RAPID COMMUNICATION

A Common Prothrombin Variant (20210 G to A) Increases the Risk of Myocardial Infarction in Young Women

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Using specimens from a population-based case control study among women ages 18 to 44 years in western Washington, we assessed the relationship between carriership of a genetic clotting factor II variant (20210 G→A) and myocardial infarction (MI) The factor II variant was previously shown to be present in 1% to 2% of the population, to increase the levels of factor II, and to be associated with venous thrombotic disease Personal interviews and blood samples were obtained from 79 women with a first myocardial infarction and 381 control women identified through random-digit telephone dialing. Polymerase chain reaction (PCR) method was used to determine the factor II genotypes. The factor II 20210 G to A transition was present more often in women with MI (5 1%) than among control women (1 6%) The age-adjusted odds ratio for MI was 4 0 (95% confidence interval 1 1 to 15 1) The relative risk was high when another major cardiovascular risk factor was also present, such as smoking (odds ratio 43.3, 95% confidence interval 6.7 to 281), and the risk seemed limited to those with other risk factors These results, in which the effect of major coronary risk factors is enhanced fourfold to sixfold by the prothrombin variant, are similar to those previously reported for another genetic clotting abnormality, factor V Leiden We conclude that factor II 20210 G to A increases the risk of myocardial infarction in young women, especially in the women with other major risk factors for coronary heart disease

PROTHROMBIN (factor II) is the precursor of thrombin, the final effector of the clotting cascade that leads to the formation of fibrin Prothrombin is a key enzyme in the balance between procoagulation and anticoagulation because it potentiates coagulation by positive feedback loops and also promotes anticoagulation by the protein C pathway [1,2]

A recently described genetic variant of prothrombin is associated with an increased risk of venous thrombosis [3] The variant is located in the 3′ untranslated region of the gene (on chromosome 11), at position 20210,[4] where one nucleotide is changed (a G to A transition) Carriership of this variant is associated with elevated levels of prothrombin in plasma, which are related to an increased risk of thrombosis Among 474 patients with a first deep-vein thrombosis we found the 20210 G → A variant in 6 2%, as opposed to 2 3% among 471 healthy control subjects From these results we concluded that carriership of the variant increases the risk of deep-vein thrombosis 2 3-fold Among patients with heritable thrombophilia, the variant was found in 18% This high proportion is consistent with the hypothesis that the variant is a genetic cause of thrombosis In a second sample of 646 unaffected individuals, we found the mutation in 1%, which led us to the conclusion that the prevalence in The Netherlands is 1% to 2% [5]

Recently we have shown that another common genetic abnormality, a mutation in clotting factor V that causes resistance to the anticoagulant effect of activated protein C (APC),[6] increases the risk of myocardial infarction (MI) in women ages 18 to 44 years This mutation, factor V R506Q or factor V Leiden,[7] was found in 4 1% of healthy control women, and 9 5% of women who suffered an MI at a young age [8] Carriership of the factor V Leiden variant increased the risk of myocardial infarction 2 5-fold The risk of myocardial infarction was high in women who carried the factor V Leiden allele in combination with other major cardiovascular risk factors For women who carried the factor V Leiden and had one or more 'metabolic risk factors' (obesity, hypertension, hypercholesterolemia, or diabetes mellitus), the risk was 25-fold increased compared to women with neither factor V Leiden nor a metabolic risk factor, for women who smoked and carried the factor V mutation, the risk was 32-fold increased relative to nonsmoking noncarriers

Our previous analyses have demonstrated that besides its relevance to public health in young women, our study also provides a model for studying the interaction of atherogenic and thrombogenic risk factors [8-10] Therefore, we set out to assess the effect of a newly described prothrombotic genetic abnormality, the prothombin 20210 G to A transition This is a fairly common variant and thus of potential importance in the etiology of myocardial infarction

MATERIALS AND METHODS

We conducted a population based case control study of myocardial infarction among women 18 to 44 years of age residing in three contiguous counties of western Washington state The methods of the study have been described extensively previously [8,11] Cases were women aged 18 to 44, without a prior history of coronary heart disease or cerebrovascular disease, who were diagnosed between July 1, 1991 and February 28 1995 with a first acute MI Criteria for myocardial infarction were defined by evidence of symptoms, elevated enzymes, and electrocardiographic changes [12] Of 165 eligible patients, 112 participated in an in person interview and 84 were willing to undergo venapuncture

Controls were identified by random-digit telephone dialing Eligible were women aged 18 to 44 years living in the same area during the time period of the study without a history of cardiovascular

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Submitted June 3 1997 accepted June 13 1997

Supported by the National Institute for Child Health and Human Development (NO1 HD 1 3107)

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0006 4971/97/9005 0044$3 00/0

disease [13] A household census was completed for 94 9% of the residences contacted Of 684 randomly chosen eligible individuals, 525 agreed to a personal interview (response 73%) and of these 391 agreed to venapunctuie

Participating cases and controls were interviewed in person regarding histories of diabetes, hypertension, or hyperlipidemia (physician's diagnosis and drug-treatment), cigarette smoking, height and weight, reproductive and contraceptive histories, and demographic characteristics All questions elicited information from a time period before each case's cardiovascular event, or an equivalent date for controls

We obtained 30 mL venous blood from the antecubital vein in EDTA-treated vacutainers, from which the cells were separated by centrifugation at 2,000*g* for 10 minutes, the buffy coat was resuspended in phosphate-buffered saline and frozen at −70°C White blood cell aliquots were shipped to Leiden, The Netherlands, where DNA analysis was performed DNA was extracted from these samples essentially as described by Miller et al [14] Analyzable DNA was available for 79 women with MI and 381 control subjects (5 women included in an earlier analysis[8] were later classified as having unstable angina and were excluded from this analysis) The technician who performed DNA analyses was blinded as to whether a specimen was from a patient or a control subject

The presence of the factor II variant (20210, G to A replacement) was first determined using an A-allele–specific polymerase chain reaction (PCR) according to the method of Glisic and Alavantic [15] The heterozygous AG status of putative positives was confirmed by the presence of a *Hin*dIII restriction site in the relevant PCR-fragment using a previously described protocol [3]

Smokers were defined as women who reported smoking currently and regularly, while all others were classified as nonsmokers A woman was classified as diabetic, hypertensive, or hypercholesterolemic if she reported that she had ever been diagnosed by a physician as such Additionally, we assessed which women were currently taking prescription drugs for these conditions We considered obese any woman with a body mass index (BMI) equal to or exceeding 27 3 kg/m^2 In some analyses the variables for hypertension, hypercholesterolemia, diabetes mellitus, and obesity were grouped together as 'metabolic risk factors'

The association of carriership of the factor II variant with MI was examined by unconditional logistic regression adjusted for the matching variable age, and expressed with the odds ratio (OR) as a measure for relative risk Effect modification was assessed through stratified analyses Confidence intervals (95%, CI95) were calculated by standard methodology

Table 1 General Characteristics of Patients and Control Subjects

	Patients With MI (n − 79)	Control Women (n − 381)
Age (yr)		
Mean	39 7	37 7
Median	41	39
Range	23 44	19 44
Current smokers	59 (74 7)	86 (22 6)
Ever diagnosed with		
Hypertension	27 (34 2)	37 (9 7)
Hypercholesterolemia	33 (41 8)	60 (15 7)
Diabetes mellitus	12 (15 2)	11 (2 9)
Currently drug treated for		
Hypertension	13 (16 5)	10 (2 6)
Hypercholesterolemia	2 (2 5)	2 (0 5)
Diabetes mellitus	5 (6 3)	3 (0 8)
Obese	46 (58 2)	102 (27 0)

Data were not available for treated hypercholesterolemia in one control, and body mass index in three controls Percentages are given for available data, in parentheses All data, including diagnosis or treatment of the conditions mentioned above, refer to dates before the MI

Table 2 Factor II 20210A Among Patients With MI and Controls and ORs in Relation to Smoking and Metabolic Risk Factors

Factor II Mutation	Other Risk Factor	Patients (n − 79)	Controls (n − 381)	OR	CI95
Smoking					
GG	No	20	291	1	
AG	No	0	4	0	0 23 2
GG	Yes	55	84	9 3	5 2 16 5
AG	Yes	4	2	43 3	6 7 281
Metabolic risk factor					
GG	No	14	210	1	
AG	No	0	4	0	0 24 5
GG	Yes	61	162	5 3	2 9 9
AG	Yes	4	2	33 8	5 5 209
Overall					
GG		75	375	1	
AG		4	6	4 0	1 1 15 1

ORs are adjusted for age, and calculated separately for each stratum with a dummy variable model, age-adjusted and relative to the reference category (OR 1) Metabolic risk factors are either obesity or a physician's diagnosis of diabetes mellitus, hyeprtension, or hypercholesterolemia

RESULTS

The 79 women with an acute MI had a mean age of 39 7 (median 41), the control women of 37 7 (median 39 0) All major risk factors for coronary disease such as smoking, obesity, hypertension, hypercholesterolemia, and diabetes were reported more often among cases than among controls (Table 1) Smoking (75%) and obesity (58%) were particularly prevalent among the patients

Five percent of women with an MI carried the factor II 20210A allele (4 of 79, 5 1%), compared to 1 6% of the control women (6 of 381) (Table 2) All carriers of the mutation were white women whereas none of the 51 nonwhite women in the study carried the mutation The OR for MI (from the age-adjusted logistic model) was 4 0 (CI95 1 1 to 15 1, P = 038) These results did not materially change when the analyses were restricted to white women (n = 409, OR = 4 2), premenopausal women (n = 389, OR = 3 7), or nonusers of oral contraceptives (n = 414, OR = 6 3) The complementary subgroups for these variables included too few women to allow risk estimation

The risk associated with factor II 20210A was particularly high when other major risk factors were also present (Table 2) Among nonsmokers or women without metabolic risk factors, the risk was not increased when women carried the factor II variant, but among smokers who carried the mutation the risk was increased 43-fold (CI95 6 7-281) compared to nonsmoking women without the factor II variant, and, among women with one or more of four metabolic risk factors (obesity, hypertension, hypercholesterolemia, or diabe-

Table 3 ORs for Cardiovascular Risk Factors and Risk of MI With and Without the Simultaneous Presence of Clotting Factor Abnormality

	OR*	CI95
Smoking		
Without FVL or FII20210A	8 6	4 7 15 5
With FVL or FII20210A	36 3	10 7 123
Obesity†		
Without FVL or FII20210A	3 8	2 2-6 6
With FVL or FII20210A	20 5	4 7 88 9
Metabolic risk factor‡		
Without FVL or FII20210A	4 6	2 4 8 6
With FVL or FII20210A	24 9	8 3 74 2

*OR adjusted for age, relative to those with neither the clotting abnormality nor the other risk factor

†BMI ≥ 27 3 kg/m^2

‡Obesity or physician s diagnosis of hypertension hypercholester olemia, or diabetes

tes mellitus) the risk was increased 34-fold (CI95 5 5 to 209) when they carried the factor II variant In contrast, women with one or more of these metabolic risk factors without the factor II variant had a 5 3-fold increased risk (CI95 2 9 to 9 9), ie, the already elevated risk among women with these risk factors was increased further by more than sixfold Restriction of this analysis to drug-treated hypertension, hypercholesterolemia, or diabetes mellitus (instead of reported physician's diagnosis of these conditions) led to similar results

These results of a striking synergy with other major cardiovascular risk factors is similar to what we have previously described for the association of factor V Leiden (factor V 1691 G to A) and MI among these young women Therefore, we combined these two mutations into one variable representing either clotting abnormality, which was present in 12 cases (15 2%) and 21 controls (5 5%) None of the 460 women carried the combination of factor V Leiden and factor II 20210A (located on chromosome 1 and 11, respectively) The OR associated with any clotting abnormality was 3 1, with a 95% confidence interval of 1 5 to 6 7 (P = 003) This relative risk indicates a moderate strength risk factor that increases the risk threefold, however, when the clotting abnormality was present combined with other cardiovascular risk factors, the relative risks ranged from 21 (obesity) to 36 (smoking), compared to women without the other risk factor or any clotting abnormality Table 3 shows the relative risks of several major determinants of coronary disease when present without the clotting abnormality, and when present in combination with the clotting abnormality The presence of either prothrombotic mutation (factor V Leiden or factor II 20210A) increased further the already elevated risk of MI associated with smoking, obesity, and metabolic risk factors by fourfold to sixfold

DISCUSSION

A common variant in the prothrombin gene, factor II 20210 G to A replacement, was associated with an overall fourfold increased risk of MI in young women The variant was found in 1 6% of healthy young women The relative risk became particularly high when other major cardiovascular risk factors were also present

MI is a rare event in young women For MI to occur in a young individual, several risk factors need to be present simultaneously, as is the case for venous thrombosis [16 20] Therefore, young individuals form an excellent group to study the effect of new risk factors as well as the interaction between risk factors We have reported previously from this study population that over 95% of cases had at least one of the risk factors smoking, hypertension, hypercholesterolemia, diabetes mellitus, obesity, or factor V Leiden [8] Smoking and obesity both were extremely frequent and present in more than half of the women with MI Because MI is uncommon in young women, the absolute individual risk of disease will remain low even when the relative risk is high However, it should be kept in mind that all major diseases are rare in the young, and that therefore even small absolute risks contribute significantly to overall morbidity and mortality among the young

The factor V Leiden mutation has a distinct racial and, to a lesser extent, geographical distribution [21 22] It is common in whites and very rare in Asians and Africans, among whites in Europe, there seems to be a North-South gradient, with prevalences ranging between 2% in Italy to 7% in Sweden Among 51 nonwhite women in this study, we did not find any carriers of the factor II mutation Because the overall prevalence of the factor II mutation was 1 6%, this is obviously well within the expected finding, nevertheless, our data suggest the possibility that this variant, like the factor V Leiden mutation, is also present mainly in whites

The findings of an association of MI with factor II 20210 G to A only among young women with other risk factors are strikingly similar to our previous reports on factor V Leiden in young women [8] Both clotting abormalities display a strong synergistic effect with other cardiovascular risk factors, in particular with smoking These two recently discovered clotting abnormalities were present in over 5% of the population, and thus may affect a large number of people

In young women, MI appears to be the result of combined atherogenic and thrombogenic factors Without a concomitant atherogenic abnormality, there was little effect of the clotting abnormality with regard to the risk of MI The reverse seemed true, too, as is shown in Table 3 the various atherogenic risk factors, such as obesity, hypercholesterolemia, hypertension, and diabetes had a modest effect on the risk of MI in these women when present solely, and this risk was greatly enhanced when a clotting abnormality as factor V Leiden or factor II 20210A was simultaneously present

Our results are based on a small number of mutant gene carriers and therefore the statistical uncertainty around the risk estimates remained considerable The confidence intervals were particularly wide for the estimates in the interaction analysis However, because the results for the overall effects of carriership of factor II 20210 A, as well as for the interaction with other major risk factors for coronary disease, are similar to those we reported previously for factor V Leiden, we feel confident about our conclusions of an increased risk and synergism with other risk factors Although we found no increased risk for gene carriers who did not

have another major risk factor for coronary heart disease, the confidence intervals for these estimate were wide and did not exclude the possibility of a mildly increased risk

A further question is whether the association between the factor II mutation and the risk of MI is causal and indeed brought about by factor II In our previous study in venous thrombosis we have shown a relation between the mutation and factor II levels, which appeared instrumental to the risk [3] Therefore, even though we could not measure clotting factor levels in the current study, it is likely that a prothrombotic effect of carriership of factor II 20210 A increases the risk, since we have shown before, among these same women with MI at a young age, a similar effect of another clotting abnormality (factor V Leiden) [8]

Although generally there is no reason to assume that risk factors in the young qualitatively differ from those in middle-aged and older adults, they may differ in their strengths [19 20 23] Therefore, our findings cannot be generalized to older or male populations It may well be that clotting factor abnormalities play a role in the etiology of MI in those populations, too, but it may be more difficult to discern because with advancing age both the presence and severity of atherosclerosis increases, which results in a higher background incidence of MI among those without a particular risk factor Additionally, large groups need to be studied to examine specific interactions of atherogenic and prothrombotic risk factors in older populations We believe that it will be worthwhile to look for specific interactions of risk factors in those populations as well

ACKNOWLEDGMENT

We thank all the women who participated in the study We are grateful to the many hospital medical record administrators and physicians who assisted in the identification of patients for this study Fran Chard, Karen Graham, and Carol Handley Dahl expertly abstracted medical records, and Judy Kaiser, Marlene Bengeult, Carol Ostergard, Denise Horlander, and Barb Twaddell recruited and interviewed the patients and control subjects Sandy Tronsdal and Jill Ashman supervised these activities We thank Esther Vogels who performed the DNA analyses

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