</td>
<td>Wild type</td>
<td>193</td>
<td>96.5</td>
</tr>
<tr>
<td>Heterozygous</td>
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<td>11.24</td>
<td>Heterozygous</td>
<td>7</td>
<td>3.5</td>
</tr>
<tr>
<td>Homozygous</td>
<td>5</td>
<td>1.51</td>
<td>Homozygous</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 1. Genotype Distribution

Not in HW equilibrium

<table>
<thead>
<tr>
<th>Allele</th>
<th>Control (658)</th>
<th>Case (400)</th>
<th>OR (95% CI) P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>611 92.85%</td>
<td>393 98.25%</td>
<td>Reference</td>
</tr>
<tr>
<td>T</td>
<td>47 7.14%</td>
<td>7 1.75%</td>
<td>0.2316 (0.10 – 0.52)</td>
</tr>
</tbody>
</table>

Table 2: Allele Distribution
References