

# Association studies between -1185A/G von Willebrand factor gene polymorphism and coronary artery disease

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## Abstract

High levels of von Willebrand factor (vWF) have been associated with cardiovascular disease. The A allele of the -1185A/G polymorphism in the 5'-regulatory region of the vWF gene was associated with the highest plasma vWF levels in a normal population. To examine the association between -1185A/G polymorphism and coronary artery disease (CAD), 173 Brazilian Caucasian subjects submitted to coronary angiography were studied. Of these, 57 (33%) had normal coronary arteries (control group) and 116 (67%) had CAD (patient group). Plasma vWF levels were higher in patients (145 U/dl) than in controls (130 U/dl), but the differences were significant only for O blood group subjects. Polymerase chain reaction amplification of the 864-bp vWF promoter region followed by *AccII* restriction digestion was used to identify the -1185A/G genotypes. The -1185A allele frequency was 43.1% in patients and 44.7% in controls. Allele and genotype frequencies were not significantly different between patients and controls. No association was observed between the -1185A/G genotypes and plasma vWF levels in patients or controls. These results suggest that -1185A/G polymorphism is not an independent risk factor for CAD.

## Key words

- von Willebrand factor
- Coronary artery disease
- DNA polymorphisms
- Risk factor

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## Introduction

von Willebrand factor (vWF) is a multi-meric glycoprotein serving as a carrier protein for coagulation factor VIII, and is involved in platelet adhesion and aggregation at the site of vessel wall injury. vWF-mediated platelet adhesion and thrombus formation are crucial under conditions of high shear stress such as those found in small blood vessels and in stenotic arteries. High

levels of vWF have been associated with cardiovascular disorders such as ischemic heart disease, peripheral and pulmonary vascular disease, cerebrovascular disease, left ventricular aneurysm, atrial fibrillation, and deep venous thrombosis, as reviewed by Lip and Blann (1). Some studies have shown that high plasma vWF levels are a significant and independent predictor of cardiovascular risk in subjects with coronary artery disease (CAD) (2-11), but few studies have exam-

ined the relationships between vWF genotypes and coronary heart disease (12-16).

Four single nucleotide polymorphisms (SNPs) in the 5'-regulatory region of the vWF gene have been recently associated with plasma vWF levels in a normal population (17,18). These polymorphisms are in strong linkage disequilibrium and are segregated in two distinct haplotypes. For one of these SNPs, the -1185A/G polymorphism, AA homozygotes were significantly associated with the highest vWF levels, GG homozygotes showed the lowest levels, and heterozygotes showed intermediate levels (17). Thus, the -1185A allele would be expected to be associated with an increased incidence of CAD and to constitute a risk factor.

The aims of this study were to evaluate the frequencies of the -1185A/G genotypes and alleles in Caucasian patients with CAD and controls and to examine the association of this polymorphism with CAD and with plasma vWF levels.

## Subjects and Methods

A total of 183 consecutive and unrelated Brazilian Caucasian individuals admitted for routine angiography, for investigation of chest pain and/or suspected CAD were recruited for the study from the Hemodynamics Service of the Hospital de Clínicas de Porto Alegre (Rio Grande do Sul, Brazil) over a period of nine months (March through November 1998). Subjects who had had a myocardial infarction in the 30 days before the investigation were excluded from the study. Individuals with angiography-proven  $\geq 50\%$  stenosis in a major coronary artery or one of their branches constituted the CAD group (116 individuals) and those without any angiographically detectable lesion constituted the control group (57 individuals). Ten subjects with  $>10$  and  $<50\%$  stenosis were excluded from the analysis.

All subjects gave written informed con-

sent to participate in the study. All individuals completed a standard questionnaire concerning their personal history and the Institutional Ethics Committee of the Hospital de Clínicas de Porto Alegre approved the study protocols.

Individuals reporting regular smoking during the last five years were considered to be smokers. Hypertension was defined as present if mean blood pressure was higher than 160/90 mmHg, or if the patient was already under treatment with antihypertensive drugs at inclusion. Diabetes mellitus was diagnosed as present if the fasting blood glucose level was  $\geq 126$  mg/dl or if the individuals were on treatment with insulin or other antidiabetic agents at the time of inclusion. Dyslipidemia was defined by levels of triglycerides  $\geq 160$  mg/dl, HDL cholesterol  $< 40$  mg/dl and LDL cholesterol  $> 100$  mg/dl, or if the individual was receiving lipid-lowering treatment at inclusion. Overweight or obesity was defined as BMI  $\geq 26$  kg/m<sup>2</sup>.

High-molecular weight DNA was extracted from whole blood using a nonenzymatic technique for DNA analysis (19). A 864-bp fragment was amplified by the polymerase chain reaction (PCR), as previously reported (20). DNA fragments were prepared with the *AccII* restriction enzyme, separated by electrophoresis on 1% agarose gel containing ethidium bromide, and visualized under ultraviolet light. In the absence of a cleavage site, *AccII* digestion yielded one 864-bp band (the A allele), while the presence of a cleavage site resulted in 668- and 196-bp bands (the G allele).

Plasma vWF antigen (vWF:Ag) was quantified by immunoelectrophoresis using a polyclonal rabbit antihuman vWF antibody (21). All assays were performed at two different plasma dilutions. Pooled human plasma from 20 normal donors was considered to contain 100 U/dl vWF:Ag. Plasma vWF:Ag levels were reported as U/dl relative to the level in the normal plasma pool.

Allele frequencies were determined by

direct count of the alleles. Departures from Hardy-Weinberg equilibrium and differences between groups were evaluated by the  $\chi^2$  test. The skewness of the plasma vWF:Ag distribution was normalized by logarithmic (log) transformation, although for convenience, back-transformed means and 95% confidence intervals are given in the tables. The unpaired Student *t*-test was used to compare age and vWF levels between patients and controls, and all other variables were analyzed by the  $\chi^2$  test. Multiple linear regression analysis was used to assess the relationship between plasma vWF levels and CAD risk factors (smoking, diabetes mellitus, hypertension, dyslipidemia, overweight/obesity, gender, age, and ABO blood group). Linear regression analysis was used to adjust plasma vWF levels for the effect of age and ABO. ANOVA was used to compare vWF levels between genotype groups in patients and controls. A P value <0.05 was considered to indicate statistical significance.

### Results

The main characteristics of the patients and control subjects are shown in Table 1. No significant differences were observed between patients and controls for some CAD risk factors (smoking, diabetes mellitus, hypertension and overweight/obesity), although patients were significantly older and there were more males and dyslipidemics in the patient group than in the control group.

The plasma vWF levels of patients and controls are shown in Table 2. Patients showed higher vWF levels than controls, but this difference was not significant. Plasma vWF levels differed significantly between O and non-O blood group individuals in both patients and controls (patients:  $t_{(114)} = 2.54$ ,  $P = 0.012$ ; controls:  $t_{(55)} = 2.79$ ,  $P = 0.007$ ). In an analysis carried out only with O blood group subjects, the patients had higher vWF levels than controls. Comparison between patients and controls only for subjects of

non-O blood groups did not show significant differences. Analysis of plasma vWF levels adjusted for effects of age and ABO blood group showed no significant differences between patients and controls.

Allele and genotype frequencies of -1185A/G polymorphism in patients and controls are shown in Table 3. The observed

Table 1. Main characteristics of coronary artery disease patients and control subjects.

	Patients (N = 116)	Controls (N = 57)
Mean age (range)	62.4 (32-84)*	55.6 (31-82)
Male sex	75.0*	40.3
Smoker (current or former)	31.9	22.8
Overweight/obesity	69.0	59.7
Hypertension	78.4	71.9
Diabetes mellitus	16.4	12.3
Dyslipidemia	50.9*	31.6

Data are reported as percentages, except for age (years).

\*P<0.05 compared to controls (unpaired Student *t*-test for age, and  $\chi^2$  test for other data).

Table 2. Plasma von Willebrand factor levels in coronary artery disease patients and control subjects.

Blood group	Patients (N = 116)		Controls (N = 57)	
	Mean	95%CI	Mean	95%CI
All subjects	145.0	134.1-156.8	130.0	117.6-143.7
O blood group	131.7*	118.5-146.4	108.7	99.0-119.3
Non-O blood group	160.2	143.1-179.3	143.2	124.7-164.5

95%CI: 95% confidence interval.

\*P<0.05 compared to controls (unpaired Student *t*-test).

Table 3. Allele and genotype frequencies of -1185A/G von Willebrand factor gene polymorphism in patients and control subjects.

	Patients (N = 116)	Controls (N = 57)
Allele		
A	100 (43.1)	51 (44.7)
G	132 (56.9)	63 (55.3)
Genotype		
AA	21 (18.1)	16 (28.1)
AG	58 (50.0)	19 (33.3)
GG	37 (31.9)	22 (38.6)

There were no statistically significant differences between allele frequencies or genotype frequencies ( $\chi^2$  test). Values in parentheses are percentages.

genotype frequencies were in Hardy-Weinberg equilibrium in the patient group, but in the control group there was a deficiency of heterozygotes ( $P < 0.05$ ), probably owing to the relatively small group size. The -1185A allele frequency was 43.1% in patients and 44.7% in controls. No significant differences were detected in allele or genotype frequencies between patients and controls. There was no statistically significant association between the -1185A/G genotypes and plasma vWF levels in either patients or controls. In spite of the absence of statistical significance, plasma vWF levels presented an increase in the same direction as observed by Keightley et al. (17), with AA homozygotes having the highest levels (Table 4). Analysis of the effect of -1185A/G polymorphism and ABO blood groups on plasma vWF levels showed no significant interaction between them (patients:  $F = 0.05$ ,  $P = 0.949$ ; controls:  $F = 0.47$ ,  $P = 0.628$ ).

In a multiple linear regression model, age and ABO blood groups were the only independent and significant predictors of plasma vWF levels. For each 5 years of increase in age there was a 10.2 U/dl increase in plasma vWF levels in patients and a 10.4 U/dl rise in plasma vWF levels in controls ( $P < 0.001$ ).

## Discussion

Several gene polymorphisms of the hemostatic system are currently identified as risk factors for thrombosis and/or athero-

sclerosis (22,23). We assessed the potential association between CAD and the -1185A/G polymorphism of vWF gene in Brazilian subjects submitted to coronary angiography. The main genetic variable known to be involved in vWF levels is the ABO blood system, that accounts for 30% of the genetic variance (24). Individuals of the O blood group have, on average, lower vWF levels than individuals of the non-O blood group (25-28). Our results agree with the well-documented relationship between the ABO blood system and vWF levels.

High vWF levels have been associated with CAD (2-11). In the present study patients showed higher plasma vWF levels than controls, but this difference was not significant. This may have been due to differences in frequencies of ABO blood groups between controls and patients, with an excess of O individuals in the patient group, at the limit of significance (50.9% in patients versus 35.1% in controls:  $\chi^2 = 3.833$ ,  $P = 0.05$ ). Significant differences in plasma vWF levels were observed only in subjects of the O blood group, with patients having higher vWF levels than controls.

The -1185A/G polymorphism is in strong linkage disequilibrium with three other polymorphisms (-1792C/G, -1234C/T and -1051G/A) in the promoter region of the vWF gene. Due to this linkage disequilibrium, these polymorphisms segregate as two distinct haplotypes (18). The influence of these polymorphisms on CAD was analyzed in three studies. Heywood et al. (12) studied the -1792C/G and -1051G/A polymorphisms in patients with ischemic heart disease compared to age-matched control subjects. The -1792C/G polymorphism was suggested as a potential risk predictor for ischemic heart disease, but was not correlated with vWF levels. Tompkins et al. (13) analyzed patients with unstable angina and ABO-matched and age-matched control subjects. The analysis of the three vWF gene regulatory sequence polymorphisms, including the

Table 4. Plasma von Willebrand factor levels according to -1185A/G genotype.

Genotype	Patients (N = 116)		Controls (N = 57)	
	Mean	95%CI	Mean	95%CI
AA	160.6	126.3-204.2	138.1	108.8-175.4
AG	144.6	128.9-162.3	124.0	105.3-146.0
GG	137.4	123.2-153.3	129.6	110.3-152.2

There were no statistically significant differences between patients and controls regarding -1185A/G genotypes (unpaired Student *t*-test). 95%CI: 95% confidence interval.

-1185A/G polymorphism, showed no significant difference between the two groups, but high levels of plasma vWF were associated with unstable angina. Di Bitondo et al. (15) investigated the -1185A/G and -1051G/A polymorphisms and the risk of myocardial infarction in a case-control study and found no association between them. Our results show that the allele frequencies of the -1185A/G polymorphism do not differ significantly between patients and controls, indicating that there is no association between this polymorphism and CAD.

However, it should be noted that the statistical power to detect differences in the present study may be limited due to the small sample size of the control group. On the basis of the conflicting results of these stud-

ies, it is possible to assume that the -1185A/G promoter polymorphism can have some effect on plasma vWF levels, and consequently on acute coronary events, but this influence may be slight and perhaps restricted to some specific environment-genotype effect.

The findings of the present study indicate that the -1185A/G vWF gene polymorphism is not an independent risk factor for CAD in the Brazilian Caucasian population and that plasma vWF levels were not associated with -1185A/G genotypes. Our data therefore are not consistent with the hypothesis that the -1185A/G vWF gene polymorphism is related to CAD, although an influence on outcome, or a link with subtypes of coronary syndromes, cannot be ruled out.

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