Association of the APOE, MTHFR and ACE genes polymorphisms and stroke in Zambian patients

Masharip Atadzhanov,1 Mwila H. Mwaba,1 Patrice N. Mukomena,1 Shabir Lakhi,1 Sruti Rayaprolu,2 Owen A. Ross,2 James F. Meschia3 1Department of Internal Medicine, School of Medicine, University of Zambia, Lusaka, Zambia; Departments of 2Neuroscience and 3Neurology, Mayo Clinic, Jacksonville, FL, USA

Abstract

The aim of the present study was to investigate the association of APOE, MTHFR and ACE polymorphisms with stroke in the Zambian population. We analyzed 41 stroke patients and 116 control subjects all of Zambian origin for associations between the genotype of the APOE, MTHFR and ACE polymorphisms and stroke. The APOE ε2ε4 genotype showed increased risk for hemorrhagic stroke (P<0.05) and also a high risk for ischemic stroke (P=0.05). There was complete absence of the APOE ε2ε2 and the MTHFR TT genotypes in the Zambian population. The difference between cases and controls was not significant for the other genetic variants when analyzed for relationship between stroke, stroke subtype and genotype. We show that genetic variation at the APOE locus affects susceptibility to stroke. No detectable association were observed for the MTHFR and ACE genotypes and stroke in the Zambian population.

Introduction

While stroke incidence has declined in developed countries over the last decade, its incidence rose during the same period in low- and middle- income countries.1 Although stroke is an ever-increasing problem in developing countries, little is known about its incidence and outcomes in sub-Saharan Africa (SSA).2 The INTERSTROKE study showed the commonality of the main risk factors for stroke worldwide, including SSA: hypertension, obesity, diabetes mellitus (DM), smoking, alcohol abuse, hypercholesterolemia, diet and physical activity.3 In addition, genetic factors may act either by predisposing to vascular risk factors, such as hypertension, heart disease, DM, hyperlipidemia or by a direct independent effect on stroke risk.4,5 Association studies have nominated a number of candidate genes associated with stroke risk.6 These genes include apolipoproteinE (APOE), plasminogen activator inhibitor 1 (SERPINE1), platelet receptor GP1BA, angiotensin-converting enzyme (ACE), and methylenetetrahydrofolate reductase (MTHFR).4 Case-control studies of stroke candidate genes in different ethnic groups have produced contrary results. A meta-analysis of Chinese, Japanese, and Korean ancestry reported significant association of polymorphisms in the APOE, MTHFR and ACE genes with ischemic stroke.6 However, in other studies, only minor or non-significant risk associations were observed,7 and it was concluded that it is a combination of several genotypes interacting which increases the odds ratio for risk of ischemic stroke.8

Studies on stroke conducted within African populations mainly focus on the prevalence, risk factors, subtypes and outcome of stroke.9,10 To date, there are only a handful of studies examining genetic association of APOE, MTHFR, ACE or other candidate genes with cardiovascular disease in African populations.11,12 These candidate genes provide some of the most likely genetic variants which are involved in stroke risk and have been among the most studied. Common APOE alleles has been shown to affect risk of cardio- and cerebrovascular disease and is considered an established genetic risk factor, however common variation in the MTHFR gene (related to folate metabolism) and the ACE gene although implicated in disease risk are not confirmed.

The aim of the present study therefore, was to investigate association of the APOE, MTHFR and ACE gene polymorphisms with stroke in Zambian patients.

Materials and Methods

Study population

Forty one consecutive patients with mean age 54±16 years admitted to the University Teaching Hospital (UTH) Lusaka, Zambia, between June and December 2010 with a diagnosis of stroke, were enrolled. We included only patients who had stroke confirmed by neurological examination and brain CT scan. Patients who had no brain imaging done and those with head injury were excluded. Daily physical checks/reviews were made of emergency room, admission ward and in-patient wards for stroke patients.

Clinical procedure

Within 24 hours of admission, we obtained medical history, conducted general and neurological examination and brain CT scan was performed. The patient's demographic details and risk factors for stroke were documented on a data collection sheet by study physician. Risk factors for stroke included: hypertension, DM, elevated cholesterol, heart disease, tobacco smoking/sniffing, alcohol consumption, history of transient ischemic attack, and a family history of stroke. As a risk factor of stroke hypertension was defined as blood pressure of ≥140/90 mmhg and current use of antihypertensive medication. DM was diagnosed when patient was taking anti-diabetic drugs prior to stroke. Elevated cholesterol was considered if serum fasting cholesterol was >5.2 mmol/L or prestroke treatment with a cholesterol lowering agent. Other risk factors included HIV infection. The severity of stroke was evaluated on admission using the National Institutes of Health Stroke Scale (NIHSS). Data on risk factors and stroke severity in our study population was published in our previous article.11 As a control population, 116 subjects (68 women, 48 men) matched for age, sex, free of cerebrovascular diseases and other risk factors including hypertension, DM and HIV, were recruited from the department of internal medicine of UTH. Only indigenous Zambian patients and control subjects were included in the study. All participants gave written informed consent and the study was approved by the Biomedical Research Ethics Committee of the University of Zambia.
Genetic analysis

Whole blood was collected in 4 mL EDTA tubes and stored at 4 degrees Celsius prior to DNA extraction which was performed using silica-gel-based membrane with vacuum technology QIAamp DNA Mini Kit (250) (QIAGen, London, UK). The common APOE epsilon alleles defined by the p.C112R (rs429358) and p.R158C (rs7412) substitutions were selected for analysis, as well as the common MTHFR C677T (p.A222V; rs1801133) variant and the ACE exonic synonymous variant p.T202T (rs4343; AG); which acts as a surrogate marker for the common intronic Alu insertion/deletion (VD); rs4646994) polymorphism. Genotyping was performed with ABI pre-designed TaqMan probes and chemistry (Applied Biosystems, Foster City, CA) with analysis performed using SDS 2.2.2 software on an ABI 7900 for TaqMan. Positive and negative controls where included on all TaqMan assay plates. Positive or ambiguous results in the TaqMan assay were also resolved with direct sequencing.

Statistical analysis

All calculations were done using Graphpad Prism version 6.0 software. The Fisher's test was used to calculate for P-value and the two tailed probability levels for statistical significance with P<0.05 being considered significant. An odds ratio (OR) was calculated as a measure of the strength of association.

Results

The genotype and allele frequencies in the study and control groups are shown in Table 1. There was no significant difference between the two groups in the frequencies of APOE, MTHFR and ACE genotypes and alleles. The APOE, MTHFR and ACE genotype frequencies for the case group were compatible with the Hardy-Weinberg equilibrium. The APOE ε2ε2 and the MTHFR TT genotypes were absent in the Zambian population. However, in the control group, the APOE, MTHFR genotype frequencies were compatible with the Hardy-Weinberg equilibrium (HWE), while the ACE genotype was not in agreement with HWE. The frequency of control subjects homozygous for APOE ε4 was 7.7% compared to 2.4% for stroke patients. The MTHFR T allele frequency was 8.6% in control subjects and was observed at a frequency of 11.0% in patients. The frequency for ACE rs4343 G allele corresponding to the D allele was 13.8% for subjects in the control group compared to 12.2% for patients.

There was significant genotypic variations between cases and controls for APOE ε2ε4 and ε2ε3 genotypes, statistical analysis demonstrated a higher risk for hemorrhagic stroke in APOE ε2ε4 carriers (OR of 4.45 (P<0.05)) while the presence of APOE ε2ε3 genotype seemed to significantly lower the risk for hemorrhagic stroke (OR 0.097; 0.006-1.67, P<0.05) (Table 2).

APOE ε2ε2 and MTHFR 677TT polymorphisms were absent in both ischemic and hemorrhagic stroke. Though not significant, the frequency of the MTHFR 677TT allele was higher (double) in ischemic stroke (17.4%) compared to hemorrhagic stroke (8.3%). The ACE genotypes showed no significant frequency differences between cases and controls.

Discussion and Conclusions

Studies conducted over the last decade has shown that among blacks, stroke is more common, more severe, and carries higher mortality when compared with other races; blacks also have increased frequency of risk factors such as DM, hypertension, and obesity. In this study we have analyzed for the first time common polymorphisms of the APOE, MTHFR and ACE genes in Zambian stroke patients and in controls. This constitutes an approach to start characterization of the genetic risk architecture of Zambian patients with cerebrovascular disease. The study also describes the prevalence of APOE, MTHFR and ACE polymorphisms corresponding to stroke in the Zambian population.

We observed a relatively high frequency of the APOE ε4 allele and a complete absence of the APOE ε2ε2 genotype in both patients and controls. We did not find any association between the APOE ε4 allele and stroke in our patients. However, there was an increased risk to hemorrhagic stroke by the APOE ε2ε4 genotype with OR at 4.45 P<0.05. This may suggest that Zambian carriers of APOE ε2 and ε4 have an increased risk of hemorrhagic stroke. Of note, in our previous study, the prevalence of hemorrhagic stroke was observed to be higher than in reported Western cohorts. A similar high frequency for hemorrhagic stroke has been reported for other African countries with estimates up to 52% in Democratic Republic of Congo, up to 60% in Ghana, and Tanzania. According to the literature, carriers of APOE ε2 and ε4 have an increased risk of intracerebral hemorrhage perhaps because of the effects of these gene variants on risk of cerebral amyloid angiopathy.

Other studies have found conflicting results regarding association of APOE alleles and stroke. Some studies report an increased risk of stroke for APOE ε4 carriers. A recent meta-analysis in Caucasian populations found APOE ε2 genotypes to be associated with increased risk of hemorrhagic stroke. We found the effect of APOE ε2 on carriers to be inconclusive as it increased hemorrhagic stroke risk in

| Alleles | Cases n=41 (%) | Control n=116 (%) | OR | P |
|---------|---------------|------------------|----|---|
| APOE    |               |                  |    |   |
| ε2ε2    | 0             | 0                | 0.62 (0.24-1.65) | 0.494 |
| ε2ε3    | 6 (14.6)      | 25 (21.6)        | 1.68 (0.46-6.089) | 0.479 |
| ε2ε4    | 4 (9.8)       | 7 (6)            | 1.121 (0.668-1.881) | 0.696 |
| ε3ε3    | 15 (36.6)     | 38 (32.8)        | 1.232 (0.584-2.597) | 0.700 |
| ε3ε4    | 15 (36.6)     | 37 (31.9)        | 0.297 (0.056-2.423) | 0.456 |
| ε4ε4    | 1 (2.4)       | 9 (7.7)          | 0.512 (0.127-1.988) | 0.885 |
| MTHFR   |               |                  |    |   |
| CC      | 32 (76.2)     | 96 (82.8)        | 0.740 (0.306-1.791) | 0.492 |
| CT      | 9 (22.0)      | 20 (17.2)        | 1.35 (0.558-3.264) | 0.492 |
| TT      | 0             | 0                | 1.307 (0.570-2.999) | 0.512 |
| ACE     |               |                  |    |   |
| AA (II) | 32 (76.2)     | 90 (77.6)        | 1.03 (0.44-2.43) | 0.951 |
| AG (ID) | 8 (19.5)      | 20 (17.2)        | 1.16 (0.47-2.89) | 0.744 |
| GG (DD) | 1 (2.4)       | 6 (5)            | 0.40 (0.05-3.43) | 0.623 |
| A (I)   | 72 (87.8)     | 200 (86.2)       | 1.152 (0.539-2.462) | 0.851 |
| G (D)   | 10 (12.2)     | 32 (13.8)        | 0.888 (0.405-1.855) | 0.851 |

[Neurology International 2013; 5:e20]
However, as genetic technologies become more available to African countries and in particular to those between the Sahara and South Africa we will gain a better understanding of how genetics will determine health outcomes. These data will help ease health-related social and economic burdens within these developing nations.

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Table 2. The genotype and allele data of ischemic and hemorrhagic cases and controls.

| Allele | Ischemic Cases (%) | Ischemic Control (%) | OR   | Hemorrhagic Cases (%) | Hemorrhagic Control (%) | OR   |
|--------|-------------------|----------------------|------|-----------------------|------------------------|------|
| APOEε2ε4 | n=23              | n=116                |      | n=18                  | n=116                  |      |
| ε2ε3   | 4 (17.4)          | 25 (21.6)            | 0.7663 (0.239-2.459) | 0                      | 25 (21.6)             | 0.097 (0.006-1.67) | ≤0.05 |
| ε2ε4   | 3 (13.0)          | 7 (6)                | 1.32 (0.524-3.321)  | 4 (22.2)               | 7 (6)                  | 4.45 (1.15-17.1)  | ≤0.05 |
| ε3ε3   | 9 (39.1%)         | 38 (32.8%)           | 0.984 (0.352-4.65)  | 6 (33.3)               | 38 (32.8)             | 1.03 (0.358-2.95) |       |
| ε3ε4   | 7 (30.4%)         | 37 (31.9%)           | 0.24 (0.014-4.286)  | 7 (38.9)               | 37 (31.9)             | 1.36 (0.487-3.79) |       |
| ε4ε4   | 0                 | 9 (7.7%)             | 1.122 (0.462-2.724) | 1 (5.6)                | 9 (7.7)               | 0.695 (0.083-5.88) |       |
| ε2     | 7 (15.2%)         | 32 (13.8%)           | 1.162 (0.604-2.34)  | 4 (11.1)               | 32 (13.8)             | 0.781 (0.259-2.36) |       |
| ε3     | 26 (60.0%)        | 138 (59.5%)          | 0.762 (0.357-1.027) | 19 (52.8)              | 138 (59.5)            | 0.761 (0.376-1.54) |       |
| ε4     | 10 (21.7%)        | 62 (26.7%)           | 1.04 (0.352-3.07)   | 13 (36.1)              | 62 (26.7)             | 1.55 (0.740-3.25) |       |
| MTHFR   | n=23              | n=116                |      | n=18                  | n=116                  |      |
| CC     | 15 (65.2%)        | 96 (82.8%)           | 0.39 (0.141-1.045)  | 15 (65.3)              | 96 (82.8)             | 1.04 (0.275-3.94) |       |
| CT     | 8 (34.8%)         | 20 (17.2%)           | 2.56 (0.957-6.85)   | 3 (16.7)               | 20 (17.2)             | 0.96 (2.54-3.63)  |       |
| TT     | 0                 | 0                    | 0                |                        | 0                      |      |
| C      | 38 (82.6%)        | 212 (91.4%)          | 0.448 (0.184-1.091) | 33 (91.7)              | 212 (91.4)            | 1.04 (0.292-3.69) |       |
| T      | 8 (17.4%)         | 20 (8.6%)            | 2.23 (0.917-5.43)   | 3 (8.3)                | 20 (8.6)              | 0.964 (0.271-3.42) |       |
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