MTHFR and ACE gene polymorphisms and risk of vascular and degenerative dementias in the elderly

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Abstract

Focal lacunar infarctions due to cerebral small vessel atherosclerosis or single/multiple large cortical infarcts lead to vascular dementia, and different genes and environmental factors have been implicated in causation or aggravation of the disease. Previous reports suggest that some of the risk factors may be common to both vascular as well as degenerative dementia. Among genetic factors, role of angiotensin converting enzyme (ACE) and methylene-tetrahydrofolate reductase (MTHFR) genes as putative risk factors has been examined but the outcome of these studies remain inconclusive. Present study attempted to see the importance of ACE alu insertion/deletion and MTHFR C677T polymorphisms as genetic predisposers to dementia. The study comprised of 80 vascular dementia patients, 90 degenerative dementia patients and 170 age matched controls. All were genotyped for ACE, MTHFR and APOE polymorphisms using PCR-RFLP method. Frequency of ACE D allele was seemingly high in dementia cases (26.7%) when compared to controls (11.2%). However, after adjusting for age and APOE E4, none of the ACE alleles showed good correlation. MTHFR genotypes or alleles also did not show any correlation. Our study suggests no true correlation of ACE or MTHR genes with dementia in elderly.

1. Introduction

Dementia is a multifactorial disorder. Besides old age, hyperlipidemia and presence of APOE E4, mid-life hypertension and hyperhomocysteinemia have also been known to contribute significantly to the disease (SBU Report, 2008; http://www.sbu.se/dementia project). Therefore, genes responsible for these risk factors such as ACE and MTHR could have some role to play in dementia.

MTHFR gene codes for enzyme methylene-tetrahydrofolate reductase which converts 5, 10 methylene tetrahydrofolate to 5 methyl tetrahydrofolate required for the conversion of homocysteine to methionine (Pandey & Pradhan, 2006). A mutation (C677T) in MTHFR gene codes for aberrant thermo-labile enzyme resulting in raised plasma homocysteine concentration (Frost et al., 1995).

Angiotensin converting enzyme (ACE) regulates systemic blood pressure and fluid electrolyte balance and is encoded by ACE gene. Alu insertion/deletion polymorphism in intron 16 of ACE gene has been found to be associated with both vascular and degenerative dementias (Alvarez et al., 1999; Kehoe et al., 1999; Wang et al., 2006). In vitro studies also indicated the involvement of ACE in degrading amyloid beta peptide and preventing its further accumulation in brains of AD patients (Hu, Igarashi, Kawamata, & Nakagawa, 2001; Kehoe et al., 2003).

Objective of the present study is to evaluate the role of ACE I/D and MTHFR C677T polymorphism in vascular and combined degenerative dementias in our regional North Indian cohort. Since, APOE E4 allele is unequivocally associated with increased susceptibility to AD (Rubinsztein & Easton, 1999), we also analysed the effect of ACE and MTHR in development of cognitive impairment in E4 stratified subset of the studied population.

2. Materials and methods

2.1. Patients

Subjects for this study were recruited from the Neurology outpatient clinic of Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow. All patients came from the State of Uttar Pradesh and other surrounding states of North India. The patients who complained of (or if their relatives gave history of) memory or other cognitive impairment, were subjected to mini mental state examination (MMSE) and those having <24/30 score were subjected to detailed neuropsychological testing for abnormalities in behavior, cognition and executive functions. In the neuropsychological examination, patients who fell under DSM IV TR criteria...
for dementia were taken in the study. Diagnosis of probable AD was made according to NINCDS-ADRDA criteria; while diagnosis of VaD was based on NINDS-AIREN criteria for vascular dementia. Modified Hachinski score was used to differentiate between AD and VaD. Familial cases of cognitive and behavior abnormality, metabolic disorders, toxic encephalopathy and major depression, dementia due to infectious diseases, and structural brain disorders were excluded. The patients were further categorized into vascular and degenerative dementia on the basis of chronology of progression of symptoms, standard neuropsychological examination and MRI of brain. Clinical symptoms of each patient were correlated with the MR image. Patients with poor correlation or those having features of mixed dementia were excluded.

The selection criteria for different dementias were as follows.

2.2. Vascular dementia

The patients who fulfilled the criteria for dementia and had in addition, evidence of one or more ischemic strokes from history and multiple large cortical infarcts, border-zone/watershed infarcts, small strategic infarcts, for example, bilateral thalamic strokes, multiple lacunar infarcts particularly basal ganglia Leukoaraisis-extensive white matter disease (sub-cortical leukoencephalopathy). Major parenchyma haemorrhage Cerebral amyloid angiopathy were included in the study. These criteria are in conformity with NINDS-CSN criteria. However, the patients with single clinical stroke and corresponding single large ischemic or hemorrhagic lesion on MRI, who were hospitalised in acute stage of the disease and had cognitive impairment at the time of discharge (2–5 weeks from admission) were excluded from this study.

2.3. Alzheimer’s disease

The patients who fulfilled the criteria for dementia and had a progressive worsening of two or more cognitive domains in the absence of other systemic or brain disease to account for the condition. The diagnosis was supported by the absence of extrapyramidal symptoms and presence of spatial disorientation in the initial phase of the illness.

2.4. Dementia with Lewy bodies

The patients who fulfilled the criteria for dementia and had a progressive course, fluctuating cognition (particularly in attention and alertness), recurrent visual hallucinations (particularly formed hallucinations of people or animals), and parkinsonian features during the early phase of dementia.

2.5. Frontotemporal dementia

The patients who fulfilled the criteria for dementia and had behavioral and personality changes early during the evolution of disease, particularly in the domains of mood and personal/social awareness. The patients were included if the cognitive profile was dominated by speech disturbance with relative preservation of practical functions and spatial orientation.

Patients with other degenerative brain diseases (such as Parkinson’s disease, CBD, PSP, etc.) were included in this study if they had cardinal features of that disease and dementia developed in late stage of the disorder.

3. Controls

Control group comprised of 170 non-related age-matched healthy volunteers aged 45–90 years. None had personal and familial history of cognitive impairment (dementia), stroke, or any neurodegenerative disorder and all of them came to the hospital for routine health check-up.

About 4 ml of blood was taken after getting informed consent from all patients and control subjects. DNA was isolated from the leucocytes using salting out procedure (Miller, Dykes, & Polusky, 1988).

4. ACE genotyping

DNA isolated by the above-said procedure was subjected to amplification using specific primers 5’CTGAGACCATCCCAT-CATTCTGCTGAGGTGTTGCTGAGGAGTGA and 5’GTATGCGGTCACATTTGTGTCAGAT3’ as described earlier (Tiret et al., 1992). Genotyping was done by running amplified PCR products on 3% agarose gel along with molecular weight markers. PCR product is a 190 bp fragment in the absence of insertion and 490 bp in the presence of the Alu insertion.

5. MTHFR genotyping

DNA was amplified using specific primers 5’TGAGAGAGACTGGTCTGGGGCA 3’ and 5’AGGCCGTTGCGGTAGTGCTG3’ as described previously by Frost et al. (1995). The amplified product of 198 bp was digested using restriction enzyme Hinf1. Restriction digestion produced digested fragment of 175 and 23 bp which was analysed by running the digested product on 12% polyacrylamide gel.

APOE genotyping was done as described by Hixson and Vernier, 1990.

6. Statistical analysis

Chi-square ($\chi^2$) test was applied for the analysis of genotypic and allelic distributions. Binary logistic regression analysis was used to find age adjusted odds ratio, using age as a covariate. In analysis of contribution of each genetic factor towards developing dementia, we calculated E4 adjusted odd’s ratio, using E4 allele as a covariate. All the analyses were done in control subjects vs. cumulative dementia, as well as control subjects vs. degenerative and vascular dementias separately using SPSS v 11.5. $p$-Value of less than 0.05 was taken as significant.

7. Results

In this study we recruited 170 patients aged 45–90 years (mean age 64.16 ± 10.02 years), and 170 controls aged 45–90 years (mean age 60.60 ± 8.48 years). There was no significant difference in the mean age of the patients and controls ($p = 0.07$). Of 90 degenerative dementia patients, 33 had AD, 25 had DLB, 14 had FTD, 13 had PD with dementia and five had progressive supranuclear palsy with dementia. MR imaging of all patients was consistent with the clinical diagnosis.

APOE E4 allele frequency was found to be significantly higher in degenerative dementia ($p = 0.0001$, OR 3.86, 95% CI 1.97–7.57) as well as vascular dementia patients ($p = 0.004$, OR 2.88, CI 1.39–5.95). The frequency of this allele was still high when we combined the two dementias ($p = 0.0001$, OR 3.39, CI 1.82–6.32). APOE E2 allele frequency was found to be significantly protective in degenerative dementia ($p = 0.02$, OR 0.17, CI 0.04–0.72) but not in vascular dementia (Table 1).

The genotype and allele frequencies of ACE gene in control subjects followed Hardy Weinberg equilibrium ($p = 0.30$). DD genotype frequency was found to be significantly high in dementia cases (26.7%) when compared to controls (11.2%), showing significant association with dementia, with an age adjusted odd’s ratio of
2.82 (p = 0.002, 95% CI = 1.47–5.41) (Table 2). Frequency of D allele was also significantly high in cases vs. controls (47.9% vs. 37.4%) (p = 0.005, OR = 1.55, 95% CI = 1.14–2.26). When patients were again sub-divided into degenerative and vascular dementia group, DD genotype and D allele were still higher in cases (26.7%, 48.3% and 25.0%, 47.5% for degenerative and vascular dementia, respectively) as against controls. The ACE I allele frequency in controls (62.6%) was taken as reference for logistic regression analysis.

Frequency of MTHFR C and T alleles in control subjects were 82.6% and 17.4%, respectively. Frequencies of CC and CT genotypes in the same were 69.4% and 26.5%, while that of TT genotype was 82.6% and 17.4%, respectively. Frequencies of CC and CT genotypes (62.6%) was taken as reference for logistic regression analysis.

Apart from high LDL, owing to E4 allele of APOE gene, previous studies clearly indicated role of high homocysteine in neurological as well as vascular disorders (Harker, Ross, Stichter, & Scott, 1976; Porter & Roberts, 1993) Several studies have identified the thermolabile variant TT to be associated with mildly elevated plasma homocysteine levels in general population (Deloughery et al., 1996; Jacques et al., 1996), which however, is not unanimous (Jacques et al., 1996; Mcllroy, Dynan, Lawson, Patterson, & Passmore, 2002). Due to contradictory data in the literature, contribution of elevated homocysteine and MTHFR polymorphisms in the etiopathogenesis of the disease is still obscure (Brunelli, Bagnoli, & Giusti, 2001; Gussekloo et al., 1999; Wakutani et al., 2002; Zuliani et al., 2001). In light of these events, we tested the hypothesis that MTHFR T allele may play a role in the adverse vascular events ultimately leading to dementia but the results contradict this hypothesis. Present study does not support the association of MTHFR gene with dementia, either in presence of confounding factors like age and APOE E4 or independent of it. The prevalence of MTHFR TT homozygotes exhibit vast diversity between different populations, ranging from 30% in Italian population (Abbate et al., 1998) to 0% in blacks (Giles et al., 1998; Mc Andrew, Brandt, Pearl, & Prior, 1996). The TT genotype and T allele frequencies in control subjects in the present study were quite low (4.1% and 17.4%) and matched the frequencies already reported in another Asian population (3% and 25%) (Abbate et al., 1998). No significant difference was found in the frequencies of wild type and variant allele or genotype in either of the two dementias when compared with control population. Frequency of C and T alleles and their respective genotypes in degenerative and vascular dementias are given in Table 3.

### 8. Effect of ACE allele D on the risk of developing dementia, after controlling for age and APOE E4 status

ACE D allele initially appeared to increase the risk for dementia (p = 0.002, OR 2.82); however, the effect got diluted when we stratified our data according to age and APOE E4 gene status (p = 0.04, OR 1.38). When we further sub-divided the patients into sub-types degenerative and vascular dementia, the susceptibility towards either of the two dementias imparted by D allele almost disappeared in both (p = 0.08 and 0.11, respectively) (Table 4).

### 9. Discussion

APOE E4 allele has been found to be consistently associated with AD as well as vascular dementia in the studies reported worldwide (Kawamata, Tanaka, Shimohama, Ueda, & Kimura, 1994; Luthra et al., 2004; Saunders et al., 1993) including our previous reports (Pandey, Pradhan, & Mittal, 2007; Pandey, Pradhan, & Mittal, 2008), thus providing a clear evidence, that APOE is a strong and independent predictor for dementia.
Table 2
ACE ins/del polymorphism genotype and allele frequency in total subjects and stratified in degenerative dementia and vascular dementia subjects.

<table>
<thead>
<tr>
<th>Genotype/allele</th>
<th>Total</th>
<th>Degenerative dementia</th>
<th>Vascular dementia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control (No. = 170)</td>
<td>Patient (No. = 170)</td>
<td>p-Value</td>
</tr>
<tr>
<td>I</td>
<td>213 (46.2)</td>
<td>177 (45.7)</td>
<td>–</td>
</tr>
<tr>
<td>D</td>
<td>127 (26.5)</td>
<td>163 (43.9)</td>
<td>0.005</td>
</tr>
<tr>
<td>II</td>
<td>62 (36.5)</td>
<td>51 (47.9)</td>
<td>–</td>
</tr>
<tr>
<td>ID</td>
<td>89 (37.4)</td>
<td>75 (30.6)</td>
<td>0.92</td>
</tr>
<tr>
<td>DD</td>
<td>19 (11.2)</td>
<td>44 (25.9)</td>
<td>0.002</td>
</tr>
</tbody>
</table>

Table 3
MTHFR C677T polymorphism genotype and allele frequency in total subjects and stratified in degenerative dementia and vascular dementia subjects.

<table>
<thead>
<tr>
<th>Genotype/allele</th>
<th>Total</th>
<th>Degenerative dementia</th>
<th>Vascular dementia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control (No. = 170)</td>
<td>Patient (No. = 170)</td>
<td>p-Value</td>
</tr>
<tr>
<td>C</td>
<td>281 (62.6)</td>
<td>278 (81.8)</td>
<td>–</td>
</tr>
<tr>
<td>T</td>
<td>59 (17.4)</td>
<td>62 (18.2)</td>
<td>0.76</td>
</tr>
<tr>
<td>CC</td>
<td>118 (94.5)</td>
<td>110 (64.7)</td>
<td>–</td>
</tr>
<tr>
<td>CT</td>
<td>58 (52.5)</td>
<td>58 (34.1)</td>
<td>0.18</td>
</tr>
<tr>
<td>TT</td>
<td>7 (26.5)</td>
<td>2 (31.0)</td>
<td>0.15</td>
</tr>
</tbody>
</table>

Table 4
Contribution of MTHFR allele T and ACE I allele in development of overall dementia after controlling for age and APOE E4 allele.

<table>
<thead>
<tr>
<th>Gene variant</th>
<th>p-Value</th>
<th>Odd's ratio 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total patients</td>
<td>MTHFR T allele</td>
<td>0.65 (0.50-1.00)</td>
</tr>
<tr>
<td>Degenerative dementia</td>
<td>ACE D allele</td>
<td>0.04 (0.03-1.00)</td>
</tr>
<tr>
<td>Vascular dementia</td>
<td>MTHFR T allele</td>
<td>0.38 (0.15-0.90)</td>
</tr>
<tr>
<td>ACE D allele</td>
<td>0.08 (0.02-0.40)</td>
<td></td>
</tr>
<tr>
<td>MTHFR T allele</td>
<td>0.78 (0.08-1.30)</td>
<td></td>
</tr>
</tbody>
</table>

ACE ins/del polymorphism genotype and allele frequency in total subjects and stratified in degenerative dementia and vascular dementia subjects. The contribution of MTHFR allele T and ACE I allele in development of overall dementia after controlling for age and APOE E4 allele.

To summarise, observations in the present study again support the APOE E4 allele as a major risk factor in late onset sporadic dementia. However, ACE I/D polymorphism is not a major contributory factor for either degenerative or vascular dementia; rather it might be playing a role in the primary ageing process either through the modulation of ischemia or through the effect on amyloid deposition. MTHFR gene polymorphism did not seem to play any role in the development of disease either alone, or in the presence of confounding factors. Also, due to absence of any biochemical, genetic or imagiological confirmatory test for the diagnosis of different sub-types of dementia, the clinical and currently available imaging criteria used in this study to differentiate these sub-types are quite arbitrary and remain an inherent weakness of this study.
Disclosure statement

Authors have no potential conflicts of interest including any financial, personal or other relationships with other people or organizations.

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References


