Environmental Mutagens & Carcinogens 21-2 : 106-112 (2001)

Association between Genetic Variation in the Human Factor VII Gene and Essential Hypertension in Korean Population

Jung Hee Shin, Byung Yong Kang[1], Kyung Ho Lee, Chung Choo Lee and Ki Tae Kim[1]*

School of Biological Sciences, Seoul National University, Seoul 151-742, Korea;
[1]Seoulin Bioscience Institute, Seoulin Bioscience, Co., Ltd, Seoul 134-030, Korea

(Received November 12, 2001 / Accepted December 10, 2001)

ABSTRACT **: In view of the effect of factor VII as a risk factor for essential hypertension, we investigated the length (I/D) polymorphism at position 323 promoter region and exon 8-*Msp* I RFLP of the human factor VII gene in the Korean patients with essential hypertension and normal controls. There were no significant differences in the allele, genotype and haplotype frequencies of these polymorphisms between normotensive and essential hypertensive subjects. The significant linkage disequilibrium was however, detected between two polymorphic sites. The *Msp* I RFLP and I/D polymorphism were also significantly associated with plasma triglyceride (TG) levels. Therefore, our results suggest that the significant association between two genetic variations in the human factor VII gene and plasma TG level may reflect the potential role of human factor VII gene as one of the genetic components for cardiovascular risk.**

Keywords : Essential hypertension, Factor VII and RFLP

Introduction

As well known as, essential hypertension is defined as high blood pressure in which secondary causes such as renovascular disease, renal failure, pheochromocytoma, aldosteronism, or other causes of secondary hypertension or mendelian forms (monogenic) are not present. Essential hypertension accounts for 95% of all cases of hypertension. Also, it affects approximately 20% of the adult population, and is an insidious disease in which afflicted person from myocardial infranction and stroke. Also, many factors contribute to the development of essential hypertension, including environments, daily stress and genetics.

The main task in essential hypertension research is to explain genetic causes of raised blood pressure. Among the different strategies that have been put to trial to identify quantitative trait loci involved in such complex clinical phenotypes, association(case-control) studies using candidate genes represent increasingly preferred methods(Lander *et al.*, 1994; Weeks *et al.*, 1995; Risch *et al.*, 1996; Lander, 1996).

Factor VII is a vitamine K-depedent coagulation factor that circulates in the blood as an inactivate zymogen and is activated during coagulation through cleavage by Factors IXa, Xa and XIIa. Factor VII is synthesized in the liver and secreted as a single-chain glycoprotein of molecular weight 48,000 (Broze *et al.*, 1980). It is subject to positive feedback regulation, at the level of transcription, by prothrombin fragment F1, itself the product of active prothrombin by Factor X (Mitropoulos & Esnouf, 1990). The gene for factor VII is on chromosome 13 and has five polymorphic sites which account for up to 30% of the variance in Factor VII levels in plasma(Green *et al.*, 1991; Marchetti *et al.*, 1993; Bernardi *et al.*, 1996). In the promoter, a decanuceotide insertion at position 323 is in strong linkage disequlibrium with *Msp* I RFLP in exon 8 (Green *et al.*, 1991).

In view of the important role of factor VII thrombosis and the extensive range of mutations causing these changes, it is important to investigate the association between essential hypertension and common polymorphisms of the factor VII gene. The present study examined a possible role of the two polymorphisms, insertion/deletion polymorphism of the promoter region and *Msp* I RFLP of exon 8, in the factor VII gene in Korean patients with essential hypertension and normal controls.

Materials and Methods

Study subjects

A total of 191 unrelated individuals were randomly chosen from the Seoul Hygiene Hospital, Seoul, Korea. We studied 90 subjects with essential hypertension. Patients

*To whom correspondence should be addressed

were classified as having essential hypertension if they had systolic blood pressures above 140 mmHg and diastolic blood pressure above 90 mmHg on at least three separate occasions, and had no clinical signs, symptoms and laboratory findings suggestive of secondary hypertension. In additon, a randomly selected normal population (101 individuals) was analysed as the control groups (blood pressure value, <140/90 mmHg). The clinical data was considered with references to determine the association between the genotypes of polymorphic sites and plasma lipid levels.

Determination of plasma lipid levels

Blood samples were obtained in EDTA tubes from individuals who had been fasting for 12-16 hr. Concentration of plasma total cholesterol (TC) and triglyceride were measured by enzymatic colorimetry methods with commercial kit (Boehringer Mannheim, Germany) and chemistry analyzer. HDL-cholesterol was determined by measuring cholesterol in the supernatant after precipitation of the plasma with $MgCl_2$ and dextran sulfate followed by automated analysis with a Gilford Impact 400E automated analyzer with reagents and calibrators from Boehringer Mannheim. LDL-cholesterol level was calculated by using the formular of Freidwald *et al.*, (1972)

DNA analysis

Blood samples obtained after isolation and determination of lipid profiles were collected in EDTA-containing tubes and centrifuged at 1,500×g for 10 min. Genomic DNA was isolated from buffy coat by the method of Sambrook *et al.*, (1989) with slight modification. Polymerase Chain Reaction (PCR) techniques were used for insertion/deletion (I/D) polymorphism and *Msp* I PFLP of factor VII gene (Heywood *et al.*, 1996). Briefly, total 50 μl of the reaction mixture contained 200-400 ng of genomic DNA, 100 ng of each primer, 200 μl of each dNTP, 2.5 U of *Taq* DNA polymerase and optimal buffers recommended by the manufacturer. The sequences of the primers for two polymorphisms studied were: I/D polymorphism; sense, 5'-GAGCGGACGGTTTTGTTGCCAGCG-3' and nonsense, 5'-GGCCTGGTCTGGAGGCTCTCTTC-3'; (b) *Msp* I RFLP; sense 5'-GGGAGACTCCCCAAATATCAC-3' and nonsense, 5'-ACGCAGCCTTGGCTTTCTCTC-3'. Amplification was carried out with automated thermocycler: The PCR reaction for the promoter polymorphism proceeded for 30 cycles, each with a denaturation step for 1 min at 94°C, annealing for 1 min at 71°C, and extension for 1 min at 72°C. The reaction proceed for *Msp* I RFLP, one cycle at 94°C for 7 min, 34 cycles at 94°C for 1 min, at 59°C for 1 min and at 72°C for 1 min with a final polymerization at 72°C for 10 min. Ten μl of PCR product of the exon 8 polymorphism was restriction-digested overnight with 5 U of *Msp* I at 37 °C. Undigested amplified product for the promoter region and digested product for exon 8 locus were size-fractionated after 2% agarose gel electrophoresis with TBE buffer for 40 min along with molecular markers. Ethidium bromide was incorporated into the gel. The gels were directly photographed on an UV transilluminator and genotyped. In the case of I/D polymorphism, DNA

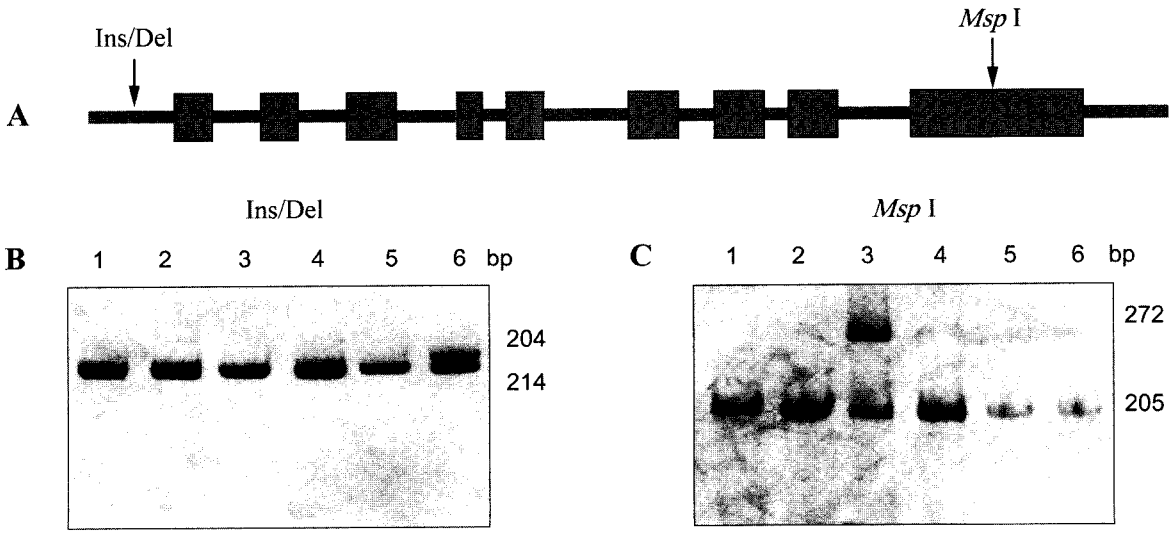

Fig. 1. Schematic diagram of the factor VII gene to show the polymorphisms within the gene. A) Restriction map of factor VII gene. B) Patterns of Ins/Del polymorphism at the factor VII gene. Lane 1~5, Del/Del genotypes; Lane 6, Ins/Del genotype. C) Patterns of *Msp* I RFLP at the factor VII gene. Lane 1, 2 and 4~6, M2M2 genotypes; Lane 3, M1M2 genotypes.

fragments were visualized yielding a band of 204 bp (allele D) in the absence of the insertion, and 214 bp (allele I) in its presence. *Msp* I RFLP yielded a constant band of 40 bp, and two bands of 205 bp and 67 bp in the presence of the cutting sites (allele M2), and only one band of 272 bp in the absence of the cutting sites (allele M1) (Fig. 1).

Statistical Analysis

Allelic frequencies were estimated by the gene counting method. Deviation in genotype distribution from that expected for Hardy-Weinberg equilibrium was estimated by χ^2-fitness test. The heterozygosity and polymorphism information content (PIC) was estimated by the methods of Bostein *et al.*, 1980. The significance of differences in allele frequencies between populations was also estimated by χ^2-independence test. The relative risk of essential hypertension associated with allelic variation was expressed in terms of an odds ratio (OR) with 95% confidence interval (CI). The Students t-test was performed to compare the mean levels of biochemical parameters among two different genotypes. Maximum likelihood estimates (MLE) of haplotypes were obtained by iterative two-steps algorithm called expectation-maximatization (EM). A Monte Carlo simulation using the Clump (version 1.6) program was performed to test the statistical significance of the association between the haplotype distribution of factor VII gene and essential hypertension (Sham and Curtis, 1995). The degree of nonrandom association was determined by calculation of the delta (Δ) (Hill and Robertson, 1968) and D (Lewontin, 1964) between the polymorphic sites in the factor VII gene. To test the significance of linkage disequilibrium, $n\Delta^2$ value was used as the χ^2 distribution with 1 df (degree of freedom). Statistical significance was accepted at the $P<0.05$. All statistical analyses were performed by the computer program of SPSSWIN (version 8.0).

Results

Genotype distribution

In the present study, we attempted to clarify the distribution of two polymorphisms in the factor VII gene in Korean population.

In the case of I/D polymorphism, the genotype and allele frequencies were not significantly different between normotensives and essential hypertensives (Table 1). The observed genotype distributions of this polymorphism were not significantly different from those expected for Hardy-Weinberg equilibrium. The frequencies of II, ID and DD genotypes were 0, 10 and 90% in normotensives, and 0, 5 and 95% in essential hypertensives, respectively. The heterozygosity and PIC values of I/D polymorphism represented the values of 0.0970 and 0.0923 for normotensives, and 0.0488 and 0.0476 for essential hypertensives, respectively. According to the heterozygosity

Table 1. Genotype and allele frequencies of the insertion/deletion polymorphism in the factor VII gene between normotensives and essential hypertensives

	Genotype No. (%)			Allele No. (%)			
	I I	I D	D D	I	D	H^1	PIC^2
Normotensives	0(0)	9(10)	79(90)	9(5)	167(95)	0.0970	0.0923
Hypertensives	0(0)	3(5)	57(95)	3(3)	117(98)	0.0488	0.0476
Chi-square		1.3080		1.2531			
Probability		0.2530		0.2630			
Odds ratio(CI)[3]			2.10(0.56-7.93)				

[1]Heterozygosity, [2]Polymorphism Information Content, [3]95% Confidence Interval.
Frequency is given as a percentage in parenthesis.

Table 2. Genotype and allele frequencies of the *Msp* I RFLP in the factor VII gene between normotensives and essential hypertensives

	Genotype No. (%)			Allele No. (%)			
	M1M1	M1M2	M2M2	M1	M2	H^1	PIC^2
Normotensives	0(0)	12(12)	89(88)	9(5)	167(95)	0.1118	0.1055
Hypertensives	0(0)	12(13)	78(87)	3(3)	117(98)	0.1244	0.1167
Chi-square		0.0910		0.0852			
Probability		0.7620		0.7704			
Odds ratio(CI)[3]			1.13(0.49 2.58)				

[1]Heterozygosity, [2]Polymorphism Information Content, [3]95% Confidence Interval.
Frequency is given as a percentage in parenthesis.

and PIC values, I/D polymorphism showed the relatively low degree of polymorphism in the both groups.

For *Msp* I RFLP, there were no significant differences in allele and genotype frequencies between two groups (Table 2). The observed genotype distribution of this RFLP was not significantly deviated from Hardy-Weinberg equilibrium. The frequencies of M1M1, M1M2, and M2M2 genotypes were 0, 12 and 88% in normotensives, and 0, 13 and 87% in essential hypertensives, respectively. The heterozygosity and PIC values of I/D polymorphism represented the values of 0.1118 and 0.1055 for normotensives, and 0.1244 and 0.1167 for essential hypertensives, respectively. According to the heterozygosity and PIC values, *Msp* I RFLP showed a relatively high degree of polymorphism in the both groups compared with the I/D polymorphism.

Association with biochemical parameters

Table 3 presents the comparision of anthropometric data and intermediate phenotypes across I/D polymorphism. I/D polymorphism was significantly associated with plasma triglyceride levels (Students t-test, $P<0.05$).

Table 4 displays the comparision of anthropometric data and intermediate phenotypes across the exon 8 *Msp* I RFLP. The *Msp* I RFLP was also significantly associated with plasma triglyceride levels (Students t-test, $P < 0.05$).

Haplotype analysis

The haplotype distribution and the linkage disequilibrium statistic reflecting the extent or significance of pair-wise nonrandom associations between the two polymorphic sites were shown in Table 5. There was no statistically significant difference in haplotype frequency between two groups (Monte-Carlo simulation, $T_2 = 0.9959$, $df = 1$, $P = 0.3183$, simulation number = 10,000). However, the significant pair-wise linkage disequilibrium between I/D polymorphism and *Msp* I RFLP in the factor VII gene was detected in the both groups by χ^2-test.

Discussion

Hypertension is one of the most common disease in civilized contries. It is currently seen as a "complex" genetic trait caused by multiple susceptibility genes which are modulated by gene-environment and gene-gene interactions.

Table 3. The comparison of the anthropometric data and intermediate phenotypes according to factor VII insertion/deletion genotypes

Variables	Genotypes		
	I I(No.)[7]	ID(No.)	DD(No.)
Age(year)	0.0 ± 0.0(0)	55.8 ± 11.0(12)	59.6 ± 11.5(134)
BMI(kg/m^2)[1]	0.0 ± 0.0(0)	22.9 ± 2.1(12)	23.6 ± 2.6(118)
*Tg(mg/dl)[2]	**0.0 ± 0.0(0)**	**93.7 ± 24.6(12)**	**133.9 ± 83.5(110)**
TC(mg/dl)[3]	0.0 ± 0.0(0)	149.1 ± 39.4(12)	153.5 ± 34.0(110)
LDL-chol(mg/dl)[4]	0.0 ± 0.0(0)	103.4 ± 37.1(12)	99.3 ± 35.6(110)
HDL-chol(mg/dl)[5]	0.0 ± 0.0(0)	26.9 ± 8.4(12)	27.2 ± 8.8(110)
Lp(a)[6]	0.0 ± 0.0(0)	20.0 ± 13.9(12)	15.9 ± 11.5(105)

[1]Body Mass Index, [2]Triglyceride, [3]Total cholesterol, [4]LDL-cholesterol, [5]HDL-cholesterol, [6]Lipoprotein(a) and [7]Number. Value are mean ± SD (standard deviation).
*There was the significant difference in plasma TG level across the genotype frequencies (by Students t-test, $P < 0.05$).

Table 4. The comparison of the anthropometric data and intermediate phenotypes according to factor VII / *Msp* I genotypes

Variables	Genotypes		
	M1M1(No.)[8]	M1M2(No.)	M2M2(No.)
Age(year)	0.0 ± 0.0(0)	58.0 ± 11.9(24)	59.5 ± 11.0(164)
BMI(kg/m^2)[1]	0.0 ± 0.0(0)	23.8 ± 2.5(23)	23.6 ± 2.4(149)
***Tg(mg/dl)[2]**	**0.0 ± 0.0(0)**	**98.7 ± 30.9(21)**	**133.4 ± 72.2(119)**
TC(mg/dl)[3]	0.0 ± 0.0(0)	150.0 ± 34.5(21)	148.5 ± 37.9(119)
LDL-chol(mg/dl)[4]	0.0 ± 0.0(0)	105.8 ± 31.5(21)	94.3 ± 35.4(119)
HDL-chol(mg/dl)[5]	0.0 ± 0.0(0)	24.4 ± 10.3(21)	27.2 ± 9.5(119)
Lp(a)[6]	0.0 ± 0.0(0)	23.0 ± 15.3(17)	16.2 ± 13.2(119)
Apo AI(mg/dl)[7]	0.0 ± 0.0(0)	105.6 ± 17.7(3)	97.9 ± 36.4(47)

[1]Body Mass Index, , [2]Triglyceride, [3]Total cholesterol, [4]LDL-cholesterol, [5]HDL-cholesterol, [6]Lipoprotein(a), [7]Apolipoprotein AI and [8]Number. Value are mean ± SD (standard deviation).
*There was the significant difference in plasma TG level across the genotype frequencies (by Students t-test, $P < 0.05$).

Table 5. Haplotype frequencies and linkage disequilibrium statistic (D',Δ) between pairs of two DNA polymorphisms in the factor VII gene

Haplotypes		Normotensives	Hypertensives
Ins/Del	*Msp* I		
Ins	M1	0.046090	0.027777
Ins	M2	0.007756	0.000000
Del	M1	0.007756	0.018519
Del	M2	0.938397	0.953703
Total chromosomes		130	108
Δ		**0.8485**	**0.7668**
D'		**0.8485**	**0.9801**
χ^2		**93.594**	**63.502**
P		**<0.001**	**<0.001**

There was no significant difference in haplotype frequency between normotensives and essential hypertensives (Monte-Carlo simulation, $T_4 = 0.9959$, df = 1, P = 0.3183, simulation number = 10,000).

Also, their etiology is complex with substantial environmental components. There is a strong indication that multiple genes are implicated. Specific candidate genes have been tested for linkage and association with a blood pressure or the diagnosis of hypertension. Nevertheless, the genetic alterations responsible for inherited "essential" hypertention remain largely unknown, and the success to date in identifying susceptibility genes has been very limited. Depending on the genetic factors of human essential hypertension, it appears that DNA polymorphisms at the candidate genes may play a significant role as useful genetic markers in the association study.

The defect of factor VII gene may occur quantitative decrease or qualitative abnormality of plasma factor VII that is accompany with frequent thrombosis. On the contrary, elevated factor VII levels have been associated with increased cardiovascular risk in some studies. Thus, the defect of factor VII gene may occur to elevate the blood pressure, and elevated blood pressure can predispose to the essential hypertension. Aiming at deciphering the genetic architecture of blood pressure regulation and essential hypertension (Emmerich *et al.*, 1994; Oquma *et al.*, 1992), we determined the distribution of the polymorphisms of factor VII gene in Korean normotensives and hypertensive groups.

The present study revealed that there were no significant differences between Korean normotensive subjects and subjects with essential hypertension in allele or genotype frequencies of the I/D polymorphism and *Msp* I RFLP in the factor VII gene. Therefore, it is unlikely that these polymorphisms are significantly associated with the etiology of essential hypertension among Korean population.

For the I/D polymorphism, there was a significant association in plasma triglyceride levels across the genotypes in Korean population. DD homozygotes is significantly higher in plasma triglycerides levels than ID genotypes. In several reports, the coagulation activity of factor VII is positively correlated to serum triglycerides (Donders *et al.*, 1993; Hoffman *et al.*, 1994; Mennen *et al.*, 1996; Green *et al.*, 1997; Hansen *et al.*, 2001). Regulation of haemostasis and thrombosis involves numerous plasma factors that contribute to procoagulant and anticoagulant pathway. Proagulant lipids and lipoproteins include triglyceride-rich particles in plasma and oxidized low density lipoprotein (LDL) which can accelerate activation of prothrombin, factor X and factor VII (Griffin *et al.*, 2001). These reports suggest that plasma triglycerides may involved in the regulation of thrombosis. Thus, the factor VII gene may influence the levels of plasma triglycerides and relate to pathogenesis of essential hypertension in Korean population.

In the case of *Msp* I RFLP, M2M2 homozygote is significantly higher in plasma triglyceride levels than M1M2 heterozygote. The *Msp* I RFLP of factor VII gene has been previously shown to modify factor VII levels (Humphires *et al.*, 1994; Hong *et al.*, 1999). Subsequently reported an interaction between genotype and plasma triglyceride levels on factor VII such that the correlation between factor VII levels and plasma triglyceride concentration was more pronounced in subjects who were M2-homozygous than among those with a M1 allele. There is also evident that there is a genotype-specific difference in the association between plasma triglyceride level and factor VII activity (Humphire *et al.*, 1994). These reports do suggest that at least some of the effects of genotype on factor VII levels may be mediated through the differential, allele-specific effects of plasma lipids on Factor VII activation. Unfortunately, we could not measure the plasma factor VII level in our subjects. Thus, further studies will be needed in order to investigate whether the correlation between factor VII levels and plasma triglyceride concentrations is modified by the *Msp* I RFLP of factor VII gene in Korean population.

By pair-wise haplotype analysis, the significant linkage disequilibrium between two polymorphic sites was detected. This finding suggests that the haplotype occurred by two polymorphisms decreases the information content for linkage analysis, while it did not require the large sample size to perform the association study. Therefore, association study may be better than linkage analysis to discover the disease susceptibility gene in the case of two polymorphisms in the factor VII gene.

The allele distribution of I/D polymorphism (Table 6) and *Msp* I RFLP (Table 7) in the factor VII gene was

Table 6. Comparison of allele frequencies of I/D polymorphism in the factor VII gene from various ethnic groups

Populations	Sample number	Allele frequencies		P[1]
		I	D	
Cancasian[2]	93	0.11	0.89	P<0.05
Korean	88	0.05	0.95	

[1]Probability, [2]Heywood *et al.*, 1996.

Table 7. Comparison of allele frequencies of *Msp* I RFLP in the factor VII gene from various ethnic groups

Populations	Sample number	Allele frequencies		
		M1	M2	
Cancasian[2]	95	0.11	0.89	P<0.05
Korean	101	0.05	0.95	

[1]Probability, [2]Heywood *et al.*, 1996.

different among various ethnic groups. The reason for this phenomenon may be explained by different genetic background of populations or various sample sizes. It seems to be important for carefully designed studies to minimize the ethnic heterogeneity of the case and control populations.

Within the limitations of the present study of about 100 normotensive and 100 essential hypertensive subjects, we were unable to demonstrate the association between the alleles of I/D polymorphism nor those of *Msp* I RFLP in the factor VII gene, and the occurrence of essential hypertension. It could be however, argued that the relatively small number of subjects may give a low probability of detecting a small effect of the polymorphism (a small gene effect is expected in the case of a disease as complex as essential hypertension). Moreover, these types of study design are prone to type II error (that is, failing to reject the null hypothesis that there is no differences in allelic distributions between the two groups when it is false). The lack of association indicates that the particular DNA changes causing the polymorphisms are not responsible for essential hypertension, and that polymorphisms are not in linkage disequilibrium with other loci that are responsible. Negative finding generated by retrospective case-control studies can in no way be advocated to rule out the gene effects in clinical phenotype under investigation. This is why we cannot exclude the possibility that factor VII gene is somehow involved in the pathogenesis of essential hypertension.

Nevertheless, In this study, there were no significant differences in the allele, genotype and haplotype frequencies of two polymorphisms in the factor VII gene between normotensives and essential hypertensives in Korean population. This might suggest that these polymorphisms are not a useful marker to investigate the relationship between the factor VII gene and essential hypertension in Korean population.

Acknowledgement

The human blood samples were kindly supplied by Dr. Seung Hee Cho, Clinical Pathology, Seoul Hygiene Hospital, Seoul, Korea.

References

Bernardi, F, Marchetti, G, Pinotti, M, Arcieri, P, Baroncini, C, Papacchini, M, Zepponi, E, Ursicino, N, Chiarotti, F, and Mariani, G (1996): Factor VII gene polymorphisms contribute about one third of the factor VII level variation in plasma, *Arterioscler. Thromb. Vasc. Biol.* **16**, 72-76.

Bostein D, White R.L, Skolnick M, and Davis R.W. (1980): Construction of a genetic linkage map in man using restriction fragment length polymorphisms, *Am. J. Hum. Genet.* **32**, 314-331.

Broze G.J. and Majerus P.W. (1980): Purification and properties of human coagulation Factor VII, *J. Biol. Chem.* **255**, 1242-1247.

Donders S.H., Lustermans F.A., and Van Wersch J.W. (1993): Coagulation factors and lipid composition of the blood in treated and untreated hypertensive patients. *Scand. J. Clin. Lab. Invest.* **53**, 179-186.

Emmerich, J., Chadeuf, G., Alhenc-Gelas, M., Gouault-Heilman, M., Toulon, P., Fiessinger, J.N, and Aiach, M. (1994): Molecular basis of antithrombin type I deficiency: the first large in-frame deletion and two novel mutations in exon 6, *Thromb. Haemost.* **72**, 534-539.

Friedwald, W.T, Levy, R.I. and Fridrickson, D.S. (1972): Estimation of the concentration of low-density lipoprotein cholesterol in plasma without the use of the preparative ultracentrifuge, *Clin. Chem.* **18**, 499-502.

Green, F., Kelleher, C., Wilkes, H., Temple A., Meade T., and Humphries, S.A. (1991): Common genetic polymorphism associated with lower coagulation factor VII levels in healthy individuals, *Arterioscler. Thromb.* **11**, 540-546.

Green D., Chamberlain M.A., Ruth K.J., Folsom A.R., and Liu K. (1997): Factor VII, cholesterol, and triglycerides. The CARDIA study. Coronary artery risk in young adults study. *Throm. Vasc. Biol.* **17**, 51-55.

Griffin J.H., Fernandez J.A., and Deguchi H. (2001): Plasma lipoproteins, hemostasis and thrombosis. *Thromb. Haemost.* **86**, 386-394.

Hansen J.B., Grimsgaard S., Huseby N., Sandset P.M., and Bonaa K.H. (2001): Serum lipids and regulation of tissue factor-induced coagulation in middle-aged men. *Thromb. Res.* **102**, 3-13.

Heywood, D.M., Mansfield, M.W., and Grant, P.J. (1996): Factor VII gene polymorphisms, factor VII:C levels and features of insulin resistance in non-insulin-dependent diabetes mellitus, *Thromb. Haemostat.* **75**, 401-406.

Hill, W.G. and Robertson, A. (1968): Linkage disequilibrium of finite populations, *Theor. Appl. Genet.* **38**, 226-231.

Hoffman C.J., Lawson W.E., Miller R.H., and Hultin M.B. (1994): Correlation of vitamin K-dependent clotting factors with cholesterol and triglycerides in healthy young adults. *Arterioscler. Thromb.* **14**, 1737-1740.

Hong Y., Pedersen, N.L., Egberg, N., and de Faire, U. (1999): Genetic effects for plasma factor VII levels independent of and in common with triglycerides, *Thromb. Haemost.* **81**, 382-386.

Humphries, S.E, Lane, A., Green, F.R., Cooper, J., and Miller, G.J. (1994): Factor VII coagulant activity and antigen levels in healthy men are determined by interaction between factor VII genotype and plasma triglyceride concentration, *Arterioscler. Thromb.* **14**, 193-198.

Lander, E.S. and Schork, N.J. (1994): Genetic dissection of complex traits, *Science* **265**, 2037-2048.

Lander, E.S. (1996): The new genomics: global views of biology. *Science* **274**, 536-539.

Lewontin, R.C. (1964): The interaction of selection and linkage. I. General considerations: heterotic models, *Genetics* **49**, 49-67.

Marchetti, G., Patracchini, P., Papacchini, M., Ferrati, M. and Bernardi, F. (1993): A polymorphism in the 5' region of coagulation factor VII gene (F7) caused by an inserted decanucleotide, *Hum Genet* **90**, 575-576.

Mennen L.I., Schouten E.G., Grobbee D.E., and Kluft C. (1996): Coagulation factor VII, dietary fat and blood lipids: a review. *Thromb. Haemost.* **76**, 492-499.

Mitropoulos, K.A. and Esnouf, M.P. (1990): The prothrombin activation peptide regulates synthesis of the vitamin K-dependent proteins in the rabbit. *Thromb. Res.* **15**, 541-549.

Oquma, Y., Sakuragawa, N. and Hiraga, K. (1992): The antithrombin III gene polymorphism in Japan: examination for haplotypes relevant to disordered antithrombin III biosynthesis, *Thromb. Res.* **65**, 519-531.

Risch, N. and Merikangas, K. (1996): The future of genetic studies of complex human diseases, *Science* **273**, 1516-1517.

Sambrook, J., Fritsch, E.F., and Maniatis, T. (1989): Molecular cloning: a Laboratory Manual. 2nd Ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, pp 9.16-9.23.

Sham, P.C. and Curtis, D. (1995): Monte Carlo tests for associations between diseases and alleles at highly polymorphic loci, *Ann. Hum. Genet.* **59**, 97-105.

Weeks, D.E. and Lathrop, G.M. (1995): Polygenic disease: methods for mapping complex disease traits, *Trends. Genet.* **11**, 513-519.