A Study on Hereditary Thrombophilia and Stroke in a Cohort from Sri Lanka

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Background: Thrombophilia is an enhanced tendency of arterial or venous blood clot formation. The frequently assessed hereditary thrombophilia mutations associated with stroke are methylenetetrahydrofolate reductase (*MTHFR*) c.677C>T, Factor V (*F5*) c.1691G>A (Leiden), and prothrombin (*F2*) c.20210G>A. The aim of this study was to describe the prevalence of the 3 mutations in ischemic stroke patients in Sri Lanka. *Methods:* A database of clinical details and genetic test results of stroke patients referred for thrombophilia screening from June 2006 to April 2014 was maintained prospectively and analyzed retrospectively. *Results:* A total of 400 ischemic stroke patients (319 arterial, 66 venous, and 15 location unreported) were screened for hereditary thrombophilia. Patients with the *MTHFR* c.677C>T, *F5* c.1691G>A, and *F2* c.20210G>A mutations were 17.3%, 3.3%, and .5% of the total cohort, respectively. *F5* mutation was present in a statistically significant number of patients with venous thrombosis ($P = .005$) compared to patients with arterial thrombosis. The *MTFHR* and *F2* mutations showed no such significant association. The mean age of patients with *MTHFR*, *F5*, and *F2* mutations was 29 (±15), 34 (±11), and 38 (±5.6) years, respectively. *Conclusion: MTHFR* c.677C>T is the predominant mutation and the only mutation that had patients with the homozygous mutant genotype. Venous thrombosis showed a significant association with the *F5* c.1691G>A mutation. **Key Words:** Stroke—hereditary thrombophilia—methylenetetrahydrofolate reductase—*F5* Leiden—prothrombin.

Introduction

Stroke is the third most common cause of death worldwide, leading to an estimated 5.7 million deaths every year.[1] Sri Lanka has a stroke prevalence of 9 in 1000 individuals.[2] Pathologically, stroke is classified into ischemic stroke, intracerebral hemorrhage, and subarachnoid hemorrhage.[3] This study focuses on ischemic stroke, which is the most common subtype.[4] Ischemic stroke is a disease with many possible causative factors.[5] The most common etiologies are large-artery atherosclerosis, cardioembolism, and small-vessel occlusions.[6] Less common etiologies are cerebral vasculitis, hypercoagulable states, and hematological disorders, whereas a considerable number of ischemic strokes come under the category of undetermined etiology.[6] Inherited thrombophilia is also a known risk factor for a proportion of ischemic strokes.[7]

Protein C, protein S, antithrombin deficiencies, activated protein C resistance, thrombophilia mutations

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methylenetetrahydrofolate reductase (*MTHFR*) 677 C>T, Factor V (*F5*) Leiden 1691 G>A, prothrombin (*F2*) 20210 G>A, and raised plasma homocysteine levels are the main inherited risk factors implicated in stroke.[8] The most commonly investigated thrombophilia mutations associated with ischemic stroke are *MTHFR* c.677C>T, *F5* Leiden c.1691G>A, and *F2* c.20210G>A.[9] A meta-analysis involving 120 case–control studies showed statistically significant associations between ischemic stroke and these 3 mutations.[9] The majority of strokes due to inherited thrombophilia are associated with venous circulation.[10]

MTHFR is a folate-dependent enzyme, involved in the conversion of the amino acid homocysteine to methionine.[11] The *MTHFR* c.677C>T point mutation causes the reduction of the normal MTHFR enzyme activity by 35% in CT genotypes and 70% in TT genotypes, leading to hyperhomocysteinemia.[11] The mechanism of hyperhomocysteinemia resulting in increased thrombosis is broadly linked to the increased oxygen free radicals that promote vascular endothelial damage.[12] Two meta-analyses involving 38 studies and 26 studies showed that *MTHFR* c.677C>T leads to an increased risk of ischemic stroke in Asian and European populations.[13,14] The frequency of the *MTHFR* c.677C>T mutation is quite variable not only across different ethnic groups of the world but also among Asians.[15,16] There is a prevalence of about 1.4% and 18% respectively, among the homozygotes and heterozygotes of the *MTHFR* c.677C>T mutation in South Asia.[17] Even though there are conflicting results, most meta-analyses show a trend toward an association between *MTHFR* c.677C>T and ischemic stroke.[13,18,19] The *MTHFR* c.677C>T mutation is more prevalent in middle-aged and elderly stroke patients.[19,20]

F5 c.1691G>A is a gain of function mutation that makes clotting *F5* resistant to proteolytic cleavage by activated protein C, which leads to a hypercoagulable state.[21] The *F5* c.1691G>A mutation is the most common inherited factor that causes venous thrombosis.[22,23] This mutation is prevalent in about 5% of the Caucasian population and infrequent in the African and Asian populations.[9,22] In a meta-analysis conducted on 17 case–control studies, 9 of the studies showed a strong association between ischemic stroke and *F5* c.1691G>A.[19] A clear trend toward decreasing prevalence of *F5* c.1691G>A in ischemic stroke is observed with increasing age.[10] This has led to the screening of the *F5* c.1691G>A mutation in stroke patients aged less than 40 years.[23,24] Furthermore, studies have shown increased risk of cerebrovascular events in women with *F5* c.1691G>A mutation.[25,26]

F2 gene mutation *F2* c.20210G>A leads to elevated *F2* levels with a resultant increase in thrombotic events.[24,27] *F2* is the precursor of thrombin, which is the final product of the clotting cascade that leads to the formation of fibrin.[20] A meta-analysis of 17 case–control studies has shown a statistically significant association between the presence of *F2* mutation and ischemic stroke.[22] Another study found a significant relationship between 20210 G>A and ischemic stroke in patients below the age of 50.[23] *F2* c.20210G>A mutation is a known risk factor for venous thrombosis and is not commonly associated with arterial thrombosis.[28-30] There is also evidence of an increased risk of stroke/transient ischemic attack in men carrying the *F2* c.20210G>A mutation.[31] This mutation is less common in South Asian populations.[32,33]

A study on the association between hereditary thrombophilia and ischemic stroke in Sri Lanka has not been conducted before. The genetic test results of the 3 thrombophilia mutations were analyzed based on age, sex, and the type of thrombosis (arterial/venous). The infarction sites, stroke etiologies as defined by the Trial of Org 10172 in Acute Stroke Treatment (TOAST)[6] guidelines, and the concomitant risk factors were also reported. This study was conducted to describe the presence of the 3 thrombophilia mutations: *MTHFR* c.677C>T, *F5* c.1691G>A, and *F2* c.20210G>A in a cohort of ischemic stroke patients from Sri Lanka.

Methods

The Human Genetics Unit receives referrals from neurology and hematology units for genetic thrombophilia testing of patients who have had ischemic stroke. All patients with ischemic stroke referred for thrombophilia screening from June 2006 to April 2014 were included in the study. Stroke patients with intracerebral and subarachnoid hemorrhages were excluded. Data were collected using a medical officer administered data collection sheet that included patient information on the thrombotic event, age of onset, sex, type of thrombosis, and concomitant risk factors. Further clinical details on stroke etiology and the infarction sites were obtained from attached investigation reports and computed tomography/magnetic resonance imaging (MRI) brain scans.

Peripheral blood of the referred ischemic stroke patients were collected in a 2-mL EDTA collection tube. Genomic DNA was extracted using blood DNA purification kit (Promega, Madison, Wisconsin, United States). The DNA pellet was stored in 50 µL of TE buffer at –20°C until the thrombophilia screening was performed. The routine multiplex polymerase chain reaction–restriction fragment length polymorphism (PCR/RFLP) procedure conducted at the Human Genetics Unit was used for the detection of the mutations. *F2* c.20210G>A and *F5* c.1691G>A duplex PCR/RFLP procedure was adapted from a previously described method by Koksal et al in 2007.[35] A different PCR/RFLP method was used to detect the *MTHFR* c.677C>T mutation, as developed by Dissanayake in 2004.[39]

The primers used to amplify the genes were as follows: *F5* primers: forward, 5'ACATCGCCTCTGGGCTAATA-3'; reverse, 5'-TTGAAGGAAATGCCCCATTA-3'; *F2* primers: forward, 5'-ATGGGGTGAAGGCTGTGACC-3';