MOLECULAR GENETICS AND METABOLISM **63,** 155–157 (1998)
ARTICLE NO. GM982697

BRIEF COMMUNICATION

Age as a Risk Factor for Myocardial Infarction in Leiden Mutation Carriers

A single factor V gene G-A mutation (Arg506Gln) underlying activated protein C (APC) resistance is a common risk factor for venous thromboembolism. It is still unclear whether the factor V Leiden predisposes patients to arterial thrombosis and myocardial infarction (MI). To determine a correlation between the factor V Leiden mutation and MI in different age categories, DNA samples from 287 patients with "early" and "late" MI were investigated. As control groups 373 young subjects (mean age 11 years) and 110 elderly ones (mean age 80 years) were studied. We found a significant difference in mutant allele distribution in the "late" MI group compared to the "early" MI group ($\chi^2 = 9.86$, OR = 13,7, $P < 0.005$) and the control group of elderly subjects ($\chi^2 = 5.92$, OR = 8.6, $P < 0.02$). The mean age of MI patients carrying the Leiden mutation was 72 years, i.e., 12 years higher than the mean age of all investigated MI patients (60 years). Thus, we found a statistically significant correlation between MI and factor V Leiden mutation in elderly subjects.

Key Words: **factor V Leiden; myocardial infarction.**

Activated protein C (APC) resistance is a recently identified thrombophilic disease due to a single factor V gene G-A mutation (Arg506Gln) known as factor V Leiden. APC is a key component in the anticoagulant system that cleaves and inactivates the prothrombotic factors Va and VIIIa. The mutation affects the site of cleavage of factor Va by APC, rendering it resistant to inactivation and increased thrombotic risk (1).

A recent study of 1690 individuals from 24 populations demonstrated that the mutation was found at a high prevalence within Europe where it varied from 2 to 7% (2).

It is known that APC resistance is the most prevalent cause of familial venous thrombosis identified so far. Among young patients with venous thrombosis but no underlying cancer, the prevalence of resistance to APC has ranged from 20 to 60% (1,3–5).

Thrombotic occlusion at the site of rupture of an atheromatous coronary plaque is the most common cause of myocardial infarction (MI). It is still unclear whether the factor V Leiden predisposes patients to arterial thrombosis and MI. In addition, an association between the factor V Leiden mutation and MI has been discussed in different studies with controversial results (6–11).

The purpose of our work was to investigate the possibility that APC resistance due to the factor V Leiden mutation could be an important predisposing factor to MI among different age categories.

MATERIALS AND METHODS

DNA samples were obtained from 287 MI patients, 373 young control subjects and 110 elderly control subjects. MI patients in the acute phase were admitted to two hospitals of St.Petersburg between May 1995 and July 1996. The mean MI patient age was 60 years (range 27–91). As a young control group we used school children aged 6 to 17 years (mean 11 years) from three secondary schools of St.Petersburg. As an elderly control group we used 110 apparently healthy persons (mean age of 80 years) from a St.Petersburg home for the elderly who had no history of MI.

Genomic DNA was isolated from whole blood by a phenol-chloroform method (12). A 223-basepair (bp) fragment spanning the exon-intron junction was amplified from genomic DNA as described by Ridker *et al.* (7). DNA analysis for the G-A mutation in exon 10 was detected by loss of a cleavage site for MnlI.

Allele frequencies were estimated by gene counting. χ^2 statistics was used to compare the distribu-

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TABLE 1
Distribution of Factor V Leiden Genotype and Allele Frequencies in MI Patients and Control Groups

Groups	Total (*N*)	Genotypes			Prevalence of mutant alleles, %
		nn	nm	mm	
Control group (young)	373	360	13	0	1.7
Control group (elderly)	110	109	1	0	0.5
MI patients	287	278	8	1	1.7
MI under 65 yrs ("early")	168	167	1	0	0.3
MI at 65 yrs and over ("late")	119	111	7	1	3.8

tion of mutant (R506Q) alleles among MI patients and the control group, and among subgroups of MI patients and control subjects.

RESULTS

Ten mutant alleles (8 heterozygote, 1 homozygote) were found among the 574 alleles tested in MI patients, resulting in an allele frequency of 1.7%. Thirteen heterozygous carriers in 746 alleles of the young control group (allele frequency, 1.7%) and 1 carrier in 220 alleles of the elderly group (allele frequency, 0.5%) were detected (Table 1). The allele frequency of this mutation among men who had MI was similar to that among the young control group. On the other hand, we have found a tendency to diminished allele frequencies in healthy elderly subjects compared to MI patients; however, this was not significant (χ^2 = 3.50, OR = 2.2, P = NS).

In detailed analysis it was found that all patients carrying mutant alleles had MI at the age of 65 and over (except for one patient with MI at the age of 40). We divided the MI patients into two groups: under and over the age of 65 ("early" and "late" MI, respectively). Allele frequency distribution of the mutation studied in patient subgroups is presented in Table 1.

The mutation frequency in the "late" MI group was 2.5 times higher than that of young control subjects and 7.5 times higher than that of elderly control ones.

We had a significant difference in mutant allele distribution among "late" and "early" MI groups (χ^2 = 9.86, OR = 13,7, $P < 0.005$) as well as "late" MI and elderly control subjects (χ^2 = 5.92, OR = 8.6, $P < 0.02$).

DISCUSSION

The R506Q factor V mutation underlies the resistance to the activated protein C observed in patients with this genotype and is prevalent in European countries where its mean frequency is in the range of 2–7% (2). The mutation frequency in the Russian population determined by investigation of 746 chromosomes in randomized pupils (1.7%) was lower than that in other European countries (4.4%).

The reported absence of the mutation in ethnic groups of Middle Eastern, Asian, and African countries has caused speculation about the possible recent appearance of the mutation in Europe and its further spread to other countries due to migration. Despite the fact that various investigations confirm the above-mentioned assumption, our previous study of Leiden mutation in Kazakhs and Uigurs (nationalities of the Central Asia) showed the mutation's existence in Asian populations as well, suggesting either a common origin of the mutation or independent mutational events in these populations (13).

It is well known today that the R506Q mutation is a predisposing factor for venous thrombosis and that its risk is seven times higher in heterozygotes and 80 times higher in homozygous carriers of the mutation (14). The mutation frequency in patients with venous thrombosis reaches 64% according to the results of some authors (3,4).

A role of the mutation in the development of arterial thrombosis (especially ishemic heart disease) is still unknown. The data from the latest investigations are contradictory. A large number of researchers found no correlation between this mutation and MI. Samani *et al.* did not report a higher frequency of factor V Leiden mutation in young men with MI (at the age up to 55 years old) compared to that of a healthy control population (6).

The investigation of 374 patients with MI by Ridker *et al.* (7) demonstrated no correlation with the mutation. Lindblad *et al.* (8) were the first who published data that APC-resistance had resulted in lethal arterial thromboembolism in a man aged 32. In addition two women, ages 33 and 34 years, with MI were reported to be homozygous for the factor V Leiden mutation.

In our study, the mutation frequency in the group with myocardial infarction (1.7%) showed no statistically significant difference compared to the control

group of children (1.8%). Patients with a history of MI were divided into two groups according to "early" and "late" MI. The mutation frequency in the control group of children was three times lower than the first group and two times lower than the second one.

The mean age of the patients with MI who are carriers of the Leiden mutation is 72 years which is 12 years higher than the mean age of all investigated patients (60 years). Martz was the first to demonstrate the increase of the mutation occurrence with aging (in patients over 55) in the group of patients with coronary artery disease (11).

For comparison, we created one more control group demonstrating the allelic distribution in the elderly. The frequency of the mutant allele in the group of the elderly was three times lower than that in the group of young patients, suggesting the loss of mutant alleles, through the death of those who have factor V Leiden with aging.

Comparison of the group of MI patients over the age of 65 with a healthy elderly population indicated a significant difference in the mutation frequency ($\chi^2 = 5.92$, $P = 0.02$, OR = 8.6). This difference was particularly distinct in the case of the comparison of two groups with "early" and "late" MI. The mutation frequency was 10 times higher in the second group than in the first one ($\chi^2 = 9.86$, $P = 0.005$, OR = 13.2).

These results allow us to assume that the influence of different risk factors for MI correlate with age. Our findings suggest that the factor V Leiden mutation is an important risk factor for MI in elderly subjects (over 65), in whom arteriosclerosis becomes the main cause of cardiovascular disease.

ACKNOWLEDGMENT

This study was supported by the Russian Foundation for Basic Research (Grant 95-04-12052).

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Received February 9, 1998

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