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# Relationship between the -455G/A and -148C/T polymorphisms in the beta-fibrinogen gene and cerebral infarction in the Xinjiang Uygur and Han Chinese populations☆

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## Abstract

We sought to investigate the correlation between the -455G/A and -148C/T polymorphisms of the β-fibrinogen gene and plasma fibrinogen levels in patients with cerebral infarction and in healthy subjects among the Xinjiang Uygur and Han Chinese populations, by using polymerase chain reaction-restriction enzyme digestion analysis. Results showed that there were no statistically significant differences in the distributions of the -455G/A genotype and allele frequency between the Uygurs and the Han. Plasma fibrinogen levels in cerebral infarction patients among the Uygurs and the Han were higher than those among healthy subjects. In particular, the frequencies of the -455G/A AA and -148C/T TT genotypes were significantly higher than in healthy subjects. Individuals carrying the A or T allele had a higher incidence of cerebral infarction compared with those carrying the G or C allele. Our experimental findings indicate that the -148C/T and -455G/A polymorphisms are associated with cerebral infarction in Xinjiang Uygur and Han Chinese subjects. The susceptibility-conferring alleles are -148T and -455A, and the susceptibility-conferring genotype is -455G/A + AA.

**Key Words:** Uygur; Han; cerebral infarction; β-fibrinogen gene; polymorphism; neural regeneration

**Abbreviations:** β-Fg, β-fibrinogen; CI, cerebral infarction; PCR; polymerase chain reaction; RFLP, restriction fragment length polymorphism

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## INTRODUCTION

Epidemiological evidence indicates that an elevated plasma fibrinogen concentration is an independent risk factor for ischemic cardiovascular and cerebrovascular diseases[1]. Plasma fibrinogen levels are affected by both environmental and genetic factors, including polymorphisms in the fibrinogen genes. In particular, the β-fibrinogen (β-Fg) gene has been found to confer susceptibility to thromboembolic diseases and elevated plasma fibrinogen levels[2-3]. However, the relationship between fibrinogen gene polymorphisms and plasma fibrinogen levels in subjects with thromboembolic diseases is not clear. For example, the frequencies of -455G/A and -148C/T polymorphisms in the β-Fg gene and their association with plasma fibrinogen levels in patients with cerebral infarction in the Xinjiang Uygur and Han Chinese communities are unknown. In this study, we recruited a total of 156 cerebral infarction patients and 143 healthy subjects from the Han and Uygur Chinese populations, and the -455G/A and -148C/T polymorphisms in the β-Fg gene and their association with cerebral infarction were investigated.

## RESULTS

### Subjects

A total of 299 cerebral infarction patients and healthy subjects were recruited from Han and Uygur Chinese in the First Teaching Hospital of Xinjiang Medical University, China from March 2007 to January 2008. They were divided into cerebral infarction patients group ($n$ = 156), including 63 Uygur Chinese and 93 Han Chinese, and healthy subjects group ($n$ = 143), including 63 Uygur Chinese and 80 Han Chinese. There was no significant difference in gender or sex ratio between the cerebral infarction patient and control groups, as determined using the chi-square test ($\chi^2$ = 1.098, $P$ > 0.05; $t$ = 1.881, $P$ > 0.05).

### Polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) identification of β-Fg-455G/A

The PCR-amplified products were 669 bp in

length. When the genotype was -455G/G homozygote, the DNA fragments were 488 and 181 bp in length. For -455G/A heterozygotes, the DNA fragments were 669, 488 and 181 bp. For -455A/A homozygotes, the DNA fragment was 669 bp (Figure 1).

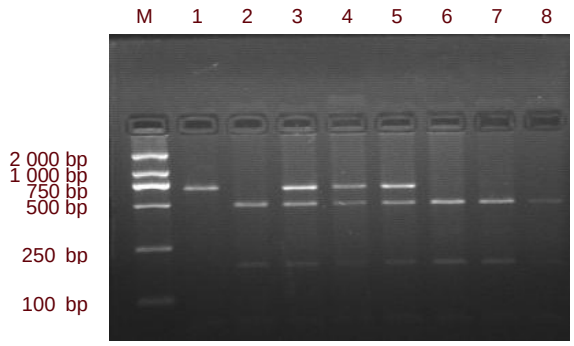


Figure 1 *Hae*III enzyme digestion identification of β-fibrinogen -455G/A (polymerase chain reaction and restriction fragment length polymorphism).

M: DL2000 marker; 1: AA (669 bp); 2, 6, 7, 8: GG (488 bp, 181 bp); 3–5: GA (455 bp).

### PCR and RFLP identification of β-Fg-148C/T

The PCR-amplified products were 669 bp in length. When the genotype was -148C/C homozygote, the DNA fragments were 202 and 98 bp. When the genotype was -148C/T heterozygote, the DNA fragments were 300, 202 and 98 bp. When the genotype was -148T/T homozygote, the DNA fragment was 300 bp (Figure 2).

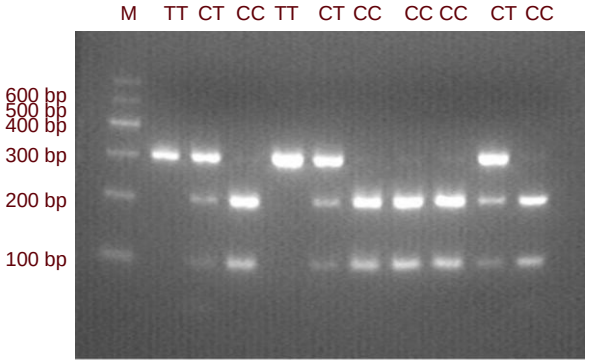


Figure 2 *Hae*III enzyme digestion identification of β-fibrinogen -148C/T (polymerase chain reaction and restriction fragment length polymorphism).

M: Marker; C/C: 202 bp, 98 bp; C/T: 300 bp, 202 bp, 98 bp; T/T: 300 bp.

### Hardy-Weinberg equilibrium testing of the genotype

The expected value and observed value in cerebral infarction patients and healthy individuals from both the Uygur and Han populations conformed to the Hardy-Weinberg equilibrium principle and results showed favorable goodness-of-fit (cerebral infarction patients: $\chi^2$ = 2.179 and 0.195 for Uygurs and Han; *df* = 1, *P* > 0.05; healthy subjects: $\chi^2$ = 0.053 and 0.065 for Uygurs and Han; *df* = 1, *P* > 0.05).

### -455G/A genotype and allele frequencies of the β-Fg gene

As shown in Table 1, cerebral infarction patients had higher frequencies of the -455A/A genotype and the -455A allele, and a lower frequency of the -455G/G genotype than healthy individuals. Significant differences in the distributions of the -455A/A and -455G/G genotypes, and in the -455A allele frequencies were observed between patients and healthy subjects (*P* < 0.05). Subjects carrying the A allele were more susceptible to cerebral infarction than those carrying the G allele. Significant differences in the -148C/T genotype frequency (*P* < 0.05), but not in allele frequency, were observed between Uygurs and Han (Table 2). Furthermore, marked differences in -148C/T genotype and allele frequency were noted between healthy subjects and Uygur and Han cerebral infarction patients. Because the number of Uygurs and Han with the -148T allele was relatively low, patients with the -148C/T genotype and those with the -148TT genotype were analyzed as one group, and dramatically significant differences were observed (Uygurs: $\chi^2$ = 6.250, *P* = 0.012; Han: $\chi^2$ = 4.061, *P* = 0.044).

Table 1 Frequencies of the -455G/A genotype and allele of the β-fibrinogen gene in patients and healthy subjects

| Group | *n* | Genotype frequency [*n* (%)] | | | Allele frequency [*n* (%)] | |
|---|---|---|---|---|---|---|
| | | GG | GA | AA | G | A |
| Patients | 156 | 83(53.21) | 66(42.31) | 7(4.48) | 232(74.36) | 80(25.64) |
| Healthy subjects | 143 | 105(73.43) | 35(24.48) | 3(2.09) | 245(85.66) | 41(14.34) |
| $\chi^2$ | | 13.149 | | | 11.816 | |
| *P* | | 0.001 | | | 0.001 | |

Table 2 Frequencies of the -148C/T genotype and allele of the β-fibrinogen gene in patients and healthy subjects

| Group | *n* | Genotype frequency [*n* (%)] | | | Allele frequency [*n* (%)] | |
|---|---|---|---|---|---|---|
| | | CC | CT | TT | C | T |
| Patients | 156 | 84(53.85) | 64(41.03) | 8(5.13) | 232(74.36) | 80(25.64) |
| Healthy subjects | 143 | 101(70.63) | 38(26.57) | 4(2.80) | 240(83.92) | 46(16.08) |
| $\chi^2$ | | 8.911 | | | 8.182 | |
| *P* | | 0.003 | | | 0.004 | |

### Frequencies of the -455G/A and -148C/T genotypes and alleles of the β-Fg gene in Uygurs and Han

As shown in Table 3, there were no significant differences in the -455G/A genotypic or allelic frequencies between Uygurs and Han. But marked differences in the frequencies of the -455G/A genotype and of the alleles were found between patients and healthy subjects among both Uygurs and Han. Because the number of Uygurs and Han with the -455A allele was

relatively low, patients with the -455GA genotype and those with the -455AA genotype were analyzed as one group, and the results showed significant differences (Uygurs: $\chi^2$ = 7.327, $P$ = 0.007; Han: $\chi^2$ = 6.382, $P$ = 0.012). As shown in Table 4, a significant difference in the -148C/T genotype frequency ($P$ < 0.05), but not in allele frequency, was observed between Uygurs and Han.

Table 3 Frequencies of the -455G/A genotype and allele of the β-fibrinogen gene in Uygurs and Han

| Group | People | *n* | Genotype frequency [*n* (%)] | | |
|---|---|---|---|---|---|
| | | | GG | GA | AA |
| Patients | Uygurs | 63 | 29(46.03) | 31(49.21) | 3(4.76) |
| | Han | 93 | 54(58.06) | 35(37.63) | 4(4.31) |
| Healthy subjects | Uygurs | 63 | 44(69.84) | 17(26.98) | 2(3.18) |
| | Han | 80 | 61(76.25) | 18(22.50) | 1(1.25) |

| Group | People | *n* | Allele frequency [*n* (%)] | |
|---|---|---|---|---|
| | | | G | A |
| Patients | Uygurs | 63 | 89(70.63) | 37(29.37) |
| | Han | 93 | 143(76.88) | 43(23.12) |
| Healthy subjects | Uygurs | 63 | 105(83.33) | 21(16.67) |
| | Han | 80 | 140 (87.50) | 20(12.50) |

$\chi^2$ test: $\chi^2$ = 2.299, $P$ = 0.317 and $\chi^2$ = 2.088, $P$ = 0.148 for -455G/A genotype frequency and allele frequency, respectively, between Uygurs and Han.

$\chi^2$ = 7.366, $P$ < 0.05 and $\chi^2$ = 5.733, $P$ < 0.05 for -455G/A genotype frequency and allele frequency, respectively, between patients and healthy subjects among Uygurs.

$\chi^2$ = 6.740, $P$ < 0.05 and $\chi^2$ = 6.512, $P$ < 0.05 for -455G/A genotype frequency and allele frequency, respectively, between patients and healthy subjects among Han.

Table 4 Frequencies of the -148C/T genotype and allele of the β-fibrinogen gene in Uygurs and Han

| Group | People | *n* | Genotype frequency [*n* (%)] | | |
|---|---|---|---|---|---|
| | | | CC | CT | TT |
| Patients | Uygurs | 63 | 28(44.44) | 31(49.21) | 4(6.35) |
| | Han | 93 | 55(59.12) | 33(35.48) | 5(5.40) |
| Healthy subjects | Uygurs | 63 | 42(66.67) | 18(28.57) | 3(4.76) |
| | Han | 80 | 59(73.75) | 20(25.00) | 1(1.25) |

| Group | People | *n* | Allele frequency [*n* (%)] | |
|---|---|---|---|---|
| | | | C | T |
| Patients | Uygurs | 63 | 87(69.05) | 39(30.95) |
| | Han | 93 | 143(76.88) | 43(23.12) |
| Healthy subjects | Uygurs | 63 | 102(80.95) | 24(19.05) |
| | Han | 80 | 138(86.25) | 22(13.75) |

$\chi^2$ test: $\chi^2$ = 5.962, $P$ = 0.015 and $\chi^2$ = 3.341, $P$ = 0.068 for -148C/T genotype frequency and allele frequency, respectively, between Uygurs and Han.

$\chi^2$ = 5.733, $P$ = 0.017 and $\chi^2$ = 4.743, $P$ = 0.029 for -148C/T genotype frequency and allele frequency, respectively, between patients and healthy subjects among Uygurs.

$\chi^2$ = 4.505, $P$ = 0.034 and $\chi^2$ = 4.933, $P$ = 0.026 for -148C/T genotype frequency and allele frequency, respectively, between patients and healthy subjects among Han.

Furthermore, marked differences in the -148C/T genotype and allele frequencies were noted between healthy subjects and cerebral infarction patients among Uygurs and Han. Because the number of Uygurs and Han with the -148T allele was relatively low, patients with the -148C/T genotype and those with the -148TT genotype were analyzed as one group, and dramatically significant differences were observed (Uygurs: $\chi^2$ = 6.250, $P$ = 0.012; Han: $\chi^2$ = 4.061, $P$ = 0.044).

**Plasma Fg levels in cerebral infarction patients and healthy subjects among Uygurs and Han**

As shown in Table 5, plasma levels of Fg were significantly higher in cerebral infarction patients compared with healthy individuals among both Uygurs and Han ($P$ < 0.05).

Table 5 Plasma levels of fibrinogen (Fg; g/L) in cerebral infarction patients and healthy subjects among Uygurs and Han

| People | Group | *n* | Fg level | *t* | *P* |
|---|---|---|---|---|---|
| Uygurs | Patients | 63 | 4.11±0.99 | 3.547 | < 0.05 |
| | Healthy subjects | 93 | 3.49±0.97 | | |
| Han | Patients | 63 | 4.14±1.26 | 4.796 | < 0.05 |
| | Healthy subjects | 80 | 3.35±0.87 | | |

Homogeneity of variance test of plasma Fg level in Uygurs indicated heterogeneity of variance ($F$ = 6.761, $P$ = 0.01), and $t$-test showed significant difference in Fg level between patients and healthy subjects ($t$ = 3.547, $P$ < 0.05) for Uygur Chinese.

Homogeneity of variance test of plasma Fg level in Han indicated heterogeneity of variance ($F$ = 6.375, $P$ = 0.012), and $t$-test showed significant difference in Fg level between patients and healthy subjects ($t$ = −4.796, $P$ < 0.05) for Han Chinese.

Data are expressed as mean ± SD, two sample $t$-test.

## DISCUSSION

The frequency of gene polymorphisms varies between different nations, different peoples and even different regions. We have examined, for the first time, the frequencies of the -455G/A and -148C/T polymorphisms of the β-Fg gene and their association with plasma fibrinogen levels in cerebral infarction patients among the Xinjiang Uygur and Han Chinese populations. We investigated the -455G/A and -148C/T polymorphisms of the β-Fg gene in Xinjiang Uygur Autonomous Region. The results demonstrate that the frequencies of the -455A and -148T alleles were 0.16 and 0.12, respectively, in Uygurs and 0.19 and 0.14, respectively, in Han, which is comparable to the frequencies reported for the Han in Beijing, but lower than those in Europe[4-5]. This discrepancy may be explained by genetic heterogeneity and small sample size.

Although numerous studies have been conducted to investigate the relationship between the -455G/A and -148C/T polymorphisms of the β-Fg gene and cerebral infarction, the results are controversial. Our results show significant differences in the -455G/A and -148C/T

genotypes and allele frequencies between cerebral infarction patients and healthy subjects. In addition, there was a marked difference in -148C/T, but not -455G/A, genotype and allele frequencies between Uygurs and Han. In Uygurs and Han, there were significant differences in -455G/A and -148C/T genotype and allele frequencies between cerebral infarction patients and healthy subjects. Because the number of subjects with the -455A allele was relatively small, patients with the -455GA and -455AA genotypes were analyzed as one group, and statistical analysis showed a significant difference (Uygurs: $\chi^2$ = 7.327, $P$ = 0.007; Han: $\chi^2$ = 6.382, $P$ = 0.012). In Uygurs, a significant difference in allele frequency was found between patients and healthy subjects, that is, cerebral infarction patients had a higher frequency of -455A than healthy individuals ($\chi^2$ = 5.733, $P$ = 0.025). In Han, a significant difference in allele frequency was found between patients and healthy subjects, that is, cerebral infarction patients had a higher frequency of -455A than healthy individuals ($\chi^2$ = 6.512, $P$ = 0.011). When patients with -148C/T and -148TT genotypes were analyzed as one group, a significant difference was observed (Uygurs: $\chi^2$ = 6.250, $P$ = 0.012; Han: $\chi^2$ = 4.061, $P$ = 0.044). In Uygurs, a significant difference in allele frequency was found between patients and healthy subjects, that is, cerebral infarction patients had a higher frequency of -148T than healthy individuals ($\chi^2$ = 4.743, $P$ = 0.029). In Han, a significant difference in allele frequency was found between patients and healthy subjects, that is, cerebral infarction patients had higher frequency of -148T than healthy individuals ($\chi^2$ = 4.933, $P$ = 0.026).

According to the results mentioned above, cerebral infarction patients had higher frequencies of the rare -455A allele and -148C/T genotype, but healthy subjects had higher frequencies of the common -455G and -148C alleles. Significant differences in the frequencies of the -455A and -148T alleles were observed between patients and healthy subjects. The -455A and -148T alleles confer susceptibility to cerebral infarction. Furthermore, the frequencies of the -455GG, -148CC, -455GA, -148C/T, -455AA and -148TT genotypes in cerebral infarction patients were markedly higher than in healthy subjects, and these also confer susceptibility to cerebral infarction. In addition, the -455G/A and -148C/T polymorphisms are closely related to the occurrence of cerebral infarction, and the -455A and -148T alleles were positively associated with cerebral infarction. Currently, the relationship between the -455G/A and -148C/T polymorphisms of the β-Fg gene and arterial thrombosis is controversial. Kessler *et al* [6] proposed that the -455G/A polymorphism within the promoter of the β-Fg gene is closely related to the plasma Fg level. The higher the allele frequency, the higher the plasma Fg level; and this positively correlates with the incidence of ischemic cerebrovascular disease. They also showed that the -455A/A genotype was more frequently observed in patients with cerebrovascular disease, being associated with stenosis of large vessels. However, Austin *et al* [7] investigated Fg gene polymorphisms in patients with ischemic stroke due to lesions in large vessels and unknown causes and those with a history of stroke, and the results did not indicate significant differences in Fg gene polymorphisms between these patients. Therefore, they speculated that Fg gene polymorphisms were not related to ischemic stroke. Color Doppler was applied in the study of Schmidt *et al* [4], with results showing that patients with the -148T/T genotype had severe carotid atherosclerosis. Therefore, they postulated that the -148T/T genotype is a risk factor for carotid atherosclerosis in the elderly. Liu *et al* [8] demonstrated that 91 male patients with ischemic stroke had a higher frequency of the H2 allele than elderly controls, and the plasma Fg level was the highest in patients with the GG genotype, followed by those with the GA genotype, and thereafter the AA genotype. These results suggest that the A allele might be a risk factor for ischemic stroke in male Chinese. Although the -455G/A polymorphism in the promoter of the β-Fg gene could affect plasma Fg levels, it does not appear to be a determinant of ischemic cerebrovascular disease among Chinese.

In this study, the relationship between the -455G/A and -148C/T polymorphisms of the β-Fg gene and cerebral infarction was investigated. Some results were consistent with previously reported findings, but others were different. These discrepancies may be explained by: (1) the fact that cerebral infarction is a polygenic disease, with several candidate genes being involved in the pathogenetic process, although it is not clear which gene is dominant; (2) there being an interaction between environmental and genetic factors, which contributes to the phenotypic heterogeneity of cerebral infarction; (3) ethnic and geographic differences, which also contribute to variability; and (4) the small sample size, which may have led to sampling error and therefore contributed to the differing results.

In summary, an elevated plasma Fg level is an important risk factor for cardiovascular and cerebrovascular diseases, which are influenced by both genetic and environmental factors. The -455A and -148T alleles may be genetic markers for ischemic cerebrovascular disease. Further research is required to clarify the relationship between Fg gene polymorphisms and cerebral infarction in the Uygur population, as it may provide crucial insight for the prevention of cerebral infarction.

## SUBJECTS AND METHODS

### Design

A human genetics case-control study.

### Time and setting

Experiments were performed at the laboratory of Xinjiang Medical University, China, from October 2007 to March 2008.

### Subjects

All cerebral infarction patients and healthy subjects were

recruited from Han and Uygur Chinese in Xinjiang Uygur Autonomous Region, at the First Teaching Hospital of Xinjiang Medical University, China, from March 2007 to January 2008.

### *Cerebral infarction patient group*

A total of 156 cerebral infarction patients were included. Among these were 39 males and 24 females from the Uygur Chinese population, with a mean age of 61.06 ± 10.44 years (range: 41–84 years), and 65 males and 28 females from the Han Chinese population, with a mean age of 61.53 ± 8.91 years (range: 38–80 years).

Inclusion criteria*:* Diagnosis of cerebral infarction was based on the *Essentials of Diagnosis of Cerebrovascular Diseases*, formulated by the Chinese Society for Neurology and Neurosurgery[9].

Exclusion criteria*:* Subjects with potential cardiogenic emboli, autoimmune disease, blood disease, neoplastic disease, liver or kidney dysfunction, and those receiving thrombolytic, anticoagulant or antiplatelet treatment were excluded.

### *Control group*

A total of 143 healthy unrelated subjects were enrolled. Among these healthy subjects, there were 35 males and 28 females from Uygur Chinese, with a mean age of 59.12 ± 10.90 years (range: 40–80 years), and 52 males and 28 females from Han Chinese, with a mean age of 60.08 ± 9.24 years (range: 38–78 years).

Inclusion criteria*:* Healthy individuals, based on physical examination in the same period.

Exclusion criteria*:* According to the standard risk factors listed in the WHO MONICA project, subjects with cardiovascular, liver, kidney or thyroid disease, those with diabetes, as well as those with blood relatives with one of these diseases, and subjects with recent inflammation were excluded.

All experimental procedures complied with the *Administrative Regulations on Medical Institution*, issued by the State Council of China[10]. Informed consent was obtained from each subject before study and the whole study was approved by the Ethics Committee of Xinjiang Medical University in China. There was no significant difference in the gender or sex ratio between the cerebral infarction patient and control groups, as determined with the chi-square test ($\chi^2$ = 1.098, $P$ > 0.05; $t$ = 1.881, $P$ > 0.05).

## Methods

### *Determination of plasma Fg* levels

Fasting venous blood, 3 mL, was treated with the anticoagulant EDTA and the plasma level of Fg was determined with an automated coagulation analyzer (Beijing, China).

### *Extraction of genomic DNA*

EDTA anticoagulated venous blood (2 mL) was stored at −70°C. The genomic DNA was extracted from antecubital vein mononuclear cells with a rapid genomic DNA extraction kit (Pel Freeze Clinical Systems, Brown Deer, WI, USA).

### *Detection of β-Fg gene fragment by RFLP-PCR*

PCR was performed to determine the expression of β-Fg mRNA. The primers for β-Fg-455G/A and -148C/T were designed by Shanghai Sangon (Shanghai, China) as previously reported[11-12].

The primers are as follows:

| Gene | | Sequence (5'–3') |
|---|---|---|
| β-Fg-455G/A | Forward | GAA CAT TTT ACC TTA TGT GAA TTA AGG |
| | Reverse | GAA GCT CCA AGA AAC CAT CC |
| β-Fg-148C/T | Forward | CCT AAC TTC CCA TCA TTT TGT C |
| | Reverse | ATG GTT TTA AGT TTG TGG AAG C |

The anticipated length of the amplified products was 669 bp and 300 bp for β-Fg-455G/A and β-Fg-148C/T, respectively. Both amplified products included a *Hae*III (New England Biolabs, Ipswich, MA, USA) restriction site, forming an RFLP (Bio-Rad, Hercules, CA, USA).

A total of 30 μL of the mixture was used for amplification and the PCR conditions for β-Fg-455G/A were: pre-denaturation at 95°C for 5 minutes; 35 cycles of denaturation at 95°C for 50 seconds, annealing at 58.2°C for 45 seconds and extension at 72°C for 60 seconds; and a final extension at 72°C for 7 minutes. Reaction was terminated by cooling to 4°C. Then, 6 μL of products were separated by 1.5% agarose gel electrophoresis (100 V) for 20 minutes and visualized with ethidium bromide staining.

A total of 25 μL of mixture was used for amplification, and the PCR conditions for β-Fg-148C/T were: pre-denaturation at 94°C for 2 minutes; 35 cycles of denaturation at 94°C for 45 seconds, annealing at 56.7°C for 45 seconds, extension at 72°C for 45 seconds; and a final extension at 72°C for 7 minutes. Reaction was terminated by cooling to 4°C. Then, 10 μL of products were separated by 2% agarose gel electrophoresis (80 V) for 35 minutes and visualized with ethidium bromide staining.

### *Restriction enzyme digestion*

The amplified products for β-Fg -455G/A were incubated with 1 μL of *Hae*III restriction enzyme (MBI, Glen Burnie, MD, USA) at 37°C overnight, and the DNA fragments were visualized under ultraviolet light after separation on a 2% agarose gel (100 V) containing ethidium bromide. The amplified products for β-Fg -148C/T were incubated with 1 μL of *Hae*III restriction enzyme at 37°C overnight, and the DNA fragments were visualized under ultraviolet light after separation on a 2.5% agarose gel (80 V). The bands were analyzed by a gel imaging system (BTS-20M; Unvitec, England).

### *Statistical analysis*

Data are expressed as mean ± SD. Statistical analysis was performed with SPSS 11.5 software (SPSS, Chicago, IL, USA). The frequencies of the different alleles were assessed by gene counting and the Hardy-Weinberg equilibrium principle was applied for goodness-of-fit testing. Allele and genotype frequencies were compared with the chi-square test. Significance level, $\alpha$ = 0.05.

Different genome Fg levels (measurement material) were compared using the two sample *t*-test. Significance level $\alpha = 0.05$.

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**Author contributions:** Xiaoning Zhang was head of the foundation, designed the research protocol, provided technical support, and wrote the manuscript. Yanyun Li and Xuebing Guo produced, integrated and analyzed the data, performed statistics, and participated in writing the manuscript. Lei Du integrated the data and participated in writing the manuscript. Jianhua Ma directed the research and statistical analysis, provided valuable material and document support for the project, and reviewed the manuscript.
**Conflicts of interest:** None declared.
**Ethical approval:** Subjects in the patient and control groups provided signed consent, under the approval of the Ethics Committee of Xinjiang Medical University in China.

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