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# Genetic determinants in ischaemic stroke subtypes: Seven year findings and a review

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ABSTRACT

Stroke is a global health problem and a leading cause of disability worldwide. There have been numerable studies undertaking research on different aspects of ischaemic stroke employing various epidemiological, clinical and molecular parameters. Nevertheless ischaemic stroke being a complex disorder with different subtypes demands equal attention towards its subtypes too. Since there has been enough evidence that disposition to certain subtype is genetically determined and there is a distinct mechanism that influences its development, association studies should focus on subtypes simultaneously while studying specific genes. Data from such studies will thus provide better and intricate findings with regard to heterogenous ischaemic stroke. In the present review we discuss the genes studied by our group over a period of seven years in association with stroke subtypes in a South Indian population and correlate the findings with similar genetic studies from other populations so as to provide an overview of various genes involved in the pathogenesis of ischaemic stroke subtypes.



## 1. Introduction

Stroke has been recognised as a multi-factorial polygenic and complex disease resulting from a combination of vascular, environmental and genetic factors (Della-Morte et al., 2012). Approximately 80–90% of strokes are ischaemic (IS), which happens when a blood vessel (artery) supplying the blood to an area of the brain becomes blocked by a blood clot (Bonita, 1992; Flossmann et al., 2004; Brown et al., 1996). The role of genetic determinants in ischaemic stroke has been demonstrated in a number of reports which include twin, family and animal model studies (Wang et al., 1997). Recent technological advancements and two major international projects i.e. 'Human Genome Project' and 'HapMap Project' have tremendously contributed in the discovery of genes associated with various complex diseases. The discovery of SNPs in the first project and the development of haplotype map of human genome in the latter have greatly influenced the role of association studies in complex diseases including cardiovascular diseases and stroke. Among the several genes reported to be associated with stroke only a few have been replicated which could be attributed to complex genetic aetiology and many loci influencing the pathophysiology of stroke. Nevertheless, the association of several identified genes with stroke still remains controversial and differences in ethnicity/race further add up to the underlying complexity of the disease, its risk and prognosis. Apart from these etiological factors ischaemic stroke is also characterised by different subtypes that have distinct pathophysiological mechanisms and different classification systems have been proposed for establishing the distinct stroke subtypes. These include The Trial of ORG 10172 in Acute Stroke Treatment (TOAST) classification, Stop-Stroke Study TOAST (SSS-TOAST) classification, the Causative Classification System (CCS), A-S-C-O (A for atherosclerosis, S for small vessel disease, C for cardiac source, O for other causes) classification and OSCP (Oxfordshire Community Stroke Project) classification (Adams et al., 1993; Ay et al., 2005, 2007; Amarenco et al., 2009; Bamford et al., 1991).

Among these the TOAST classification has been extensively used in majority of the studies, and it is also the first system based on stroke mechanism and currently the most preferred one, although with certain limitations. It classifies ischaemic stroke into 5 categories: large artery atherosclerosis (occlusion or stenosis with ≥50% diameter reduction of a brain-supplying artery with location and morphology typical of atherosclerosis); small artery occlusion (the presence of one of the traditional lacunar syndromes — pure motor stroke, pure sensory stroke, sensory motor stroke, ataxic hemiparesis, and dysarthria-clumsy hand syndrome additionally infarction <1.5 cm of diameter or normal CT/MRI examination, the absence of acute cerebral cortical dysfunction,

*Abbreviations:* TOAST, The Trial of ORG 10172 in Acute Stroke Treatment; CCS, Causative Classification System; OCSP, Oxfordshire Community Stroke Project; RAAS, Renin Angiotensin Aldosterone System; CT, computed tomography; MRI, magnetic resonance imaging; LAA, large artery atherosclerosis; ILA, intracranial large artery; ELA, extracranial large artery; SAO, small artery occlusion; CE, cardioembolism; ODE, other determined Etiology; UDE, undetermined Etiology.

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and the absence of signs of cardiac embolisms); cardio-embolism (the presence of a high- or medium-risk source of cardiac embolism); other determined aetiologies (show some rare causes of stroke e.g. non-atherosclerotic vasculopathies, hypercoagulable states or haematologic disorders, genetic disorders and metabolic disorders and moreover diagnostic procedures, including blood tests or arteriography should reveal one of the unusual causes of stroke) and stroke of undetermined aetiology. The undetermined category is a heterogeneous group with no cause found despite proper investigation. Although a recent study undertaken by Marnane et al. (2010) found both the CCS and ASCO system to be good enough when compared with TOAST, they do suggest for a feasible single combined classification system (Marnane et al., 2010). Such a system if well-established will provide a uniform platform to harmonise the heterogenous ischaemic stroke to an extent and also for optimising the stroke treatment.

The genetic contribution to multifactorial stroke is polygenic. However, identifying the underlying genes has been a major challenge. Most studies have focussed on polymorphic variants promoting stroke, predisposing phenotypes or mediators. The polyetiologic ischaemic stroke shows marked variation in its subtypes, therefore studies focussing on genetic risk factors should equally pay attention to aetiological ischaemic stroke subtypes.

Significant research is being conducted to establish the relationship between the functional variants of a number of genes including genes involved in Renin Angiotensin Aldosterone System (RAAS), homocysteine metabolising gene, nitric oxide synthase metabolising gene, lipid metabolising gene, fibrinolytic/thrombotic genes, pro-inflammatory/anti-inflammatory genes and other classes of genes. However, very few studies have evaluated the role of various candidate genes in the development of specific stroke subtypes. Therefore, in the present study we aim to document the various genes involved in progression of different stoke subtypes in a South Indian population from Andhra Pradesh and also review the genes involved in the pathogenesis of stroke subtypes reported in other populations.

## 2. Materials and methods

### 2.1. Subjects

One thousand and five hundred ischaemic stroke patients (males: females = 1069:431) presenting with new stroke evaluated in the neurology department of Nizam's Institute of Medical Sciences (NIMS), Hyderabad (A.P., India) between June 2007 and March 2014 were enrolled for the study. The study was approved by the ethical committee of the study hospital as well as the Institutional Ethical Committee. All the patients were examined by a qualified stroke neurologist and ischaemic stroke was differentiated by computed tomography (CT) scans and magnetic resonance imaging (MRI). All the patients underwent CT scan as well as MRI. Patients with major cardiac, renal, hepatic, endocrinological disorders, skeletal disorders and cancerous diseases were excluded from this study. As a control group healthy individuals matched for sex and age were recruited from the same geographic area with no clinical evidence of any cerebrovascular disease. Information on demographic characteristics and risk factors was collected using a structured questionnaire and samples were collected only after obtaining the written informed consent. Ischaemic stroke was classified into subtypes according to the TOAST classification (Adams et al., 1993) and hypertension, alcohol use, diabetes and smoking were defined as reported previously (Munshi et al., 2008).

### 2.2. DNA isolation and genotyping

A total of 5 ml of blood was collected in EDTA tubes and genomic DNA was extracted from blood samples using standard phenol–chloroform method. The polymorphisms in various genes reported in this study were detected as reported earlier (Munshi et al., 2008, 2009a, 2009b, 2010a, 2010b, 2010c, 2010d, 2011, 2012a, 2012b, 2012c; Babu et al., 2012; Das et al., 2014; Roy et al., 2014; Sharma et al., 2013).

### 2.3. Statistical analysis

Hardy–Weinberg equilibrium was tested for the various gene polymorphisms and the association between genotypes and ischaemic stroke was examined by odds ratio with 95% confidence interval (CI) and chi-square analysis using Open EPI6 software (Open Epi Version 2.3.1 from the Department of Epidemiology, Rollins School of Public Health, Emory University, Atlanta, GA 30322, USA). Allelic frequencies were calculated according to the number of different alleles observed and the total number of alleles examined. Statistical significance was defined as $p < 0.05$.

## 3. Results

A total of 1500 ischaemic stroke patients were collected over a period of seven years. The clinical characteristics of all the patients have been given in Table 1. The mean age was 54.6 years for ischaemic stroke patients and the profiles of the patients for the various risk factors revealed hypertension in 57.5%, diabetes in 47.9%, smoking in 47.1%, alcohol use in 42.8% and family history of stroke in 21.6% of patients. The distribution of patients belonging to different subtypes according to TOAST classification has been given in Table 2. A total of 669 (44.6%) patients were found to be diagnosed with large artery atherosclerosis (LAA) of which 431 (64.4%) and 238 (35.6%) patients were found to be classified as intracranial and extracranial large artery respectively (ILA and ELA). Small artery occlusion (lacunar) (SAO) was diagnosed in 232 (15.5%), cardioembolism (CE) in 206 (13.7%), other determined aetiologies (ODA) in 82 (5.5%) and undetermined aetiology (UDA) in 311 (20.7%) of ischaemic stroke patients.

We have been studying the association of various candidate genes involved in various pathways with stroke and its subtypes for the past seven years (Munshi et al., 2008, 2009a, 2009b, 2010a, 2010b, 2010c, 2010d, 2011, 2012a, 2012b, 2012c; Babu et al., 2012; Das et al. 2014; Roy et al. 2014; Sharma et al., 2013). In the present study we have given a holistic picture of all these genes in association with stroke subtypes and have also evaluated all the 1500 IS samples because the sample size in some of our previous studies was low (Munshi et al., 2008, 2009b, 2010a, 2010b, 2010c, 2010d, 2012b; Das et al. 2014). The various genes found to be associated with IS subtypes from different pathways in multiple ethnicities have been depicted in Figs. 1 and 2. The different genes studied in association with IS subtypes by us have been summarised in Table 3. The genes studied in RAAS system include ACE and CYP11B2. In ACE the I/D polymorphism studied revealed a significant association with subtype ILA [$p = 0.007$, OR = 1.78 (95% CI; 1.05–3.03)]. On the other hand the -344C/T polymorphism of CYP11B2 was found to be associated with ILA [$p < 0.001$, OR = 3.07

**Table 1**
Clinical characteristics of ischaemic stroke patients.

| Characteristics | Patients (n = 1500) |
|---|---|
| Age | 54.6 (16.4) |
| Male:female | 1069:431 |
| Systolic BP (mm Hg) (mean ± S.D.) | 149 (14.8) |
| Diastolic BP (mm Hg) (mean ± S.D.) | 90.7 (17.6) |
| Total cholesterol (mean ± S.D.) | 198.56 (40.2) |
| Triglycerides (mean ± S.D.) | 181.6 (39.42) |
| Random glucose (mean ± S.D.) | 132.7 (9.4) |
| HDL cholesterol (mean ± S.D.) | 58.3 (20.6) |
| Hypertension | 57.5% |
| Diabetes | 47.9% |
| Smoker | 47.1% |
| Alcohol use | 42.8% |
| Family history of stroke | 21.6% |

Age, systolic BP, diastolic BP, total cholesterol, high density lipoprotein (HDL) cholesterol, random glucose and triglycerides are given as mean (SD).