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Original Article

Coagulation factor XII gene C46T polymorphism and risk of cerebral hemorrhage in a Chinese population

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Abstract: Factor XII (F12) plays a complex role in the coagulation system. T allele of F12 C46T polymorphism was shown to lead to reduced F12 plasma levels in an allele-dose-dependent manner. This functional F12 C46T polymorphism was probably a risk factor for ischemic stroke. We tested whether this genetic variant is associated with the risk for cerebral hemorrhage (CH). We performed a case-control study including 195 patients with CH and 116 healthy population controls, which were all of southern Han-Chinese origin. The F12 C46T genotype was assessed using Touchdown PCR and Multiplex SNaPshot analysis. No statistically significant differences were found in the allele or genotype distributions of the F12 C46T polymorphism among CH patients and control subjects, even after adjusting for different confounding variables ($P > 0.05$). Our results demonstrated that the F12 C46T polymorphism has no major role in genetic susceptibility to cerebral hemorrhage. The highest T allele distribution in our population among different populations increased the reliability of this conclusion.

Keywords: Cerebral hemorrhage, coagulation factor XII, C46T polymorphism, case-control study

Introduction

Stroke is the second leading cause of death worldwide. Studies showed that from 1990 to 2010, the incidence of stroke has increased by 12% in low-income and middle-income countries [1]. Unlike only 10% are cerebral hemorrhage (CH) of all strokes in the United States [2], CH accounts for 17.1-55.4% of all cases of stroke in China [3, 4]. This makes it important to further understand the causes of CH. Now, stroke is wildly regarded as a complex disease that involves genetic and environmental factors [5, 6].

Factor XII (F12) plays a complex role in the coagulation system, because it initiates both the procoagulatory cascade and the fibrinolytic pathways [7]. A common variant in the F12 gene (C46T, rs1801020) results in decreased plasma levels of F12 [8]. Moreover, an allele-dose-dependent manner was found between carriers of the T allele of F12 C46T polymorphism and reduced F12 plasma levels [9, 10]. Association studies have shown the relationship of F12 levels and the C46T polymorphism in the development of ischemic stroke, but the results are controversial [11-14]. Recent reports indicate that ischemic and hemorrhagic stroke have a shared genetic basis [5, 6]. However to our knowledge, no study has explored the association between CH and F12 C46T polymorphism.

We therefore tested the hypothesis that the F12 C46T polymorphism may be associated with the risk of CH.

Materials and methods

Study population

The study population and subject characteristics were previously described [15]. This is an ongoing molecular epidemiologic study of CH conducted in Changsha, Hunan, China and the subject recruitment was approved by the Ethics Committee of Xiangya Hospital, Central South University. Briefly, all subjects were genetically

Table 1. Basic information and clinic features in CH patients and controls

	CH patients (n = 195)	Controls (n = 116)
Mean age, y (SD)	58.40±11.21	54.97±10.41
Male, no. (%)	133 (68)	75 (65)
BMI, mean (SD)	24.38±4.01	23.41±4.25
Hypertension, no. (%)	97 (50)[a]	26 (22)
Ischemic heart disease, no. (%)	19 (10)[a]	0 (0)
Diabetes mellitus, no. (%)	15 (8)[a]	0 (0)
Alcohol consumption, no. (%)	57 (29)[a]	15 (13)
Smoking, no. (%)	18 (10)	7 (7)
TC, mmol/L (SD)	2.02±4.89	1.65±1.60
TG, mmol/L (SD)	4.53±1.04[a]	4.83±1.16
LDL, mmol/L (SD)	2.45±0.86	2.46±0.87

Notes: [a]$P < 0.05$, considered to be statistic significant. [b]BMI indicates body mass index, TC, total cholesterol; TG, triglycerides; LDL, low density lipoprotein.

unrelated southern Han Chinese. Submission of the individuals to the study was conditioned by an obtained written informed consent form. Blood samples were collected from 195 CH patients seen in the department of Neurology, Xiangya Hospital, Central South University. During the same time, 116 unrelated healthy subjects who entered the hospital for health check-ups were enrolled as the control group and matched with CH patients for age and gender. Brain CT and/or MRI were performed in all patients. Patients with CH related to trauma, neoplasms, coagulation disorders or thrombolytic therapy, aneurysms, or other vascular malformations were ruled out. Controls had no symptoms or history of stroke, coronary artery disease, autoimmune disease, peripheral atherosclerotic disease, or hematologic disease. All participants were interviewed using a structured questionnaire to obtain information on demographic factors and classical stroke risk characteristics.

Genotyping

Genomic DNA was extracted from a peripheral whole blood sample from each subject. Touchdown PCR and Multiplex SNaPshot analysis were performed to determine the genotype of the C46T polymorphism of F12 gene. The 227 base pair (bp) fragment encompassing the C to T polymorphic site in F12 region was amplified using specific primers 5'-CCCACCCACAAACTCCCAACT-3' and 5'-ACCAGCAGGAACCCCAGGAG-3'. The extension primer used for SNaPshot of F12 gene C46T polymorphism is 5'-TTTTTTTTTTTTTTTTTTTTTTTTAGCTGGACC AACGGACGGA-3'. SNaPshot reactions were performed as illustrated by the producer (Applied Biosystems Co., USA). The mixture of 15 μl of touchdown PCR product, 1 U shrimp alkaline phosphatase (SAP) and 1 U Exonuclease I (ExoI) was incubated at 37°C for 1 hour. After a 15-min incubation at 75°C to inactivate the enzyme, 2 μl of digested PCR product was mixed with 5 μl of prepared reaction premix, 1 μl of primers mixture, and 2 μl of ultrapure water. The following PCR cycling conditions were used: 96°C for 1 min, followed by 28 cycles of 96°C for 10 s, 50°C for 5 s and 60°C for 30 s, with a final extension at 60°C for 1 min. When finished, 1 U SAP was added and the reaction mixture was incubated at 37°C for 1 hour, and then inactivated at 75°C for 15 min. Before loading onto the ABI3130XL (Applied Biosystems Co., USA), 0.5 μl of Liz120 SIZE STANDARD and 9 μl of Hi-Di was added to 0.5 μl of reaction mixture and samples were heated to 95°C for 5 min. To ensure quality control, genotyping was performed without knowledge of the subjects' case/control status and five randomly-selected samples of each genotype were genotyped twice by different people. The genotyping rates for the C46T polymorphism was 98.3%, and the reproducibility was 100%.

Statistical analysis

Data analysis was performed by the computer software Statistical Package for Social Sciences (SPSS) for Windows (version 11.5). The Hardy-Weinberg equilibrium (HWE) was assessed using χ^2 test. χ^2 test was also used to determine whether there was any significant difference in allele and genotype frequencies between CH patients and controls. The association between F12 gene C46T genotypes and the risk of CH were estimated by computing the odd ratios (OR) and 95% confidence intervals (CI) from logistic regression analysis. Probability levels less than 0.05 were used as a criterion of significance.

Results

General characteristics of the subjects

A total of 311 Chinese subjects were enrolled in this study. No significant difference was found

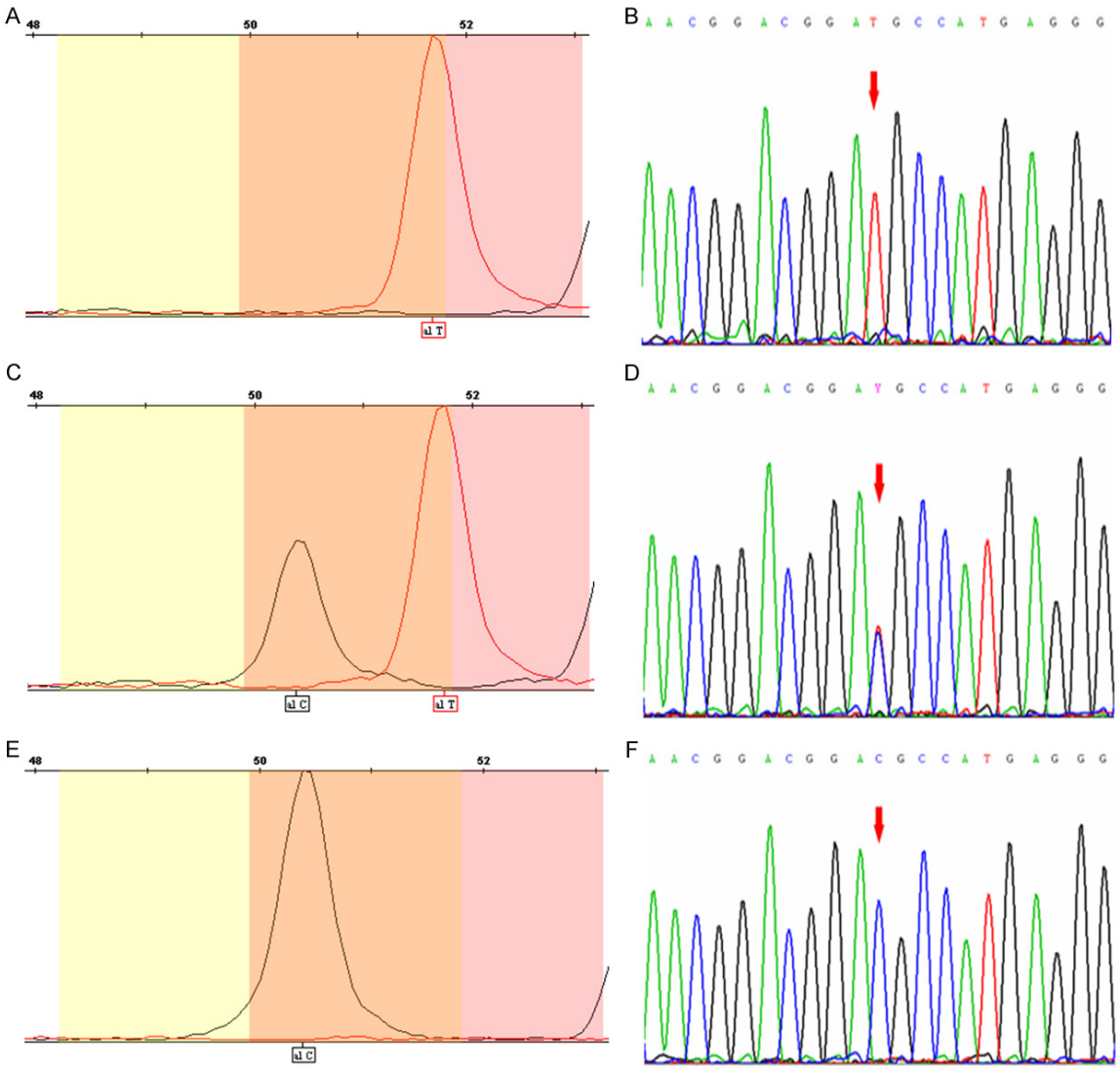

Figure 1. Genotyping pictures and forward sequencing graphs of F12 C46T polymorphism. Note: A and B are for TT genotype; C and D are for TC genotype; E and F are for CC genotype of F12 C46T polymorphism.

between CH patients and controls with regard to age and sex. Similarly, there were no significant differences in body mass index, smoking status, serum total cholesterol level and serum low-density lipoprotein level between case and control group. But, there were more subjects with hypertension, ischemic heart disease, diabetes mellitus, alcohol consumption in case patients compared with control subjects. The serum triglycerides level in CH patients was lower than that in controls (**Table 1**).

Genotype frequency distribution of F12 C46T polymorphism

Genotyping pictures and forward sequencing graphs of F12 C46T polymorphism are shown in **Figure 1**. The frequency distributions of the different genotypes for F12 C46T polymorphism are shown in **Table 2**. The genotypic frequencies were in Hardy- Weinberg equilibrium. The allelic and genotypic frequencies of case subjects were not significantly different from those of the controls ($P > 0.05$).

In addition, we analyzed the allele distribution of F12 C46T polymorphism between populations in this study and other areas (data from the National Center for Biotechnology Information, http://www.ncbi.nlm.nih.gov/projects/SNP, chosen from the largest population) (**Table 3**). It shows that there are significant differences between the southern Han-Chinese population in this study and most other populations:

Table 2. Alleles and genotype frequency distribution of the F12 C46T polymorphism among cases and controls and their associations with cerebral hemorrhage risk

	Patients, n (%)	Controls, n (%)	*p* value (2 d.f.)	Logistic Regression			
				Crude OR (95% CI)	Crude *p* value	Adjusted OR (95% CI)	Adjusted *p* value
Allele frequency							
T	298 (76.8)	175 (76.8)	0.989	1.00 (Reference)		1.00 (Reference)	
C	90 (23.2)	53 (23.2)		1.003 (0.681-1.477)	0.989	1.005 (0.678-1.479)	0.987
General genotype							
TT	121 (62.4)	66 (57.9)	0.438	1.00 (Reference)		1.00 (Reference)	
TC	56 (28.9)	43 (37.7)		0.539 (0.190-1.527)	0.245	0.536 (0.192-1.530)	0.243
CC	17 (8.8)	5 (4.4)		0.383 (0.131-1.121)	0.080	0.381 (0.128-1.123)	0.077

Logistic regression adjusted for hypertension, ischemic heart disease, diabetes mellitus, alcohol intake, and serum level of triglycerides. d. f. = degrees of freedom, OR = odds ratio, CI = confidence interval.

Table 3. Comparison of allele frequencies of the F12 C46T polymorphism between populations in this study and in other ethnics

Population	n	T	C	χ^2	*p* value
Ours	228	76.8	23.2		
HCB	90	61.1	38.9	5.984	0.014
JPT	172	69.8	30.2	1.258	0.262
European	226	18.6	81.4	67.388	0.000
African American	46	52.2	47.8	13.648	0.000
Sub-Saharan African	226	58.0	42.0	8.228	0.004

Reported data from the National Center for Biotechnology Information, chosen from the largest population, HCB = Han Chinese background, JPT = Japanese from Tokyo, Japan.

Discussion

F12 is an 80 kDa protein of the serine-protease family which initiates the coagulation cascade by the intrinsic pathway and also participates in the kinine activation system [16, 17]. F12 used to be considered to have little to no effect on coagulation in vivo [18]. But recent studies suggested that F12 deficiency prevents pathological thrombus formation, while not affect regular hemostasis [11, 19]. F12 is then thought to be "a neglected player in stroke pathophysiology" [11].

Stroke is a complex disease caused by a combination of genetic and environmental factors [5, 6]. F12 gene is located on 5q33-qter which comprises 13 introns and 14 exons [20, 21]. Although several polymorphisms in the F12 gene have been found to be associated with F12 deficiency, the association of C46T polymorphism and ischemic stroke is of special interest [8, 22-26]. This functional C46T polymorphism has been known to influence F12 levels and increase the risk of several thrombosis diseases because it causes a new start codon (ATG) for the transcription of the mRNA and a frame shift that produces a truncated protein [8, 27, 28]. But the results of studies on the relationship of the C46T polymorphism and F12 levels with the risk of ischemic stroke are still controversial [11-14]. Contrary to our expectation, the present case-control study in southern Han-Chinese population showed for the first time that no significant association appeared between risk of CH and F12 C46T polymorphism in overall statistical analyses.

Han Chinese background (HCB), European, African American and Sub-Saharan African. The only population shares the same allele distribution with ours is the Japanese from Tokyo, Japan (JPT).

F12 C46T polymorphism and risk of cerebral hemorrhage

To evaluate the risk of CH according to the F12 C46T polymorphism genotype, logistic regression analysis was conducted (**Table 2**). Using the TT genotype as reference, TC genotype and CC genotype both decreased nearly half of the CH risk (OR = 0.539, 95% CI: 0.190-1.527, *P* = 0.245; OR = 0.383, 95% CI: 0.131-1.121, *P* = 0.080, respectively) although neither association reached statistical significance. These results remained not significant after adjusting for different confounding variables (adjusted OR = 0.536, 95% CI: 0.192-1.530, *P* = 0.243; adjusted OR = 0.381, 95% CI: 0.128-1.123, *P* = 0.077, respectively).

Our findings also added evidences to the complex ethnic or geographic differences of the F12 C46T polymorphism. For instance, according to the National Center for Biotechnology Information database, T allele frequency of F12 C46T polymorphism among the different ethnicities is as follows: 0.186 in Caucasians, 0.522/0.580 in Africans, 0.611 in Chinese and 0.698 in Japanese. This study found that the T allele frequency of F12 C46T polymorphism was 0.768 among our southern Han-Chinese population, only similar to the reported allele frequencies in Japanese, even not similar to the reported Chinese data from different districts of China. Since the allele-dose-dependent manner was found in carriers of the T allele of F12 C46T polymorphism to reduce F12 plasma levels [9, 10], the highest T allele distribution in our population in turn increased the reliability of our conclusion.

Our study has several limitations. First, our sample size was not large enough. Second, we did not include F12 haplotype or gene-gene interaction in our study design. Considering these additional effects would promise further power implications.

In summary, our results indicated that the functional F12 C46T polymorphism is unlikely to be a major CH susceptibility gene locus in the studied population. The highest T allele distribution in our population among different populations increased the reliability of this conclusion. However, further independent studies are needed to validate our findings and to better understand the association of F12 C46T polymorphism and CH susceptibility.

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Disclosure of conflict of interest

None.

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References

[1] Feigin VL, Forouzanfar MH, Krishnamurthi R, Mensah GA, Connor M, Bennett DA, Moran AE, Sacco RL, Anderson L, Truelsen T, O'Donnell M, Venketasubramanian N, Barker-Collo S, Lawes CM, Wang W, Shinohara Y, Witt E, Ezzati M, Naghavi M and Murray C. Global and regional burden of stroke during 1990-2010: findings from the Global Burden of Disease Study 2010. Lancet 2014; 383: 245-254.

[2] Go AS, Mozaffarian D, Roger VL, Benjamin EJ, Berry JD, Blaha MJ, Dai S, Ford ES, Fox CS, Franco S, Fullerton HJ, Gillespie C, Hailpern SM, Heit JA, Howard VJ, Huffman MD, Judd SE, Kissela BM, Kittner SJ, Lackland DT, Lichtman JH, Lisabeth LD, Mackey RH, Magid DJ, Marcus GM, Marelli A, Matchar DB, McGuire DK, Mohler ER 3rd, Moy CS, Mussolino ME, Neumar RW, Nichol G, Pandey DK, Paynter NP, Reeves MJ, Sorlie PD, Stein J, Towfighi A, Turan TN, Virani SS, Wong ND, Woo D, Turner MB; American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Heart disease and stroke statistics--2014 update: a report from the American Heart Association. Circulation 2014; 129: e28-e292.

[3] Yang QD, Niu Q, Zhou YH, Liu YH, Xu HW, Gu WP, Tian FF, Xie YQ, Zhang L and Xia J. Incidence of cerebral hemorrhage in the Changsha community. A prospective study from 1986 to 2000. Cerebrovasc Dis 2004; 17: 303-313.

[4] Zhang LF, Yang J, Hong Z, Yuan GG, Zhou BF, Zhao LC, Huang YN, Chen J and Wu YF. Proportion of different subtypes of stroke in China. Stroke 2003; 34: 2091-2096.

[5] Bersano A, Ballabio E, Bresolin N and Candelise L. Genetic polymorphisms for the study of multifactorial stroke. Hum Mutat 2008; 29: 776-795.

[6] Hademenos GJ, Alberts MJ, Awad I, Mayberg M, Shepard T, Jagoda A, Latchaw RE, Todd HW, Viste K, Starke R, Girgus MS, Marler J, Emr M and Hart N. Advances in the genetics of cerebrovascular disease and stroke. Neurology 2001; 56: 997-1008.

[7] Colman RW and Schmaier AH. Contact system: a vascular biology modulator with anticoagulant, profibrinolytic, antiadhesive, and proinflammatory attributes. Blood 1997; 90: 3819-3843.

[8] Kanaji T, Okamura T, Osaki K, Kuroiwa M, Shimoda K, Hamasaki N and Niho Y. A common genetic polymorphism (46 C to T substitution)

in the 5'-untranslated region of the coagulation factor XII gene is associated with low translation efficiency and decrease in plasma factor XII level. Blood 1998; 91: 2010-2014.
[9] Bertina RM, Poort SR, Vos HL and Rosendaal FR. The 46C-->T polymorphism in the factor XII gene (F12) and the risk of venous thrombosis. J Thromb Haemost 2005; 3: 597-599.
[10] Endler G, Exner M, Mannhalter C, Meier S, Ruzicka K, Handler S, Panzer S, Wagner O and Quehenberger P. A common C-->T polymorphism at nt 46 in the promoter region of coagulation factor XII is associated with decreased factor XII activity. Thromb Res 2001; 101: 255-260.
[11] Pham M, Stoll G, Nieswandt B, Bendszus M and Kleinschnitz C. Blood coagulation factor XII--a neglected player in stroke pathophysiology. J Mol Med (Berl) 2012; 90: 119-126.
[12] Santamaria A, Mateo J, Tirado I, Oliver A, Belvis R, Marti-Fabregas J, Felices R, Soria JM, Souto JC and Fontcuberta J. Homozygosity of the T allele of the 46 C->T polymorphism in the F12 gene is a risk factor for ischemic stroke in the Spanish population. Stroke 2004; 35: 1795-1799.
[13] Reiner AP, Siscovick DS and Rosendaal FR. Hemostatic risk factors and arterial thrombotic disease. Thromb Haemost 2001; 85: 584-595.
[14] Oguchi S, Ito D, Murata M, Yoshida T, Tanahashi N, Fukuuchi Y, Ikeda Y and Watanabe K. Genotype distribution of the 46C/T polymorphism of coagulation factor XII in the Japanese population: absence of its association with ischemic cerebrovascular disease. Thromb Haemost 2000; 83: 178-179.
[15] Zeng Y, Zhang L, Hu Z, Yang Q, Ma M, Liu B, Xia J, Xu H, Liu Y and Du X. Fibrinogen polymorphisms associated with sporadic cerebral hemorrhage in a Chinese population. J Clin Neurosci 2012; 19: 753-756.
[16] Furie B and Furie BC. The molecular basis of blood coagulation. Cell 1988; 53: 505-518.
[17] Griffin JH. Role of surface in surface-dependent activation of Hageman factor (blood coagulation factor XII). Proc Natl Acad Sci U S A 1978; 75: 1998-2002.
[18] Renne T, Schmaier AH, Nickel KF, Blomback M and Maas C. In vivo roles of factor XII. Blood 2012; 120: 4296-4303.
[19] Renne T, Pozgajova M, Gruner S, Schuh K, Pauer HU, Burfeind P, Gailani D and Nieswandt B. Defective thrombus formation in mice lacking coagulation factor XII. J Exp Med 2005; 202: 271-281.
[20] Cool DE and MacGillivray RT. Characterization of the human blood coagulation factor XII gene. Intron/exon gene organization and analysis of the 5'-flanking region. J Biol Chem 1987; 262: 13662-13673.
[21] Royle NJ, Nigli M, Cool D, MacGillivray RT and Hamerton JL. Structural gene encoding human factor XII is located at 5q33-qter. Somat Cell Mol Genet 1988; 14: 217-221.
[22] Oguchi S, Ishii K, Moriki T, Takeshita E, Murata M, Ikeda Y and Watanabe K. Factor XII Shizuoka, a novel mutation (Ala392Thr) identified and characterized in a patient with congenital coagulation factor XII deficiency. Thromb Res 2005; 115: 191-197.
[23] Kondo S, Tokunaga F, Kawano S, Oono Y, Kumagai S and Koide T. Factor XII Tenri, a novel cross-reacting material negative factor XII deficiency, occurs through a proteasome-mediated degradation. Blood 1999; 93: 4300-4308.
[24] Ishii K, Oguchi S, Moriki T, Yatabe Y, Takeshita E, Murata M, Ikeda Y and Watanabe K. Genetic analyses and expression studies identified a novel mutation (W486C) as a molecular basis of congenital coagulation factor XII deficiency. Blood Coagul Fibrin 2004; 15: 367-373.
[25] Kanaji T, Kanaji S, Osaki K, Kuroiwa M, Sakaguchi M, Mihara K, Niho Y and Okamura T. Identification and characterization of two novel mutations (Q421 K and R123P) in congenital factor XII deficiency. Thromb Haemost 2001; 86: 1409-1415.
[26] Mordillo C, Martinez-Marchan E, Fontcuberta J and Soria JM. Molecular analysis of multiple genetic variants in Spanish FXII-deficient families. Haematologica 2007; 92: 1569-1572.
[27] Girolami A, Sartori MT, Lombardi AM and Pellati D. Rebuttal: factor XII levels, factor XII 46 C>T polymorphism and venous thrombosis: a word of caution is needed. Thromb Haemost 2004; 92: 892-893; author reply 894-895.
[28] Calafell F, Almasy L, Sabater-Lleal M, Buil A, Mordillo C, Ramirez-Soriano A, Sikora M, Souto JC, Blangero J, Fontcuberta J and Soria JM. Sequence variation and genetic evolution at the human F12 locus: mapping quantitative trait nucleotides that influence FXII plasma levels. Hum Mol Genet 2010; 19: 517-525.