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**Research Article**

# Molecular and Biochemical Characterization of Ischemic Stroke

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**Abstract:** Stroke or cerebral infarction is a condition that arises due to the obstruction in the flow of blood (ischemic stroke) or due to rupture in the blood vessels supplying blood to the brain (hemorrhagic stroke). This results in lack of oxygen and nutrients to the brain cells causing their failure to perform the metabolic function. Stroke is the third largest killer in the World. The total damage arising from stroke depends on the area of the brain affected and the amount of blood obstructed to the brain. In the present study we aimed to study the single nucleotide polymorphism in phosphodiesterase 4D (PDE4D) SNP56 genes and LPL gene. 25 patients and 25 healthy individual's blood samples were collected and DNA was isolated. The presence of PDE4D SNP56 gene was determined by PCR-Sequencing methods and LPL gene polymorphism was determined by PCR-RFLP methods. Same numbers of patient and control samples were used to estimate the nitric oxide levels and uric acid levels. The results indicate that the PDE4D SNP56 gene and LPL gene polymorphism is significantly associated with stroke in the study population. The serum nitric oxide and uric acid levels were found to be significantly increased in patients as compared to controls.

**Key words:** Ischemic stroke, Atherosclerotic plaque, Nitric oxide

## INTRODUCTION

Stroke is also called as cerebrovascular attack (CVA) is characterized by the sudden loss of blood circulation to a particular area of the brain which results in the loss of neurological function. Strokes can be divided into 2 major categories: ischemic and hemorrhagic[1,2]. Ischemic stroke is the condition where the artery of the brain is blocked by a blood clot or fatty deposit occurred due to atherosclerosis. Typically, a cholesterol plaque in a small blood vessel with in the brain that has gradually caused blood

vessel narrowing ruptures and starts the processes of forming a small blood clot [3]. Hemorrhagic stroke is the one which causes bleeding within the brain.

Stroke is one of the leading causes of mortality and morbidity worldwide. Approximately 20 million people each year will suffer from stroke and of these 5 million will not survive[4]. Of these, 5 million die and another 5 million are permanently disabled. Developing countries like India account for about 85% of the global deaths from stroke[5]. Stroke is one of the leading causes of mortality and morbidity worldwide. Approximately 20 million people each year will suffer from stroke and of these 5 million will not survive.

Symptoms of stroke may occur suddenly without any warning, this often happens during the occurrence of stroke. The most common symptoms of stroke are sudden weakness, numbness of face, arm or leg, confusion, trouble speaking, dizziness and headache. Transient ischemic attack (TIA) shows similar symptoms of stroke but lasts for 1-2 hours. There are mainly two types of risk factors for stroke, modifiable and non-modifiable factors[6]. Modifiable are those which can be modified by changing our lifestyle which includes smoking, hypertension, diabetes, alcoholism, migraine, obesity, diet and physical activity. Non-modifiable are those which cannot be modified which includes age, ethnicity and gender.

In twin studies, the concordance rates for stroke are 17.7% in monozygotic twins and 3.6% in dizygotic twins[7]. In the Framingham study, using information obtained across 3 generations, including the original and offspring cohorts, a parental history of stroke was associated with an approximately 2-fold increase in stroke risk[8]. Stroke is a multifactorial polygenic disorder but some cases occurred due to single gene disorder. Genetic factor seems to be more important in large vessel stroke and small vessel stroke[9]. There are two major approaches to find genes related to stroke the candidate gene approach and the genome-wide approach (GWA)[10].

The single gene disorders are uncommon and account for less than 1% of stroke. CADASIL is characterized by recurrent subcortical ischemic strokes starting at ages 30 to 40 years, progressive or stepwise cognitive decline, and white matter changes on magnetic resonance imaging. Migraines with aura and mood disturbances[11].Studies have identified polymorphism in a number of candidate genes and their association with stroke[12]. Significant research is being conducted to establish the relationship between the functional variants of a variety of genes and the risk of stroke.

Treatment of stroke depends on where the stroke has occurred and whether it's ischemic or hemorrhagic. FDA approved tPA (tissue plasminogen activator) as the first treatment of stroke which is used to treat ischemic stroke. Antiplatelet agents like aspirin and abciximap have been reported to reduce the risk of recurrent stroke and death in some patients[13]. "Prevention is better than cure". Strokes can be prevented by reducing the blood pressure, quitting smoking, maintaining optimal weight, maintaining proper diet, physical exercising. The present has been taken to investigate the association of PDE4D (SNP 56) gene and LPL gene polymorphisms with ischemic stroke.

## MATERIAL AND METHODS

A total of 25 specimens of EDTA blood samples were obtained from randomly selected clinically diagnosed stroke patients. 25 Control blood samples were collected from different communities with informed consent. The disease was diagnosed by clinical and hematological data.Genomic DNA was isolated from whole blood in anticoagulant (EDTA) by using sodium dodecyl sulphate and proteinase K, and digested overnight at 37$^{o}$C. DNA was purified using phenol-chloroform-isoamyl alcohol and precipitated in ethanol. After DNA extraction, PCR reactions were set up for each sample.

**PCR Conditions for PDE4D (SNP56):** A total of 50 µl of reaction volume was used for this purpose. The reaction volume was composed of 2µl of the genomic DNA template, 1µl of each of the two primers (forward primer, reverse primer), 5µl of taq buffer, 2µl of dntp's, 2µl of 25mM $Mgcl_2$, 1µl of taq polymerase, 36µl of distilled water. The thermal cycling regimen consisted of 34 cycles: Initial denaturation at 94˚C for 6 minutes, denaturation at 94˚C for 1 minute, annealing at 60˚C for 1 minute, elongation at 72˚C for 1 minutes and Final extension at 72˚C for 6 minutes.

**PCR Conditions for LPL:** A total of 50 µL of reaction volume was used for this purpose. The reaction volume was composed of 5µl of the genomic DNA template, 1µl of each of the two primers (forward primer, reverse primer), 5µl of taq buffer, 2µl of dntp's, 2µl of 25mM $Mgcl_2$, 1µl of taq polymerase, 33µl of distilled water. The thermal cycling regimen consisted of 34 cycles: Initial denaturation at 94˚C for 6 minutes, denaturation at 94˚C for 1 minute, annealing at 57˚C for 1 minute, elongation at 72˚C for 1minute and Final extension at 72˚C for6 minute.

## RESULTS

**DNA amplification and LPL digestion:** RFLP is a widely used technique to detect known mutations and variations using specific Restriction endonucleases. The LPL polymorphism at 140 & 210 position of the LPL gene was confirmed by HindIII restriction enzyme digestion of a 350bp amplified DNA sequence from the promoter region of the LPL gene.

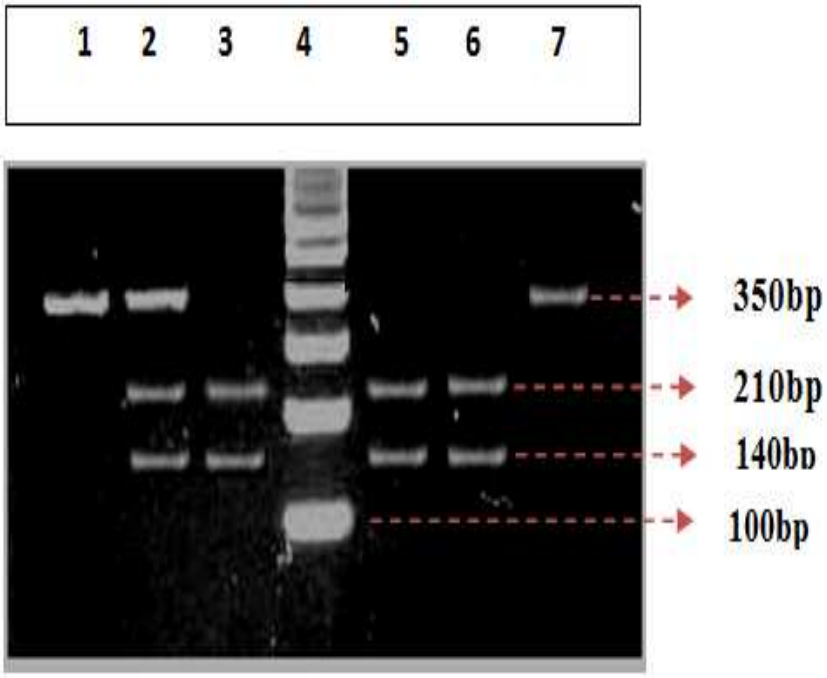



**Figure 1: RFLP of the LPL PCR product**

Successful amplification was confirmed by electrophoresis on an ethidium bromide-impregnated 1.5% agarose gel. For RFLP analysis, LPL PCR products were digested withHindIII and visualized on a 3% agarose gel. Analysis of lpl status in patients indicated that 25 H (+/+) Homozygous, 4(16%), H(+/-)-Heterozygous 15 (60%),H(-/-)Homozygous mutant 6(24%).where as in controls H(+/+)-Homozygous 8.

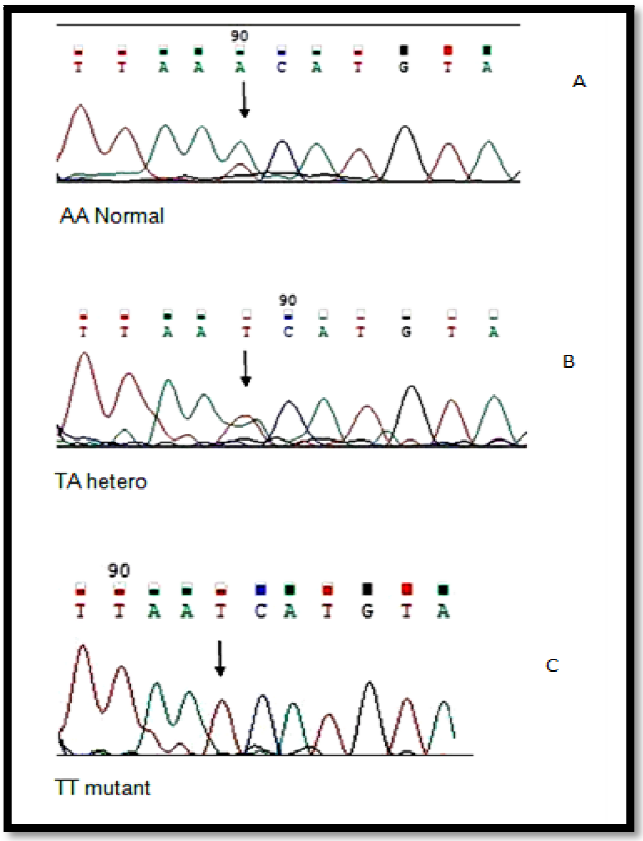
PCR products (LPL) digested with HindIII restriction enzyme produced bands of 210bp and 140bp when both chromosomes possessed the LPL polymorphic site homozygous mutant (lanes 3, 5 and 6). Bands of 650bp, 450bp and 200bp were observed when only one chromosome possessed the site Heterozygous

(lane 2) and lane 4 represents 100 bp ladder absence of the LPL site in both chromosomes Homozygous (lane 1,7).The mean values of nitric oxide levels and uric acid acid levels in the serum are shows in table1.

**Mean values of uric acid and nitric oxide in controls and patients**:

| Study group | Mean value of uric acid | Mean value of Nitric oxide |
|---|---|---|
| Controls (n=25) | 3.9 mg/dl | 3.33 µM/ml |
| Patients (n=25) | 7.5 mg/dl | 8.132 µM/ml |

**Sequencing results of PDE4D gene:**




A represents AA: Homozygous normal
B represents TA: Heterozygous
C represents TT: Homozygous mutant

After completion of DNA sequencing of PDE4D (SNP56). In case of patients the genotypic distribution are AA homozygotes were 9, AT heterozygotes were 11 and TT homozygotes were 5. whereas in controls the AA homozygotes were 13.

## DISCUSSION

High throughput genotyping are rapidly developing, as are the statistical methods to analyze increasingly complex data. The technical developments are likely to outpace the collections of large carefully phenotyped samples. The future of stroke genetics will depend on the samples available and on close collaborations between clinicians and geneticists. Genetic testing has become a valuable tool in diagnosing single gene disorders associated with ischemic stroke, whereas, it is currently not recommended in patients with multi factorial stroke. Much progress has been made in the identification of genes for Mendelian conditions associated with stroke. However, comparatively little is known about the genes involved in multifactorial stroke. There are various methodological approaches, yet careful phenotyping and large simple sizes remain effective. Collaborative efforts from multiple centers are needed to elucidate the genetic basis of common multifactorial ischemic stroke.

It is clear that the frequency of hypertension was more in patients as compared to the controls. The frequency of hypertension in patient samples was 68% whereas in controls it was 56%, followed by smoking which was 52% in patients and 44% in controls, alcoholism was 40% in patients and 36% in controls, diabetes was 52% in patients and 32% in controls.

Identification of key genetic variants involved in stroke will provide a better platform for further research. Here, we have analyzed the PDE4D (SNP 56) and LPL gene variants in ischemic stroke. The results were obtained using 25 patients and 25 control samples which revealed the PDE4D and LPL genes are risk factors for stroke. In case of PDE4D gene the frequency of homozygous mutants (20%) and heterozygous genotypes (42.5%) is higher and the mutant allele is more frequent in patients (0.42) as compared to the control samples (0). In case of LPL the frequency of homozygous mutants (24%) and heterozygous genotypes (60%) is higher and the mutant allele is more frequent in patients (0.54) as compared to the control samples (0). The nitric oxide levels were elevated in case of patients (8.132µM/ml) than in controls (3.33µM/ml). The uric acid levels were also found to be elevated in the patients 7.5 mg/dl as compared to healthy controls 3.9 mg/dl. The elevated nitric oxide levels and uric acid levels in patient's serum indicate the stress level in stroke patients.

## CONCLUSION

Therefore, the conclusion of the analysis is that the nitric oxide levels, uric acid levels and PDE4D gene, LPL gene polymorphisms are significantly associated with stroke in the study population.

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