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Original Research Article

Apo E Gene Polymorphism in Stroke Patients: A Hospital Based Study

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Abstract

Introduction: Apolipoprotein E gene polymorphism is associated with chance of occurrence and the outcome of cerebrovascular accident. Different alleles of APO E gene show different outcomes in the stroke patients
This study was done to compare the genetic polymorphism of APOE in stroke patients with healthy population and also to find out any difference in genetic polymorphism in different types of strokes.

Materials and Methods: total 300 stroke patients were compared with equal number of controls from the Department of Neuromedicine, Bangur Institute of Neurosciences, Kolkata. DNA extraction and gene amplification were done from the samples collected from all the study subjects and the different alleles were finally identified by the method of restriction fragment gene polymorphism.

Result: The homozygous E3 genotype was the most common (85.5%) followed by E3/E4 (14.5%) in our study group. The APOE4 allele showed a 1.14-fold odd for developing ischemic stroke whereas the E3 allele is showed protection with odds ratio 0.53. The E3 allele showed protection from developing hemorrhagic stroke with odds ratio 0.366. and higher frequency of E4 allele with odds 1.08.

Conclusion: there is significant association of APOE gene polymorphism in ischemic as well as haemorrhagic stroke patients of ethnic Bengali population and the E3 allele had been the protective factor from developing the stroke.

Keywords: Apolipoprotein E, Cerebrovascular Accident, Gene Polymorphism.

Introduction

Stroke is the focal or global neurological disturbance which is vascular in origin lasting for more than 24 hours if the patient survives till then. Much advances have been made in the acute management of stroke till date with much remaining to attain. Like all other neurological diseases stroke or cerebrovascular accident has strong genetic predisposition. [1]

The greatest advances in stroke genetics have been for monogenic disorders such as cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) and mitochondrial encephalopathy with lactic acidosis and stroke-like episodes (MELAS), and these disorders are of direct clinical relevance. [2]

Apolipoprotein E (Apo E) is essential for the normal catabolism of triglyceride-rich lipoprotein constituents. Gene for Apo E is mapped to chromosome 19 (19q13.2) [3]. It consists of 4 exons and 3 introns, totalling 3597bp. Apo E is polymorphic with three major isoforms e2, e3 and e4. [4].

The isoforms are different in their physiological functions. e2 may lead to hyperlipoprotinemia type III and hence more chance of vascular disease. E3 isomer is associated with Alzehimer's disease.

They have different physiological consequences eg. e2 – This isoform may lead to hyperlipoproteineamia type III and with both increased and decreased risk of atherosclerosis. They will be at greater risk for early vascular disease [5]. e3 – This is a neutral Apo E genotype. 4 – This may lead to Alzheimer's disease, Stroke, impaired cognitive function and reduced neurite outgrowth [6].

Genetic predisposition of stroke has been documented in many studies. Though the concrete evidence is still lacking, but multifactorial polygenic aetiology is responsible for it according to the available literatures. The factor V Leiden G1691A, the angiotensin converting enzyme D allele, the methylene tetrahydrofolate reductase C677T and APOE4 allele are considered as unfavourable genetic risk for ischemic stroke

ApoE genotype is also a strong predictor of recurrent hemorrhagic stroke. Cognitive impairment is common among stroke patients. Here also, the apolipoprotein E e4 allele is an interesting candidate gene because it is a known risk factor for late onset Alzheimer's disease [7].

However, there is a lacuna in the study on Apo E4 gene in cerebrovascular disease and no such concrete evidence of association of it with the severity of stroke.

Objective

The study was done to compare the genetic polymorphism of APOE in stroke patients with healthy population and also to find out any difference in genetic polymorphism in different types of stroke

Materials and Methods

This cross sectional observational study was done in the department of Neuromedicine of Bangur Institute of Neurosciences, Kolkata and in the department of Biochemistry, IPGMER and SSKM Hospital, Kolkata after approval of Institutional Ethics Committee, IPGMER.

Total 300 patients of stroke (both hemorrhagic and ischemic) were compared with 300 healthy controls. Controls were chosen who were non-kin neighbours of the patients who used to come along with the stroke patients during the period of February 2016 to December 2019.Open EPI version 3 software was used to calculate the sample size. All cases of acute stroke aged more than 18 years with a diagnosis of ischemic/haemorrhagic stroke based on clinical and imaging causing neurological deficit were included in the study and cases with significant head trauma or prior stroke in previous 3 months, intracranial neoplasm, arteriovenous malformation, or aneurysm, recent intracranial or intraspinal surgery and acute bleeding diathesis were excluded.

From all the cases and controls we collected 10 ml blood from which we transferred 5 ml in the EDTA vial for the APOE gene polymorphism from the antecubital vein with all aseptic precautions. The rest 5 ml of blood were transferred in a clotted vial. After formation of clot we put the vial in centrifuge at 1500 rpm and the serum samples were kept at -20^0C until further analysis

The DNA were isolated from the EDTA blood by Phenol Chloroform method (Mosquer GT et al) and stored at -20°C. [8]

The PCR amplification was then carried out in a final reaction volume of 25 µl containing 2µl of genomic DNA, 1x PCR buffer, 2mM MgCl2, 0.5µl dNTPs, 1µl of forward primer, 1µl of reverse primer. Reaction was carried out for 30 cycles using a melting temperature of 95°C, an annealing temperature of 65°C and a reaction temperature of 72°C.PCR products are stored at -20°C. [9]

Restriction digests containing 10µl of PCR product and 1µl of restriction enzymes were incubated for 16-17hr at 37°C. [9] The products of enzyme digestion were analysed on a 4% agarose gel. The agarose gel slab was visualized under gel documentation system.

Serum Lipid profile parameters were done in ERBA XL 640 automated analyser in the laboratory using validated kits from the samples collected from both cases and controls.

Statistical Tools

Differences in genotype frequency were examined by chi-square analyses. A P- value of <0.05 (two tailed) was considered as significant. For the association study the data were evaluated for p-value, odds ratio (OR), and 95% confidence interval (CI).Independent t tests were used to analyse the significance of lipid profile values between ischemic and haemorrhagic strokes and between patients and control group.

Results

Out of 300 cases of stroke, 172 (57.2%) had ischemic stroke and 128 (42.7%) had hemorrhagic type stroke. Genetic analyses were performed to identify the frequency distribution of three alleles and six possible APOE genotypes among patients and controls.

The E3/E3 genotype was identified as a predominant one in both patients and controls groups [81%]. Only 2 individuals from patient group found to harbor E4/E4 genotype.

Table 1: Distribution of allele and the genotypes among the study population

Allele	Number of cases	Number of controls
E2	0	0
E3	298	279
E4	57	21
Genotypes	Total number of cases	Total number of controls
E3/E3	243	258
E3/E4	55	21
E4/E4	2	0

E3 allele has been found to be the most frequent allele followed by the E4 allele. The case/ control association study was done to identify the E3 and E4 as risk factor [O.R:0.093 and 1.26 respectively] for stroke. The genotype of E3E3 was most prevalently found to be the protective one [O.R:0.796, p=0.18] and the genotype E3E4 was found to have as risk factor [O.R:1.25] for stroke [p=0.12].

Table 2: The association of different genotype allele between cases and controls

Association (Cases/controls)	Odds ratio	P value
E3 vs others	0.093	0.2
E4 vs others	1.26	0.01
E3/E3 vs others	0.796	0.18
E3/E4 vs others	1.25	0.12

We further subdivided the cases into ischemic and haemorrhage groups with an aim to identify the differential involvement of APOE genotype in particular disease pathogenesis. A statistical significant negative association was found for APOE3 allele with disease pathogenesis when patients from both groups were compared with controls separately. [P = 0.002, O.R 0.538 for ischemic and P = 0.007, O.R 0.366 for haemorrhage]. In contrast the APOE4 allele was found to be risk factors for both ischemic stroke patients group [P0.06 O.R 1.143] and haemorrhage group [p=0.382 O.R 1.086].

Table 3: Associations of different genotype alleles between hemorrhagic and ischemic stroke and between each type of strokes with the controls

Association (Haemorrhagic/Ischemic)	Odds ratio	P value
E3 vs others	0.347	<0.05
E4 vs others	1.148	<0.05
E3/E3 vs others	0.286	<0.001
E3/E4 vs others	1.179	<0.05
Association (Haemorrhagic/controls)		
E3 vs others	0.366	<0.05
E4 vs others	1.086	0.382
E3/E3 vs others	0.305	<0.05
E3/E4 vs others	0.206	0.279
Association (Ischemic/controls)		
E3 vs others	0.538	<0.05
E4 vs others	1.143	0.067
E3/E3 vs others	0.416	<0.05
E3/E4 vs others	1.139	0.073

The lipid profile parameters were compared between the stroke patients and the control group. Statistically significant difference has been found between the 2 groups have been found

Table 4: Comparison of lipid profile parameters between the stroke patients and the control group

Parameters	Stroke patients (mean ± SD)	Controls (mean ± SD)	P value
Total cholesterol	180.05±31.77	167.77±33.16	<0.05
Triglyceride	133.26±14.45	119.77±5.94	<0.05
LDL	116.05±18.89	105.02±15.31	<0.05
HDL	32.19±4.12	49.98±6.12	<0.05
VLDL	47.72±13.29	68.27±12.68	<0.05

Discussion

Stroke, popularly known as brain attack, comprises a mix of different risk profiles, incidence rates, management and outcomes. Though polygenic in nature, the precise definition of genetic factors responsible for stroke is still lacking. The ApoE gene polymorphism has been found with ApoE mutation allele E4 is associated with the increased risk of ischaemic stroke, which has been found in the Chinese population in a recent study.

The apoE genotype is also associated with the occurrence of hemorrhagic stroke according to another study. The E4 allele is also a known predictor of Alzheimer's disease. [10,11]

In the recent study the E3/E3 genotype is the most frequently found one among the all genotypes not only the stroke patients but also in the control population (table 1).

In this study, we have seen that, the APOE E3 and E4 allele as a protective and risk factors respectively for developing stroke among the ethnic Bengali population of West Bengal. The homozygous E3 genotype was the most common (85.5%) followed by E3/E4 (14.5%), which is similar to the other reports from India (table 1 and table 2). [12,13] In contrary to the other population of the world, the E4/E4 genotype was absent in our eastern Indian study cohort, this may be due to the small number of sample size. The homozygous E4 genotype was also rare in the studies from Northern India14 and absent in Koch & Maria Gond population of India.[13]

The APOE4 allele showed a 1.14-fold odd for developing ischemic stroke whereas the E3 allele is showed protection with odds ratio 0.53. Our result is consistent with a meta-analysis report described by Gu et al., 2013. Our results are similar to results in the ischemic stroke patients in some previous and recent studies in different countries[14,15,16,17] (Table3).

The E3 allele showed protection from developing hemorrhagic stroke with odds ratio 0.366. and higher frequency of E4 allele with odds 1.08 (table 3).

The study also showed there is significantly increased level of the lipid profile parameters like Cholesterol, LDL, VLDL and triglyceride whereas the the HDL is significantly decreased in the stroke patients in comparison to the control group. (table 4). It has already been proven in many other studies that dyslipidemia is a well-established risk factor for developing atherosclerosis, myocardial infraction and the cerebrovascular accidents.[18,19]

Conclusion

There is significant association of APOE gene polymorphism in ischemic as well as haemorrhagic stroke patients of ethnic Bengali population and the E3 allele had been the protective factor from developing the stroke.

Limitation

Our study was done in the eastern Indian population and involving the patient attending a tertiary care hospital mostly from the urban surroundings. So, it could possible to have different scenario if we could involve the people from rural areas also.

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