SCIENTIFIC ARTICLE

F11 rs2289252T and rs2036914C Polymorphisms Increase the Activity of Factor XI in Post-trauma Patients with Fractures Despite Thromboprophylaxis

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Objective: To evaluate the association between *F11* rs2289252, rs2036914 polymorphisms and the activity of clotting factor XI in post-trauma patients with fractures receiving routine anticoagulation therapy for deep venous thrombosis (DVT).

Methods: A case-control study involving 110 consecutive post-trauma patients with fractures and DVT in our hospital was conducted from April 2014 to October 2015; these patients comprised a DVT group. Another 40 sex- and age-matched patients with fractures but without DVT served as controls. Additionally, 40 sex- and age-matched healthy people were chosen as a normal group. Venous blood samples (2 mL) were drawn from all participants and genomic DNA extracted from the leukocytes of the patients with fracture-related DVT, whose genotype and allele frequency distribution of *F11* gene rs2089252 and rs2036914 single nucleotide polymorphism were then assessed by a sequencing method. The activity of factor XI was measured by a solidification method in all participants, including those in control and normal groups.

Results: The activity of factor XI in patients with fracture-related DVT and *F11* rs2089252 CT was 1.16 times that of those with CC genotypes ($P < 0.0001$), whereas in patients with fracture-related DVT and *F11* rs2089252 TT genotypes it was 1.32 times that of those with CC genotypes ($P < 0.0001$), in patients with fracture-related DVT and *F11* rs2089252 T allele it was 1.24 times that of those with C allele ($P < 0.05$), in patients with fracture-related DVT and *F11* rs2036914 CC it was 1.35 times that of those with TT genotypes, in patients with fracture-related DVT and *F11* rs2036914 CT genotypes it was 1.12 times that of those with TT genotypes ($P < 0.05$), and in patients with fracture-related DVT *F11* and rs2036914 C allele it was 1.22 times that of those with T allele ($P < 0.05$). The activity of factor XI was significantly higher in the control than in the normal group ($P < 0.05$).

Conclusions: High activity of factor XI indicates a risk of occurrence of DVT in post-trauma patients with fractures. *F11* rs2089252 and rs2036914 (single nucleotide polymorphisms) are associated with activity of factors XI in such patients despite prophylaxis.

Key words: Deep vein thrombosis; Factor XI; Fractures; Polymorphism

Introduction

Deep venous thrombosis (DVT) has a high morbidity and mortality and is widely acknowledged as a complicated and multifactorial disorder in which both environmental and genetic factors have been implicated[1]. In most cases the mechanism(s) underlying development of DVT remain unclear because its pathogenesis is multifactorial. DVT is one of the commonest complications of fractures, its incidence ranging from 50% to 70% in trauma patients not receiving thromboprophylaxis[2–4]. Virchow's triad comprises venous stasis, endothelial injury and hypercoagulability: the three factors that are traditionally considered harbingers of development of venous thrombosis[5]. Venous stasis can be caused by limitation of activity, long-term immobility,

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insufficiency of venous valves and release of cytokines from damaged tissue[6].

Because of their prolonged rest and coagulation abnormalities, patients who sustain fractures are at high risk of developing DVT. Adequate thromboprophylaxis substantially reduces the rate of fracture-related DVT; the most studied and frequently used drug in prophylaxis of thrombosis is heparin[7]. Low molecular weight heparin (LMWH) is recognized as an effective method of anticoagulation and is widely used in hospitalized patients with fractures[8]. However, many such patients still develop DVT during the post-injury period, even when they have been receiving thromboprophylaxis[9–11].

The first genome-wide association study found an association between polymorphisms located in cytochrome P450 (CYP4V2 rs13146272) and DVT[12]. Subsequent genotyping of loci containing kallikrein B and factor XI (F11) genes, which are located near CYP4V2, identified a correlation between single nucleotide polymorphisms (SNPs) rs2289252 and rs2036914 in the F11 gene and DVT[13,14]. El-Galaly *et al.* replicated the genome-wide association study findings and found that the CC genotype for rs2036914 and the CT and TT genotypes for rs2289252 were associated with a significantly higher risk of venous thromboembolism.[15] *F11* rs2289252 is also associated with pregnancy-related venous thrombosis[15]. Another study found a significantly higher frequency of *F11* rs2289252 polymorphism in patients with DVT and further observed the tendency for *F11* rs2289252 to have higher odds ratios (*OR*) in patients with repeated episodes of DVT than in those with a single episode of DVT[16]. A recent study found an association between *F11* rs2289252 and rs2036914 and DVT in women, and furthermore found SNP–SNP interactions between F11 rs2289252 and ABO rs514659[17].

Because many patients with fractures routinely receive LMWH, it is essential to identify the mechanism(s) for failure of LMWH to prevent DVT in some of these patients. This current study aimed to: (i) investigate the association between F11 SNPs rs2289252, rs2036914 and plasma factor XI (FXI) clotting activity in patients with fractures despite receiving thromboprophylaxis; (ii) identify the risk factors associated with the occurrence of DVT in trauma patients to enable better targeting of specific thromboprophylaxis modalities; and (iii) provide additional resources for the patients who can benefit the most when anticoagulation fails to prevent venous thrombus embolism and reduce bleeding complications caused by excessive thromboprophylaxis.

Materials and Methods

Study Participants

DVT Group

Between April 2014 and October 2015, a case-control study involving 110 consecutive post-trauma patients with fractures was conducted at a general referral hospital in Tianjin, China. The inclusion criteria were as follows: (i) age >20 years; (ii) documented DVT in the leg or arm (with or without pulmonary embolism); and (iii) initial admission to our hospital. Patients were excluded if they: (i) had undergone cardiac surgery; (ii) had a former personal or family history of DVT; or (iii) were pregnant. No patient had isolated pulmonary embolism. The included patients had been examined by Doppler sonography before surgery to exclude DVT.

Control and Normal Groups

Forty sex- and age-matched patients with fractures but without DVT were chosen as controls. All patients in the control group received the same standardized antithrombotic prophylactic treatment with s.c. enoxaparin sodium as those in the DVT group. Forty sex- and age-matched healthy people attending our hospital for physical examination were chosen as the normal group.

Thus 190 patients were enrolled in this study: 110 with DVT, 40 matched controls and 40 normal subjects. Relevant patient characteristics were comparable in the DVT, control and normal groups: they were matched according to sex, age, weight and medications (Table 1). This study was approved by the Tianjin Hospital Medical Ethics Committee. All participants provided written informed consent. All patients in all groups were of Chinese origin.

DNA Extraction

Venous blood samples (2 mL) were drawn from all participants into Vacutainer tubes containing ethylenediaminetetraacetic acid disodium. Genomic DNA was extracted from leukocytes using a MagNA Pure LC 2.0 (Roche, Basel,

TABLE 1 Characteristics of the study subjects

Groups	Age (years, mean ± SD)	Sex: female [cases (%)]	Body mass index (kg/m^2, mean ± SD)
DVT group (110 cases)	43.5 ± 12.7	52 (47)	26.0 ± 14.5
Control group (40 cases)	44.2 ± 11.5	20 (50)	29.0 ± 13.2
Normal group (40 cases)	40.5 ± 13.6	20 (50)	27.0 ± 13.5
P value	NS	NS	NS

Note: NS, Not Significant.

TABLE 2 Nucleotide sequence of primers used for genotype screening

Alleles	Primer sequence	PCR product (bp)
F11 rs2289252	5′TGAGTCGTCTTCAGGTGCATAG3′ 3′TTTGCCTTGTTGTCGTGTATTT5′	346
F11 rs2036914	5′CGCCCACTCCGCATTATTA3′ 3′TCTCATAAAGGCAAGGCAAGTA5′	380

Note: bp, base pair.

Switzerland), according to the manufacturer's instruction and stored at −80 °C using standardized procedures.

F11 rs2289252 and rs2036914 Genotyping

Amplification of DNA was performed using two primers for each polymorphism, one forward and one reverse primer (Table 2). Polymerase chain reaction (PCR) amplifications were performed using 25 μL solution containing 80 ng of DNA template, 2 μL of 10× PCR buffer, 0.25 μL of Taq polymerase (Takral, Beijing, China), 2.5 mmol of dNTPs and 10 μmol of each primer. The PCR cycling conditions were 5 min at 95 °C followed by 35 cycles of 30 s at 95 °C, 30 s at 56 °C and 30 s at 72 °C and with a final step at 72 °C for 5 min to allow the complete extension of all PCR fragments. The PCR fragments of *F11* gene rs2089252 and rs2036914 single nucleotide polymorphism were then directly sequenced. The gene sequencing pattern is shown in Fig. 1.

Measurement of FXI Clotting Activity

FXI clotting activity was measured by one-stage clotting assay with FXI deficient plasma using a ACL 700 (Werfen, Bedford, MA, USA) according to the manufacturer's instructions[18].

Statistical Analysis

Continuous data are expressed as the mean ± standard deviation (SD). Categorical data are expressed with percentages. Statistical analysis was performed using SPSS software version 16.0 (SPSS, Chicago, IL, USA). $P < 0.05$ was considered significant.

Results

The associations between rs2289252 and rs2036914 genotype and FXI activity in the DVT group are shown in Table 3.

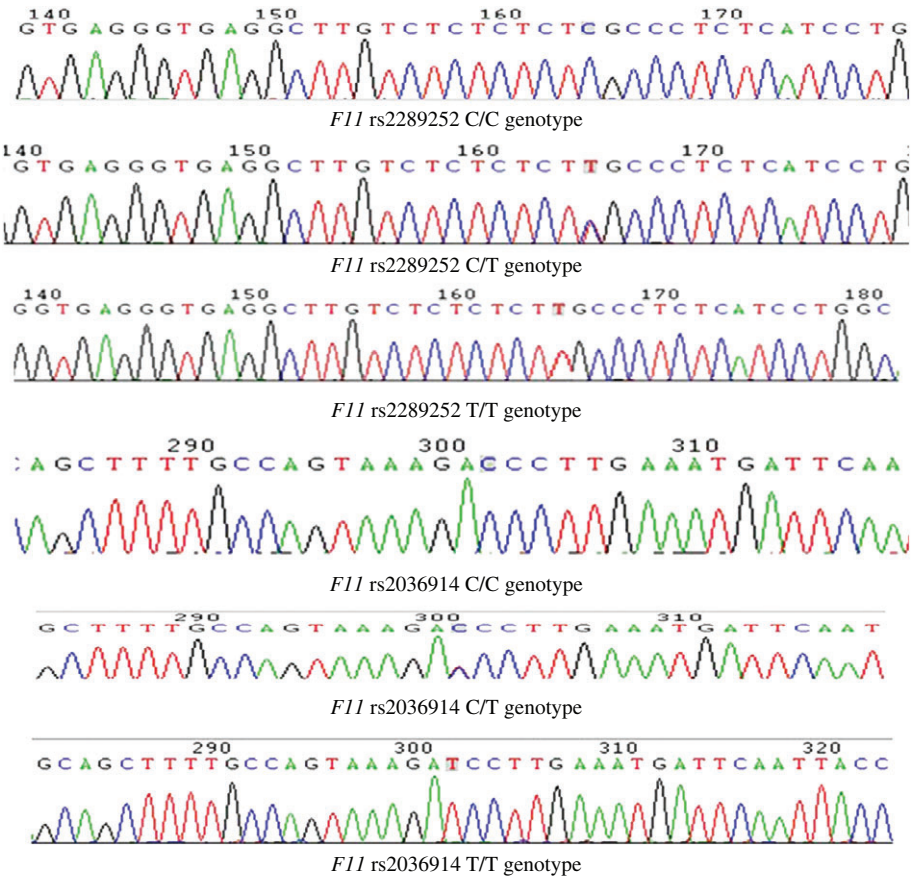

Fig. 1 The sequencing results of F11 rs2289252 and rs2036914 genotypes.

TABLE 3 Association between *F11* polymorphism and FXI activity in DVT group

F11 polymorphism	Genotype	Cases	Activity (mean ± SD, %)	*P* value
rs2289252	CC	76	118.4 ± 18.1	<0.0001
	CT	16	142.1 ± 13.1	
	TT	18	161.4 ± 17.1	
	C	92	122.6 ± 19.5	<0.0001
	T	34	152.3 ± 18.0	
rs2036914	CC	20	161.3 ± 17.1	<0.0001
	CT	14	133.0 ± 15.9	
	TT	76	119.6 ± 18.5	
	C	34	149.7 ± 21.6	<0.0001
	T	90	121.1 ± 18.7	
rs228925252TT+rs2036914CC		14	165.9 ± 16.7	<0.0001
rs228925252CC+rs2036914TT		66	116.8 ± 17.9	
rs2289252(TT+CT)+rs2036914(CC+CT)		24	158.2 ± 17.3	<0.0001
rs228925252CC+rs2036914TT		66	116.8 ± 17.9	

Activity of FXI in Control and Normal Groups

The activity of FXI was significantly higher in the control (108.10 ± 20.99) than in the normal group (86.28 ± 18.13, $P < 0.05$).

Association between rs2289252 Genotype and FXI Activity

In the DVT group, the prevalence of rs2289252 TT genotypes was 1.16 times that of CC genotypes ($P < 0.0001$). The activity of FXI in patients with fracture-related DVT with *F11* rs2089252 TT genotypes was 1.32 times that of those with CC genotypes ($P < 0.0001$) (Fig. 2A). Furthermore, FXI activity was 1.24 times greater in rs2289252 T carriers than in C carriers (Fig. 2B).

Association between rs2036914 Genotype and FXI Activity

In the DVT group, the prevalence of *F11* rs2036914 CC genotypes was 1.35 times that of TT genotypes. The activity of FXI in patients with fracture-related DVT with *F11* rs2036914 CT genotypes was 1.12 times that of those with TT genotypes ($P < 0.05$) (Fig. 2C). The activity of FXI in patients with fracture-related DVT and *F11* rs2036914 C allele was 1.22 times that of those with T allele ($P < 0.05$, Fig. 2D).

Association between rs2289252 and rs2036914 Genotype and FXI Activity

When we combined rs2289252 and rs2036914 genotypes, we found that the prevalence of rs2289252 TT and rs2036914 CC was 1.42 times that of rs2289252 CC and rs2036914 TT ($P < 0.0001$). The median FXI activity of patients with rs2289252 TT and rs2036914 CC was 165.9%, which is 1.35 times that of those with rs2289252 TT and rs2036914 CC ($P < 0.0001$) (Fig. 2E). The FXI activity of patients who were both rs2289252T carriers (TT, CT) and rs2036914C carriers (CC, CT) was 1.35 times that of those with rs2289252 CC and rs2036914 TT ($P < 0.0001$) (Fig. 2F).

Discussion

Among surgical patients, DVT is very common and can cause significant morbidity and mortality in patients with fractures. Most orthopedic surgeons are aware of the importance of preventing thrombosis in such patients. However, many of these patients develop DVT despite receiving thromboprophylaxis.

Optimization of antithrombotic management in trauma patients is important in their clinical management and therapy. However, intensifying anticoagulation is associated with a higher risk of bleeding. Therefore, more aggressive antithrombotic measures should be instituted only in patients at particularly high risk of DVT. Identification of such patients would provide evidence for targeting them with intensified antithrombotic measures and help trauma surgeons to balance the risks of bleeding and thromboembolic events.

To determine whether high FXI activity influences the risk of DVT in trauma patients who develop DVT despite prophylaxis, we measured the activity of plasma FXI in a DVT, control and normal group. We found that patients with fractures (DVT and control groups) had high FXI activity than normal subjects (normal group). This finding further supports the contention that the trauma of fracture is a risk factor for DVT and can lead to thrombus formation via a series of pathophysiological changes. We also found that trauma patients with DVT (DVT group) had higher FXI activity than those without DVT (control group) and that high activity of FXI seems to contribute to the occurrence of DVT in trauma patients despite LMWH prophylaxis. A lot of work had been done in the past 25 years to clarify the role of FXI in thrombin generation. FXI is a component of the intrinsic pathway of coagulation and involved in amplification of thrombin generation[19]. Meijers *et al.* found that high concentrations of FXI are a risk factor for DVT and that there is a dose–response relationship between FXI concentration and the risk of DVT[20]. Data from FXI-deficient animal models indicate that FXI deficiency results in a relatively

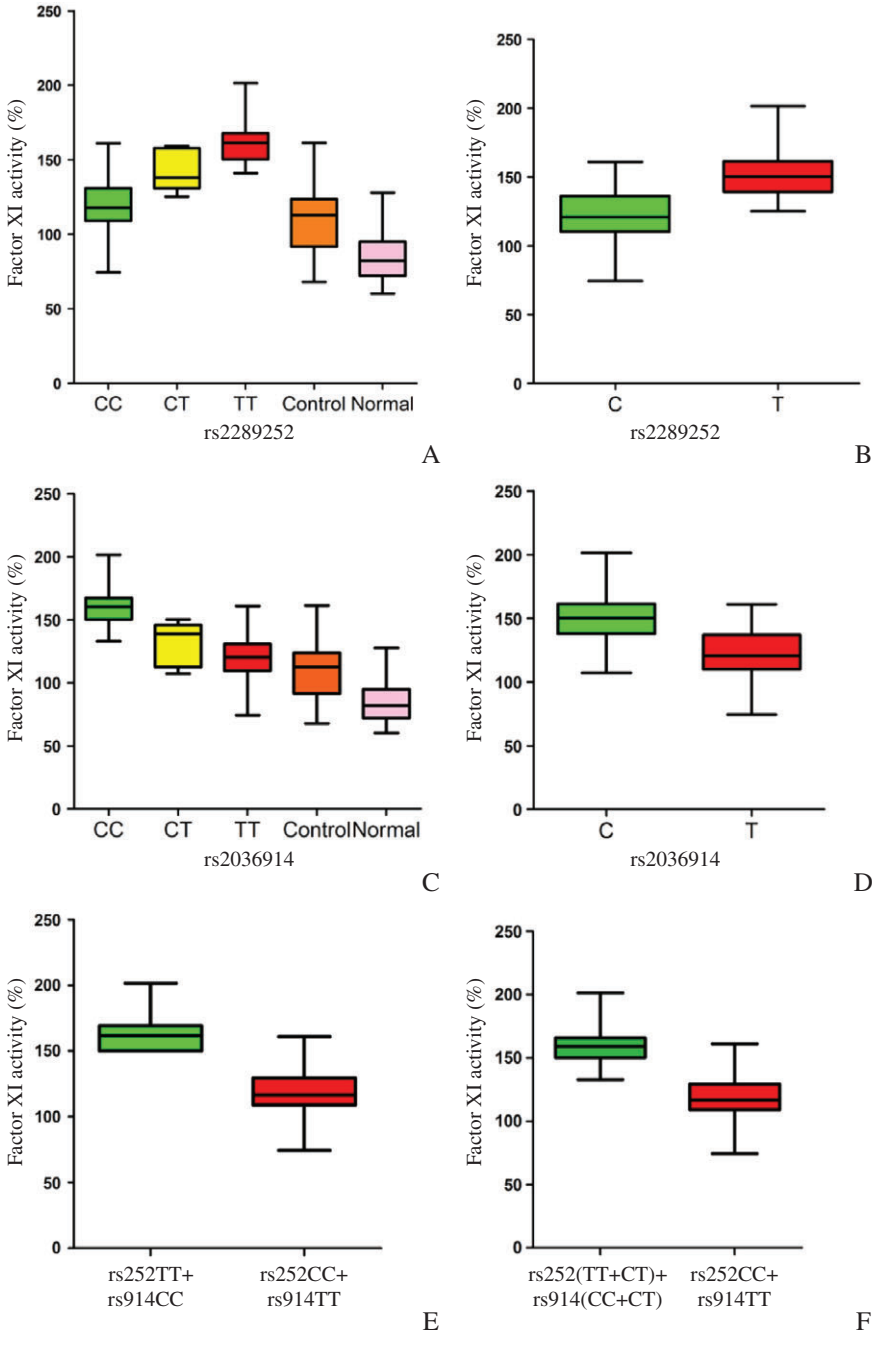

Fig. 2 Association of FXI activity (%) with combined genotypes and allele of rs2289252 and rs2036914 in the *F11* gene. Association of FXI activity (%) with (A) genotypes of rs2289252 in the *F11* gene; (B) allele of rs2289252 in the *F11* gene; (C) genotypes of rs2036914 in the *F11* gene; (D) allele of rs2036914 in the *F11* gene; (E) combined allele of rs2289252 and rs2036914 in the *F11* gene rs252: rs2289252, rs914: rs2036914; and (F) combined genotypes of rs2289252 and rs2036914 in the *F11* gene rs252: rs2289252, rs914: rs2036914.

mild bleeding disorder[21]. A recent study demonstrated that FXI contributes to postoperative thrombosis and that reducing FXI concentrations in patients undergoing total knee arthroplasty helped to prevent DVT and appeared to be safe, doing so without increasing bleeding[22]. These findings support the concept that inhibition of FXI may serve as a safe, specific, novel therapeutic approach to thrombosis treatment and prevention[23]. Our results suggest that identification of trauma patients with high FXI activity can help orthopedic surgeons to choose suitable means of thromboprophylaxis; inhibition of FXI activity in trauma patients may be a promising antithrombotic strategy in the future.

In this study, we also investigated the association of *F11* gene rs2089252 and rs2036914 SNPs with plasma FXI activity and their contribution to risk of thrombosis. We genotyped all patients in the DVT group for these two SNPs. Several recent studies have reported an association between these two SNPs and DVT[24,25]. However, there are currently few published data that focus on these two SNPs and their association with FXI activity and failure of

prophylactic anticoagulation in trauma patients with fractures. Bezemer *et al.* and Li *et al.* found that rs2289252 and rs2036914 are associated with FXI plasma antigen concentrations in subjects who have undergone Molecular Evolutionary Genetics Analysis[12,14]. *F11* rs2089252 is reportedly associated with increased FXI activity in Italian women with thrombosis independent of age[13]. We hypothesized that these two SNPs in *F11* are associated with FXI activity in trauma patients regardless of LMWH prophylaxis. Our results indicate that these two SNPs may influence the risk of trauma-related DVT by modulating FXI activity. Furthermore, we found that rs2289252 T and rs2036914 C alleles are predictors of higher FXI activity, which is consistent with the findings of previous studies. When we combined rs2289252 and rs2036914 SNPs, we found that rs2289252 TT and rs2036914 CC homozygosity predicted high FXI activity. It thus seems that these two SNPs have a combined effect.

Our study has several limitations: (i) Many factors increase the risk of DVT despite prophylaxis, such as type of intervention, fixation device and surgeon. (ii) We genotyped only patients in the DVT group and our study was relatively small because if the high cost and insufficiency of financial support.

This study showed that high FXI activity is a risk factor for DVT in patients with fractures despite thromboprophylaxis. Additionally, *F11* SNPs rs2289252 and rs2036914 polymorphism is associated with FXI activity, which further supports the possibility of a relationship between genotypes and thrombotic status-associated factors contributing to risk of thrombosis. However, our findings need to be further confirmed by more studies with higher accuracy.

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