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Relationship of apolipoprotein (APOE) ε4 gene polymorphism with the risk of ischemic stroke: A hospital based case-control study

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ABSTRACT

Background and purpose: The apolipoprotein E (*APOE*) ε4 allele has been considered as an important gene variant which may lead to elevated level of cholesterol and thereby related with higher risk of ischemic stroke (IS). The present study was undertaken to investigate the relationship of *APO-ε4* gene polymorphism with the risk of IS in North Indian population.

Methods: A total of 250 IS subjects and 250 gender and age matched control subjects were included from inpatient and outpatient Department of Neurology, All India Institute of Medical Sciences, New Delhi, India from period of October 2012 to November 2014.

Results: The average age and standard deviation was 52.8 ± 12.6 years for IS cases and for control subjects 50.9 ± 12.7 years. A significant association was found between *APO-ε4* gene polymorphism and IS risk under dominant model (adjusted OR, 2.67; 95% CI, 1.49 to 4.77), recessive model (unadjusted OR, 3.33; 95% CI, 1.33 to 8.30) and allelic model (OR, 2.31; 95% CI, 1.65 to 3.22) for overall stroke. Further carrying out stratified analysis as per TOAST classification, demonstrated a significant association for Large Vessel Disease (LVD) under recessive model was observed.

Conclusion: The present study suggests that *APO-ε4* gene variant may be an important genetic risk factor for IS. Further large genome wide studies are needed to validate the present findings.

1. Introduction

Studies suggest that stroke is the second most leading cause of death and number one cause of adult disability (Feigin et al., 2014). Stroke has accounted for almost 5.7 million deaths worldwide and 87% of these deaths occured in low and middle income countries (Strong et al., 2007). In the last four decades, incidence of stroke have been increased by > 100% in low and middle income countries while this is decreased by 42% in the developed European countries (Abegunde et al., 2007). A study suggested that the incidence of stroke in South India has been reported to be 13 per 100,000 (Abraham et al., 1970; Shrivastava et al., 2016) while in North India it is known to be 33 per 100,000 (Bansal et al., 1973; Prasad et al., 2012). The estimated prevalence of stroke in India has been reported to be 44–843/100,000 (Prasad et al., 2012). This increase in incidence of stroke in developing countries could have been influenced by environmental, life style and genetic factors (Bevan and Markus, 2011; Hassan and Markus, 2000).

Stroke is a polygenic, multi-factorial disease in which genetic and environmental factors together significantly influence the pathophysiology of stroke. Epidemiological and twin studies have strongly suggested the genetic contribution in the pathogenesis of ischemic stroke (IS). The genetic influences are probably polygenic, and IS itself has a number of different subtypes which may each have different pathogenesis (Della-Morte et al., 2012).

Apolipoprotein E (*APOE*) gene is one of the commonly studied genes in vascular and neurodegenerative diseases (Ribalta et al., 2003). Apo-E gene has major role in lipid transport and metabolism and is also found to be extensively expressed in the brain. Its protein products are composed of glycoproteins with three isoforms, E2, E3, and E4, coded by the respective alleles ε2, ε3, and ε4, giving rise to total six genotypes. Literature suggests that there is considerable indication regarding the relationship between *APO-ε4* allele and elevated Low Density Lipoprotein Cholesterol (LDLC) levels and it thereby increases the probability of developing cardiovascular disease including stroke (Al-Khedhairy, 2004, p.; Anthopoulos et al., 2010; Laskowitz et al., 1998; Mahley, 1988). Variation in Apo-E gene leads to an increased inflammatory state, in addition to the influence on leakage of blood brain barrier in combination with NMDAR1-AB which contributes to the deleterious

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effects and increases the risk for stroke (Nishitsuji et al., 2011). A recent review published by Tai et al. (2016) has shown that the ApoE4 allele induces harmful cerebrovascular changes which include reduction in cerebral blood flow (CBF), conditions which may lead to cerebral amyloid angiopathy, and improper transport of essential nutrients and toxins. It has been observed that higher level of Apo-E protein in plasma is a significant risk factor for developing stroke in Polish and Dutch populations particularly at an older age (Slowik et al., 2003; van Vliet et al., 2007). Several studies have shown inconsistent results regarding the association between *APO-ε4* polymorphism and risk of IS (Abboud et al., 2008; Giassakis et al., 2007; Konialis et al., 2016; Luthra et al., 2002; Saidi et al., 2007). Various meta-analyses have also demonstrated that *APOE* is a risk factor for stroke. A recent meta-analysis published by Kumar et al. (2016) has emphasized the role of *APO-E4* allele in causing risk of IS (Kumar et al., 2016). Genetic risk differs in the different ethnic populations. Only one pilot study with a small sample size of 63 stroke patients and 50 healthy controls from North India has shown preliminary evidence for *APO-ε4* allele as a predictor for stroke. But power of this study was not sufficient to detect the small effect caused by Apo-E polymorphism for increasing risk of stroke. Therefore, there is a clear need to conduct the study to know the association between Apo-E and risk of stroke in North Indian Population. Thus, we carried out this case-control study to investigate a relationship between *APO-ε4* gene polymorphism and risk of IS in North Indian population.

2. Materials and methods

2.1. Study participants

The inclusion, exclusion criteria and definition of variables of cases and control subjects were same as were in our published study protocol (Kumar et al., 2013). The methods section was similar as published previously by our group (Kumar et al., 2016b).

2.2. Genotype determination

Four milliliter blood samples in an ethylene diamine tetra acetic acid vial from patients and control subjects were taken from antecubital vein. Genomic Deoxyribose Nucleic Acid (DNA) was isolated from whole blood through standard phenol-chloroform method (Sambrook and Russell, 2006).

The *APO-ε4* promoter regions were amplified by using the following primers: Forward 5′-TCCAAGGAGCTGCAGGCGGCGCA -3′, Reverse 5′-ACAGAATTCGCCCCGGCCTGGTACACTGCCA-3′ and SNaPshot primer 5′-ATGCCGATGACCTGCAGAAG-3′. The PCR amplification was performed in a total volume of 10 μl mixture containing: 1 μl (50 ng) genomic DNA, 1 μl 10× buffer solution, 0.1 μl 1.5 unit Taq DNA polymerase, 0.2 μl (20 pmol) of each primer, and 0.2 μl (200 μmol/l) of each deoxynucleotide triphosphate. Determination of genotypes was carried out using the SNaPshot method on 3130xl automated DNA sequencer.

2.3. Statistical analysis

The statistical analysis section was similar to our previously published paper (Kumar et al., 2016b).

3. Results

The baseline and demographic characteristics are similar to the previously published article by our group (Kumar et al., 2016b) and is also depicted in Table 1

Tables 2 and 3 show the distribution of the APO-ε genotypic and allelic frequencies among cases and controls. Genotypic frequency distributions were in accordance with HWE in both cases and controls. Baseline and demographic variables for which P value of association with stroke was less than 0.25 obtained in the univariate analysis were included in the multivariate analysis to determine the independent association between Apo-E and risk of IS.

Multivariable conditional logistic regression analysis demonstrated a statistically significant association of *APO-ε4* gene variant and the risk of IS under dominant model (OR, 2.67; 95% CI, 1.49 to 4.77; $p = 0.001$) in case of adjusted analysis, and under the allelic model (OR, 2.31; 95% CI, 1.65 to 3.22; $p < 0.001$). Further carrying out stratified analysis based on TOAST classification, a significant association was discerned in Large Vessel Disease (LVD) subtype of IS in dominant (OR, 2.63; 95% CI 1.44 to 4.80, $p = 0.002$), recessive (OR, 4.29; 95% CI 1.43 to 12.83, $p = 0.009$) and allelic (OR, 2.57; 95% CI 1.72 to 3.85, $p = 0.001$) models. A significant association was also discerned in the Small Vessel Disease (SVD) subtype of IS under dominant (OR, 2.04; 95% CI 1.08 to 3.84, $p = 0.02$) and allelic (OR, 2.05; 95% CI 1.31 to 3.22, $p = 0.001$) models. In the others subtype (stroke of determined and undetermined etiology) of IS, a significant association was observed under the dominant model (unadjusted OR, 3.07; 95% CI 1.47 to 6.42, $p = 0.003$).

4. Discussion

This hospital based case-control study in the North Indian population was carried out to determine the relationship between *APO-ε4* gene polymorphism and IS risk and our results suggested that there is a higher risk of IS in subjects who are carrier of ε4 allele of *APOE* gene. The genotype frequencies of the *APO-ε4* gene in the control group were in accordance with Hardy-Weinberg Equilibrium (HWE) which shows that all the samples were appropriate for genetic analysis. Based on TOAST classification, our study is the first study from North India representing the risk of *APO-ε4* gene polymorphism in LVD, SVD and others but not in the cardioembolic (CE) subtype of IS. The reason might be small number of samples observed in the CE subtype of IS. The etiology of ε4 allele in stroke is not fully understood but has been related to lipid transport and metabolism. IS is a complex entity which may consist of many levels and is caused by the interaction of several factors including environmental and lifestyle and genetic makeup, each one exerting a mild to moderate risk.

Apo-e has been one of the most comprehensively studied genetic polymorphisms, specifically for its role in lipid profiles and CHD risk. In comparison for establishing the risk, *ε3ε3* is considered as the reference genotype. In general, ε2 lowers the blood cholesterol and ε4 raises the cholesterol levels (Eichner et al., 2002).

There are different interactions of various isoforms of apo E with particular lipoprotein receptors, finally altering circulating levels of cholesterol. Carriers of ε2 allele are less effective at making and transferring chylomicrons and VLDLs from the blood plasma to the liver compared to ε3 and ε4 alleles (Siest et al., 1995).

Therefore, genotypic differences of Apo-E lead to differential level of total and LDL cholesterol levels. Elevated LDL cholesterol and triglyceride levels have been related to higher risk of CVD. A study which included nine populations estimated approximately 40% of CHD mortality for ε4 carrier compared to ε3ε3 carriers or ε2 carriers (Stengård et al., 1998). A number of studies have demonstrated that ε4 carriers are prone to developing coronary lesion or to have increased risk of death from CHD (Eichner et al., 1993; Lehtinen et al., 1995). Studies have shown that population with higher cholesterol levels and with higher CHD mortality rate also have higher number of ε4 alleles (Schiele et al., 2000; Stengård et al., 1998), however, few studies have observed an association between ε2 allele and higher CHD risk (Eichner et al., 1993).

Numerous studies have depicted the association of various models with different IS subtypes in varied ethnic groups. A study published by Lai et al. (2007) in Taiwanese population, showed an association of ε3/ε4 genotype with small vessel disease but not with large vessel disease infarcts (Lai et al., 2007). Another study by Paternoster et al. (2008)