# Study on the association of platelet membrane glycoprotein IaC807T gene polymorphism with the susceptibitity to ischemic cerebrovascular disease in Han population of Henan province.

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**Absract**

**Objective**：To investigate the association of the platelet membrane glycoptein IaC807T gene polymorphism and the genetic susceptibility to ischemic cerebrovascular disease and its mechanism in Han poplulation of Henan province. **Methods**: Platelet membrane glycoprotein Ia gene C807T polymorphism in 317 samples of cerebral infarction and 311 samples of healthy control from Henan Han population was detected using PCR-restriction fragment length polymorphism (RFLP) technique. **Results**: There were two alleles (C and T)and 3 genotypes (C/C C/T T/T). The frequencies of GpIa T allele were significantly higher in cerebral infarction group among individuals yonger than the mean age of 55 years ($x^2$= 10.01 $p<0.05$) and individuals with high-risk factors ($x^2$=4.183 $p<0.05$) than those in the control group. **Conclusion**: The platelet collagen receptor GpIa-IIaT807 allele might be an independent risk factor for the development of cerebral infarction in younger patients and high-risk patients of Henan province. Life Science Journal. 2009; 6(4): 69 – 73] (ISSN: 1097 – 8135)

**Key words**：Platelet membrane glycoprotein Ia; genetic polymorphsim; ischemic cerebrovascular disease; Henan province ; Han population.

According to the medicine research in the latest years, the dysfunction of platelet aggregation and adhesion plays an important role in the process of occurrence and development in the ischemic cerebrovascular disease. platelet membrane glycoprotein Ia-IIa is an important collagen receptor of platelet , which belongs to integrin -family. it is made up of α and β subunit , also called α2β1 integrin, GpIa-IIa plays a critical role in the process of platelet activation and thrombosis through the GpIa-IIa platelet adheres to the exposed endothelial collagen [1,2], then was activated. In recent years,two types of polymorphism had been found in the GpIa gene transcription district 807 (C or T) and 873 (G or A).

Both 807T and 873A were completely chained [3]. It was reported that the polymorphism was significantly related to the level of GpIa-IIa on the surface of platelet [4] .So GpIa gene polymorphism may be a genetic risk factor of thrombotic disease.

In this study, the GpIaC807T genotypes of cerebral infarction and the normal controls from Han population in Henan province were detected and analysed the allele frquency in order to explore the relationship and mechanism between the gene polymorphism and ischemic cerebrovascular disease.

## 1. Materials and Methods

**1.1 Clinical materials** According to the diagnosis criteria of cerebrovascular disease made on the Fourth National Symposium on cerebrovascular disease, a total of 317 patients with IVCD from unrelated kindred of Chinese Han ethnicity in Henan area were inpatients or outpatients admitted to the top two hospitals of Henan

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province from March 2008 to February 2009.In the IVCD group, there were 171 males and 146 females aging from 38 to 75 years old, with an average age of 58.3±17.6 years old.They were diagnosed as IVCD by clinical symptoms and CT or MRI scan. 311 healthy outpatients admitted to the top two hospitals in Henan province as controls, including 311 healthy individuals (165 males and 146 femals) aging from 39 to 74 years old, with an average age of 58.2±16.8 years old. All subjects in the research were of the Chinese Han ethnicity, severe systemic diseases such as cerebral infarction(CI) 、myocardial Infarction (MI) 、liver or renal diseases were excluded . There was no significant difference($p>0.05$) in the composition of gende、age、lipid level、blood glucoses and smoking history between the two groups ,so they can be comparable .

### 1.2 Methods

#### 1.2.1 Samples collection and gene group DNA abstraction

Venous blood samples were drawn from the patients and controls in the fasting states in the morning . 5ml and 3ml blood were respectively collected with common biochemistry tube and EDTA-2Na anticoagulation tube. Biochemical indictator such as TG、BS were tested in the biochemical specimen. Gene group DNA was extracted with phenol/chloroform extraction proledure from peripheral blood leckocytes.

#### 1.2.2 Measurement of GPIaC807T gene polymorphism

PCR-RFLP is used ,the amplification of aim fragment refers to a pair of primer designed by Reiner, method: the forward primer 5'-GTGTTTAACTTGAACACATAT-3',reverse primer 5'-ACCTTGCATATTGAATTGCTT-3', aim fragment is about 184bp. PCR reaction volume 25ul: 2xTaq Master Mix( contained 0.1U Taq DNA polymerase, 20mM Tris-HCl,100mM KCl,3mM MgCl2, 500uM each dNTP )8ul、upstream primer and downstream primer were respectively 2ul (10mmol / L) 、DNA template 2ul、ddH2O added to 25ul. PCR amplification condition: PCR was carried out with 35 cycles at 95℃for 5 min, 94℃ for 30 s, 55℃for 30S, 72℃ for 1 min and finally 72℃ for 5min. PCR product 10ul was detected in 20g/L agarose gel electrophoresis under ultraviolet rays lamp with reference of Marker I. the digestion reaction system 20ul, including PCR product 10ul, TaqI restriction enzyme 5$^{u}$, ddH2O 8ul was immersed in 65℃ water for 90 min, the digestion was detected in 20g/L agarose gel electrophoresis under ultraviolet rays gel imaging system with reference of Marker I took photograph, judging the length of PCR digestion by PCR brand.

**1.3** Statistical analysis was performed by the spss 11.0 for windows Stastical package. Genotype frequencies were compared between the cases and conrtols by using chi-square test .The relative risk of genotypes and alleles was described by odds rations (OR) and 95%confidence intervals(95% CI). Logistic regression was used to analyse independent risk factors. Hardy-weinbery equilibrium was conformed with chi-square test. Pvalues were two-tailed and statistical significance was accepted as $p<0.05$.

## 2 Results

**2.1** PCR amplification fragment is 184bp.According to the digestion results , there were 3 types of genotypes, The product 115bp in length was genotype TT, and 92bp in length was genotype CC, if there were two bands in the length of 115bp and 92bp,known as CT type.

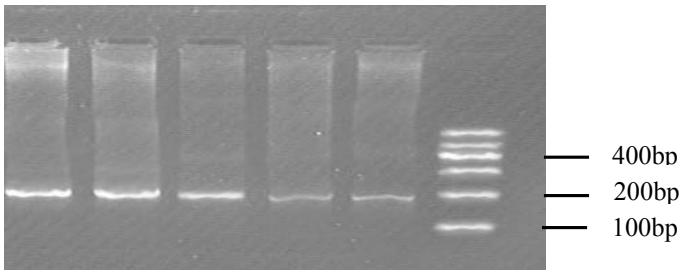


Figure 1. PCR amplification result

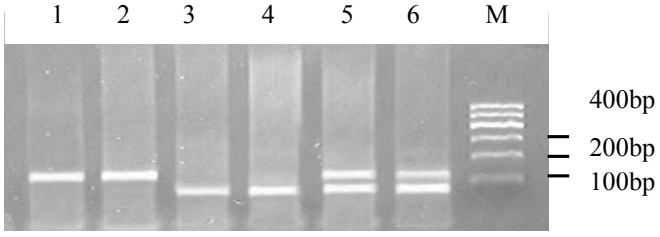


Figure 2. Result of PCR-RFLP

( 1,2 were genotype TT; 3,4 were genotype CC; 5,6 were genotype CT;M was 100bp DNA ladder)

**2.2 Hardy-weinberg test of gene GPIaC807T**

Patients and controls' genotype distribution ratio was in accordance with Hardy-Weinberg equilibrium (P>0.05), With the representation of population. Two group genotypes and frequencies of alleles（Table 1）.

**2.3** Distribution of GpIa genotype and ratio of allomorph of patients and controls. (See table 2),The difference of gene GpIa allele T ratio between patients and controls is not significant （$\chi 2=2.615$, $p>0.05$）

**2.4** The distribution of GpIa genotype and ratio of allele of stroke patients aged below 55 and controls ,the difference gene GpIa allele T ratio between stroke patients aged below 55 and controls was significant（$\chi 2=10.01$, $p<0.05$）.(Table 3) allele T was significantly correlated with stroke patient aged below 55 [OR= 1.611, 95% CI is（1.198-2.167）].

**2.5** The stroke patients were divided into high risk group and low risk group by whether with hypertension、diabetes mellitus、BMI≥26kg/㎡、hyperlipidemia .The difference ratio of gene GpIa allomorph T between high risk stroke patients and controls was significant（$\chi 2=4.183$，$p<0.05$）.(see table 4), allomorph T was significant correlation to the high risk stroke patients [OR=1.271,95% CI was （1.010-1.600）].

## 3 Discussion

Now, the effect of GpIa-IIa compound on platelet membrane is being payed more and more attention .GpIa-IIa, as the important collagen receptor on platelet membrane, belongs to a member of terminal antigen subfamily of the cell adhesion molecules integrin, it can mediate platelet to adhere to Ⅰ-Ⅷ type collagen. Kunicki [5, 6] ,et found the density of GpIa-IIa on platelet membrane differs among healthy persons about 4 times. Also some research found that gene polymorphism of GpIa is related to the expression rate of GpIa-IIa [7], so the variation of density of GPIa on platelet maybe the risk factor of thrombus disease. persons with T807 allelomorph express high level of GpIa-IIa, otherwise , persons with C807 allelomorph express low level of GpIa-IIa. high level expression of GpIa-IIa only relies on the existence of T807 allelomorph. Comparing to homozygote TT, heterozygote CT expresses the similar amount of GpIa-IIa. Therefore, the clinical meaning of gene polymorphism of GpIa is being payed more and more attention.

We analyzed the polymorphism of platelet gene GpIa C807T in ischemic stroke patients in han population of henan province with the method of PCR-RFLP, research results were as follows:(1)the difference of ratio of allele T807 between cerebral infarction patient group and the control is not significant （P>0.05）.(2) T807 allele in cerebral infarction patients beneath 55 years old showed a significant higher ratio than the control, stroke risk adds 1.6 times.(3) high risk cerebral infarction patient have significant higher ratio of GpIa gene T807 allele than the control (P<0.05), stroke risk adds 1.27 times, results shows: in cerebral infarction patients aged beneath 55 and high risk stroke patients, allele T807 maybe the independent risk factor of cerebral infarction.

Reiner's[8] research found that, female stroke patients beneath 45 years are significant related to GpIa gene allele T807.In one case-control study, Carlsson[9, 10], et analyzed alleles T807 of 227 stroke patients confirmed by CT or MRI and the control group (patients with cerebra vascular disease history、acute myocardial infarction and deep vein thrombus are excluded). After matching other risk factors, we found that in the population beneath 50 years, allele T807 is the independent heredity risk factor. Allele T carriers likely to have stroke 3 times more than the allele C carriers. Our result is the same as the statement above-mentioned, which showed GpIa gene allele T807 might be the independent risk factor of cerebral infarction of han population in henan province,and palyed an important part in the arterial thrombus disease.

Research indicates that allele T807 may be the independent risk factor in young ischemic infarction patients[11]. Also because ischemic stroke is a kind of muti-gene disease influenced by heredity、environment and so on ,the effect of allomorph T807 and other risk factors may accumulate. We can presume that the conclusion that allele T807 is correlated with high risk ischemic stroke patients is reasonable.

Our research found that young ischemic stroke group and high risk ischemic stroke group carried more TT and CT genotypes and T allomorph than the control, indicating allomorph GPIa807T carrier of han population in henan province maybe a heredity risk factor of ischemic stroke. The concrete mechanism needs further

investigation, the probable mechanism is that GPIa in plasma affects the formation of arteriosclerotic thrombus[12],then results in ischemic cerebrovascular disease.our research provided the relationship between allele GpIaC807T of han population in henan province and ischemic stroke. Indicated that allele T807 may be the heredity risk factor in young ischemic infarction patients and high risk ischemic infarction. But among the whole population the frequency of T allele was not significant correlation（P>0.05）.This result cound be explained in this way:GPIa807T may be the genetic susceptibility marker, however, with the age growing, many environment risk factors gradually become prominent[13], which may hide the potential risk of GPIa807T. So the carrier of GPIa807T in the young people should be discovered as early as possible. Because the number of cases is still relatively small,conclusion still needs confirmation by large-sample prospective research. Our research is valuable in the early recognition and intervention of the people who have the heredity risk factor of ischemic stroke,Also it provided theoretical basis for whether patients should receive anti-platelet treatment.

**Table 1** Heredity equilibrium test of distribution of patients and controls GPIaC807T genotype

| genotypes | controls | | Patients | |
|---|---|---|---|---|
| | Real subjects(%) | Expected subject* | Real subjects (%) | Expected subject * |
| T/T | 79 (25.40) | 74.29 | 83（26.18） | 74.33 |
| C/T | 146（46.95） | 155.42 | 141（44.48） | 158.34 |
| C/C | 86（27.65） | 81.29 | 93（29.34） | 84.33 |
| | $\chi2$＝1.143，P＞0.05 | | $\chi2$＝3.802，P＞0.05 | |

Notice: * was figured by each allele frequency according to Hardy-weinberg equilibrium law

**Table2** Distribution of GpIa genotype and ratio of allele of patients and controls

| groups | cases | frequencies of genotypes（n,%） | | | frequencies of alleles（n,%） | |
|---|---|---|---|---|---|---|
| | | CC | CT | TT | C807 | T807 |
| stroke | 317 | 93（29.34） | 141（44.48） | 83（26.18） | 327（51.58） | 307（48.42） |
| control | 311 | 86(27.65) | 146(46.95) | 79(25.40) | 318(51.13) | 304（48.87） |
| | | | | | $\chi2$＝2.615 P＞0.05 | |

**Table 3** GpIa genotype ratio of stroke patients aged below 55 and controls

| Groups | cases | frequencies of alleles（n,%） | |
|---|---|---|---|
| | | T807 | C807 |
| Stroke | 131 | 166 (63.36) | 96(36.64) |
| Control | 311 | 304 (48.87) | 318(51.13) |
| | | $\chi2$＝3.859 P<0.05 OR=1.337 | 95%CI（1.001-1.787） |

**Table 4** Ratio of gene GpIa allomorph T of high risk stroke patients and controls

| Groups | cases | frequencies of alleles（n,%） | |
|---|---|---|---|
| | | T807 | C807 |
| Stroke | 279 | 322 (57.71) | 236(42.29) |
| Control | 311 | 304 (48.87) | 318(51.13) |
| | | $\chi2$＝3.859 P<0.05 OR=1.298 | 95%CI（1.0321.633） |

**References**

[1].Jiangyan, platelet membrane glycoprotein and its research of polymorphism [J]. China Diagnostic Test ,2003,7:281-283.

[2].Yeh PS,Lin HJ,Li YH,et al.Prognosis of young ischemic stroke in Taiwan: impact of prothrombotic gen etic polymorphisms[J]. Thromb Haemost, 2004; 92(3):583~589.

[3].Kunicki TJ,Kritzik M,Annis DS,et al.Hereditary variation in platelet integrin alph 2 beta 1 density is associated with two silent poly-morphisms in the alpha 2 gene coding sequence[J]. Blood, 1997; 89: 1939-1943.

[4].Kritzik BM, SavageB,Nugent DJ, etal. Nucleotide polymorphism in the a2 gene define multiple alleles that are associated with differences in platelet a2b1 density[J].Blood, 1998,92(7):2382-2388.

[5].Kunicki TJ,Orckekowski R,Annis D,et al.Viariability of integrin alpha2 beta 1a activity on human platelets[J].Blood, 1993,82(9):2693-2703.

[6].ikolopoulos GK,Tsantes AE,Bagos PG et al.Integrin,alpha 2 geneC807Tpolymorphism and risk of ischemic stroke:a meta-analysis[J].Thromb Res.2007 ; 119:501-510.

[7].Hsieh K,Funk M ,Schillinger M,et al.Vienna StrokeRegisty. Impact of the platelet glycoprotein Ib alpha Kozak，polymorphism on therisk of ischemic cerebrovascular events :a case-controlstudy[J]. Blood Coagul Fibrinolysis,2004;15(6)：469~473.

[8].Reiner AP,Kumar PN,Schwartz SM,et al.Genetic variants of platelet glycoprotein receptors and risk of srotke in young women. Stroke[J] .Blood, 2000, 31: 1628-1633.

[9].Carlsson LE,Santoso S,Spitzer C,et al.The α2 gene coding sequence T807/A873 of the platelet collagen receptor integrin α2β1 might be a genetic risk factor for the decelopment of stroke in younger patients[J]. Blood,1999,93:3583-3586.

[10].Gao XC,Huo Y,Liu XZ,et al.Gene Polymorphism of Platelet Glycoprotein I balpha in Chinese Patients with Large-and Small Artery Subtypes of Ischemic Sroke[J].Eur Neurol, 2005;54(2):73-77.

[11].Loncar R,Stold V ,Thomas V ,et al:The 807 C/T polymorphism in the a-subunit of integrin a2ß1 modulates platelet adhesion onto immobilized collagen under arteral flow conditions[J]. Transfus Med –Hemother, 2004 ;31(suppl 3):12

[12].Ringleb PA. Thrombolytics, anticoagulants, and antiplatelet agents.Stroke,2007;38:1113-1114.

[13].Luzak B, Golanski J,Rozalski M,et al.Effect of the 807 c/t polymorphism in glycoprotein is on blood Platelet reactivity .J Biomed Sci.2003,10(6 Pt 2):731-737.