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Thrombosis

Homozygosity of the *T* allele of the 46 C→T polymorphism in the *F12* gene is a risk factor for acute coronary artery disease in the Spanish population

Following new guidelines that contain recommendations on the desirable features of a genetic association study, we performed a case-control study to establish the risk of acute coronary artery disease (CAD) related to the polymorphism (46 C→T) in the *F12* gene. We found a 6-fold higher risk of acute CAD associated with the homozygosity of the *T* allele of the *F12*, *46C→T* polymorphism in the Spanish population.

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Acute coronary artery disease (CAD) is complex and results from interactions between environmental and genetic factors.[1,2] Association studies have shown that there is a relation between factor XII levels and the 46 C→T polymorphism in the development of CAD, but the results are controversial.[3-6]

Our association study is unique because it was designed to avoid the usual biases of association studies in regard to establishing a polymorphism as a risk factor. We followed the new guidelines containing recommendations on the desirable features of a genetic association study.[7,8] From the GAIT Project (a family-based study), we demonstrated a high heritability (h^2 = 67%) of factor XII, and we also reported that the structural *F12* gene influenced both susceptibility to thrombosis and plasma levels of factor XII.[9-10] Following these results, we conducted an age-gender-ethnic matched, case-control study of an independent sample of Spanish individuals to assess the risk of acute CAD associated with the 46 C→T polymorphism of the *F12* gene and factor XII levels.

We included 174 patients who were diagnosed as having acute CAD, and 211 control subjects. Patients with acute CAD were admitted to the Cardiology Department of our hospital between 1998 and 2003 at their first episode of acute CAD. Acute CAD was confirmed on the basis of definitive ischemia or necrosis of the myocardium. Control subjects were friends and spouses of patients; they were included only if they had no personal history of thromboembolic disease, including venous and arterial thrombosis, cirrhosis, nephrotic syndrome or active cancer. Patients and controls gave informed consent to participation in the study. A limitation of our study is that it included the survivors of the acute event. Blood samples were obtained from the antecubital vein no earlier than 6 months after the acute episode. Fibrinogen was measured by the Clauss method as described elsewhere. Assays for factor VIII were performed on fresh plasma samples. The remaining plasma samples were stored at –80°C until used. Factor VIIIc and factor XIIc were assayed using deficient plasma from Diagnostica Stago (Asnières, France).[9,10] The 46 C→T variant was determined using the primers described previously , with minor modification in the reaction conditions.[10] For statistical analysis, using ROC curves, we considered factor VIII levels to be elevated if they were higher than 151% and fibrinogen levels elevated if they were higher than 3.5 g/L. For factor XIIc, we used levels lower than the 10th percentile (lower than 68%) as a cut-off. Odds ratios (OR) were calculated as risk for acute CAD adjusted for age and sex and other co-variables by logistic regression. We considered the genotypes C/C and C/T as the reference group. *p* values <0.05 were considered statistically significant.

The basic characteristics of the sample population are given in Table 1. All of the major cardiovascular conventional risk factors, such as hypercholesterolemia, hypertension, and diabetes mellitus, were associated with a signifi-

Table 1. Basic characteristics of patients and controls.

	ACAD (n=174) n (%)	*Controls (n=211) n (%)*	*Unadjusted OR (95% CI) and p values*
Sex (female/male)	54/120	91/120	NS
Age (years, mean, range)	57 (21-78)	57 (26-80)	NS
Smoking	96 (55)	78 (37)	2.1 (1.4-3.2)*
Hypercholesterolemia	92 (53)	44 (21)	4.3 (2.7-6.7)*
Family history of arterial thrombosis	84 (48)	38 (18)	4.3 (2.7-6.7)*
Hypertension	92 (53)	40 (19)	4.8 (3.0-7.5)*
Obesity	19 (11)	7 (3)	3.5 (1.5-8.7)*
Alcohol intake	11 (6)	11 (5)	1.7 (0.5-2.9)
Diabetes mellitus	34 (20)	11 (5)	4.4 (2,2-9.0)*
Factor VIIIc levels, % (mean, range)	186.9 (74-526)	159.4 (48-360)	*p*<0.0001
Fibrinogen, g/L, mean, range)	3.7 (1.9-6.9)	3.5 (2.1-7.8)	*P*=0.05

**Differences are statistically significant (p<0.05).*

Table 2. Risk of acute CAD (ACAD) ssociated with factor XIIc plasma levels and with T/T genotype.

	ACAD n=174	*Controls n=211*	*OR**	*OR°*
Factor XIIc >68 %, n (%)	158 (91)	207 (98)	1#	1#
Factor XIIc <68 %, n (%)	16 (9)	4 (2)	5.1 (95% CI: 1.7-15.5)	5.9 (95%CI: 1.7-20.5)
C/C and C/T, n (%)	164 (94.3)	208 (98.6)	1@	1@
T/T, n (%)	10 (5.7)	3 (1.4)	4.2 (95% CI:1.2-15.6)	4.8 (95%CI: 1.2-20.5)

**Unadjusted. °Adjusted by age, sex, smoking, hypertension, obesity, hypercholesterolemia, diabetes, fibrinogen and factor VIIIc levels. #Reference group: subjects with factor XIIc >68%. @Reference group: subjects with C/C and C/T genotype.*

cantly increased risk of acute CAD. Levels of factor VIIIc higher than 151% were associated with a higher risk of acute CAD.

The prevalence of the C/C genotype was about 60% in both patients and in controls; the prevalence of the C/T genotype was 35% in patients and 38% in controls (*p=ns).* In contrast, the prevalence of genotype T/T was almost 6% in patients and less than 2% in controls ($p<0.001$). Factor XIIc levels, analyzed as a function of the 46 C→T polymorphism, were statistically significantly different among groups with the different genotypes. Subjects with *T/T* genotype showed lower levels of factor XIIc [52.5% (range: 27-74)] than those with the other genotypes [*C/C* genotype: 124.1% (range: 58-180) and *C/T* genotype: 98.1 (range: 52-164)].

The risk of acute CAD associated with plasma factor XIIc levels and with genotype T/T is shown in Table 2. The crude OR of acute CAD associated with low levels of factor XIIc (<68%) in patients compared within controls was 5.1 (CI 95%: 1.7-15.5), when we adjusted for covariables, the OR was 5.9 (95% CI: 1.7-20.5). The crude OR of acute CAD associated with the 46 C→T polymorphism for the T/T genotype was 4.2 (95% CI: 1.2-15.6); when we adjusted for co-variables, the OR was 4.8 (95% CI: 1.2-20.5). The risk was not statistically significant when adjusted by factor XII levels as a covariate.

In conclusion, we found a 6-fold higher risk of acute CAD associated with homozygosity of the *T* allele of the *F12*, 46 C→T polymorphism in the Spanish population. We also found that low levels of factor XIIc (<68%) are associated with a higher risk of acute CAD. Knowledge of genetic variants related to the risk of acute CAD should allow us to understand arterial disease better, and ultimately lead us to improve the management of patients with acute CAD.

Amparo Santamaría, Antonio Martínez-Rubio, José Mateo, Isabel Tirado, José M. Soria, Jordi Fontcuberta*

*Unitat d´Hemostasia I Trombosis, Hematology Department, *Cardiology Department, Hospital de la Santa Creu i Sant Pau, Universitat Autónoma de Barcelona, Barcelona, Spain*

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Correspondence: Dr. Amparo Santamaría Ortiz, Department of Hematology, Hospital de la Santa Creu i Sant Pau, C/ Sant Antoni Mª Claret, 167, 08025-Barcelona, Spain. Phone: international +34.93.2919193. Fax: international +34.93.2919192. E-mail: msantamaria@hsp.santpau.es

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