Original Article

Association of G−33A Polymorphism in the Thrombomodulin Gene with Myocardial Infarction in Koreans

Hyun-Young PARK*,**, Toru NABIKA*, Yangsoo JANG**, Hyuck Moon KWON**, Seung Yun CHO**, and Junichi MASUDA*

Thrombomodulin (TM), a thrombin receptor expressed on the endothelial surface, is known to play an important role in the anti-thrombogenic system *in vivo*. In this study, we examined the effects of 3 single-nucleotide polymorphisms (SNPs) in the TM gene (G−33A, C1418T and C1922T) on the development of myocardial infarction (MI) in Koreans. We found that G−33A was a common SNP (the minor allele frequency was 0.09) in Koreans. Eighty-five MI patients who had received coronary angiography were enrolled and were divided into 3 groups according to the number of coronary arteries in which stenosis was found angiographically (1-vessel disease (1VD) to 3-vessel disease (3VD)). The criterion of coronary stenosis was 50% or more stenosis on angiography. In addition, 102 controls (CONT) who had no significant stenosis were employed. The number of AA/GA genotypes of G−33A was found to be significantly greater in the 1VD than in the CONT (p=0.004 by X^2-test) while no significant difference was found between the multivessel disease (2−3VD) and the CONT. Multiple logistic analysis showed that G−33A was an independent risk factor for the 1VD with an odds ratio of 4.63 (95% confidence interval; 1.62−13.3). C1418T and C1922T were both in linkage disequilibrium with G−33A; however, they were not independent risks for either the 1VD or the 2−3VD. A reporter gene assay showed that G−33A had a significant effect on the TM promoter activity. These results indicated that G−33A polymorphism in TM might be a genetic risk factor for myocardial infarction.
(*Hypertens Res* 2002; 25: 389−394)

***Key Words*: thrombomodulin, myocardial infarction, case-control study, single-nucleotide polymorphism, genetics**

Introduction

Thrombomodulin (TM) is a thrombin receptor expressed on the luminal surface of endothelial cells. The TM-thrombin complex can activate protein C, which then acts as a potent anticoagulant (*1*). Studies on mice without functional TM (*2*) as well as on rare mutations in the TM gene reported in patients of myocardial infarction (MI) (*3, 4*) have suggested that dysfunction of TM plays an important role in the pathogenesis of MI. These observations raised the question of whether more common polymorphisms in the TM gene might have predisposing effects on MI in the general population. And in fact, recent studies have indicated that such genetic polymorphisms do have predisposing effects in MI; Norlund *et al*. showed that a common single-nucleotide polymorphism (SNP), C1418T, which results in Ala^{455} to Val substitution, was associated with MI in their case-control study (*5*). Ireland *et al*. found 3 SNPs in the 5′-untranslated region (5′UTR) of the TM gene, one of which might be a

From the *Department of Laboratory Medicine, Shimane Medical University, Izumo, Japan, and **Department of Cardiology, Yonsei University College of Medicine, Seoul, Korea.

This work was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture of Japan and a grant from Shimane Institute of Health Science.

Address for Reprints: Toru Nabika, M.D., Department of Laboratory Medicine, Shimane Medical University, Izumo 693−8501, Japan. E-mail: nabika@shimane-med.ac.jp

Received January 30, 2002; Accepted in revised form February 12, 2002.

Table 1. Demographic Data of Studied Populations

	CONT(102)	1VD(30)	2VD(29)	3VD(25)
Age (years)*	55±10	56±9	57±11	61±10
Sex (% male)†	51.0	76.7	93.1	84.0
BMI (kg/m^2)	24.3±3.9	24.3±3.6	24.2±5.6	24.4±2.4
Hypertension (%)	44.1	26.7	34.5	56.0
Diabetes mellitus (%)†	9.8	20.0	27.6	44.0
Smoking (%)†	27.5	73.3	86.2	68.0
TC (mg/dl)	187±41	203±39	201±44	198±36
HDL-C (mg/dl)	38.7±9.5	38.4±13.8	37.4±8.6	36.1±8.7
TG (mg/dl)‡	149±92	145±60	185±94	152±58

CONT, control population; 1VD, 2VD, 3VD, 1-, 2-, 3-vessel disease, respectively; BMI, body-mass index; HDL-C, high-density lipoprotein cholesterol; TC, total cholesterol; TG, triglyceride. * $p<0.05$ between CONT and 3VD by one-way analysis of variance (ANOVA). † $p<0.001$ by χ^2-test. ‡ $p<0.05$ between CONT and 2VD by one-way ANOVA.

risk for MI (*6*). According to the latter study, the three SNPs may be more common in Asians than Caucasians, although the authors did not provide precise frequencies of the polymorphisms in clearly defined ethnic populations. This observation prompted us to study TM polymorphisms in Koreans with MI. In Korea, coronary artery diseases (CAD) are one of the leading causes of premature death among the middle-aged. Identification of a genetic predisposition to MI would thus have a beneficial impact on its prevention in the Korean population.

In this study, we newly identified an SNP in the 3′-untranslated region (3′UTR) of the TM gene using the single-strand conformation polymorphism (SSCP) method. Further, we found that G−33A polymorphism in the 5′UTR was common in the Korean population and may be a risk factor for a subpopulation of MI. We also demonstrated that this polymorphism affects the TM promoter activity, suggesting that it is functionally important.

Methods

Subjects

Eighty-six MI patients who had received coronary angiography at the University Hospital of Yonsei University were recruited. They were divided into 4 subgroups according to the number of coronary arteries showing significant stenosis (0-vessel disease (0VD) to 3-vessel disease (3VD))(Table 1). The criterion of coronary stenosis was 50% or more stenosis on angiography. Because only one MI patient with 0VD was found, this patient and the 0VD subgroup were excluded from the present study. As a control population (CONT), we employed 102 patients who had neither significant coronary stenosis (less than 30% stenosis on angiography) nor clinical evidence of MI. Informed consent was obtained from all participants. To estimate genotype frequencies in the general Korean population, 291 subjects were employed as a reference population from participants of a health examination done in the Seoul area. Because these subjects were anonymous, no clinical information on the reference population was available. The study was approved by the ethical committee of Yonsei University College of Medicine.

Polymerase Chain Reaction (PCR)-SSCP

Genomic DNA was extracted from white blood cells using a commercial kit. SSCP detection was done in 48 CAD patients as described previously (*7*, *8*). Briefly, fifteen overlapping fragments with sizes between 246 and 136 bps were amplified by PCR using ^{32}P-endlabeled primers. They covered 2,240 bp of the 5′UTR and the exon of the TM gene. PCR products were denatured by heating at 96ºC for 2 min and then loaded onto 0.5% Mutation Detection Enhancement (MDE) gel (FMC Bioproducts Co., Rockland, USA). Electrophoresis was done at room temperature for 15–25 h at 5 W of constant power. Gels were dried and autoradiograms were analyzed on a BAS 2500 system (Fujifilm, Tokyo, Japan). PCR fragments showing polymorphism on SSCP gels were then sequenced to identify the polymorphic sites.

Genotyping

Allele-specific oligonucleotide (ASO) hybridization was employed to genotype 3 SNPs in the TM gene. The primers and conditions used for amplification are listed in Table 2. PCR products were denatured in 200 μl of 0.4 eq/l NaOH and transferred onto nylon membranes. The membranes were then hybridized with ^{32}P-endlabeled ASOs, and rinsed with the buffer under the conditions indicated in Table 2. Autoradiograms were analyzed using the BAS 2500 system.

Promoter Activity

Four constructs (pTM−355 with −33G or −33A, pTM −125 with −33G or −33A) were made for the evaluation of the TM promoter activity. Four PCR fragments of the TM

Table 2. PCR and Hybridization Conditions for ASO Genotyping

SNP	PCR primers	PCR condition*	ASO**	Hybridization temp.(ºC)	Washing condition
G−33A	5′-CCTTTTCCCGAACGTCC-3′	1.0/60	G: 5′-TAAGTGCCCGGCCCTC-3′	45	G: 2×SSC/0.2% SDS, 60ºC
	5′-GCCTCTCCTGTCCGTCC-3′		A: 5′-GAGGGCCAGGCACTTA-3′		A: 2×SSC/0.2% SDS, 60ºC
C1418T	5′-CTAGCTCCGGGGTGTGC-3′	1.0/65	C: 5′-GCCCTTGCCCGCCACA-3′	45	C: 2×SSC/0.2% SDS, 60ºC
	5′-CGCAGATGCACTCGAAGGT-3′		T: 5′-TGTGGCGGACAAGGGC-3′		T: 2×SSC/0.2% SDS, 60ºC
C1922T	5′-CTGGTGGTGGCGCTTTTGGC-3′	1.0/65	C: 5′-CCTGGCTCCGTCC-3′	35	C: 2×SSC/0.2% SDS, 45ºC
	5′-CAAAGCTGGGGGTGAGGAGGCA-3′		T: 5′-CCTGGCTTCGTCC-3′		T: 2×SSC/0.2% SDS, 43ºC

PCR, polymerase chain reaction. * PCR condition is expressed as $MgCl_2$ (mmol/l)/anneal temperature (°C). ASO, allele-specific oligonucleotide. ** Underlines indicate polymorphic nucleotides.

Table 3. Genotype Frequencies of 3 Single-Nucleotide Polymorphisms in the Thrombomodulin Gene

	CONT	1VD	2VD	3VD	REF
G−33A					
GG	86 (84.3)	18 (60.0)*	22 (75.9)	20 (80.0)	247 (85.0)
GA/AA	16 (15.7)	12 (40.0)*	7 (24.1)	5 (20.0)	44 (15.0)
C1418T					
CC	68 (66.7)	13 (43.3)†	15 (51.7)	17 (68.0)	175 (60.0)
CT/TT	34 (33.3)	17 (56.7)†	14 (48.3)	8 (32.0)	116 (40.0)
C1922T					
CC	98 (96.1)	26 (86.7)‡	28 (96.6)	24 (96.0)	186 (98.4)
CT/TT	4 (3.9)	4 (13.3)‡	1 (3.4)	1 (4.0)	3 (1.6)

Values are *n* (%). CONT, the control population; 1VD, 2VD, 3VD, 1-, 2-, 3-vessel disease, respectively; REF, the reference population. * $p=0.004$ *vs*. CONT and $p<0.001$ *vs*. REF by χ^2-test. † $p=0.02$ *vs*. CONT by χ^2-test. ‡ $p<0.001$ *vs*. REF by χ^2-test.

gene promoter spanning either −355 to 64 (pTM−355) or −125 to 64 (pTM−125) were obtained using genomic DNA of AA or GG homozygotes for G−33A as templates. These fragments were then cloned into the Picagene vector (Wako, Tokyo, Japan).

Chinese hamster ovary (CHO) cells and bovine aortic endothelial (BAE) cells were used for the reporter gene assay. Cells were transfected with one of the four constructs described above. The plasmid pcDNA3.1-LacZ was used in cotransfection to estimate the transfection coefficiency. All the transfection experiments were performed in triplicate. Cells were plated at 10^5/well in 6-well plates and grown to a subconfluent state. Mixtures of the promoter constructs (0.8 μg) and pcDNA3.1-LacZ (0.2 μg) were transfected using LipofectaminePlus (Gibco BRL, Gaithersburg, USA) following the manufacturer's instructions. Transfected cells were then cultured for 48 h and luciferase activities were measured using the Luciferase Reporter Assay system (Promega, Madison, USA). Luciferase activity was standardized with galactosidase activity to correct the transfection efficiencies between the experiments.

Statistics

One-way analysis of variance (ANOVA) and χ^2-test were used in the comparison of demographic data. Univariate analysis of the genetic effects of SNPs was done using the χ^2-test. Multivariate tests of risk factors were performed using logistic regression analysis with the SPSS package. Linkage disequilibrium between SNPs was estimated as described by Hill (*9*) and Thompson *et al*. (*10*).

Results

Clinical profiles of the subjects are shown in Table 1. A higher age of onset and a higher prevalence of diabetes mellitus (DM) were observed in those with multivessel disease (2−3VD) than in those with the 1VD.

Through the SSCP study, we identified a new SNP, C1922T, in the 3′UTR of the TM gene. In addition, we confirmed two SNPs, G−33A and C1418T ($Ala^{155}Val$), in the Korean population. Although several other nucleotide-substitutions in the TM gene have been reported in patients with MI or venous thrombosis (*2*, *3*, *11*), we could not confirm these mutations in the present cohort. In addition, the −9/10AT and −133A alleles, which were identified in MI patients by Ireland *et al*. (*6*), were not found on further analysis of an additional 86 Koreans (46 MI and 40 references) using direct sequencing. This result suggested that these two nucleotide substitutions are rare in the Korean

Table 4. Multiple Logistic Analyses of Risk Factors for the Single-Vessel (1VD) and the Multivessel Diseases (2−3VD)

	β	χ^2	p	OR [95% confidence interval]
1VD				
History of smoking	2.13	17.5	<0.0001	8.44 [3.10−22.9]
TM G−33A	1.53	8.03	0.005	4.63 [1.62−13.3]
2−3VD				
Age	0.084	10.4	0.001	1.09 [1.03−1.14]
Sex	1.63	4.34	0.03	5.08 [1.11−23.6]
DM	1.60	8.24	0.004	4.97 [1.65−14.9]
History of smoking	2.11	12.5	0.0004	8.26 [2.53−26.8]
TC	0.018	7.71	0.006	1.02 [1.01−1.03]

Variables included in the analyses were age, sex, history of smoking, history of hypertension, history of diabetes mellitus (DM), body-mass index, total cholesterol (TC), high-density lipoprotein cholesterol, triglyceride and thrombomodulin (TM) G−33A. OR, odds ratio.

population.

Among the SNPs studied, we found G−33A and C1418T to be most common in the Korean population. The allele frequencies of the minor alleles, −33A and 1418T, were 0.09 and 0.12, respectively, in the reference population. In contrast, the allele 1922T is much more rare (0.008) in Koreans. These three SNPs were in strong linkage disequilibrium; the allele −33A was almost exclusively associated with 1418T (the coefficient of linkage disequilibrium was 0.972). The coefficient of linkage disequilibrium between G−33A and C1922T was 0.941, which indicated significant linkage disequilibrium between these two SNPs as well.

The genotype frequencies of the three SNPs are shown in Table 3. The observed genotype frequencies in all groups were in Hardy-Weinberg's equilibrium. The frequency of AA/AG genotypes at −33 was significantly higher in the 1VD than in the CONT or the reference population ($\chi^2=8.2$, $p=0.004$ and $\chi^2=11.7$, $p<0.001$, respectively) while the 2VD and the 3VD showed genotype frequencies similar to that of the CONT. The G−33A genotype frequency of the CONT was not significantly different from that of the reference population. A similar tendency was observed for C1418T and C1922T.

Multivariate analyses indicated that the −33A allele was an independent risk factor for the 1VD, but not for the 2−3VD (Table 4). Among the other classical risk factors, smoking was a common risk for both the 1VD and the 2−3VD, while serum cholesterol level, male sex, age, and diabetes mellitus (DM) were independent risks only for the 2−3VD. C1418T and C1922T were not independent risk factors for either the 1VD or the 2−3VD.

The activity of the TM promoter was studied using constructs possessing either G or A at −33. Both in BAE and CHO cells, pTM−355 with −33A showed significantly less luciferase activity than did pTM−355 with −33G (Fig. 1). In contrast, the promoter activity was not significantly different between pTM−125 with −33A and that with −33G.

Discussion

In this study, we showed that G−33A polymorphism in the TM gene might be a risk factor for the 1VD. Further, we provided *in vitro* evidence that this SNP itself is functional, and affects the promoter activity.

Ireland *et al*. identified 3 SNPs, C−133A, G−33A and GG−9/10AT, in the 5′UTR of the TM gene (*6*). They found 6 of 208 individuals harboring one of these polymorphisms. Interestingly, 5 of them were of Asian origin, even though only 38 Asians were included in their study population (*6*). This observation suggested that these polymorphisms were more common in the Asian population than in Caucasians. In the present study, however, we found that G−33A is a common polymorphism in the Korean population. The minor allele frequency was 0.09 in our Korean cohort, similar to that in Japanese (Park *et al*., unpublished data). In contrast, Ireland *et al*. found no occurrence of the −33A allele among 168 Caucasians. Le Flem *et al*. confirmed that −33A was rare in Caucasians; they found only 3 heterozygotes among 600 individuals with and without venous thrombosis (*12*). Therefore, G−33A is much more common in East Asian populations than in Caucasians. This result emphasizes that the ethnic background of the studied population should be considered carefully when genetic risk factors for CAD are evaluated. In contrast to G−33A, we could not identify the −9/10AT or −133A allele in 190 Korean individuals either by SSCP or direct sequencing, suggesting that these two nucleotide-substitutions are very rare in Koreans. These two mutations have therefore only a small impact on the general Korean population even if they have potent predisposing effects.

Clinical and epidemiological observation distinguished the 1VD from the 2−3VD. The 2−3VD showed a higher age of onset as well as a higher prevalence of DM (*13–16*). In accordance with these previous observations, we found that age and DM were both independent risk factors for the 2−3VD (see Table 4). We thus propose that the 2−3VD pro-

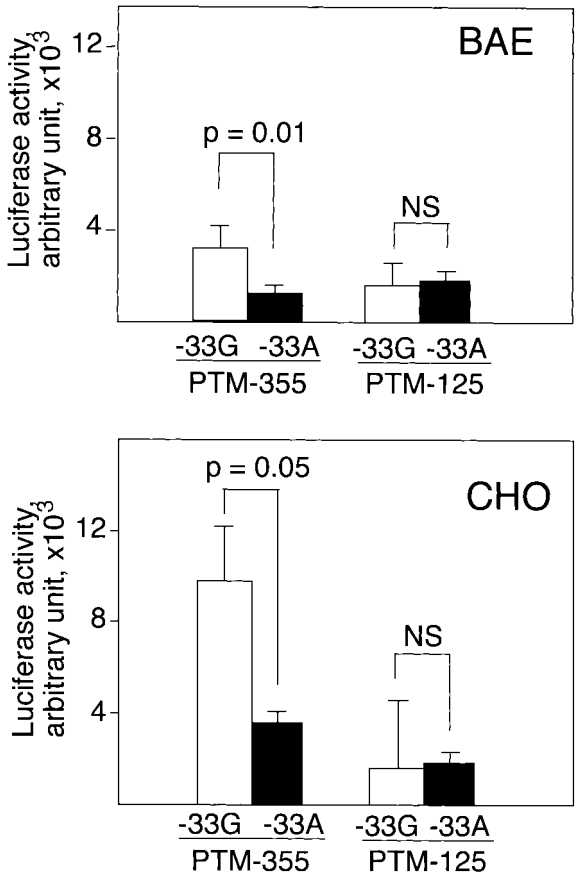

Fig. 1. *Luciferase assay using 4 different constructs for the thrombomodulin promoter. Four different constructs constructed, one each with the promoter region between −355 and 64 (pTM−355) with −33G or A, and one each with the promoter region between −125 and 64 (pTM−125) with −33G or A. Each construct was transfected into Chinese hamster ovary (CHO) cells or bovine aortic endothelial (BAE) cells. The luciferase assay was performed in triplicate and the galactosidase activity of cotransfected pcDNA3.1-LacZ was used to correct transfection efficiencies between the experiments. Columns and bars show the means±SD of 3 independent experiments.*

gresses gradually without significant thrombotic accident in its earlier stage. DM probably promotes arteriopathy, particularly in smaller arteries, in this process. By contrast, thrombotic accidents may play a more critical role in the development of the 1VD. In support of this idea, several studies have pointed out that severe coronary stenosis was found less frequently in younger patients of MI (*13*, *16*). Our observation raises the possibility that TM plays a major role in the development of thrombosis before the multivessel disease becomes established. This idea is supported by the observation that the prevalence of the AA/AG genotype in the younger patients (below the median age of 58 years) with the 1VD was as high as 50%, which was in marked contrast to that in the 2–3VD (13%). Reduced TM activity, as suggested by the *in vitro* promoter assay (see Fig. 1), in the local environment of coronary arteries may accelerate thrombogenesis once triggered, resulting in the occlusion of the coronary arteries. However, as multiple factors are necessary prior to the development of MI, MI incidence may still be lower in Koreans than in Caucasians even though TM−33A is much more frequent in the former population.

Recently, the concept of an acute coronary syndrome has attracted much interest because it proposes an interpretation based on MI without prior coronary stenosis (*17*, *18*). During this study, we found 5 patients with unstable angina or MI without significant coronary stenosis. In three of these cases, the patient was heterozygous for G−33A. Although this number is too small to draw any conclusion, it may be interesting to study the effect of TM G−33A on such a pathological condition.

The promoter assay revealed that −33A significantly decreased the TM promoter activity. This result basically confirmed the observation by Le Flem *et al.* (*12*), and seems compatible with the association of −33A with MI; we can expect that, with a lower level of expression of TM, the intravascular environment will tend to be thrombogenic. In contrast to Le Flem *et al*., however, we found no significant difference in the promoter activity when the shorter constructs were employed. This discrepancy might be due to differences in the cell culture systems used in these experiments. In addition, we might have missed small differences in the activity, because the decrease of promoter activity was only 15% in the previous study (*12*). Our results indicated that the effect of G−33A was at least more obvious in the longer construct than in the shorter one.

Yu *et al.* suggested that the TM promoter region between −375 and −225 had domains binding with unknown trans-acting factors (*19*). Tazawa *et al.* indicated that a similar region had an enhancer element that is different from Sp1 binding sites (*20*). G−33A itself or a protein binding to an element including G−33A may interact, directly or indirectly, with the putative trans-acting factors binding to this upstream region. Because of the lack of this interaction, we might not be able to observe the difference between the shorter constructs with −33G and A. It is known that TM expression can be affected by shear stress as well as by several inflammatory cytokines, such as interleukin−1β and tumor necrosis factor−α (*21–23*). Hence, it may be interesting to observe the effects of G−33A in cells stimulated by these cytokines or by shear stress.

Numbers of studies have used association analysis to evaluate effects of genetic polymorphisms on heart diseases (*24–26*). This analysis is, however, thought to be susceptible to sampling biases (*27*). It is thus important to employ appropriate control populations. In this study, we employed a reference population to estimate the allele frequency of the SNPs in the general Korean population. The frequency was similar between CONT and the reference population, imply-

ing that the observed difference in genotype frequencies between 1VD and CONT was not due to a bias in CONT. However, the sample sizes were too small to draw conclusive results. We are now collecting a larger cohort of CAD to perform a replication study. Despite its limitations, our case-control study together with the *in-vitro* study of the TM promoter activity indicated that G−33A was a functional polymorphism acting as a risk factor for MI.

References

1. Esmon CT: Thrombomodulin as model of molecular mechanisms that modulate protease specificity and function at the vessel surface. *FASEB J* 1995; **9**: 946–955.
2. Christie PD, Edelberg JM, Picard MH, *et al*: A murine model of myocardial microvascular thrombosis. *J Clin Invest* 1999; **104**: 533–539.
3. Doggen CJM, Kunz G, Rosendaal FR, *et al*: A mutation in the thrombomodulin gene, 127G to A coding for Ala25Thr, and the risk of myocardial infarction in men. *Thromb Haemost* 1998; **80**: 743–748.
4. Kunz G, Ireland HA, Stubbs PJ, Kahan M, Coulton GC, Lane DA: Identification and characterization of a thrombomodulin gene mutation coding for an elongated protein with reduced expression in a kindred with myocardial infarction. *Blood* 2000; **95**: 569–576.
5. Norlund L, Holm J, Zoller B, Ohlin A-K: A common thrombomodulin amino acid dimorphism is associated with myocardial infarction. *Thromb Haemost* 1997; **77**: 248–251.
6. Ireland H, Kunz G, Kyriakoulis K, Stubbs PJ, Lane DA: Thrombomodulin gene mutations associated with myocardial infarction. *Circulation* 1997; **96**: 15–18.
7. Nabika T, Bonnardeaux A, James M, *et al*: Evaluation of the Sa locus in human hypertension. *Hypertension* 1995; **25**: 6–13.
8. Park H-Y, Nabika T, Jang Y, Kim D, Kim H-S, Masuda J: Identification of new single-nucleotide polymorphisms in the thrombin receptor gene and their effects on coronary artery diseases in Koreans. *Clin Exp Pharmacol Physiol* 2000; **27**: 690–693.
9. Hill WG: Estimation of linkage disequilibrium in randomly mating populations. *Heredity* 1974; **33**: 229–239.
10. Thompson EA, Deeb S, Walker D, Motulsky AG: The detection of linkage disequilibrium between closely linked markers: RFLPs at the AI-CIII apolipoprotein genes. *Am J Hum Genet* 1988; **42**: 113–124.
11. Ohlin A-K, Marlar RA: The first mutation identified in the thrombomodulin gene in a 45-year-old man presenting with thromboembolic disease. *Blood* 1995; **85**: 330–336.
12. Le Flem L, Picard V, Emmerich J, *et al*: Mutations in promoter region of thrombomodulin and venous thromboembolic disease. *Arterioscler Thromb Vasc Biol* 1999; **19**: 1098–1104.
13. Zimmerman FH, Cameron A, Fisher LD: Myocardial infarction in young adults: angiographic characterization, risk factors and prognosis (Coronary Artery Surgery Study Registry). *J Am Coll Cardiol* 1995; **26**: 654–661.
14. Melidonis A, Dimopoulos V, Lempidakis E, *et al*: Angiographic study of coronary artery disease in diabetic patients in comparison with nondiabetic patients. *Angiography* 1999; **50**: 997–1005.
15. Dortimer AC, Shenoy PN, Shiroff RA, *et al*: Diffuse coronary artery disease in diabetic patients: fact or fiction? *Circulation* 1978; **57**: 133–136.
16. Gallet B, Leroy G, Saudemont JP, *et al*: Myocardial infarction in the young subject: a medium-term clinical and coronary angiographic study in 40 patients under 36 years of age: comparison with coronary angiographic data of myocardial infarction in patients after 50 years of age. *Arch Mal Coeur Vaiss* 1984; **77**: 27–36.
17. Fuster V, Badimon L, Badimon JJ, Chesebro JH: The pathogenesis of coronary artery disease and the acute coronary syndromes (1). *N Engl J Med* 1992; **326**: 242–250.
18. Fuster V, Badimon L, Badimon JJ, Chesebro JH: The pathogenesis of coronary artery disease and the acute coronary syndromes (2). *N Engl J Med* 1992; **326**: 310–318.
19. Yu K, Morioka H, Fritze LMS, Beeler DL, Jackman RW, Rosenberg RD: Transcriptional regulation of the thrombomodulin gene. *J Biol Chem* 1992; **267**: 23237–23247.
20. Tazawa R, Hirosawa S, Suzuki K, Hirokawa K, Aoki N: Functional characterization of the 5′-regulatory region of the human thrombomodulin gene. *J Biochem* 1993; **113**: 600–606.
21. Takada Y, Shinkai F, Kondo S, *et al*: Fluid shear stress increases the expression of thrombomodulin by cultured human endothelial cells. *Biochem Biophys Res Commun* 1994; **205**: 1345–1352.
22. Nawroth PP, Handley DA, Esmon CT, Stern DM: Interleukin 1 induces endothelial cell procoagulant while suppressing cell-surface anticoagulant activity. *Proc Natl Acad Sci U S A* 1986; **83**: 3460–3464.
23. Lentz SR, Tsiang M, Sadler E: Regulation of thrombomodulin by tumor necrosis factor-α: comparison of transcriptional and post-transcriptional mechanisms. *Blood* 1991; **77**: 542–550.
24. Wu S, Hong J, Li H, *et al*: No correlation of polymorphism of angiotensin-converting enzyme genes with left ventricular hypertrophy in essential hypertension. *Hypertens Res* 2000; **23**: 261–270.
25. Obineche EN, Frossard PM, Bokhari AM: An association study of five genetic loci and left ventricular hypertrophy amongst Gulf Arabs. *Hypertens Res* 2001; **24**: 635–639.
26. Cambien F, Poirer O, Lecerf L: Deletion polymorphism in the gene for angiotensin-converting enzyme is a potent risk factor for myocardial infarction. *Nature* 1992; **359**: 641–644.
27. Khoury MJ, Beaty TH: Applications of the case-control method in genetic epidemiology. *Epidemiol Rev* 1994; **16**: 134–150.