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Association of PS gene polymorphism and soluble P-selectin levels in atrial fibrillation thromboembolism population in Xinjiang ☆

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ABSTRACT

To investigate the association between the polymorphism of P choose element (p. selectin, PS) and soluble P-selectin levels in atrial fibrillation (AF) thromboembolism in Han and Uigur population of Xinjiang. *Method:* Using ELISA method determination of plasma level of sPs. The frequency distributions of SNP sP-selectin gene promoter (−2123C/G) and SNP in exon region (Thr715Pro) were investigated by polymerase chain reaction (PCR)-restriction fragment length polymorphism and direct DNA sequence analysis among 302 Xinjiang Uigur and 340 age- and sex-matched Han people. *Results:* Cases sPs exist significant difference serum level and the control group. The frequencies of the −2123C/G allele among the Uigur population had no significant differences from those of the Han population. Thr715Pro did not show any polymorphism in the two populations. *Conclusions:* The sP-selectin gene polymorphisms are associated with serum sP-selectin levels or thromboembolic events, suggesting that the patients with nonvalvular AF and thromboembolic events may have genetic susceptibility.

1. Introduction

Atrial fibrillation or AF is the most common clinical harm serious arrhythmia, it is atrial electrical activity loss rules orderly, lose the shrinkage and expansion, the effective pump function deterioration or loss. Cause some angina pectoris, low blood pressure, shock or cardiac insufficiency, seriously affecting the quality of life, is particularly important is can lead to thromboembolism, stroke and other clinical consequences. The incidence of atrial fibrillation in recent decades shows continuous increase [1], China currently has about 8 million or more [2]. Atrial fibrillation continued existence leads to the incidence of ischemic stroke and systemic embolism events increased significantly. Recent studies have found that the sPs gene polymorphism and cycle level or thrombosis has certain relations [3–6]. Some gene polymorphisms located in the promoter region of the P-selectin gene have been described, as well as some polymorphisms that affect the protein sequence. These Thr715Pro, 2123 C/G, 1817 T/C gene polymorphism in some other countries have related reports [6,7]. Haven't seen China Xinjiang Han and Uigur PS gene polymorphism and atrial fibrillation thromboembolism formation correlation studies. Considering Ps gene loci polymorphism distribution in different areas, different population frequency have differences, these differences will inevitably sPs nonvalvular atrial fibrillation and the role of the occurrence and development of thromboembolic events cause certain influence. This article discussed with Xinjiang Uigur and Han nonvalvular atrial fibrillation thromboembolism and patients with nonvalvular atrial fibrillation as the research object, for PS gene 2123 C/G, Thr715Pro polymorphism loci and atrial fibrillation whether there is a connection between thromboembolism, whether there are ethnic differences are analyzed.

2. Materials and methods

2.1. Object of study

The Han nationality patients with atrial fibrillation complicated by thromboembolism (Han cases) 121 cases, male 65 cases, female 56 cases, age from 40 to 83 years, mean age (61.02 ± 10.43) years, Uigur patients with atrial fibrillation complicated by thromboembolism (Uigur cases) 103 cases, male 56 cases, female 47 cases, age from 41 to 78 years, mean age (54.8 ± 9.61) years. These are all between January 2011 and January 2012 in the first affiliated hospital of Xinjiang medical university hospital patients.

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Evaluation of AF was carried out by expert cardiologists and based on the standard diagnostic criteria according to ACC/AHA/ESC 2006 guidelines for the management of patients with AF [8] Clinical examinations were carried out using rest electrocardiograms (ECG), and sometimes aided with bedside telemetry or ambulatory Holter ECG recordings. And confirmed by echocardiography with thrombosis of valvular heart disease. Han atrial fibrillation group (Han control group) 219 cases, male 68 cases, female 61 cases, age from 32 to 87 years, mean age (60.75 ± 9.45) years, Uigur atrial fibrillation group (Uigur control group) 199 cases, male 50 cases, female 51 cases, age from 40 to 73 years, mean age (56.72 ± 9.36) years. Are all in the same period in the hospital and has not concurrent thromboembolism in patients with atrial fibrillation. Patients with infectious or non-infectious inflammatory disease, acute coronary syndrome, severe liver and kidney dysfunction, cancer, immune system diseases, tissue damage within a month and vascular events, surgery and stroke within six months were all excluded. They had physical examination, routine laboratory tests, electrocardiogram (conventional, 24 h dynamic), chest X-ray, thyroid function tests, and echocardiography. Transthoracic and/or transesophageal echocardiography examination were carried out to exclude potential patients with structural heart disease during the same time. There was no statistically significant difference in age, gender, BMI, smoke, history of hyperthyroidism, hypertension, diabetes between the two groups, and all patients have signed an informed consent.

2.2. Experimental methods

2 mL fasting quiet state peripheral cubital vein blood were collected, and then placed in EDTA anticoagulant tube.

2.2.1. Peripheral blood of sPs detection

Plasma sPs measured by ELISA method (bought kits from the Invitrogen trade co., LTD), operating according to specifications. SPs normal reference value: 0.97 pg/mL.

2.2.2. Isolation of genomic DNA

Peripheral whole blood samples from participants were drawn by atraumatic and sterile antecubital venipuncture into vacuum tubes containing the anticoagulant ethylene-diaminetetracetic acid. Use to buy in TIANGEN Biochemical technology co., LTD (Beijing) of genomic DNA extraction kit to extract genomic DNA, and diluted to 10 ng/μL for analysis. Extracted DNA samples were stored at −20 °C.

2.2.3. SNP genotyping

Polymerase chain reaction (PCR) amplifications as well as the HRM procedures for the variants of these SNPs were carried out in 96-well plates in the C1000 Thermal Cycler PCR System. In both populations, at least 1 DNA sample of each genotype of each SNP was previously genotyped by sequencing. Primers synthesized from ShengGong biological engineering co., LTD (Shanghai), Primer sequences for PS gene polymorphism and reaction conditions (Table 1).

The PCR reaction system were 20 μL, including: 2 μL purified genomic DNA, 10 μL PCR mix, upstream primers 0.5 μL, downstream primers 0.5 μL, ddwater 7 μL. PCR was performed as the following conditions: initial denaturation at 95 °C for 3 min, amplification for 34 cycles by denaturing at 95 °C for 30 s, annealing at 54.7 °C (2123 C/G) and 52.8 °C (Thr715Pro) for 30 s, and extension at 72 °C for 1 min. After amplification, PCR products were denatured at 72 °C for 5 min and cooled to 4 °C for 1 min to form double strand DNA. Then the HRM analyses were performed by gradually increasing the temperature from 65 °C to 95 °C at a rate of 0.01 °C/s. After the melting procedure, the instrument was cooled down to 4 °C. Two positive controls for each genotype were included in each run. PCR amplification products were taken 10 μL, sPs gene 2123 C/G site of the enzyme as BpmI 10 U put in 30 °C constant temperature water bath digest 5 h. Thr715Pro site of the enzyme HincII 10 U, 37 °C incubation 16 h. Reaction after termination, the digestive fragments on 2% agarose gel electrophoresis. Substitute EB dyeing, dyeing after judgment result by gel imaging system.

In order to verify the genotyping results, we randomly selected 40 cases and controls for direct DNA sequence analysis. DNA sequence analysis done by ShengGong biological engineering co., LTD (Shanghai). The sequencing result for the 40 samples perfectly matched that by the HRM method.

2.3. Statistical analysis

Using the SPSS17.0 statistical software to analyze data processing. Clinical parameters measurement data mean comparison between the two groups used independent samples t-test, t'-test, counting data with χ^2-test. Genotype and allele frequency utilizing gene counting method to calculate directly. Then the Hardy–Weinberg genetic laws of balance test. Showed that the genotype frequencies have reached its genetic balance, has a group representative. Genotype and allele frequency distribution between groups compares with χ^2, and inspection, with $P < 0.05$ for the difference is statistically significant.

3. Results

In order to detect whether SNPs in the P-selectin gene are associated with AF in a non-European ancestry population, we carried out a case–control association study with a samples from Chinese Han (121 cases) and Uigur (103 cases) patients with atrial fibrillation complicated by thromboembolism patients and AF controls (Han 219 cases, Uigur 199 cases).

3.1. Comparison of clinical features

Case group and the control group, two groups in age, gender, BMI, serum triglycerides, total cholesterol, HDL cholesterol, LDL cholesterol, plasma fibrinogen levels, with or without smoking, hypertension, diabetes, coronary heart disease, heart failure and other differences were not statistically significant.

Table 1
Primer sequences for genotyping P-selectin polymorphisms.

Polymorphism	Reference SNP ID	Ref SNP alleles	Primer sequences	Incision enzyme
−2123C/G	rs1800807	C/G	F: 5′-CCGTTTAATTAGC CAGTAGTGATG-3′ R: 5′-CCGAAGTGTGGTATGTAGACTAG TAG-3′	BpmI
Thr715Pro	rs 6136	A/C	F: 5′-ATGAACTGCT CCAACCTCTG-3′ R: 5′-CCCACATGAAAATTG TACCTT-3′	HincII

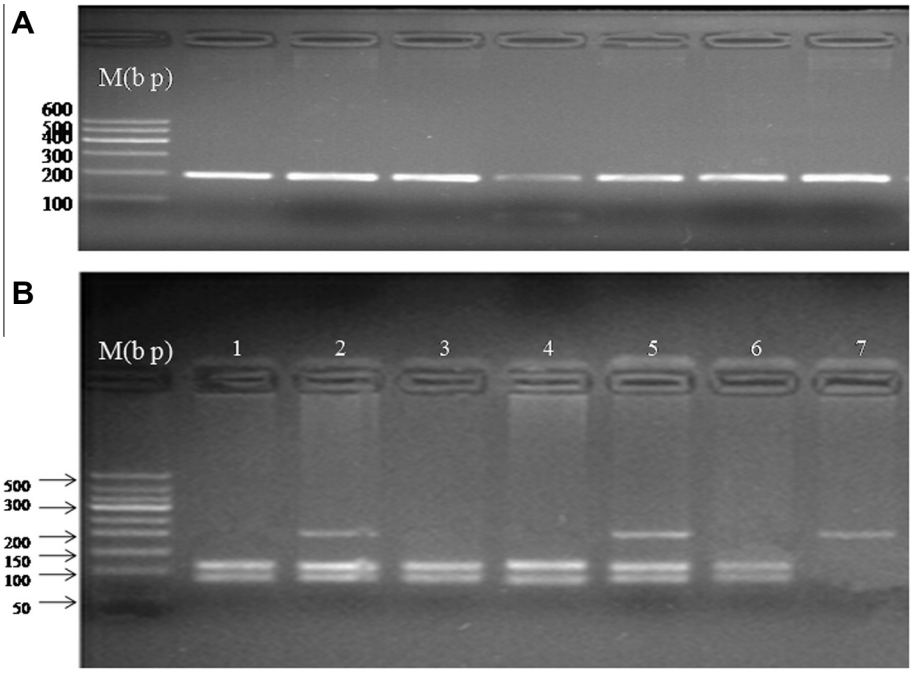

Fig. 1. The PCR and digestion products of −2123C/G (M: marker) A: −2123C/G PCR product with product length of 189 bp; B: −2123C/G digestion products. 2, 5 were heterozygous CG genotype; 1, 3, 4, 6 were mutated homozygous CC genotype; 7 was homozygous GG wild-type.

3.2. PS genotypic test results

Ps gene 2123 C/G polymorphism loci, PCR amplification products fragment size is 189 bp (Fig. 1), Pst I restriction fragment of the situation in accordance with restriction endonucleases. There are three kinds of genotypes, CC-type (106 and 76 bp 2 bands). CG type (189, 106 and 76 bp 3 bands), GG-type (189 bp 1 band). The experiment was not detected Thr715Pro polymorphism (Fig. 2).

3.3. sPs test results

In this experiment by ELISA method for determination of cases of Chinese Han and Uigur groups (the Han nationality in 100 cases, 100 cases of Uigur) and control group (the Han nationality in 100 cases, 100 cases of Uigur) the concentration of sPs, four groups of comparative differences are significant ($P < 0.001$), respectively (42.4 ± 8.2) μg/mL and (36.3 ± 8.4) μg/mL.

3.4. Case and control groups

2123 C/G genotype distribution and allele frequencies are compared in Table 2.

3.5. Relations Ps gene polymorphism and sPs concentration

Through the comparison between 2123 C/G genotype and the relationship between the concentration of sPs, found 2123 C/G gene polymorphism of GG genotype sPs is significantly higher than CG + CC genotype (Table 3).

4. Discussion

Chromosome encoding gene location of Ps is in the human l (1 q21–24) on the DNA sequence, it is 50 KB's long, and contains 16 exons and 17 introns, there are 13 polymorphism loci [7]. In Europeans, 2 SNPs in the promoter region of P-selectin gene (−2123C/G, −1969A/G) and 1 SNP in the exon 13 (Thr715Pro), are considered to be strongly associated with sP-selectin level [9], nevertheless, polymorphism of location Val599Leu is more strongly associated with sP-selectin among African-Americans [10]. Meanwhile, 4 P-selectin polymorphisms at positions −2123C/G, −1969A/G, −1817T/C and Ser290Asn are in tight linkage disequilibrium in Europeans [11]. Parts of our country AF case retrospective investigation data, the prevalence of atrial fibrillation in patients with cerebral apoplexy was 17.5% [12]. Ma et al. [13] on the incidence of atrial fibrillation in patients with non-valvular ischemic stroke in Beijing's report, the annual average of ischemic stroke in patients with non-valvular atrial fibrillation non-anticoagulant state incidence of 5.3%, although it is difficult to prove in patients with atrial fibrillation endothelial dysfunction is the precise mechanism of thrombosis. However, endothelial dysfunction and blood clotting mechanism will work together to cause high blood coagulation status, some blood clotting active tags can reaction dynamics have risk factors, for example exists in patients with atrial fibrillation sPS and fibrinogen gene changes on the development of high coagulation state has played a role.

In this study, the frequencies of the −2123G allele among the Xinjiang population were 0.741, and there are no significant differences between Uigur and Han populations (0.707 and 0.792, respectively). But the frequencies were different from those of Northern Ireland and French populations in an ECTIM study (0.386; 0.470, respectively) [11]. The frequencies of the Pro715 allele among the healthy subjects in Europeans and European-Americans were about 10% [11,14], meanwhile the Pro715 variant allele of P-selectin was found to correlate with lower sPs levels and was reported that plays a protective role of myocardial infarction in European Caucasians [15]. P-selectin polymorphisms at positions −2123C/G, −1969A/G, −1817T/C and Ser290Asn were reported to be in tight linkage disequilibrium with each other in Europeans [16]. Several studies have been done to study P-selectin gene SNPs in Chinese Han population, one of them had explored P-select in gene −2123C/G, −1969A/G, −1817T/C and Thr715Pro in a Han population in Guangxi, a Southern province in China [17]. The G allele frequencies of −2123C/G

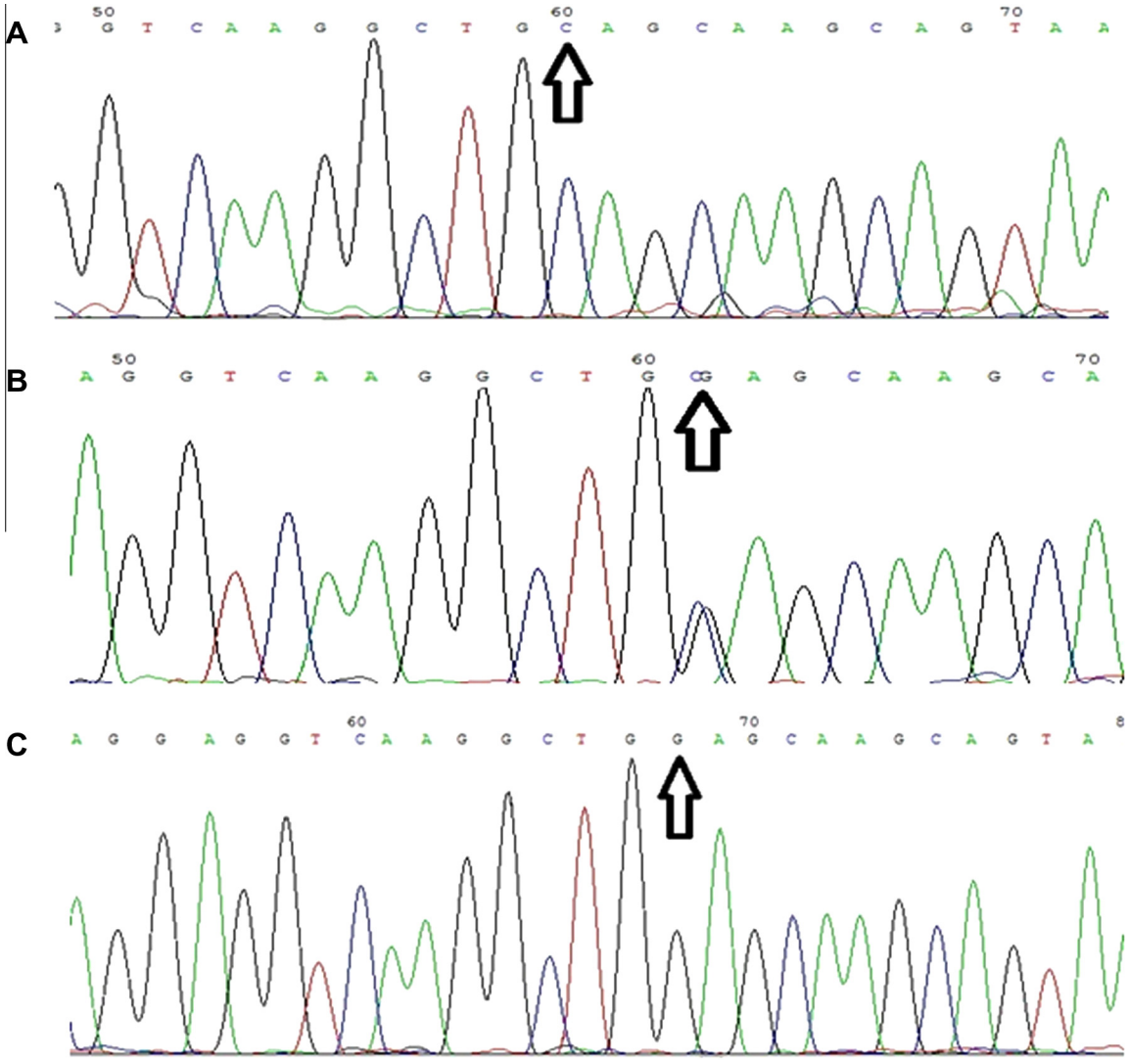

Fig. 2. The sequence of CC, CG, and GG genotype, the arrow showed the mutation (A: CC, B: CG, C: GG).

Table 2
Allele and genotype analysis of −2123C/G in Han and Uigur populations.

	N	Allele				Genotype					HWE	
		F		χ^2	*P*	*F*			χ^2	*P*	*C*	*P*
		C	G			G/G	C/G	C/C				
Han	340	236 (0.347)	444 (0.653)	0.901	0.343	164 (0.412)	140 (0.482)	36 (0.106)	5.089	0.079	0.557	0.455
Uigur	302	225 (0.373)	379 (0.627)			121 (0.401)	137 (0.453)	44 (0.146)				

N: Numbers genotyped successfully; *F*: frequencies; *P*: *P*-value (1 df); *C*: Chi square value.

Table 3
sPs Concentration of −2123C/G in Han and Uigur populations.

	Group	Frequencies	GG	CG + CC	sPs(GG) ($X \pm S$, μg/l)	sPs(CG + CC) ($X \pm S$, μg/l)	*P*-value (1 df)
Han	Case	121	42 (0.347)	79 (0.653)	48.6 ± 9.7	40.1 ± 7.2	<0.001
	Control	219	98 (0.447)	121 (0.553)	44.5 ± 6.7	35.5 ± 8.3	0.001
	Case + control	340	140 (0.4)	210 (0.6)	47.4 ± 9.0	37.8 ± 8.1	<0.001
Uigur	Case	103	32 (0.311)	71 (0.689)	49.1 ± 8.3	42.3 ± 8.4	<0.001
	Control	199	89 (0.447)	110 (0.553)	44.9 ± 7.3	38.3 ± 7.8	<0.001
	Case + control	302	121 (0.401)	181 (0.599)	48.5 ± 9.2	40.4 ± 8.2	<0.001

in the healthy Han population of Guangxi had no statistical differences from those of the Han population in this study. And Thr715Pro did not show any polymorphism in the Guangxi Han population, neither in Xinjiang Han and Uigur population in our study.

Whether these putative consensus sites are involved in the regulation of the P-selectin expression, and if so, how these polymorphisms affect their function, that remains unknown. In the present study, there are some slight differences between Europeans and Chinese Uigur population as well as Han population. PS is a highly polymorphic gene, according to the current reports more than 13 points, the rest of the site and the concentration of sPs correlation still need further research. This study sample size is small, still need to increase the sample size to do further research. To test it, in patients with atrial fibrillation may early prevention, diagnosis and treatment of atrial fibrillation thromboembolism provides a new platform.

Conflict of interest

We declare that we have no conflict of interest.

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