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Research Article
Association of Fibrinogen Receptor (Integrin αIIbβ3) Polymorphism in Sudanese Ischemic Stroke Patients

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Abstract

Background and Objective: The fibrinogen receptor the human platelet antigen (HPA1 and HPA3) have an essential role in Atherothrombosis. This study aimed to detect the association of αIIbβ3 polymorphism with ischemic stroke in Sudanese patients and its association with the common risk factors. **Methodology:** This is a case-control study. Fifty atherosclerotic with ischemic stroke Sudanese patients were included in present study and were compared to apparently 50 healthy Sudanese subjects at the same ages. The ages of both groups were ≥18 years. About 5 mL of venous blood sample was taken from each patient and control. The laboratory analyses were done for HbA1c, lipid profile and DNA genotyping by polymerase chain reaction (PCR) followed by *FokI* and *ScrFI* digestion. **Results:** The result showed that, the risk factors (TRI.G, HDL, HbA1C, and body mass index were associated with the increased risk of ischemic stroke). None of the cholesterol levels and LDL increased the risk of stroke. The risk of ischemic stroke was higher with B/B genotype in HPA3 (p-value 0.009) and A/B genotype in HPA1 (p-value 0.041) and HPA1 (p-value 0.041). **Conclusion:** The αIIbβ3 polymorphism were with ischemic stroke in Sudanese patients.

Key words: Platelet, fibrinogen receptor, atherothrombosis, integrin, αIIbβ3 polymorphism, ischemic stroke, Sudan

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Stroke is the second most typical explanation for death[1] and the disability-adjusted life-years worldwide[2]. Most strokes are ischemic in origin and up to 67.3-80.5% of which are due to ischemic stroke (IS) in developed countries[3]. Such events are induced by a blood clot that blocks a vessel supplying blood to the brain. Nevertheless, the etiology and pathogenesis of ischemic stroke are not totally understood. Genetics could play a crucial role in these events[4]. Risk factors included hypertension "smoking" diabetes" lack of exercise, obesity "high blood cholesterol" poor diet, depression and excessive alcohol[5,6]. Platelets have an important role in thrombosis and homeostasis[7]. The primary platelet receptor's glycoprotein (GP)VI, that binds collagen and GPIba, the major ligand-binding sub-unit of the GPIb-IX-V complex, that binds Von Willebrand factor (VWF), initiates platelet aggregation (thrombus formation) by recognition of exposed VWF/collagen in the damaged blood vessel wall[8]. Downstream signals lead to platelet activation, secretion of ADP and other agonists, spreading and platelet aggregation mediated by the platelet integrin αIIbβ3[9]. Platelets have a crucial role in the pathogenesis of thromboembolic diseases[10]. Therefore, the realization of appointed disease-associated genetic agents might widen recognition of disease pathogenesis and might also be helpful for distinguish subjects at high risk of developing it[11]. Platelet thrombosis is going between various platelet receptor complexes, involving glycoprotein, IIb/IIIa (integrin $\alpha_{IIb}\beta3$)[12], which arrange important reactions in acute and chronic processes of atherogenesis[13]. This glycoprotein constitutes the fibrinogen receptor, whose engagement represents the final common pathway for platelet aggregation[14]. It considers the most abundant platelet-specific glycoprotein[15]. This integrin is one of the adhesion molecules that caused aggregation of platelet and providing the development of blood clots. This role labels it interesting candidates in the pathological process of ischemic stroke[16]. Clinical studies done within the last 10 years suggest the possibility of genetically association with hyperaggregability which may be induced by polymorphic receptors takes into consideration integrin αIIbβ3 that engaged in platelet adhesion and aggregation. The human platelet antigen HPA-1 and HPA-3 are the most widely thoughtful polymorphisms of GPIIIa and GPIIb, respectively[17]. The genes encryption the platelet IIb and IIIa glycoprotein are found on chromo-some[17]. There are many point mutations in these genes which cause disorders of platelet binding[18]. A transversion or exchange from thymine (T) to cytosine (C) was found at codon 33 of the GpIIIa, resulting in a Leu33 (PlA1) to Pro (PlA2) change[19]. Human platelet antigen-3 (HPA-3) (Baka/Bakb) is a wide-spread polymorphism of platelet GpIIb, resulting from an isoleucine-to-serine substitution (thymine (T) to guanine (G) base change) at position 843 of the GpIIb heavy chain[18]. The precise contribution to atherothrombotic process, including stroke, provide pre-dominantly conflicting results. the Association of integrin αIIbβ3 polymorphisms with atherosclerotic ischemic stroke in Sudanese patients were not done before, however, this polymorphism has been involved in the pathological process of ischemic stroke, in many studies done outside Sudan[20,21], so the extensively study of this polymorphism may help to control the fatal attack of Ischemic stroke and may introduce GpIIb/IIIa antagonist basically in the treatment. This study aimed to determine the association of fibrinogen receptor (Integrin αIIbβ3) Polymorphism with ischemic stroke which might upgrade clinical outcome and reduce the risk of ischemic stroke in Sudanese patients by early identifying subjects at increased risk.

MATERIALS AND METHODS

This is a case-control hospital-based study that was carried out during the period between February, 2016 to June, 2018 in Khartoum state. Fifty Sudanese patients with acute ischemic stroke who were admitted to Alshaab Hospital at Khartoum state were included in this study and were compared to apparently 50 healthy Sudanese subjects at the same ages. All control subjects with hypertension, diabetes mellitus, dyslipidaemia or other recognized risk factors for ischemic stroke were eliminated from the study. The age of study population $\geq$18 years. Questionnaires were filled from them that included (age, sex, weight, height, smoking and blood pressure). The study protocol was reviewed and approved by the Deanship of Scientific Research Ethical Committee of the University of Science and Technology. An informed oral consent was obtained from all participants after full explanation of the purpose of the study. About 5 mL of Venous blood was collected from each patient and control into two containers (2.5 mL) in EDTA to assess (HbA1c, DNA genotyping) and 2.5 mL in lithium heparin for lipid profile. The laboratory analysis was done for HbA1c used Clover A1c™ self-analyzer, for lipid profile used Vitros 250 machine and for DNA genotyping used PCR and RFLP. Genomic DNA was isolated from EDTA anti-coagulated blood by using Qiagen DNA extraction protocol. Extracted DNA was kept below -20°C for further analysis.

Polymerase chain reaction (PCR): Oligonucleotide primers selected for the polymerase chain reaction (PCR) were used to

amplify those parts of the genomic DNA that contain the polymorphic sequences corresponding to the HPA-1 and HPA-3 alleles. The HPA-3 polymorphism was identified by PCR amplification of a 253-bp fragment with the use of the forward primer ('5 -CTC AAG GTA AGA GCT GGG TGG AAG AAA GAC-3') and the reverse primer ('5 -CTC ACT ACG AGA ACG GGA TCC TGA AGC CTC-3'). The HPA-1 polymorphism was discovered by PCR amplification of a 338 bp fragment with the use of the forward primer ('5 -CTG CAG GAG GTA GAG AGT CGC CAT AG-3') and the reverse primer ('5 -CTC CTC AGA CCT CCA CCT TGT GCT CT-3')[22]. PCR reaction mixture 20 µL was prepared by adding 5 µL of DNA template, 1 µL from each forward and reverse primer and 13 µL of D.W with master mix (Maxime PCR Premix kit (i-*Taq*). For *GpIIb*, 28 cycles of PCR were run at 94°C for 5 min, 94°C for 45 sec, 63°C for 45 sec, 72°C for 1 min and 72°C for 7 min, for *GpIIIa*, 30 cycles of PCR were run at 95°C for 5 min, 95°C for 45 sec, 62°C for 45 sec, 72°C, for 1 min and 72°C for 5 min[22]. The PCR products for both genes were hold at 4°C. The PCR products were analyzed by used 1.5 Agarose gel with 10 µL of ethidium bromide. About 10 µL of PCR products and 5 µL DNA ladder size marker 100 pb (Intron-Korea) were transferred to agarose gel and after 45 min for electrophoresis, the DNA bands 253 pb for *GpIIb* and 338 pb for GpIIIa were visualized and detected by using high performance ultra violet trans-illuminator documentation system.

Restriction-enzyme digestion: The PCR products were digested by using restriction enzyme Fok I (Cut Smart-NEB) for determination of *HPA3* and ScrFI (cut smart NEB) for determination of HPA1. The total of 20 µL of enzymes mixture prepared by adding 0.4 µL Of FoK1, 2 µL buffer, 10 µL PCR product, 7.6 µL H_2O and 1 µL of ScrF1, 2 µL Buffer, 10 µL PCR product, 7 µL H_2O). These mixtures were incubated in 37°C for 16 h over night and inactivated of enzyme reaction by 65°C for 20 min, then 4°C holding temperature. About 10 µL of the digested DNA fragments with 5 µL of DNA ladder were run out in to 2% agarose gel containing ethidium bromide and the results were read against DNA ladder 100 pb and identified under high the high-performance Ultra-violet gel documentation system. For HPA-3 HPA-3, presence of Ile at position 843 caused a cleavage of the 253 bp fragment into a 126 and 127 bp fragment, while the presence of Ser was identified by the uncleaved 253-bp fragment. Genotypes were classified as AA (Ile, Ile), AB (Ile, Ser) and BB (Ser, Ser). For HPA-1, the presence of Leu at position 33 caused the cleavage of the 338 bp fragment into a 214, 46 and 78 bp fragment, respectively, whereas the presence of Pro resulted in the cleavage of the 338 bp fragment into a 77, 137, 46 and 78 bp fragment, respectively. Genotypes were classified as AA (Leu, Leu), AB (Leu, Pro) and BB (Pro, Pro)[10,22].

Data analysis: Statistical analyses were conducted using SPSS (version 20, SPSS Inc., Chicago, IL) software. Data were expressed as percentage. Descriptive analyses of percentages of categorical variables were reported using chi-square x2. Comparisons of continuous variables made using the Student's t-test for parametric data. It constructed a logistic regression model to estimate odds ratios and their 95% confidence intervals for the association between genotypes and risk of ischemic stroke. The Hardy-Weinberg equilibrium was tested to compare genotypes and allele's frequencies among patients and controls. An α value of $p<0.05$ denoted a statistically significant difference in all statistical comprises.

RESULTS

The study included 50 patients met the eligibility criteria and 50 control subjects. Compared to controls group the association between TRI. The G and HDL with Ischemic stroke patients was highly statistically significant ($p<0.001$). The magnitude of body mass index in relation to stroke patients was changed compared to the controls group ($p<0.001$). Total cholesterol levels and LDL were not significantly increased the risk of stroke ($p\geq0.05$), while HbA1c level had a role in increasing of stroke ($p<0.05$) as shown in Table 1. Patients in the control group consisted of 76 males and 24% females as shown in Table 2, but this was not significantly different. Compared to the control group, more patients had a smoking history of 54.0% and patients had highly been statistically significant for hypertension (90.0%) ($p<0.001$). This was associated significantly ($p<0.001$). In addition, three main HPA3 genotypes in patients and controls have been identified as shown in Table 3. The genotype A/A was the most common genotype in patients with stroke (64.0%) and controls (90.0%). A/B genotype was higher in stroke patients than in controls (8.0%) but this was not statistically significant. The B/B genotype frequency in patients with stroke was higher than in controls (28.0 and 8.0%, respectively) and all these differences were statistically significant ($p<0.05$). Furthermore, the A allele frequency was lower in stroke patients (68.0%) than in controls. About 32.0% of patients with stroke had B allele and this difference was very statistically significant ($p<0.001$). In patients and controls, three main HPA1 genotypes have been identified as shown in Table 4. The genotype A/A was the most common genotype in patients with stroke (92.0%) and controls

Table 1: Distribution of lipid profile and HbA1c among Ischemic stroke patients and control

	Mean±SD		
Characteristics	Stroke patients (n = 50)	Control (n = 50)	p-value
TRI.G (mg dL^{-1})	114.48±61.94	81.32±18.39	0.001**
CHOL (mg dL^{-1})	132.08±35.23	141.68±48.01	0.257
LDL (mg dL^{-1})	86.78±38.43	74.46±29.02	0.074
HDL (mg dL^{-1})	39.44±13.53	55.0±11.250	0.000**
HbA1c (%)	5.44±2.590	4.66±0.830	0.047
BMI	29.55±6.760	22.94±4.760	0.000**

SD: Standard deviation, *p<0.05 statistical significant, **p<0.001 highly statistical significant

Table 2: Risk factors associated with ischemic stroke among patients and control

	N (%)		
Risk factors	Stroke patient	Control	p-value
Gender			
Male	38 (76.0)	38 (76.0)	1.000
Female	12 (24.0)	12 (24.0)	
Smoking status			
Yes	27 (54.0)	0	0.000*
No	23 (46.0)	50 (100.0)	
Hypertension			
Yes	45 (90.0)	0	0.000*
No	5 (10.0)	50 (100.0)	

N: Number of study subject, *p<0.001 highly statistical significant

Table 3: HPA3 genotype and allele distributions between ischemic stroke patients and control

	N (%)			
HPA3	Stroke patient	Control	p-value	OR (95% CI)
Normal homozygous A/A	32 (64.0)	45 (90.0)	-	1
Heterozygous A/B	4 (8.0)	1 (2.0)	0.130	5.63 (0.60-52.72)
Mutant homozygous B/B	14 (28.0)	4 (8.0)	0.009*	4.92 (1.48-16.34)
Allele A	68 (68.0)	91 (91.0)	0.000**	4.76 (2.13-10.63)
Allele B	32 (32.0)	9 (9.0)		

*p<0.05 statistical significant **p<0.001 highly statistical significant

Table 4: HPA1 genotype and allele distributions between ischemic stroke patients and control

	N (%)			
HPA1	Stroke patient	Control	p-value	OR (95% CI)
Normal homozygous A/A	46 (92.0)	50 (100)	0.041*	-
Heterozygous A/B	4 (8.0)	0		
Mutant homozygous B/B	-	-		
Alleles A	96 (96.0)	100 (100)	0.043*	-
Alleles B	4 (4.0)	0		

*p<0.05 statistical significant

(100%). The risk of stroke was higher in A/B genotype individuals (8.0%) compared to controls and all these differences were statistically significant (p<0.05). In addition, the A allele frequency was slightly lower in stroke patients (96.0%) than in controls. About 4.0% of stroke patients had B allele, a statistically significant difference (p<0.05). The data in Fig. 1 showed HPA1 338 pb the human platelet A1 PCR product detected by PCR amplification of a 338-bp fragment using the forward and reverse primers as illustrated. Results of Fig. 2 showed the digestion of the HPA1 PCR product using ScrF1 enzyme, indicated by the 214 pb (leu/leu) genotype of AA. Digestion of the HPA1 PCR product using ScrF1 enzyme, leading to heterozygotes A/B genotype ((214 pb, 137 pb) (leu, lue) as shown in Fig. 3, respectively. The Fig. 4 showed the digestion of HPA3 PCR product by using of Fok1 enzyme, that resulted in the cleavage of the 253 pb fragments into 126 and 127 pb, which were appeared as one lane that shown above in Fig. 4, whereas the presence of serine resulted in un-cleavage of 253 pb fragment.

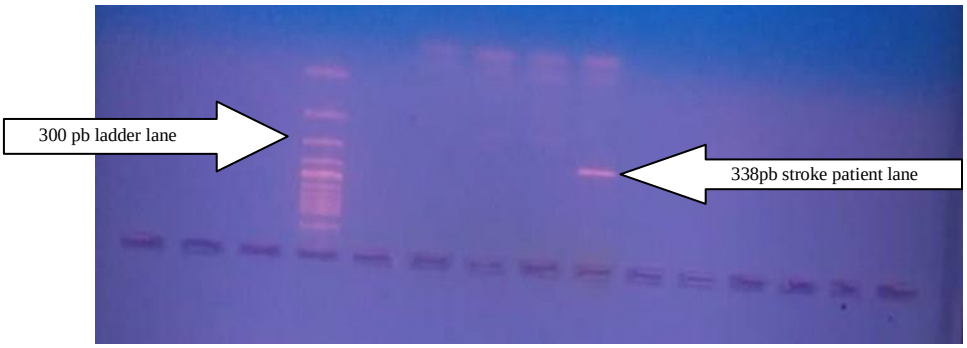

Fig. 1: HPA1 338 pb human platelet A1 PCR product

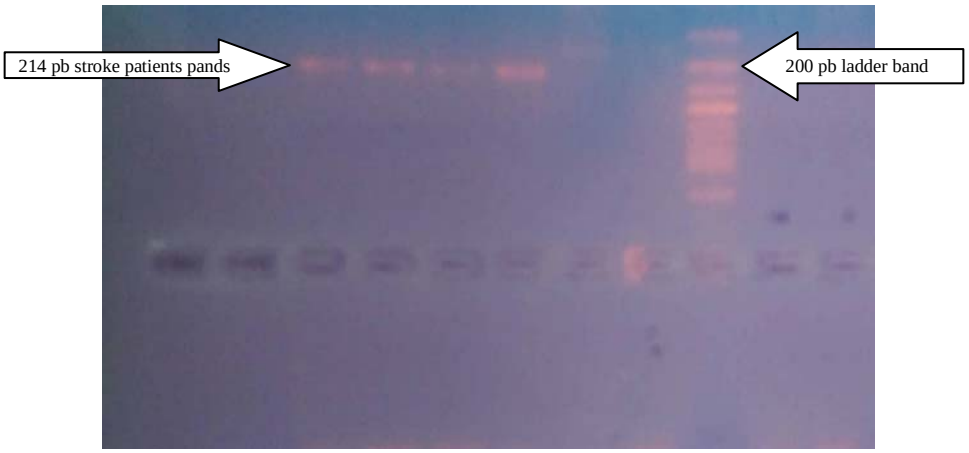

Fig. 2: Digestion of HPA1 PCR product by using ScrF1 enzyme show homozygous (leu/leu) AA genotype 214 Pb

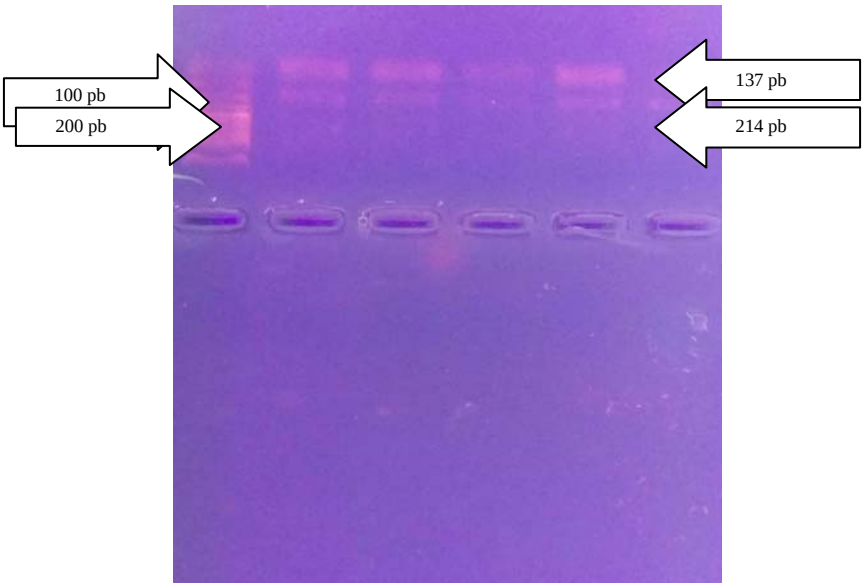

Fig. 3: Digestion of HPA1 PCR product by using ScrF1enzyme showed heterozygous A/B

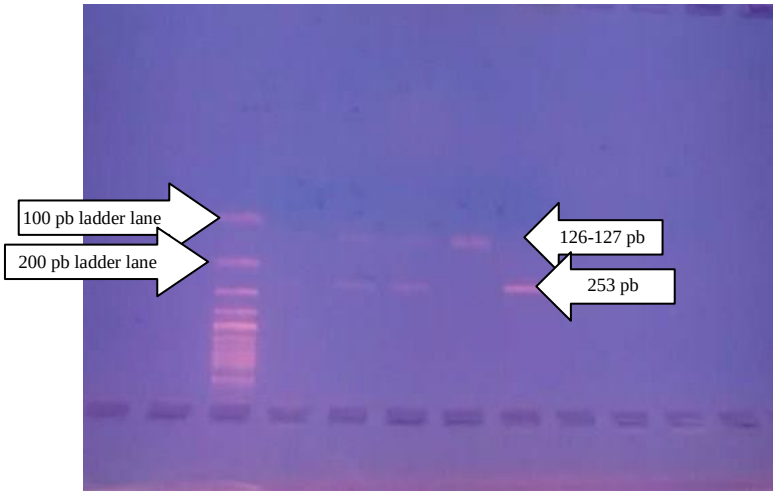

Fig. 4: Digestion of HPA3 PCR product by using Fok1 enzyme show heterozygous A/B

DISCUSSION

Glycoprotein IIIa and GPIIb, establish the fibrinogen receptor (integrin αIIbβ3), whose attachment represents the last common pathway for platelet aggregation[14]. The genes encryption for it are placed on chromosome seventeen[18]. This study aimed to detect the association of αIIbβ3 polymorphisms with Ischemic stroke and to find out if there is any correlation between the risk factors and platelet integrin αIIbβ3 polymorphisms. Among the studied ischemic stroke patients and control subjects, the association between TRI.G and HDL was highly significant. The magnitude of body mass index was changed compared to control groups. None of the total cholesterol levels, LDL showed significant association while HbA1c level had a role in increasing of stroke ($p<0.05$). This is because all patients were under medications. Compared to control groups, more patients were smokers and hypertensive. These were highly statistically significant. The present findings were consistent with the suggested risk factors for stroke[23]. Three main genotypes of HPA3 were identified in this study, the A/A genotype was the most common genotype in patients and controls. The A/B genotype was higher in stroke patients than in controls but this was not statistical significant. The frequency of B/B genotype in stroke patients was more than in controls and this was considered significant variation, that agreed with the study done by Duan *et al.*[12], who identified a notable variation in the genotype distributions of HPA-3 between patients and control. Furthermore, they found that B/B genotype was increased the risk of ischemic stroke. Moreover, current study matches up with the study done by Saidi *et al.*[21], who showed the first proof for demonstrating an association of the wide spread five human platelet antigens (HPA-1, HPA-3, HPA-2, HPA-4 and HPA-5) with stroke[21]. Reiner *et al.*[20] also found that B/B genotype was common in ischemic stroke patients. Carter *et al.*[18] disagreed with the present study by finding that there was no important change in the genotype distribution of HPA3 between patients and control[18]. This study showed that, the frequency of isoleucine was less in stroke patients than in controls. These were matches up with the study done by Hagous and Ibrahim[24]. In this study, three main genotypes of HPA1 were identified. The A/A genotype was the most common genotype in stroke patients and control. Moreover, the susceptibility of having an ischemic stroke was found in individuals with A/B genotype. The frequency of an A allele was a bit less in stroke patients than in control. These findings were consistent with Saidi *et al.*[21], who showed that the HPA-1b represented a powerful genetic hazard for ischemic stroke[21] and with Pongracz *et al.*[25], which showed that, Leu Pro 33 appears to increase the risk for stroke in patients more than 50 years[25]. This study also agreed with Rothwell *et al.*[26] suggested that the PLA2 allele was common in brain infarcts associated with occlusion of large-vessel[26] and disagreed with Duan and *et al.*[10] who found the GP111a plA1 had no relation with ischemic stroke. There were many studies in which no association with ischemic stroke was detected[27,28] or do not support these polymorphisms as risk factors for thrombotic stroke[29]. Due to racial diversity in the distribution of the HPA1 and HPA3, polymorphic variants and their potential pathogenic capability, This study recommend further studies, including considerable numbers of subjects, together with diverse population. Effectiveness and functional studies are necessary to estimate the significance of these associations in clinical practice.

CONCLUSION

Finally, the findings of the present study showed that, TRI.G and HDL, HbA1c and body mass index raised the risk of ischemic stroke. In HPA3 not only were the risks of ischemic stroke higher with (B/B) genotype but also the frequency of (A/B) was higher than that found in controls. In HPA1, the risk of stroke was increased by (A/B) genotype.

SIGNIFICANCE STATEMENT

This study discovered the association of integrin αIIbβ3 polymorphisms with ischemic stroke in Sudanese patients, which can be beneficial for the early identification of subjects at risk in order to prevent the fatal attack of ischemic stroke and to help researchers to introduce GpIIb/IIIa antagonist basically in the treatment of ischemic stroke that many researchers were not able to explore. Thus, a new theory in gene therapy may be arrived at.

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