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**Patients with type 3 severe von Willebrand disease are not protected against atherosclerosis - Results from a multicenter study in 47 patients**

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# Patients With Type 3 Severe von Willebrand Disease Are Not Protected Against Atherosclerosis

## Results From a Multicenter Study in 47 Patients

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**Background**—The results of a number of studies in pigs and mice suggest that absence of von Willebrand factor (vWF) protects against the development of atherosclerosis. We studied whether patients with a complete deficiency of vWF (type 3 von Willebrand disease [vWD]) develop fewer atherosclerotic vessel wall changes than healthy controls.

**Methods and Results**—This study included 47 individuals with type 3 vWD and 84 healthy controls. Early atherosclerotic changes were assessed by measuring the thickness of the intima-media in the carotid and femoral arteries by B-mode ultrasonography. Advanced atherosclerotic changes were quantified by summing the maximal thickness of atherosclerotic plaques in the carotid and femoral arteries and were expressed as a plaque score. Established risk factors were determined to adjust for possible differences between the groups. We found no substantial difference in intima-media thickness between vWD patients and controls (adjusted difference for carotid artery 0.007 mm, 95% CI  $-0.022$  to 0.036 mm; femoral artery 0.069 mm, 95% CI  $-0.056$  to 0.19 mm). Similar proportions of patients and controls had atherosclerotic plaques (19% and 17%, respectively). No difference was found in the plaque score between groups (adjusted difference  $-0.22$  mm, 95% CI  $-0.69$  to 0.26). Among vWD patients, we found no effect of treatment with vWF concentrates on intima-media thickness or plaque score.

**Conclusions**—The results of this study indicate that vWF does not play a substantial role in human atherogenesis. (*Circulation*. 2004;109:740-744.)

**Key Words:** atherosclerosis ■ coagulation ■ von Willebrand factor ■ cardiovascular disease ■ carotid arteries

Myocardial infarction is caused by occluding arterial thrombi on top of plaques.<sup>1</sup> Hemostasis and coagulability therefore play an important role during the formation of occluding arterial thrombi. In the activation of the coagulation system, 2 separate processes can be distinguished: adhesion and aggregation of platelets, which results in a clot of platelets (primary hemostasis), and a cascade of activating clotting factors that results in the formation of insoluble fibrin from fibrinogen (secondary hemostasis). For an adequate primary hemostasis, von Willebrand factor (vWF) is essential.<sup>2</sup> Furthermore, vWF stabilizes factor VIII, one of the clotting factors involved in secondary hemostasis. A deficiency of vWF therefore results not only in an impaired primary hemostasis but also in an impaired secondary hemostasis.

The role of hemostasis in ischemic cardiovascular disease is generally thought to be in the formation of occluding

arterial thrombi. In the process of arterial thrombus formation, vWF has a key role. In particular, in vessels with high shear stress (eg, arterioles and stenosed arteries), the presence of vWF is required for mural thrombi to be formed.<sup>3</sup> Furthermore, increased vWF levels have been shown to be associated with coronary artery thrombosis.<sup>4</sup> A number of studies, however, suggest that vWF might also play a role in the development of atherosclerotic plaques. Less atherosclerosis was found in pigs and mice that had a complete deficiency of vWF.<sup>5-9</sup>

The goal of the present study was to examine whether vWF plays a role in atherogenesis. Atherosclerotic vessel wall changes were determined in patients with a complete deficiency of vWF (ie, type 3 von Willebrand disease [vWD]) and in individuals with a normal coagulation profile.

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## Methods

### Study Design

We included 47 patients with type 3 severe vWD and 84 healthy controls. Selection of the patients with severe vWD was based on the Italian part of a registry that was started in 1984 to register all patients with severe vWD in Europe.<sup>10</sup> The patients were examined at the medical centers where they were used to be treated for their bleeding tendency (ie, the medical centers in Milan, Bologna, Vicenza, Florence, Rome, Naples, and Bari) and were all enrolled in the Italian National Registry of vWD.<sup>11</sup> The controls were healthy individuals with a similar age and gender distribution. Each patient was asked to provide 2 healthy controls if possible (partner, friends, neighbors, or colleagues). For those who failed to provide the controls, healthy volunteers (colleagues, nurses, or friends) were asked to participate instead. The selection of the healthy volunteers was based on the distributions of age, gender, and the region of origin of the patients. The diagnosis of severe vWD was confirmed in all patients and was based on the original criterion of the registry in 1984, ie, vWF:Ag below 1 U/dL, determined by an immunoradiometric assay.<sup>10</sup> For both groups, only individuals aged 15 years and older were included. The Medical Ethics Committee at our institution approved the study, and all individuals gave their informed consent for participation.

### Ultrasound

The intima-media thickness of the carotid and femoral artery was measured to quantify atherosclerotic vessel wall changes. For this purpose, an Aloka SSD 1200 ultrasound machine with a 7.5-MHz linear transducer was used. To avoid variability caused by use of different ultrasound machines, one machine was used and transported to the various medical centers through Italy. The carotid arteries were scanned with the subject in supine position. On either side, 3 frozen images (double magnified) of the intima-media complex in the far wall of the common carotid artery and carotid bifurcation were visualized in the longitudinal direction and recorded on a super-VHS videotape. All images were frozen during the R-phase of the heart cycle with an ECG triggering module that was attached to the ultrasound machine. If carotid plaques were present in the measurement areas of the common carotid artery or the carotid bifurcation, the thickest plaque in each of these areas was recorded separately. Subsequently, the left and right common femoral arteries were scanned according to a similar procedure.

### Image Analysis

At a later stage, the images that were recorded on the videotapes were digitized in a personal computer and saved on CD-recordable media. The images of an individual were saved under a unique random number to ensure blindness to the subject's identity during the actual measurements. Measurements were performed with the analytical software package of the Cardiovascular Measurement System (CMS) on a personal computer.<sup>12</sup> Images were loaded from the CD into the CMS and were again magnified 4 times to visualize clearly the intima-media complex in the far wall, as has been defined by Pignoli et al.<sup>13</sup> Measurements in the common carotid artery were performed in the area 2 cm proximal from the bifurcation. In the common femoral artery, measurements were performed in the area 2 cm proximal to the bifurcation of the common femoral artery into the superficial and deep branch of the femoral artery. In all images, intima-media thickness was measured 6 times alongside a marker line at constant intervals and averaged. Mean intima-media thickness in the carotid artery was calculated by averaging the measurements of the common carotid artery and the carotid bifurcation in both sides (average of 72 measurements). Mean intima-media thickness in the femoral artery was based on measurements in the common femoral artery of both sides (average of 36 measurements). When atherosclerotic plaques were present in 1 or more of the measurement areas, the maximal thickness of each of the recorded plaques was measured 3 times and averaged. Subsequently, the obtained thicknesses of all the recorded plaques were summed, which resulted in a single plaque

score. Image acquisitions and offline measurements were all performed by 1 individual (A.S.).

### Measurement of Risk Factors

By a standardized questionnaire, we obtained information from the subjects about their smoking habits and presence of cardiovascular disease in first-degree relatives before the age of 65 years. Smoking habits were expressed as the number of pack-years an individual had smoked. Body fat distribution was determined by measuring the waist-to-hip ratio. Systolic and diastolic blood pressures were determined 3 times. Fasting total cholesterol of each individual was determined on site, after collection of blood by a finger puncture, with a Lipotrend C device with Lipotrend test strips (Boehringer Mannheim). Data about the treatment of patients with severe vWD with clotting factor concentrates were obtained from the records of the treatment centers.

### Statistical Analysis

Comparison of vessel wall thickness and exposure to various risk factors between patients with severe vWD and healthy controls was performed by the Student *t* test for continuous variables and by the  $\chi^2$  test for discrete variables. Variables that were not normally distributed were transformed logarithmically. The relation between the studied variables and intima-media thickness was determined by linear regression analysis. The regression coefficient of a continuous variable indicates the increase or decrease of the intima-media thickness (in millimeters) for every unit increase of the studied variables (eg, age and blood pressure). Discrete variables (eg, family history for cardiovascular disease or vWD status) were coded as 0 or 1 (negative=0, positive=1) and inserted as such into the model. The regression coefficient of these variables indicates the difference between the categories. In univariate regression analysis with the systolic and diastolic blood pressures as independent variables, 12 individuals were excluded because they used antihypertensive drugs. Multivariate regression analysis was used to adjust for differences in exposure to the established risk factors (ie, age, smoking habits, waist-to-hip ratio, systolic blood pressure, total cholesterol, family history of cardiovascular disease, use of cholesterol-lowering drugs, and use of antihypertension drugs) between the study groups. To examine whether there is an effect of clotting factor treatment on vessel wall thickness, we performed univariate and multivariate regression analysis among the vWD patients with mean annual vWF concentrate treatment as an independent variable.

## Results

General characteristics and presence of established risk factors for atherosclerosis in patients and controls are shown in Table 1. Age and gender distribution and exposure to most of the risk factors were similar between both groups. A larger proportion of vWD patients used drugs to control blood pressure (difference 12%, 95% CI 1% to 24%). Hypertension (defined as either a systolic blood pressure  $\geq 140$  mm Hg or a diastolic blood pressure  $\geq 90$  mm Hg or use of antihypertensive drugs) was slightly more common in vWD patients (difference 10%, 95% CI -5% to 25%). Subdivision into grades of hypertension according to World Health Organization guidelines indicated that blood pressure among individuals with hypertension might be slightly higher in patients with severe vWD than in healthy controls (difference between patients and controls when moderate and severe hypertension are taken together 5%, 95% CI -2% to 13%). Lifetime treatment with vWF concentrate among the vWD patients ranged from 0 to 845 000 U (median 43 500 U). The mean annual treatment ranged from 0 to 18 667 U (median 1733 U).

A crude comparison of intima-media thickness between patients and healthy controls is shown in Figure 1 (carotid

**TABLE 1. General Characteristics of Patients With Type 3 VWD and Controls**

Variables	VWD (n=47)	Healthy Controls (n=84)	95% CI of Difference
Age, y	35	37	-6 to 3
Smoking, pack-years	9.3	8.3	-0.4 to 1.2*
Waist-to-hip ratio	0.85	0.85	-0.04 to 0.04
Systolic blood pressure, mm Hg	123.2	118.0	-0.01 to 0.08*
Diastolic blood pressure, mm Hg	79.0	74.7	-0.002 to 0.1*
Total cholesterol, mmol/L	4.6	5.0	-0.8 to 0.02
Male gender, n (%)	20 (43)	32 (39)	-13 to 22%
Positive family history, n (%)	5 (11)	12 (15)	-15 to 8%
Use of cholesterol-lowering drugs, n (%)	1 (2)	0 (0)	-2 to 6%
Use of antihypertension drugs, n (%)	8 (17)	4 (5)	1 to 24%
Hypertension (overall), n (%)	13 (28)	15 (18)	-5 to 25%
Hypertension (mild), n (%)	2 (4)	10 (12)	-17 to 1%
Hypertension (moderate and severe), n (%)	3 (6)	1 (1)	-2 to 13%

Overall hypertension was defined as either a systolic blood pressure  $\geq 140$  mm Hg or a diastolic blood pressure  $\geq 90$  mm Hg or use of antihypertension drugs. Mild hypertension was defined as either a systolic blood pressure between 140 and 159 mm Hg or a diastolic blood pressure between 90 and 99 mm Hg (individuals using antihypertension drugs were excluded). Moderate and severe hypertension was defined as either a systolic blood pressure  $\geq 160$  mm Hg or a diastolic blood pressure  $\geq 100$  mm Hg (individuals using antihypertension drugs were excluded).

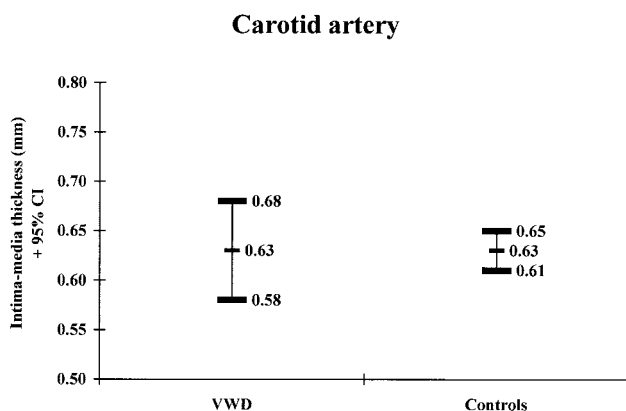
\*After logarithmic transformation.

artery) and Figure 2 (femoral artery). For both arteries, we found no difference between groups.

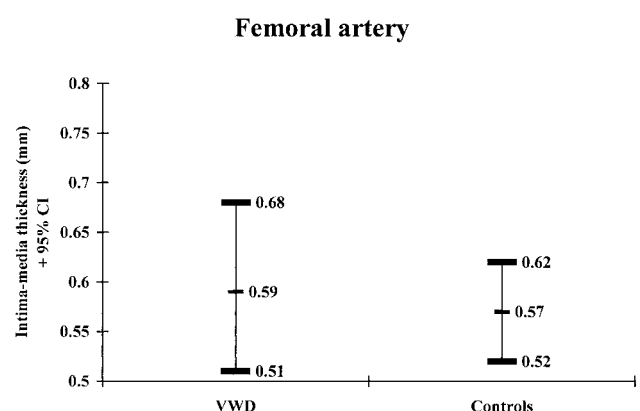
To examine the relation between vessel wall thickness in the carotid and femoral artery and the various risk factors, regression analysis was performed. First, univariate regression analysis was performed (Table 2). For both arteries, a clear relation was found between vessel wall thickness and most of the risk factors. We did not find an effect of gender (regression coefficient for carotid artery 0.002 mm, regression coefficient for femoral artery -0.06 mm) or of the presence of severe vWD (regression coefficient for carotid artery -0.005 mm, regression coefficient for femoral artery 0.022 mm) with regard to either artery. Furthermore, we found no effect of annual vWF concentrate treatment among vWD patients (regression coefficient for carotid artery -0.007 mm, regression coefficient for femoral artery

-0.015 mm). Subsequently, we performed multivariate regression analysis to adjust for the minor differences in exposure to the established risk factors. Multivariate regression analysis revealed no difference in intima-media thickness in the carotid artery between patients and controls (regression coefficient 0.007 mm, 95% CI -0.022 to 0.036). For the femoral artery, however, a minor tendency for a thicker intima-media emerged among healthy controls compared with patients (regression coefficient 0.069 mm, 95% CI -0.056 to 0.19). Among vWD patients, we found no relation between annual vWF concentrate treatment (per 1000 U) and intima-media thickness (regression coefficient for carotid artery -0.002 mm, 95% CI -0.007 to 0.004; regression coefficient for femoral artery -0.005 mm, 95% CI -0.013 to 0.003).

To examine whether a complete deficiency of vWF protects against the development and growth of advanced atherosclerotic



**Figure 1.** Comparison of mean intima-media thickness in carotid artery between patients with severe vWD and healthy controls.



**Figure 2.** Comparison of mean intima-media thickness in femoral artery between patients with severe vWD and healthy controls.

**TABLE 2. Univariate Regression Analysis of Intima-Media Thickness in Carotid and Femoral Artery**

Variables	Carotid Artery		Femoral Artery	
	Coefficient	95% CI	Coefficient	95% CI
Age (10 years)	0.075	0.06 to 0.09	0.19	0.14 to 0.24
Smoking (10 pack-years)	0.038	0.02 to 0.05	0.10	0.05 to 0.14
Waist-to-hip ratio	0.38	0.17 to 0.60	0.64	−0.050 to 1.33
Systolic blood pressure (10 mm Hg)	0.026	0.01 to 0.04	0.056	0.03 to 0.08
Diastolic blood pressure (10 mm Hg)	0.029	0.01 to 0.05	0.061	0.03 to 0.10
Total cholesterol (mmol/L)	0.029	0.013 to 0.044	0.080	0.021 to 0.14
Gender (males vs females)	0.002	−0.044 to 0.048	−0.06	−0.20 to 0.082
Positive family history	0.12	0.058 to 0.19	0.16	0.044 to 0.37
Hypertension	0.13	0.076 to 0.18	0.37	0.22 to 0.53
vWD vs controls	−0.005	−0.052 to 0.042	0.022	−0.12 to 0.17
Annual vWF concentrate treatment (1000 U)*	−0.007	−0.017 to 0.004	−0.015	−0.032 to 0.003

Hypertension was defined as either a systolic blood pressure  $\geq 140$  mm Hg or a diastolic blood pressure  $\geq 90$  mm Hg or use of antihypertension drugs.

\*Analysis among vWD patients only.

lesions, we compared the presence of atherosclerotic plaques in the carotid and femoral arteries between study groups. A similar proportion of vWD patients and controls had 1 or more visible atherosclerotic plaques in the measurement areas of the carotid and femoral arteries (19% and 17%, respectively). Subsequently, the thickness of the thickest plaque in every measurement area was summed, which resulted in a plaque score that ranged from 0 to 11.3 mm. No adjusted difference in plaque score between the 2 groups was found (difference  $-0.22$  mm, 95% CI  $-0.69$  to  $0.26$ ). Furthermore, we found no effect of annual vWF concentrate treatment (per 1000 U) on plaque score among vWD patients (regression coefficient  $-0.017$  mm, 95% CI  $-0.107$  to  $0.072$ ).

## Discussion

In the present study, we examined whether a hereditary complete deficiency of vWF results in fewer atherosclerotic vessel wall changes. Patients with type 3 vWD have no measurable vWF levels in plasma, platelets, and endothelial cells<sup>14</sup> and therefore form the ideal group to study the pathogenic role of vWF in human atherogenesis. The results show that individuals with a complete deficiency are not protected against the development of early and advanced atherosclerotic lesions.

We measured intima-media thickness in the carotid and femoral artery by B-mode ultrasonography as an indicator of generalized and coronary atherosclerosis. Histological studies have shown that quantification of the intima-media thickness in the far wall of arteries by ultrasonography corresponds well with the true histological thickness.<sup>13,15</sup> The results of the present study and numerous other studies showed clear relationships between ultrasonographically determined intima-media thickness in the carotid and femoral artery and exposure to the established risk factors of atherosclerosis, such as arterial hypertension and hypercholesterolemia, even in young individuals.<sup>16–19</sup> Furthermore, it has been shown that intima-media thickness of the carotid and femoral artery is a good indicator of atherosclerotic lesions in the coronary and peripheral arteries.<sup>20–22</sup> In a recent study, we have shown

that the technique we have used is reliable and accurate to determine intima-media thickness in the carotid and femoral artery.<sup>23</sup> One might wonder, however, whether intima-media thickness in relatively young individuals represents a true stage in the development of atherosclerosis. Atherosclerotic plaques (present in nearly 20% of the individuals) were therefore also quantified and included in the analysis.

Experimental animal studies in pigs with vWD showed a protection against aortic atherosclerosis in pigs with homozygous (severe) vWD, whereas no protection was found in pigs with heterozygous (mild) vWD.<sup>6,24</sup> These findings are supported by the results of a recently published study in mice with a complete deficiency of vWF.<sup>9</sup> Because vWF plays a role in primary and secondary hemostasis, protection against atherosclerosis could be explained by decreased adhesion and aggregation of platelets to endothelial and subendothelial structures<sup>25–27</sup> and by decreased plasmatic coagulability.<sup>28</sup> In a recent study, we found no substantial protection against atherosclerosis among individuals with hemophilia.<sup>22</sup> That result suggests that the protection that was found in pigs and mice with severe vWD is caused by an impaired adhesion and aggregation of platelets to the vessel wall. Postmortem studies of individuals who had severe vWD, however, showed that patients with a complete deficiency of vWF developed advanced atherosclerotic lesions.<sup>29,30</sup>

The results of the present study do not support the protection that was found in the pigs and mice with severe vWD and confirm previous autopsy findings in men.<sup>30</sup> An explanation for this difference in results might be that the patients in the present study were treated with clotting factors during bleeding episodes, whereas the pigs and mice were never treated. Among the patients with severe vWD, however, we found no relation between treatment with clotting factors and atherosclerotic vessel wall changes. Furthermore, platelet and endothelial vWF are not modified by transfused concentrates.<sup>14</sup> Another explanation might be the different arteries in which we have quantified the atherosclerotic

changes. In the pigs, protection against aortic atherosclerosis was found, whereas no protection was found in the coronary arteries.<sup>8,24,31,32</sup> It is known that the pace of development of atherosclerosis is different for the various sites in the vessel tree, depending on some recognized factors, such as shear stress, and some as yet unknown factors.<sup>33</sup> In the present study, we found advanced atherosclerotic lesions and found no differences between groups. Furthermore, we showed in a previous study that intima-media thickness measurements in the carotid and especially in the femoral artery are a good indicator of advanced atherosclerotic lesions in the coronary arteries.<sup>22</sup> Because the major role of vWF is to promote thrombus formation in stenosed vessels,<sup>2</sup> we can speculate that type 3 vWD patients, who lack vWF in plasma, platelet, and subendothelium, might be protected against arterial occlusion even in the presence of atherosclerotic lesions.

The data suggest a slightly higher prevalence and severity of hypertension among patients with severe vWD. In a study that was performed in patients with hemophilia, a higher prevalence of hypertension was found.<sup>34</sup> The results of the present study are based on a relatively small number of subjects. Our results, however, warrant a larger study on the prevalence of hypertension in patients with vWD. The results of the present study show no protection against atherosclerosis in patients with type 3 vWD and suggest that vWF does not play a significant role in the origin of human atherosclerosis.

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