The Role of Polymorphism Gen *Methylene Tetra Hydrofolate Reductase* (*MTHFR*) C677T in Ischaemic Stroke Patients with and Without Hypertension

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Abstract

**BACKGROUND:** Stroke is a leading cause of disability and remains the second leading cause of death in the world. Some of the pathogenesis of stroke are interactions between genetic and acquired risk factors, the interaction is related with the atherosclerotic which is the main pathogenesis of ischaemic stroke. Previous studies demonstrated an association between methylene tetrahydrofolate reductase (*MTHFR*) genotype and ischaemic stroke; the *MTHFR* C677T genotype is one of the independent risk factor.

**AIM:** This study aims to know about the role of polymorphism gen *MTHFR* C677T in ischaemic stroke patients with and without hypertension.

**METHODS:** This study is a cross-sectional study, the sample was taken consecutively, after approval by the Medical Faculty Science’s Ethics Committee at University Sumatera Utara. All sample matched with inclusion and exclusion criteria, demographic data and blood sample were taken. Demography data were analysed using descriptive statistic.

**RESULTS:** Of the 106 ischaemic stroke patients were divided into two groups, the first group is patients with hypertension (53 patients), and the second group is without hypertension (53 patients). We have done the PCR-RFLP to all the patients, we got 78 patients with 677CC of *MTHFR* genotype, 23 patients with 677CT genotype and 5 patients with 677TT genotype. We found polymorphism C677T is more frequent in ischaemic stroke patients with hypertension (16 patients; 69.5%), and all the patient with 677TT genotype are an ischaemic stroke with hypertension (5 patients; 100%).

**CONCLUSION:** We concluded that polymorphism *MTHFR* C677T have an important role in hypertension and ischaemic stroke.

Introduction

Stroke remains a health problem in the world and as the major cause of morbidity, mortality and disability in developing country [1], [2], also in Asia, Russia, East Europe [3] and as multifactorial disease and complex caused by vascular, environment and genetic [4], [5]. The incidence of stroke increased in lower and middle-income countries. WHO predict in the year 2050, 80% of cases of stroke happened in that countries [4].

Aetiology and pathogenesis of ischaemic stroke are complex and involved some risk factor [6], many evidence showed the role of genetic in stroke [4], but identification of the gen that related to stroke is still controversy [7]. *A cohort* study showed that family history with stroke would increase the risk of stroke [8], [9], and case-control study in twins also related to the inherited risk [10].

Over the past decades, the role of a different candidate gene in the pathogenesis of stroke has been examined in numerous association studies [11]. The epidemiologic study shows the existence of basic polygene in stroke incidence and many stroke
pathogenesis, they act as interactions between genetic, and others risk factors [5], [7], the interaction related to atherosclerosis which is major pathogenesis of stroke [12]. Many gene and polymorphism have been studied as the cause of hypertension and act as a major risk factor for stroke [13].

The methylenetetrahydrofolate reductase C677T mutation is one of the most common gene polymorphisms. Mapped to chromosomal region 1p36.3, the MTHFR gene spans a 2.2-kb length with 11 exons and 10 introns [14] and encodes an enzyme composed of 656 amino acids [15]. The product, known as MTHFR, is a critical enzyme in homocysteine metabolism. The C677C to T mutation in the catalysing region of the MTHFR gene may induce the displacement of alanine by valine [16]. This change may lead to the thermolability of the enzyme and the inhibition of MTHFR activity, thus decreasing the transformation from 5,10 methylenetetrahydrofolate to 5 methyltetrahydrofolates [11], which act as a co-substrate for the conversion from homocysteine to methionine [14].

A lot of case-control studies have shown the role of C to T mutation in 677 nucleotides in the MTHFR gene; they found a significant relationships between C677T with hypertension in Caucasian and Asian population [13]. The MTHFR C677T polymorphism has previously been found to associate with various vascular disease including stroke, hypertension and congestive heart failure [11].

Prevalence MTHFR C677T relatively high in general population, while the prevalence of T allele is about 0.34 (0.29-0.39) in whites, 0.42 (0.34-0.50) in Japanese and 0.08 (0.06-0.12) in African [17]. Other research shows that T allele frequency is much varied in different ethnic and country, 22.6% in the Guangxi Yao population, 32.2% in Australia, and 63.1% in Shandong [18].

People with MTHFR 677TT gene only have 30% of enzyme activity, while the MTHFR C677T has 60% of enzyme activity compared with the wild type [6]. T allele from MTHFR gene polymorphism is an independent risk factor of idiopathic ischaemic stroke [19], and prognostic factor for cardiovascular disease [20].

Genetic studies have a role in optimized stroke prevention and therapy [9], also can be used to predict the risk of the disease, but the results of the studies did not mention about the type and numerous gene that act as a stroke risk factor [7]. Significant improvement in the last few years about the role of genetic in stroke, giving deep knowledge about the basic molecular disease that can be used for the management patients [21] and to get new therapy target [10]. Identification of stroke risk factor can be modified to get stroke prevention more effective and decrease the mortality [3].

This study aims to know about the role of polymorphism gene MTHFR C677T in ischaemic stroke patients with and without hypertension.

Material and Methods

This study is a cross-sectional study; the sample was taken consecutively. The study was conducted with the approval of the ethics committee of the institutions involved, and informed consent was obtained from all subjects or their relatives (for comatose patients). Inclusion criteria were the presence of ischaemic stroke at the present hospital admission, age > 18 years old; the neurological deficits were confirmed in all cases by computerised tomography (CT) scan. We analysed the MTHFR genotypes in subjects with ischaemic stroke. The polymorphism was classified into three groups, (1) allele CC, (2) allele CT, (3) allele TT.

A polymerase chain reaction followed by the restriction fragment length polymorphism was used to genotype the C677T polymorphism of MTHFR. The conditions of amplification and digestion have been well documented previously in our laboratory. The digested PCR products after separation on a 3% agarose gel, stained with ethidium bromide, showed one band of 198bp corresponding to the wild-type homozgyous (CC), three bands of 198, 175, and 23bp for the heterozygous (CT), two bands of 175 and 23bp for the mutated homozgyous (TT).

Results

This study includes 106 ischaemic stroke patients were divided into two groups, with and without hypertension. A total of 55 men had an ischaemic stroke, while 51 women had an ischaemic stroke, 60 patients at the age below 60 y.o, 46 patients at the age above 60 y.o. Batakne 33 patients, family history was found in 32 patients.

| Table 1 Distribution of characteristic frequency ischaemic stroke with and without hypertension |
|---|---|---|---|---|---|
| Variable | Category | Ischaemic Stroke | | | |
| | | Hypertension | Non-hypertension | Total |
| | N | % | N | % | N | % |
| Age | | | | | | |
| < 60 y.o | 30 | 28.3 | 30 | 28.3 | 60 | 56.6 |
| > 60 y.o | 23 | 21.7 | 23 | 21.7 | 46 | 43.4 |
| Gender | Female | 25 | 23.6 | 26 | 24.5 | 51 | 48.1 |
| | Male | 28 | 26.4 | 27 | 25.4 | 55 | 51.9 |
| Ethnicity | Batakne | 33 | 31.1 | 29 | 27.3 | 62 | 58.4 |
| | Javane | 11 | 10.4 | 10 | 9.43 | 21 | 19.8 |
| | Acehnese | 4 | 3.77 | 3 | 2.83 | 7 | 6.60 |
| | Minangkese | 2 | 1.88 | 2 | 1.88 | 3 | 2.83 |
| | Melayumese | 2 | 1.88 | 9 | 8.49 | 11 | 10.3 |
| | Niasnese | 1 | 0.94 | - | - | 1 | 0.94 |
| Family | history | 32 | 30.1 | 17 | 16.0 | 49 | 46.2 |

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The differences in the characteristics of patients with and without hypertension are shown in Table 1.

| Variable    | Category | Ischaemic Stroke | Non-Ischaemic Stroke | Total | p-value* |
|-------------|----------|------------------|----------------------|-------|----------|
| Polymorphism|          |                  |                      |       |          |
|             |          | Hypertension     | Non-Hypertension     | Total |          |
| CC          |          | 32               | 60.3                 | 43    | 81.1     | 76      | 71.7 | 0.035 |
| CT          |          | 16               | 30.2                 | 10    | 18.9     | 26      | 24.5 |          |
| TT          |          | 5                | 9.5                  | 0     | 0        | 5       | 3.8  |          |
|             |          |                  |                      |       |          |

*Fisher Exact Test

This study found that among 106 patients ischaemic stroke with hypertension and without hypertension, the wild-type homozygous (CC) was found in 76 patients; 71.7% (32 patients with hypertension, 43 patients without hypertension). The heterozygous (CT) was found in 26 patients; 24.5% (16 patients with hypertension, 10 patients without hypertension). The mutated homozygous (TT) was found only in ischaemic stroke patients with hypertension (5 patients).

Fisher exact test was done for this variable category; the result showed a significant correlation (p = 0.035) (Table 2).

Discussion

Stroke is a common complex trait and does not follow Mendelian pattern of inheritance: Gene–gene or gene-environment interactions may be responsible for the complex trait. How the interactions contribute to stroke is still under research [12].

Now we know single polymorphism may have a weak effect on the risk of stroke when analysed individually, but their influence may be more pronounced in the presence of a permissive background. Many cases of stroke are due to a complex interaction between lifestyle factors and genetic susceptibilities. Genetic predisposition for stroke may result from synergistic co-effects [11].

Many studies have shown that T allele frequencies in different ethnicities and countries vary widely. The Guangxi Yao population rate was 22.6%, and in Australia, it was 32.2%. The Yang et al., the study of 15,357 Han patients found that the mutation frequency in Hainan was the lowest with T allele of 245 and in Shandong it was the highest (63.1%) [18].

This study found that polymorphism C677T is more frequent in ischaemic stroke patients with hypertension (16 patients), and all the patient with 677TT genotype are an ischaemic stroke with hypertension (5 patients).

This gene is responsible for producing Methylenetetrahydrofolate reductase (MTHFR) enzyme that functional. The function of this enzyme is to transform 5, 10 methyltetrahydrofolates to 5 methylytetrahydrofolates [22]. The Val form of MTHFR encoded by 677 T allele is thermolabile and has reduced enzymatic activity [23]. The mutated homozygous (TT) is the risk of thrombotic disease in young age [24].

The result of this study showed a significant correlation between the polymorphism and ischaemic stroke with and without hypertension (p = 0.035).

There is a strong association between polymorphism gen MTHFR with atherosclerosis; even the mechanism of it is indirect. Patients with MTHFR genotype 677TT, have a vascular occlusion, and infarct, had increased level of blood homocysteine. Those are the reason for the association between polymorphism and hypertension [12].

In conclusion, polymorphism MTHFR C677T has an important role in hypertension and ischaemic stroke, because both of the diseases is caused by atherosclerotic vascular disease.

There are several limitations to our study. First, the study enrolled hospital-based stroke patients rather than patients from a community based general population. Secondly, this study did not collect data on other several major risk factors of ischaemic stroke.

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References

1. Rahman A, Gupta RD, Quraishi FA, Saha UK, Miah MN, Ali Z. Relationship Between Homocysteine and Ischemic Stroke. Journal of Medicine. 2013; 14(1):47-51. https://doi.org/10.3329/jom.v14i1.14536

2. Aw D, Sharma JC. Antiplatelets in secondary stroke prevention: should clopidogrel be the first choice? Postgraduate medical journal. 2012; 88(1035):34-7. https://doi.org/10.1136/postgradmedj-2011-130100 PMid:22121248

3. Shi Z, Guan Y, Huo YR, Liu S, Zhang M, Lu H, Yue W, Wang J, Ji Y. Elevated total homocysteine levels in acute ischemic stroke are associated with long-term mortality. Stroke. 2015: STROKEAHA-115.

4. Kumar A, Sagar R, Kumar P, Sahu JK, Grover A, Srivastava AK, Vivekanandan S, Prasad K. Identification of genetic contribution to ischemic stroke by screening of single nucleotide polymorphisms in stroke patients by using a case control study design. BMC neurology. 2013; 13(1):138. https://doi.org/10.1186/1471-2277-13-
15. Nassereddine S, Kassogue Y, Korchi F, Habbal R, Nadifi S. Association of methylenetetrahydrofolate reductase gene (C677T) with the risk of hypertension in Morocco. BMC research notes. 2015; 8(1):775. https://doi.org/10.1186/s13104-015-1772-x PMid:26654251 PMCid:PMC4677044

16. Yadav S, Hasan N, Marjot T, Khan MS, Prasad K, Bentley P, Sharma P. Detailed analysis of gene polymorphisms associated with ischemic stroke in South Asians. PLoS one. 2013; 8(3):e57305. https://doi.org/10.1371/journal.pone.0057305 PMid:2350425 PMCid:PMC3591429

17. Akizhanova A, Takamura N, Kusano Y, Karazhanova L, Yamashita S, Saito H, Aoyagi K. Association between C677T/MTHFR genotype and homocysteine concentration in a Kazakh population. Asia Pacific journal of clinical nutrition. 2008; 17(2):325-9. PMid:18586655

18. Cai W, Yin L, Yang F, Zhang L, Cheng J. Association between Hcy levels and the CBS844ins88 and MTHFR C677T polymorphisms with essential hypertension. Biomedical reports. 2014; 2(6):861-8. https://doi.org/10.3892/br.2014.357 PMid:25279160 PMCid:PMC4179705

19. Salas IS, Altatz FB, Miranda AL, Sanchez GB, Ojeda JB, Chavez JG, et al. The C677T Polymorphism of the Methylenetetrahydrofolate Reductase Gene is Associated with Idiopathic Ischemic Stroke in the Young Mexican-Mestizo Population. Cerebrovasc Dis. 2010; 29:454-9. https://doi.org/10.1159/0002748949 PMid:20203488

20. Ge gene polymorphism and plasma homocysteine levels. Rheumatology international. 2013; 33(6):1519-24. https://doi.org/10.1007/s00296-012-2552-8 PMid:23247802

21. Sharma P, Yadav S, Meschia JF. Genetics of ischaemic stroke. J Neurol Neurosurg Psychiatry. 2013; 84(12):1302-8. PMid:23803072

22. Yang B, Liu Y, Li Y, Fan S, Zhi X, Lu X, Wang D, Zheng Q, Wang Y, Sun G. Geographical distribution of MTHFR C677T, A1298C and MTRR A66G gene polymorphisms in China: findings from 15357 adults of Han nationality. PloS one. 2013; 8(3):e57917. https://doi.org/10.1371/journal.pone.0057917 PMid:23472119 PMCid:PMC3589470

23. Dong Q, Tang G, He M, Cai Y, Cai Y, Xing H, Sun L, Li J, Zhang Y, Fan F, Wang B. Methylenetetrahydrofolate reductase C677T polymorphism is associated with estimated glomerular filtration rate in hypertensive Chinese males. BMC medical genetics. 2012; 13(1):74. https://doi.org/10.1186/1471-2350-13-74 PMid:22893498 PMCid:PMC3589470

24. Kim SJ, Lee BH, Kim YM, Kim GH, Yoo HW. Congenital MTHFR deficiency causing early-onset cerebral stroke in a case homozygous for MTHFR thermolabile variant. Metabolic brain disease. 2013; 28(3):519-22. https://doi.org/10.1007/s11011-013-9398-x PMid:23526309