Associations of laboratory parameters and genetic polymorphisms with ischemic stroke in Chinese Han population

Jiayong Wang
Tongji University School of Medicine
Zujun Sun
Tongji University School of Medicine
Yibao Yang
Tongji University School of Medicine
Junlu Wu
Tongji University School of Medicine
Wenqiang Quan
Tongji University School of Medicine
Anquan Shang
Tongji University School of Medicine
Ping Ji
Tongji University School of Medicine
Xingcai Chen
Guangxi Medical University
Peihua Ni
Shanghai Jiao Tong University
Dong Li (lidong@tongji.edu.cn)
Tongji University School of Medicine

Research article

Keywords: Ischemic stroke, Genetic polymorphism, Laboratory parameter, hyperlipidemia, Diabetes

Posted Date: January 20th, 2020

DOI: https://doi.org/10.21203/rs.2.21344/v1

Version of Record: A version of this preprint was published at Experimental and Therapeutic Medicine on March 16th, 2021. See the published version at https://doi.org/10.3892/etm.2021.9921.

Abstract

Background Many genetic polymorphisms and clinical laboratory parameters have been shown to be associated with ischemic stroke (IS). However, these results are often inconsistent. Therefore, the aim of the study was to evaluate the correlation between clinical laboratory parameters, the risk genetic polymorphisms and IS in Shanghai population.

Methods The clinical laboratory parameters were measured by automatic biochemical analyzer. The genotype frequencies and allelic frequencies of the polymorphisms gene encoding angiotensin-converting enzyme (ACE D/I), gene encoding methylene tetrahydrofolate reductase (MTHFR C677T), gene encoding β-fibrinogen 455/148 (β-Fg -455/148) were characterized by restricted fragment length polymorphisms-polymerase chain reaction (RFLP-PCR). The gene encoding plasminogen activator inhibitor (PAI -1 4G/5G) and gene encoding apolipoprotein E 2,3,4 (ApoE ε2,3,4) were characterized by allele specific PCR. The genotype frequency and allele frequency of the 6 risk genes of IS were analyzed by chi-square test in different groups of laboratory indexes. The distribution maps of the polymorphisms ACE D/I, MTHFR C677T, β-Fg -455 A/G, β-Fg -148 T/C, PAI -1 4G/5G, ApoE ε2,3,4 and clinical laboratory paramaters were compared between healthy people and patients with IS.

Results The laboratory parameters were related to ACE I/D, β-Fg -455 A/G, and PAI -1 4G/5G genes. D allele of ACE I/D was associated with high levels of total cholesterol (TC) and Low-density lipoprotein (LDL). High levels of fasting blood glucose (FBG), triglyceride (TG), and LDL were risk factors for IS. There were significant differences in genotype frequencies of ACE I/D, β-Fg -455 A/G, and β-Fg- 148 T/C genes between IS and control group.

Conclusions Clinical laboratory paramaters are associated with the risk genes polymorphisms of IS. It is suggested that control value of clinical laboratory paramaters should be determined according to the genotype carried by the patients with diabetes and hyperlipidemia when preventing IS.

Background

Ischemic stroke (IS) is a group of syndromes with great harmfulness, high incidence rate, high disability rate, and high mortality rate, which has become the second leading cause of disability and death in the world [1]. It is caused by multiple factors, including age, sex, body mass index, hyperlipidemia, hypertension, diabetes, smoking, PM2.5 pollution [2, 3]. However, there has been no effective treatment and prevention for IS in clinic.

Recent studies have found that many genetic polymorphisms have been shown to be associated with IS, such as C-C motif chemokine 11 (*CC11)*, paraoxonase 1, angiotensin converting enzyme (*ACE*), and methylenetetrahydrofolate reductase (*MTHFR*) [4-6]. *ACE* plays an important role in vascular remodeling in atherosclerosis and IS [7]. It has been reported that *ACE* I/D polymorphism is significantly associated with IS in different ethnic groups [8], while other studies failed to observe this association [9, 10]. Studies have shown that *ACE*2 exists widely in the central nervous system and current studies mainly focused on

the relationship between *ACE*2 gene polymorphism and cardiovascular disease, such as hypertension and coronary heart disease [11-15]. There is a significant correlation between the *MTHFR* polymorphism and IS with or without hypertension (p=0.035) [16]. Patients with an *MTHFR* genotype of *677TT* have vascular occlusion, infarct, and increased level of blood homocysteine, which is the reason of genetic polymorphism association with hypertension [17]. Polymorphisms of *MTHFR* play an important role in hypertension and IS, both of which are caused by atherosclerotic vascular disease [18]. Fibrinogen consists of 3 different subunits known as α, β, and γ chains linked by disulfide bonds. The synthesis of the β-Fg (for the β chain) subunit is a crucial step in the assembly of mature fibrinogen, which seems to be the most important factor affecting the level of plasma fibrinogen [19]. The data from adult patients have shown that 148 C/T, 448 G/A, and 455 G/A polymorphisms of the *β-Fg* gene are associated with IS of large vessels in adults [20]. Studies have demonstrated that 148 C/T and 455 G/A polymorphisms of *β-Fg* may affect plasma fibrinogen levels and these two polymorphisms are also associated with an increased risk of thrombotic disorders [21]. Recently, numerous studies have been conducted to investigate the potential association between *β-Fg* polymorphisms and the risk of IS. However, the results of these studies are inconsistent and the sample size of individual studies is inadequate to draw definite conclusions [22-24]. Kopyta et al. reported that there are not any relationships between polymorphisms of the *b-Fg* genes and pediatric arterial IS in family-based and case control models [25]. The plasminogen activator inhibitor-1 (*PAI*-1) polymorphism of 4G/5G is one of the most frequently studied [26]. The 4G allele is considered to be a risk factor for coronary artery disease and the 4G/4G genotype is thought to increase the risk of coronary artery disease [27, 28]. Several studies addressed the association between the 4G/5G polymorphism and stroke, but the results are also inconsistent [29, 30]. Although *PAI*-1 may be an important factor in the occurence of IS, the association between *PAI*-1 gene polymorphism and the risk of IS has not been elucidated. Apolipoprotein E (*ApoE*) polymorphism involves a single amino substitution and lead to three major alleles (ε2, e3, and e4) and their 6 corresponding phenotypes (ε2/ε2, ε3/ε3, ε4/ε4, ε2/ε3, ε2/ε4, and ε3/ε4) [31]. *ApoE* was proved to involve in lipid metabolism and transport of cholesterol in brain [32]. It was reported that ε2 allele is an independent risk factor for echolucent and ulcerated carotid plaque [33]. The relationship between *ApoE* genotype and stroke has been proposed, but the results are inconsistent. The contribution of *ApoE* gene polymorphism as a regulator of lipid metabolism to stroke pathogenesis also produced conflicting results. Some reports indicated a positive association between *ApoE4*-containing genotypes and stroke, while others found no relationships between *ApoE* isoforms, dyslipidemia, and stroke [34, 35]. Therefore, the aim of this study was to evaluate the potential association between the genes of *ACE* D/I, *MTHFR* C677T, *b-Fg*-455, *b-Fg*-148, *PAI-1* 4G/5G, *ApoE* ε2,3,4 polymorphisms and clinical laboratory parameters for IS in Shanghai population, which may provide reasonable control rang of laboratory parameters for prevention of IS in patients with diabetes and hyperlipidemia as early as possible.

Methods

Patients

Medical records of all newly diagnosed proven patients with diabetes and hyperlipidemia admitted to Shanghai Tongji Hospital from October 2016 to November 2018. This study was approved by the ethics committee of Shanghai Tongji Hospital and was conducted according to guidelines of the Declaration of Helsinki. All participants provided informed consent. If the subjects were unable to communicate, written consent was obtained from their legal relatives.

Patients who met the following criteria were included: (1) fasting blood glucose ≥7.0 mmol/L or OGTT 2 h≥11.1 mmol/L, (2) TG≥1.7 mmol/L or LDL-C≥3.37 mmol/L or CHOL≥5.18 mmol/L, (3) diagnosis of IS meets the standards of Chinese guidelines for diagnosis and treatment of acute ischemic stroke 2014, and (4) all the patients were Shanghai han population and unrelated.

Patients who met the following criteria were excluded: (1) diagnosis of other types of cerebrovascular disease (e.g., intracranial hemorrhage, subarachnoid hemorrhage, cerebrovascular malformation, or cerebral aneurysm) and (2) severe systemic diseases such as cancer, severe inflammatory diseases, and serious chronic diseases (e.g., hepatic failure or renal failure).

In the control group a total of 336 individuals without history of cerebrovascular disease were included. Age, sex, use of oral contraceptives and history of thrombotic events or drug abuse were recorded. There were no statistical difference in age and sex compared with IS group. The baseline characteristics of patients and controls were shown in S-table 1.

Serum Lipid and Glucose Measurement

Approximately 3 mL blood samples of fasting blood were collected from each study participant then centrifuged at 2500 rpm for 10 min (Backman Coulter, SPINCHRON^{TM} DLX Centrifuge, USA). Serum levels of total cholesterol (CHOL), triglyceride (TG), low-density lipoprotein cholesterol (LDL-C) were analyzed by automatic biochemical analyzer (Backman Coulter, DXC800, USA).

DNA extraction and genetic analysis

Genomic DNA was extracted from peripheral blood leukocytes using the TIANamp Genomic DNA Kit (TIANGEN Biotech, Beijing). Polymorphisms were genotyped using the restriction fragments length polymorphism (RFLP) or allele specific Polymerase chain reaction (PCR) method. PCR using the sequence of primers are shown in S-table 2 (Sangon Biotech Co., Ltd., Shanghai). The composition of PCR mixes includes Ex Taq enzyme, dNTP and 10x buffer (TaKaRa). Amplified PCR products of intron 16 of the polymorphic *ACE* gene were separated on 2% agarose gel (BIO-RAD Laboratories, Hercules, California, USA) (Fig. 1**)**. The presence of 191 bp fragments represented the D allele the presence of 480 bp fragments represented I allele, and the presence of 191 bp and 480 bp fragments represented D/I allele. PCR was performed by denaturation at 95℃ for 30 s, annealing segment at 55℃ for 30 s, and extension at 72℃ for 30 s, repeated for 35 cycles. Amplification products of polymorphic *b-Fg*-455 gene were digested by Hae III (Thermo, FD0154) and separated on 2% agarose gel (Fig. 1**)**. The presence of 343 bp, 383 bp, 575 bp fragments represented the GG allele, the presence of 343 bp and 958 bp

fragments represented AA allele, the the presence of 343 bp, 383 bp, 575 bp, 958 bp fragments represented G/A allele. PCR was performed by denaturation at 94℃ for 30 s, annealing segment at 55℃ for 30 s, and extension at 72℃ for 80 s, repeated for 35 cycles. Amplification products of polymorphic *b-Fg*-148 gene were digested by Hind III (Thermo, FD0505) and separated on 2% agarose gel (Fig. 1). The presence of 100 bp and 200 bp fragments represented the CC allele, the presence of 300 bp fragments represented TT allele, the presence of 100 bp, 200 bp, 300 bp fragments represented T/C allele. PCR was performed by denaturation at 94℃ for 30 s, annealing segment at 58.5℃ for 30 s, and extension at 72℃ for 30 s, repeated for 35 cycles. Amplification products of polymorphic *MTHFR* gene were digested by Hinf (Thermo, FD0804) and separated on 2% agarose gel (Fig. 1). The presence of 100 bp and 200 bp fragments represented the TT allele, the presence of 300 bp fragments represented CC allele, the presence of 100 bp, 200 bp, 300 bp fragments represented T/C allele. PCR was performed by denaturation at 94℃ for 30 s, annealing segment at 58℃ for 30 s, and extension at 72℃ for 30 s, repeated for 35 cycles. Amplification products of polymorphic *PAI-1* gene were separated on 2% agarose gel. The PCR products were recovered and submitted to sequencing. PCR was performed by denaturation at 94℃ for 30 s, annealing segment at 58.5℃ for 45 s, and extension at 72℃ for 30 s, repeated for 35 cycles. Amplification products of polymorphic *ApoE* e2,3,4 gene were separated on 2% agarose gel. The PCR products were recovered and submitted to sequencing. PCR was performed by denaturation at 95℃ for 30 s, annealing segment at 60℃ for 45 s, and extension at 72℃ for 55 s, repeated for 35 cycles. First-generation sequencing technique was used in this study (Jinweizhi Biotech co. LTD, Suzhou, China).

Statistical analysis

We used SPSS statistical software version 21.0 for the statistical analysis. Pearson chi-square test or Fisher's exact test were used for counting data. Continuous variables were displayed as mean ± SD. Single factor of variance was used to analysis patient's age, GLU, CHOL, TG, LDL-C. We used odds ratio (OR) and 95% confidence intervals (95% CI) to assess the correlation between polymorphism sites and abnormal risk of laboratory parameters. Anova was used to calculate the best control value of laboratory parameters. $P<0.05$ was considered to be obviously statistical significance.

Results

The relationship between laboratory parameters and genotype frequencies, allele frequencies of ACE I/D, MTHFR C677T, β-Fg-455 A/G, β-Fg-148 T/C, PAI-1 4G/5G and ApoE ε2-4 Genes

Table 1 showed that the laboratory parameters were correlated with *ACE* I/D, *β-Fg*-455 A/G, and *PAI*-1 4G/5G genes. And frequency of I/D genotype of *ACE* gene was the highest in fasting blood glucose (FBG) ≥7.00 mmol/L group, while frequency of II genotype was the highest in FBG <7.00 mmol/L group. Apart from that, D allele of *ACE* I/D is associated with high levels of TC and LDL as shown in Table 2. In addition, we also observed the significant association of *β-Fg*-455 A/G gene with LDL, and the association of *PAI*-1 4G/5G with TG in Table 1. The frequency of 4G5G genotype of *PAI*-1 4G/5G gene is the highest in TG <1.70 mmol/L group and the frequency of 4G5G genotype is equal to that of 4G4G

genotype in TG ≥1.70 mmol/L group, which is 2 percentage points higher than that of 5G5G genotype. However, there is no significant difference in genotype frequency distribution of *MTHFR* C677T, *β-Fg*-148T/C, and *ApoE* ε2-4 genes among FBG, TC, TG, and LDL.

The prediction value of laboratory parameters for ischemic stroke

The results in Table 3 showed that high levels of FBG, TG, and LDL were risk factors for IS. We found that the risk of IS increased by 5.47, 3.64, and 6.62-folds along with each 1 mmol/L increment of FBG, TG, and LDL, respectively. After adjusting for age and sex, it increases by 4.38, 4.41, and 5.21-fold, respectively.

The ROC curve analysis of laboratory parameters for ischemic stroke

According to Table 4, the area under the curve with 95% confidence interval AUC (95% CI) of IS predicted by FBG, TC, TG, and LDL were 0.828 (0.784–0.867), 0.595 (0.541–0.648), 0.702 (0.650–0.751) and 0.638 (0.584–0.689), respectively. We observed that FBG has a better specificity (0.833) and sensitivity (0.694) in predicting IS compared with other parameters, and its optimal cut-off point in predicting IS is 5.27 mmol/L. Furthermore, LDL has a higher specificity (0.999) compared to that of FBG, and its optimal cut-off point in predicting IS is 3.36 mmol/L. However, the sensitivity of TC and TG is poor (0.265 and 0.471, respectively) in predicting IS, although their specificity are better.

The relationshiop between ischemic stroke and allele frequencies of ACE I/D, MTHFR C677T, β-Fg-455A/G, β-Fg-148T/C, PAI-14G/5G, and ApoE ε2-4 Genes Table 5 and Fig.2 showed that there are significant differences in genotype frequencies of *ACE* I/D, *β-Fg*-455 A/G and *β-Fg*-148 T/C genes between IS and control group. However, only allele frequencies of *ACE* I/D and *β-Fg*-148 T/C are significantly different between the two groups. In addition, the frequencies of both D allele of *ACE* I/D and C allele of *β-Fg*-148 T/C in IS group are higher than that in control group. However, the frequencies of both I allele of *ACE* I/D and T allele of *β-Fg*-148 T/C in IS group are lower.

Discussion

Stroke is the second leading cause of human death and the leading cause of permanent disability in adults worldwide [36], and IS accounts for 85% of the total number of strokes [37]. Previous studies have reported that IS is closely related to some genes, blood glucose and blood lipid levels [38-41]. However, it can hardly control or treat stroke at the gene level. Therefore, this study would explore the relationship between IS and genetic and laboratory parameters (including FBG, TC, TG and LDL-C).

IS is caused by the occlusion of the main arteries or branches of the brain, which leads to vascular occlusion and deprivation of oxygen and energy, followed by the formation of reactive oxygen species, then the release of glutamate, the accumulation of intracellular calcium, and the induction of inflammatory processes [42]. The results of this study displayed the correlation of IS with *ACE* I/D, *β-Fg*-455 A/G, and *β-Fg*-148 T/C polymorphisms. Frequencies of both D allele of *ACE* I/D and C allele of *β-Fg*-148 T/C in IS group were greater than those in control group, suggesting that the IS is closely related to

both D allele of *ACE* I/D and C allele of *β-Fg*-148 T/C, which is consistent with the studies conducted by Zhao et al. [43] and Wu et al [44].

Our results showed that FBG is one of effective parameter in predicting IS, which is in accordance with the results done by Lin et al [40], suggesting that routine monitoring for FBG could effectively control and prevent the progression of IS. According to a study conducted by Anderson et al [45], hyperglycemia affects mitochondrial function in the ischemic penumbra, which results in cortical acidosis and cell death. It impaired cerebrovascular reactivity in the microvasculature, which may disturb reperfusion after recanalization [46]. Hyperglycemia or diabetes may alter blood-barrier permeability and induce blood-barrier disruption, which may aggravate brain edema formation and lead to hemorrhagic transformation [47]. In addition, the results in this study also showed that hyperlipidemia is a risk factor for IS. Lee et al. pointed out that high triglyceride level is a risk factor for IS compared with normal triglyceride level, and the risk is 1.28-fold higher than that of normal subjects. Lee et al. also noted that people with LDL ≥ 130 mg/dL may increase the risk of IS [41]. Furthermore, Pawelczyk et al. found that there is a significant increasing in soluble P-selectin (sP-selectin) plasma concentration in stroke patients with hyperlipidemia and hyperglycemia compared to normolipidemic and normoglycemic stroke patients [48]. On the one hand, the strongest positive correlation was observed between hyperglycemia and sP-selectin levels, which emphasizes the leading role of hyperglycemia in atherothrombosis progression. On the other hand, hyperlipidemia was associated with an increase of plasma sP-selectin level [48]. The sP-selectin plays a role in stimulating the release of procoagulant microparticles, which induces a procoagulant state [49]

It was reported that the methods to treat IS mainly include initial treatment with intra-arterial thrombolysis, endovascular mechanical thrombectomy, and antiplatelet treatment [50]. However, those treatments could rarely avoid the possibility of recurrence of IS. Therefore, studying the association of stroke-related genes with blood glucose and blood lipids may be one new approach. Our findings showed that LDL and TG are closely related to *ACE* I/D, *β-Fg*-455 A/G, and *PAI*-1 4G/5G, consistenting with the results of Li [51] and Guney et al. [52]. In addition, our results displayed that hyperlipidemia is correlated with D allele of *ACE* I/D gene, which was confirmed by Suzuki et al. and Lee et al., they also demonstrated the positive association of hyperlipidemia with DD genotype and D allele frequency of *ACE* I/D gene [53, 54].

There are certain limitations in the present study. First, the study enrolled hospital-based IS patients rather than patients from a community based general population. Second, the study was a single population study with a limited sample size, so the results still need be confirmed in multicenter, different populations, races, and larger samples. Third, this study did not collect data on other several major risk parameters of IS, so further research will be needed to study other several major risk parameters of IS.

Conclusions

In summary, the present study showed that D allele of *ACE* I/D gene and C allele of *β-Fg*-148 T/C gene are significantly associated with IS, and frequency of D allele of *ACE* I/D is significantly high in individuals

with hyperlipidemia. Further, high levels of GLU, TG, LDL-C are risk factors for IS, and their optimal cut-off points are 5.27mmol/L, 1.32mmol/L, 3.36mmol/L, respectively, which suggest that people with hyperlipidemia or high frequency of D allele of *ACE* I/D at a risk of IS. However, there is no significant difference in the total cholesterol and *MTHFR* C677T, *PAI*-1 4G/5G, and *Apo E* ε2-4 genes between IS and controls. Identifying the relationship among IS, stroke-related genes, blood lipid or blood glucose may be a better understanding of pathophysiology of IS in Chinese han population, which can provide reasonable control rang of laboratory parameters for prevention of IS in patients with diabetes and hyperlipidemia as early as possible.

Abbreviations

IS: ischemic stroke; TC: total cholesterol; LDL: Low-density lipoprotein; FBG: High levels of fasting blood glucose; TG: triglyceride; RFLP: restriction fragments length polymorphism; PCR: allele specific Polymerase chain reaction; sP-selectin: soluble P-selectin

Declarations

Ethics approval and consent to participate

This study was approved by the ethics committee of Shanghai Tongji Hospital and was conducted according to guidelines of the Declaration of Helsinki. All participants provided informed consent. If the subjects were unable to communicate, written consent was obtained from their legal relatives.

Consent for publication

All the authors gave their consent for the publication of this article. The results presented in this paper have not been published previously in whole or part.

Availability of data and material

The datasets analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

We declare no potential conflict of interest.

Funding

This work was supported by the National Natural Science Foundation of China (81974314, 81873975, 81802084, 81902984), the Excellent Academic Leader Training Program of Shanghai Health System (2018BR31), the Medical Guidace Science and Technology Support Project of Shanghai (19411964800),

the Natural Science Foundation of Shanghai (19ZR1448800), the Clinical Research and Cultivation Project of Shanghai Tongji Hospital [ITJ(ZD)1803, ITJ(ZD)1905, ITJ(QN)1905].

Author contributions

WJY and CXC collected data, did the statistical analysis and interpretation, SZJ wrote the manuscript. YYB, WJL, QWQ, SAQ and JP collected data. NPH and LD designed the overall study, revised the manuscript. All authors read and approved the manuscript.

Acknowledgement

Not applicable

References

1. Feigin VL, Lawes CM, Bennett DA, et al. Worldwide stroke incidence and early case fatality reported in 56 population-based studies: a systematic review. Lancet Neurol. 2009; 8(4): 355-69. https://org/10.1016/S1474-4422(09)70025-0.
2. Liu L, Wang D, Wong KL, Wang Stroke and stroke care in China: huge burden, significant workload, and a national priority. Stroke. 2011; 42(12): 3651-4. https://doi.org/10.1161/ STROKEAHA.111.635755.
3. Feigin VL, Roth GA, Naghavi M, et al. Global burden of stroke and risk factors in 188 countries, during 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet Neurol. 2016; 15(9): 913-24. https://org/10.1016/S1474-4422(16)30073-4.
4. Liang C, Ni G, Ma J, et al. Impact of tag single nucleotide polymorphisms (SNPs) in CCL11 gene on risk of subtypes of ischemic stroke in Xinjiang Han populations. Med Sci Monit. 2017; 23: 4291-4298. https://org/10.12659/msm.905942.
5. Bersano A, Ballabio E, Bresolin N, et al. Genetic polymorphisms for the study of multifactorial stroke. Human mutation. 2008; 29(6): 776-95. https://org/10.1002/ humu.20666.
6. Huen K, Yousefi P, Street K, et al. PON1 as a model for integration of genetic, epigenetic, and expression data on candidate susceptibility genes. Environ Epigenet. 2015; 1(1): 1-11. https://org/10.1093/eep/dvv003.
7. Schieffer B, Schieffer E, Hilfiker-Kleiner D, et al. Expression of angiotensin II and interleukin 6 in human coronary atherosclerotic plaques: potential implications for inflammation and plaque instability. Circulation. 2000; 101(12): 1372-8. https:// org/ 10.1161/01.cir.101.12.1372.
8. Zhang Z, Xu G, Liu D, et al. Angiotensin-converting enzyme insertion/deletion polymorphism contributes to ischemic stroke risk: a meta-analysis of 50 case-control studies. PloS One. 2012; 7(10):e46495. https://org/10.1371/journal.pone.0046495.
9. Tuncer N, Tuglular S, Kılıç G, et al. Evaluation of the angiotensin-converting enzyme insertion/deletion polymorphism and the risk of ischaemic stroke. J Cli Neurosci. 2006; 13(2): 224-7.

https://org/10.1016/j.jocn.2005.08.005.

10. Pera J, Slowik A, Dziedzic T, Wloch D, Szczudlik A. ACE I/D polymorphism in different etiologies of ischemic stroke. Acta Neurol Scand. 2006; 114(5):320-2. https://org/ 10.1111/j.1600-0404.2006.00672.x.
11. Phillips MI. Oliveira EM. Brain renin angiotensin in disease. J Mol Med. 2008; 86(6):715-22. https://org/10.1007/s00109-008-0331-5.
12. Chen J, Hao ZY, Chen S, et al. Neuronal over-expression of ACE2 protects brain from ischemia-induced damage. Neuropharmacology. 2014; 79: 550-8. https://org/10.1016/ j.neuropharm.2014.01.004.
13. Bennion DM, Haltigan EA, Irwin AJ, et al. Activation of the neuroprotective angiotensin-converting enzyme 2 in rat ischemic stroke. Hypertension. 2015; 66(1): 141-148. https://org/10.1161/HYPERTENSIONAHA.115.05185.
14. Yang M, Zhao J, Xing L, Shi The association between angiotensin-converting enzyme 2 polymorphisms and essential hypertension risk: A meta-analysis involving 14,122 patients. J Renin Angiotensin Aldosterone Syst. 2015; 16(4): 1240-4. https://doi.org/10.1177/ 1470320314549221.
15. Burrell LM, Harrap SB, Velkoska E, Patel SK. The ACE2 gene: its potential as a functional candidate for cardiovascular disease. Cli Sci(Lond). 2013; 124(2): 65-76. https://org/ 10.1042/CS20120269.
16. Kim SJ, Lee BH, Kim YM, et al. Congenital MTHFR deficiency causing early-onset cerebral stroke in a case homozygous for MTHFR thermolabile variant. Metab Brain Dis. 2013; 28(3): 519-522. https://org/10.1007/s11011-013-9398-y.
17. Liu J, Sun K, Bai Y, et al. Association of three-gene interaction among MTHFR, ALOX5AP and NOTCH3 with thrombotic stroke: a multicenter case–control study. Hum Genet. 2009; 125(5-6): 649-656. https://org/10.1007/s00439-009-0659-0.
18. Arina CA, Amir D, Siregar Y, Sembiring The Role of polymorphism gen methylene tetra hydrofolate reductase (MTHFR) C677T in ischaemic stroke patients with and without hypertension. Open Access Maced Jo Med Sci. 2019; 7(1): 29-32. https://doi.org/10.3889/ oamjms.2019.026.
19. Iacoviello L, Vischetti M, Zito F, et al. Genes encoding fibrinogen and cardiovascular risk. Hypertension. 2001; 38(5):1199-1203. https://org/10.1161/hy1101.099478.
20. Kessler C, Spitzer C, Stauske D, Mende S, Stadlmüller J, Walther R, et al. The apolipoprotein E and β-fibrinogen G/A-455 gene polymorphisms are associated with ischemic stroke involving large-vessel disease. Arterioscle Thromb Vasc Biol. 1997; 17(11):2880-4. https:// org/10.1161/01.atv.17.11.2880.
21. Yuan XD, Wang SJ, Xu YR, et al. Relationship between multi-locus fibrinogen polymorphisms and fibrinogen concentration, molecular reactivity and cerebral infarction. Zhonghua Xue Ye Xue Za Zhi. 2009; 30(9):582-7. (in Chinese)
22. Coen Herak D, Lenicek Krleza J, Radic Antolic M, et al. Association of polymorphisms in coagulation factor genes and enzymes of homocysteine metabolism with arterial ischemic stroke in children. Clin Appl Thromb Hemost. 2017; 23(8):1042-51. https://org/10.1177/ 1076029616672584.

23. Kopyta I, Niemiec P, Balcerzyk A, et al. Fibrinogen alpha and beta gene polymorphisms in pediatric stroke–Case–control and family based study. Eur Jo Paediatr Neurol. 2015; 19(2): 176-80. https://org/10.1016/j.ejpn.2014.11.011.
24. Kumar A, Misra S, Kumar P, Sagar R, Prasad K. Association between beta-fibrinogen C148T gene polymorphism and risk of ischemic stroke in a north Indian population: a case-control study. Pulse (Basel). 2017; 4(4):165-171. https://org/10.1159/000449361.
25. Isordia-Salas I, Santiago-Germán D, Cerda-Mancillas MC, et al. Gene polymorphisms of angiotensin-converting enzyme and angiotensinogen and risk of idiopathic ischemic stroke. Gene 2019; 688: 163-70. https://org/10.1016/j.gene.2018.11.080.
26. Hu X, Zan X, Xie Z, et al. Association between plasminogen activator inhibitor-1 genetic polymorphisms and stroke susceptibility. Mol Neurobiol. 2017; 54(1):328-341. https://org/10.1007/s12035-015-9549-8.
27. Eriksson P, Kallin B, Van't Hooft FM, Båvenholm P, Hamsten A. Allele-specific increase in basal transcription of the plasminogen-activator inhibitor 1 gene is associated with myocardial infarction. Pro Natl Acad Sci USA. 1995; 92(6): 1851-5. https://org/10.1073/ pnas.92.6.1851.
28. Iacoviello L, Burzotta F, Di Castelnuovo A, Zito F, Marchioli R, Donati MB. The 4G/5G polymorphism of PAI-1 promoter gene and the risk of myocardial infarction: a meta-analysis. Thromb Haemost. 1998; 80(6):1029-30.
29. Jood K, Ladenvall P, Tjärnlund-Wolf A, et al. Fibrinolytic gene polymorphism and ischemic stroke. Stroke. 2005; 36(10):2077-81. https://org/10.1161/01.STR.0000183617.54752.69.
30. Wiklund PG, Nilsson L, Ardnor SN, et al. Plasminogen activator inhibitor-1 4G/5G polymorphism and risk of stroke: replicated findings in two nested case–control studies based on independent cohorts. Stroke. 2005; 36(8): 1661-1665. https://org/10.116/ 01.STR.0000174485.10277.24.
31. Mahley RW, Rall SC Apolipoprotein E: far more than a lipid transport protein. Annu Rev Genomics Hum Genet. 2000; 1:507-537. https://doi.org/10.1146/annurev.genom.1.1.507.
32. Schipper Apolipoprotein E: implications for AD neurobiology, epidemiology and risk assessment. Neurobiol Aging. 2011; 32(5): 778-790. https://doi.org/10.1016/ j.neurobiolaging.2009.04.021.
33. Blazejewska-Hyzorek B, Gromadzka G, Skowronska M, Czlonkowska APOE ϵ 2 allele is an independent risk factor for vulnerable carotid plaque in ischemic stroke patients. Neurol Res. 2014; 36(11):950-4. https://doi.org/10.1179/1743132814Y.0000000385.
34. Pezzini A, Grassi M, Del Zotto E, et al. Synergistic effect of apolipoprotein E polymorphisms and cigarette smoking on risk of ischemic stroke in young adults. Stroke. 2004; 35(2): 438-442. https://org/10.1161/ 01.STR.0000112973.00867.98.
35. Sturgeon JD, Folsom AR, Bray MS, Boerwinkle E, Ballantyne Apolipoprotein E genotype and incident ischemic stroke: the aherosclerosis rsk in cmmunities sudy. Stroke. 2005; 36(11):2484-6. https://doi.org/10.1161/01.STR.0000185687.28520.3f.
36. Murray CJ, Lopez AD. Measuring the global burden of disease. N Engl J Med. 2013; 369(5): 448-57. https://org/10.1056/NEJMra1201534.

37. Beal Gender and stroke symptoms: a review of the current literature. J Neurosci Nurs. 2010; 42(2):80-7.
38. Li G, Liu Y, Li X, et al. Association of PAI-1 4G/5G polymorphism with ischemic stroke in Cinese patients with type 2 diabetes mellitus. Genet Test Mol Biomarkers. 2018; 22(9): 554-560. https://org/ 10.1089/gtmb.2018.0130.
39. Zhao LL, Su G, Chen LX, et al. Apolipoprotein E polymorphisms are associated with ischemic stroke susceptibility in a Northwest China Han population. Biosci Rep. 2017; 37(6): 1-8. https://org/10.1042/BSR20171088.
40. Lin CC, Yang CP, Li CI, et al. Visit-to-visit variability of fasting plasma glucose as predictor of ischemic stroke: competing risk analysis in a national cohort of Taiwan diabetes study. BMC Med. 2014; 12:1-12. https://org/10.1186/ s12916-014-0165-7.
41. Lee JS, Chang PY, Zhang Y, et al. Triglyceride and HDL-C dyslipidemia and risks of coronary heart disease and ischemic stroke by glycemic dysregulation status: the strong heart study. Diabetes Care. 2017; 40(4):529-537. https://org/10.2337/dc16-1958.
42. Fluri F, Schuhmann MK, Kleinschnitz Animal models of ischemic stroke and their application in clinical research. Drug Des Devel Ther. 2015; 9:3445- 54. https://doi.org/ 10.2147/DDDT.S56071.
43. Zhao J, Qin X, Li S, Zeng Z. Association between the ACE I/D polymorphism and risk of ischemic stroke: An updated meta-analysis of 47,026 subjects from 105 case-control studies. J Neuro Sci. 2014; 345(1-2):37-47. https://org/10.1016/j.jns.2014.07.023.
44. Wu G, Cai H, Cai H, et al. Effect of the -148C/T, 448G/A, and -854G/A Polymorphisms of the β-Fibrinogen gene on the risk of ischemic stroke in Chinese population. J Stroke Cerebrovasc Dis. 2015; 24(7):1577-90. https://org/10.1016/ j.jstrokecerebrovasdis.2015.03.029.
45. Anderson RE, Tan WK, Martin HS, Meyer FB. Effects of glucose and PaO2 modulation on cortical intracellular acidosis, NADH redox state, and infarction in the ischemic penumbra. Stroke. 1999; 30(1):160-70. https://org/10.1161/01.str.30.1.160.
46. Kawai N, Keep RF, Betz AL. Hyperglycemia and the vascular effects of cerebral ischemia. Stroke. 1997;28(1):149-54. https://org/ 10.1161/01.str.28.1.149.
47. Dietrich WD, Alonso O, Busto Moderate hyperglycemia worsens acute blood-brain barrier injury after forebrain ischemia in rats. Stroke. 1993; 24(1):111-6. https://doi.org/10.1161/ 01.str.24.1.111.
48. Pawelczyk M, Kaczorowska B, Baj The impact of hyperglycemia and hyperlipidemia on plasma P-selectin and platelet markers after ischemic stroke. Arch Med Sci. 2017; 13(5): 1049. https://doi.org/10.5114/aoms.2017.65816.
49. Nomura S, Inami N, Iwasaka T, Liu Platelet activation markers, microparticles and soluble adhesion molecules are elevated in patients with arteriosclerosis obliterans: therapeutic effects by cilostazol and potentiation by dipyridamole. Platelets. 2004; 15(3):167-72. https://doi.org/10.1080/09537100410001682779.
50. Powers WJ, Rabinstein AA, Ackerson T, et al. 2018 guidelines for the early management of patients with acute ischemic stroke: a guideline for healthcare professionals from the American heart

association/american stroke association. Stroke. 2018; 49(3):e46-e110. https://org/10.1161/STR.0000000000000158.

51. Li Plasminogen activator inhibitor-1 4G/5G gene polymorphism and coronary artery disease in the Chinese Han population: a meta-analysis. PLoS One. 2012; 7(4):e33511. https://doi.org/10.1371/journal.pone.0033511.
52. Guney AI, Ergec D, Kirac D, et al. Effects of ACE polymorphisms and other risk factors on the severity of coronary artery disease. Genet Mol Res. 2013; 12(4): 6895-906. https://org/10.4238/2013.December.19.8.
53. Suzuki T, Yokota H, Yamazaki T, Kitamura K, Yamaoki K, Nagai R, et al. Angiotensin converting enzyme polymorphism is associated with severity of coronary heart disease and serum lipids (total cholesterol and triglycerides levels) in Japanese patients. Coron artery Dis. 1996; 7(5): 371-75. https://org/10.1097/00019501-199605000-00007.
54. Lee YJ, Tsai ACE gene insertion/deletion polymorphism associated with 1998 World Health Organization definition of metabolic syndrome in Chinese type 2 diabetic patients. Diabetes Care. 2002; 25(6):1002-8. https://doi.org/10.2337/diacare.25.6.1002.

Tables

Table 1 The association of laboratory parameters with genotype frequencies of ACEI/D, MTHFRC677T, β-Fg-455A/G, β-Fg-148T/C, PAI-14G/5G and ApoE ε2-4 genes

	FBG (mmol/L)		TC (mmol/L)		TG (mmol/L)		LDL (mmol/L)	
	≥7.00	<7.00	≥5.18	<5.18	≥1.70	<1.70	≥3.37	≤3.37
ACE I/D								
II	23(51.1)	142(48.5)	14(32.6)	151(51.2)	21(42.0)	144(50.0)	15(31.3)	150(51.7)
ID	17(37.8)	108(36.8)	20(46.5)	105(35.6)	19(38.0)	106(36.8)	24(50.0)	101(34.8)
DD	5(11.1)	43(14.7)	9(20.9)	39(13.2)	10(20.0)	38(13.2)	9(18.8)	39(13.4)
χ^2/P	0.414/0.813		5.448/0.066		1.964/0.374		6.915/0.032	
MTHFR C677T								
CC	15(33.3)	96(32.8)	12(27.9)	99(33.5)	19(38.0)	92(31.9)	15(31.3)	96(33.1)
TC	25(55.6)	144(49.1)	21(48.8)	148(50.2)	21(42.0)	148(51.4)	23(47.9)	146(50.3)
TT	5(11.1)	53(18.1)	10(23.3)	48(16.3)	10(20.0)	48(16.7)	10(20.8)	48(16.6)
χ^2/P	1.431/0.489		1.445/0.485		1.503/0.472		0.532/0.767	
β-Fg-455A/G								
GG	22(48.9)	187(63.8)	22(51.2)	187(63.4)	30(60.0)	179(62.2)	25(52.1)	184(63.4)
GA	21(46.7)	95(32.4)	21(48.8)	95(32.2)	17(34.0)	99(34.4)	23(47.9)	93(32.1)
AA	2(4.4)	11(3.8)	0(0.0)	13(4.4)	3(6.0)	10(3.5)	0(0.0)	13(4.5)
χ^2/P	3.761/0.152		5.828/0.054		0.741/0.690		6.026/0.049	
β-Fg-148T/C								
CC	26(57.8)	166(56.7)	23(53.5)	169(57.3)	29(58.0)	163(56.6)	26(54.2)	166(57.2)
TC	17(37.8)	106(36.2)	19(44.2)	104(35.3)	18(36.0)	105(36.5)	21(43.8)	102(35.2)
TT	2(4.4)	21(7.2)	1(2.3)	22(7.5)	3(6.0)	20(6.9)	1(2.1)	22(7.6)
χ^2/P	0.461/0.794		2.371/0.306		0.073/0.964		2.734/0.255	
PAI-1 4G/5G								
4G4G	14(31.1)	92(31.4)	14(32.6)	92(31.2)	17(34.0)	89(30.9)	15(31.3)	91(31.4)
4G5G	23(51.1)	144(49.1)	22(51.2)	145(49.2)	17(34.0)	150(52.1)	26(54.2)	141(48.6)
5G5G	8(17.8)	57(19.5)	7(16.2)	58(19.7)	16(32.0)	49(17.0)	7(14.5)	58(20.0)
χ^2/P	0.088/0.957		0.276/0.871		7.925/0.019		0.885/0.642	
ApoE ε2-4								
E2/2	0(0.0)	5(1.7)	1(2.3)	4(1.4)	1(2.0)	4(1.4)	1(2.1)	4(1.4)
E2/3	7(15.6)	44(15.0)	5(11.6)	46(15.6)	10(20.0)	41(14.2)	5(10.4)	46(15.9)
E2/4	0(0.0)	5(1.7)	1(2.3)	4(1.4)	1(2.0)	4(1.4)	1(2.1)	4(1.4)
E3/3	33(73.3)	195(66.6)	29(67.5)	199(67.5)	31(62.0)	197(68.4)	32(66.7)	196(67.6)
E3/4	5(11.1)	43(14.7)	7(16.3)	41(13.9)	6(12.0)	42(14.6)	9(18.7)	39(13.4)
E4/4	0(0.0)	1(0.3)	0(0.0)	1(0.3)	1(2.0)	0(0.0)	0(0.0)	1(0.3)
χ^2/P	1.471/0.965		2.631/0.708		6.470/0.229		3.307/0.622	

Laboratory parameters of this study included FBG, TC, TG and LDL. FBG=Fasting blood glucose; TC=Total cholesterol; TG=Triglyceride; LDL=Low-density lipoprotein

Table 2 The association of laboratory parameters with allele frequencies of ACEI/D, MTHFRC677T, β-Fg-455A/G, β-Fg-148T/C, PAI-14G/5G and ApoE ε2-4 genes

	FBG (mmol/L)		TC (mmol/L)		TG (mmol/L)		LDL (mmol/L)	
	≥7.00	<7.00	≥5.18	<5.18	≥1.70	<1.70	≥3.37	≤3.37
ACE I/D								
I	63(70.0)	392(66.9)	48(55.8)	407(69.0)	61(61.0)	394(68.4)	54(56.3)	401(69.1)
D	27(30.0)	194(33.1)	38(44.2)	183(31.0)	39(39.0)	182(31.6)	42(43.7)	179(30.9)
$\chi 2/P$	0.324/0.559		5.916/0.015		2.122/0.145		6.217/0.013	
MTHFR C677T								
C	55(61.1)	336(57.3)	45(52.3)	346(58.6)	59(59.0)	332(57.6)	53(55.2)	338(58.3)
T	35(38.9)	250(42.7)	41(47.7)	244(41.4)	41(41.0)	244(42.4)	43(44.8)	242(41.70
$\chi 2/P$	0.456/0.500		1.229/0.268		0.065/0.799		0.318/0.573	
β-Fg-455A/G								
G	65(72.2)	469(80.0)	65(75.6)	469(79.5)	77(77.0)	457(79.3)	73(76.0)	461(79.5)
A	25(27.8)	117(20.0)	21(24.4)	121(20.5)	23(23.0)	119(20.7)	23(24.0)	119(20.5)
$\chi 2/P$	2.869/0.090		0.692/0.406		0.281/0.596		0.588/0.443	
β-Fg-148T/C								
C	69(76.7)	438(74.7)	65(75.6)	442(74.9)	76(76.0)	431(74.8)	73(76.0)	434(74.8)
T	21(23.3)	148(25.3)	21(24.4)	148(25.1)	24(24.0)	145(25.2)	23(24.0)	146(25.2)
$\chi 2/P$	0.154/0.695		0.018/0.894		0.063/0.802		0.065/0.799	
PAI-1 4G/5G								
4G	51(56.7)	328(56.0)	50(58.1)	329(55.8)	51(51.0)	328(56.9)	56(58.3)	323(55.7)
5G	39(43.3)	258(44.0)	36(41.9)	261(44.2)	49(49.0)	248(43.1)	40(41.7)	257(44.3)
$\chi 2/P$	0.015/0.902		0.172/0.678		1.222/0.269		0.234/0.629	
ApoE ε2-4								
E2	7(7.8)	59(10.1)	8(9.3)	58(9.8)	13(13.0)	53(9.2)	8(8.3)	58(10.0)
E3	78(86.7)	477(81.4)	70(81.4)	485(82.2)	78(78.0)	477(82.8)	78(81.3)	477(82.2)
E4	5(5.5)	50(8.5)	8(9.3)	47(8.0)	9(9.0)	46(8.0)	10(10.4)	45(7.8)
$\chi 2/P$	1.533/0.465		0.192/0.908		1.607/0.448		0.959/0.619	

Laboratory parameters of this study included FBG, TC, TG and LDL. FBG=Fasting blood glucose; TC=Total cholesterol; TG=Triglyceride; LDL=Low-density lipoprotein

Table 3 The prediction of ischemic stroke by laboratory indexes

	Model 1				Model 2			
	OR	95% CI		*P*	OR	95% CI		*P*
		Lower	Upper			Lower	Upper	
FBG (mmol/L)	6.47	3.95	10.61	<0.001	5.38	3.25	8.91	<0.001
TC (mmol/L)	0.34	0.10	1.13	0.077	0.44	0.15	1.28	0.130
TG (mmol/L)	4.64	2.09	10.32	<0.001	5.41	2.28	12.81	<0.001
LDL (mmol/L)	7.62	1.60	36.40	0.011	6.21	1.59	24.16	0.008

Model 1 unadjusted; model 2 is adjusted for age and gender. FBG=Fasting blood glucose; TC=Total cholesterol; TG=Triglyceride; LDL=Low-density lipoprotein

Table 4 The ROC curve analysis of laboratory indexes for ischemic stroke

	Cut-off point	Specificity	Sensitivity	AUC	95% CI	
					Lower	Upper
FBG (mmol/L)	5.27	0.833	0.694	0.828	0.784	0.867
TC (mmol/L)	5.12	0.994	0.265	0.595	0.541	0.648
TG (mmol/L)	1.32	0.833	0.471	0.702	0.65	0.751
LDL (mmol/L)	3.36	0.999	0.284	0.638	0.584	0.689

ROC=Receiver operating characteristic; AUC= Area under the curve; FBG=Fasting blood glucose; TC=Total cholesterol; TG=Triglyceride; LDL=Low-density lipoprotein.

Table 5 The relationshiop between ischemic stroke and allele frequencies of ACEI/D, MTHFRC677T, β-Fg-455A/G, β-Fg-148T/C, PAI-14G/5G and ApoE ε2-4 genes

	ACE I/D		MTHFR C677T		β-Fg-455A/G	
	I	D	C	T	G	A
IS	216(63.5)	124(36.5)	202(59.4)	138(40.6)	261(76.8)	79(23.2)
Control	239(71.1)	97(28.9)	189(56.3)	147(43.7)	273(79.0)	63(21.0)
χ^2	4.438		0.693		2.049	
P	0.035		0.405		0.152	
	β-Fg-148T/C		PAI-1 4G/5G		ApoE ε2-4		
	C	T	4G	5G	E2	E3	E4
IS	268(78.8)	72(21.2)	194(57.1)	146(42.9)	34(10.0)	280(82.4)	26(7.6)
Control	239(71.1)	97(28.9)	185(55.1)	151(44.9)	32(9.5)	275(81.9)	29(8.6)
χ^2	5.334		0.274		0.246		
P	0.021		0.601		0.884		

Ischemic stroke: IS

Figures

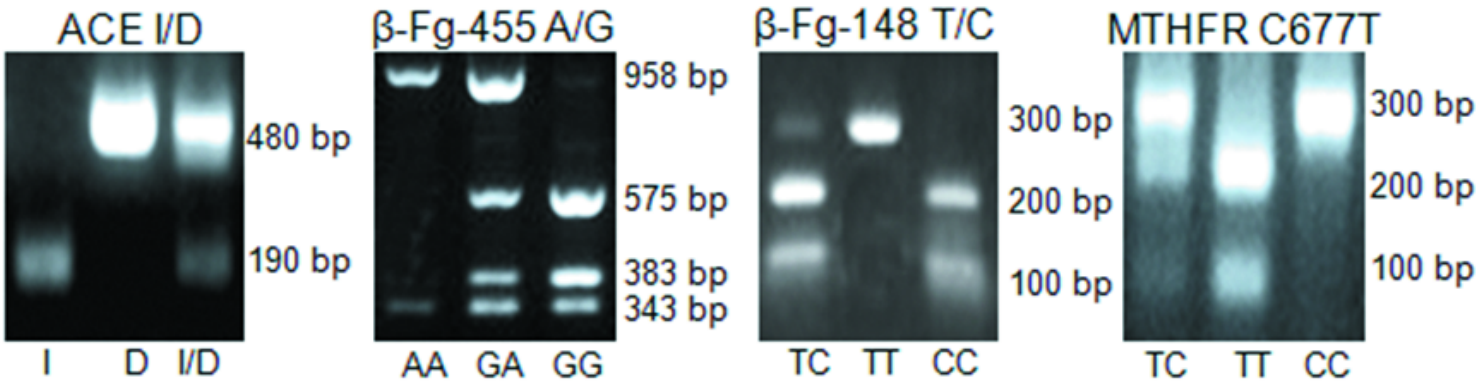

Figure 1

Genotypic determination of the polymorphism of ACE I/D, β-Fg-455 A/G, β-Fg-148 T/C, and MTHFR C677T. Agarose gel electrophoresis of PCR products.

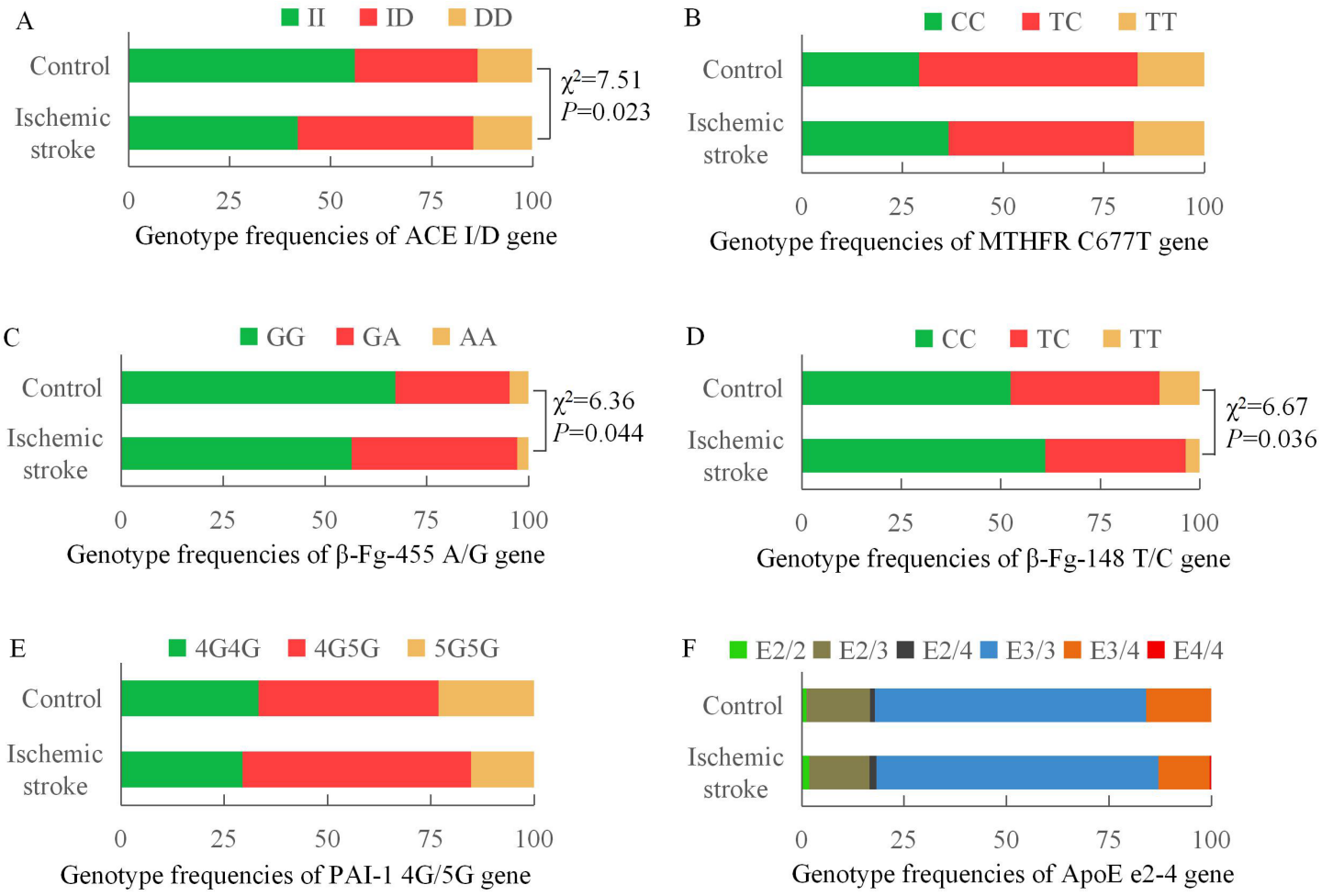

Figure 2

The association of ischemic stroke with genotype frequencies of ACE I/D (A), MTHFR C677T (B), β-Fg-455 A/G (C), β-Fg-148 T/C (D), PAI-14 G/5G (E) and ApoE ε2-4 (F) genes.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementalTables.doc