Lower levels of vWF are associated with lower risk of cardiovascular disease

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Abstract

Objective: The current study was undertaken to prospectively explore whether having low levels of von Willebrand factor (vWF) antigen and vWF activity reduce the risk for cardiovascular disease and death.

Methods: VWF antigen and vWF activity were measured by enzyme-linked immunosorbent assay and an immunological-based assay, respectively, in a subsample of 4857 individuals aged between 35 and 74 years old, enrolled between April 2007 and October 2008 in the population-based Gutenberg Health Study. VWF antigen and activity below the 20th percentile was set as a measure of “low vWF.” Adjusted robust Poisson regression models were used to analyze the relation between low vWF and the incidence of cardiovascular disease (CVD). Consequent adjusted cox regression models as well as cumulative incidence plots were calculated to explore the relation between all-cause and cardiovascular mortality and low vWF.
INTRODUCTION

Von Willebrand factor (vWF), a multimeric glycoprotein present in plasma, plays an important role in hemostasis. In vivo synthesis of vWF occurs in endothelial cells or megakaryocytes. Subsequently, depending on the site of the synthesis, vWF is either stored within endothelial cells in organelles known as Weibel-Palade bodies or within platelet α-granules. Following damage to the vascular wall, agonists (e.g., on endothelial cells: histamine, estrogen, fibrin; on platelets: ADP) will trigger the release of vWF from the Weibel-Palade bodies and α-granules.\(^1\) VWF mainly affects hemostasis by facilitating platelet adhesion and by stabilizing coagulation factor VIII, thereby optimizing its function to enhance fibrin formation.\(^2\) In addition, by recruiting platelets to the damaged vessel wall, via the interaction with the platelet glycoprotein 1b, vWF stimulates platelet adhesion and aggregation contributing to proinflammatory effects that may enhance atherosclerosis.\(^3,4\) In studies of the general population, vWF plasma levels showed weak positive associations with cardiovascular disease (CVD), whereas a more convincing association between vWF levels and CVD were observed in a high-risk population.\(^5,6\) In addition, results from a recent study showed that individuals with high vWF antigen levels have a higher risk of cardiovascular mortality than those with the lowest levels.\(^7\) Elevated levels of vWF activity were found in subclinical atherosclerosis in a study population of rheumatoid arthritis patients.\(^8\)

VWF levels are genetically determined by single-nucleotide polymorphisms (SNPs) in the vWF gene. To date, four SNPs have been identified to affect the vWF gene; rs7964777, rs7954855, rs7965413, and rs7966230. Numerous studies demonstrated that the genetic variations in the vWF promoter region resulted in higher levels of vWF.\(^9,10\) Higher levels of vWF were repeatedly associated with cardiovascular disease, in particular arterial thrombosis; thus, SNPs affecting the vWF may be directly linked to the incidence of arterial thrombosis.\(^11–13\) In addition, novel genes that affect the vWF levels were identified, such as ADAMTS-13, TSP-1, and most recently soluble NSF attachment protein receptor genes. Although more extensive research is required, the first results showed an association between these genes, vWF, and the risk of arterial thrombosis.\(^7,14,15\)

A quantitative or qualitative deficiency of vWF, known as von Willebrand disease (VWD), poses an individual at greater risk of bleeding, most commonly manifested as epistaxis or menorrhagia.\(^16\) Lower plasma levels of vWF were associated with reduced development of aortic atherosclerosis in pigs.\(^17,18\) Studies investigating the effect of VWD on CVD in humans demonstrated a significantly lower prevalence of CVD in VWD patients.\(^19–21\) VWD is an underdiagnosed condition because of the mild manifestation of symptoms, as well as a physiological wide range of vWF plasma levels in the population.\(^22\) Therefore, exploring the association between physiologically low levels of vWF rather than extreme low levels of vWF as in VWD, may give different findings than the observed studies.

We hypothesized that the lower risk for CVD is not limited to VWD patients but also occurs in individuals at the lower end of the vWF activity and antigen range. Therefore, we carried out the present analysis within a subsample of the Gutenberg Health Study (GHS) to
prospectively explore whether having low levels of vWF antigen and vWF activity reduces the risk for cardiovascular disease and death.

2    METHODS

2.1    Research design

The GHS is a population-based, prospective, observational, single-center cohort study and included 15,010 individuals at baseline examination. The aim of the GHS is to improve individual cardiovascular risk stratification. The sample was drawn randomly from the governmental local registry offices in the City of Mainz and the district of Mainz-Bingen and was stratified 1:1 for sex and residence (urban and rural) and in equal strata for decades of age. Individuals between 35 and 74 years of age were enrolled, and written informed consent was obtained from all participants for laboratory analyses, clinical examinations, sampling of biomaterial, and use of data records for research purposes. At the baseline visit, study participants underwent an extensive 5-h standardized clinical examination program as further discussed elsewhere. Next to this, a large biobank for further biochemical and genetic analyses was established. The study was designed in line with the tenets of the revised Helsinki protocol and the study protocol and sampling design were approved by the local ethics committee and by the local and federal data safety commissioners.

Primary outcomes were incidence of myocardial infarction (MI) and death related to CVD (MI, coronary artery disease [CAD], heart failure [HF], atrial fibrillation [AF], venous thromboembolism [VTE], stroke, peripheral artery disease [PAD]). Secondary outcomes were the incidence of CAD, stroke, HF, AF, PAD, or all-cause mortality. Primary and secondary outcomes were assessed during the follow-up period through collection of the data on the participants from the University Medical Center in Mainz, as well as during the telephone interview after 2.5 years and the follow-up examination after 5 years. Afterward, the data on primary outcome were discussed by a research panel consisting of two physicians and one epidemiologist. Cardiovascular death was defined as mortality from MI, CAD, stroke, PAD, HF, AF, and VTE was assessed through the medical death certificate.

2.2    Follow-up

As part of the follow-up, a standardized computer-assisted telephone interview and an inventory of primary and secondary outcomes were done 2.5 years after baseline visit. In addition, participants underwent a quinquennial, extensive clinical examination in the research facility similar to the baseline visit. During the follow-up period until June 2020 (median follow-up 10.1 years) 741 of 5000 participants were lost to follow-up. Reasons for loss to follow-up were death (126 individuals), discontinuation of participation by participant (420 individuals), relocation (59 individuals), and lack of success in contacting the participant (136).

2.3    Blood sampling and laboratory assessment

Venous blood sampling was performed according to standard operating procedures and the blood was collected in 3.2% (w/v) trisodium citrate (0.109 M, 1:9 vol:vol) monovette plastic tubes, while the subject was in fasting state (i.e., overnight fast, if subject was examined before 12 a.m. and 5-h fast, if the subject was examined after 12 a.m.). Platelet poor plasma was prepared by one-step centrifugation at 2000g at room temperature for 10 min. After preparation the platelet poor plasma was aliquoted and immediately stored at −80°C. VWF antigen was measured by enzyme-linked immunosorbent assay in the Laboratory for Clinical Thrombosis and Hemostasis at Maastricht University Medical Center. VWF activity was measured by means of immunological-based assay at the departments of Clinical Epidemiology and Systems Medicine at University Medical Center Mainz. Factor (F)-VIII (%) was measured by means of the Siemens BCS XP System, using the clotting-based coagulation methodology at the latter institution.

2.4    Study population

For this study, a subsample of the first 5000 participants of the population-based GHS enrolled between April 2007 and October 2008, were included. Because of the lack of material sent to Maastricht University Medical Center, resulting in a discrepancy between the total number of samples assessed at the two locations, 4857 individuals remained for further analysis. The reference group was defined as apparently cardiovascular healthy subjects (i.e., not suffering from CVD [MI], heart failure, presence of cardiovascular risk factors or use of antithrombotic agents, oral contraceptives or hormonal replacement therapy).

2.5    Definition of traditional cardiovascular risk factors

Diabetes mellitus and dyslipidemia were defined as individuals with a definite diagnosis of the respective disease by a physician. Additional definition of diabetes was a blood glucose level of ≥126 mg/dl in the baseline examination after an overnight fast of at least 8 h or a blood glucose level of ≥200 mg/dl in the baseline examination after a fasting period ≥8 h. Dyslipidemia was additionally defined as a low-density lipoprotein/high-density lipoprotein ratio >3.5. Hypertension was diagnosed, if antihypertensive drugs are taken, or a mean systolic blood pressure of ≥140 mmHg, or a mean diastolic blood pressure of ≥90 mmHg (in the second and third standardized measurement after 8 and 11 min of rest). Smoking was classified into nonsmokers (never smokers and former smokers) and smokers (occasional smoker [i.e., <1 cigarette/day] and smoker [i.e., ≥1 cigarette/day]). Obesity defined as a body-mass index ≥30 kg/m². Self-reported CAD, MI, HF, stroke, deep vein thrombosis, pulmonary embolism (PE), and PAD indicated personal history of CVD. A positive family history was defined as history of myocardial infarction...
or stroke in a female first-degree relative ≤65 years or a male first-degree relative ≤60 years.

2.6 | Data management and statistical analysis

A central data management unit conducted quality control on all data in this study. Statistical analysis was performed with software program R, version 3.3.1 (www.R-project.com). To study whether low levels of vWF affect the risk for cardiovascular disease and death, vWF antigen and activity below the 20th percentile were set as a measure of “low vWF.” Robust Poisson regression models, excluding subjects with prevalent CVD and adjusted for age, sex, traditional cardiovascular risk factors, and F-VIII were used to analyze the relationship between low levels of vWF antigen and activity (i.e., below the 20th percentile) and the incidence of CVD.

Consequent Cox regression models, each additionally adjusted for age, sex, cardiovascular risk factors, CVD, and F-VIII were calculated to explore the relation between all-cause and cardiovascular mortality and vWF antigen and activity below the 20th percentile. Cumulative incidence plots of cardiovascular mortality were calculated according to the Gray test, correcting for competing events. Because of the explorative nature of the current study, p values were not set as a means of statistical significance.

3 | RESULTS

3.1 | Sample characteristics

Details on the study population are reported in Tables 1 and 2. Data on the ethnic background of the study population are provided in Table S1. Overall, there was a balanced sex ratio in the study population across all the individual percentiles of vWF antigen and vWF activity. As shown in Tables 1 and 2, both vWF antigen and activity increased with age (e.g., mean age was 60.4 years in the subgroup of vWF antigen >80% compared with a mean age of 51.1 years in the subgroup of vWF antigen <20%). Arterial hypertension was the most prevalent cardiovascular risk factors across all the percentiles of vWF antigen and activity, followed by dyslipidemia. The prevalence of all cardiovascular risk factors, except for smoking, increased with higher vWF levels (Tables 1 and 2). Of the preexisting comorbidities, CAD (4.6%) was predominant across all percentiles of vWF antigen and vWF activity, followed by PAD and VTE. Similarly, the incidence of CAD was highest across all the percentiles of vWF antigen and vWF activity, followed by PAD.

As shown in Tables 1 and 2, the group of vWF antigen and activity below the 20th percentile consisted of the highest number of subjects with blood group O (67.9% and 72.6%, respectively).

3.2 | Distribution of vWF

In general, minimum and maximum measured values in the study population of vWF antigen were 28% and 369% and of vWF activity these were 22.8% and 300%, respectively, as demonstrated in Table 3. Furthermore, the median value of vWF antigen was 112% (Q1, 88%; Q3, 141%) and vWF activity was 107% (Q1, 81.6%; Q3 138%). The 20th percentile, which was the threshold set to be considered “low vWF,” corresponded to vWF antigen levels of 83% and vWF activity levels 76.2% as shown in Table 3.

3.3 | Low levels of vWF and incidence of cardiovascular disease

Results from the multiple robust Poisson regression analyses for vWF antigen and vWF activity are presented in Table 4. vWF activity level below the 20th percentile of the reference group (i.e., below 76.2%) was associated with a decreased relative risk for

| Variable                  | <20% (N = 942) | 20%–80% (N = 2974) | >80% (N = 941) |
|---------------------------|---------------|--------------------|---------------|
| Sex (women)               | 50.1% (472)   | 49.1% (1460)       | 47.0% (442)   |
| Age, y                    | 51.1 ± 10.3   | 55.4 ± 10.8        | 60.4 ± 9.9    |
| BMI, kg/m²                | 25.7 (23.4/28.3) | 26.5 (23.9/29.8)  | 27.7 (24.7/31.5) |
| Blood group (0-type)      | 67.9% (549)   | 37.0% (914)        | 15.6% (122)   |
| Prevalent CVD             | 7.3% (616)    | 11.6% (345)        | 21.8% (203)   |

Prevalence of cardiovascular risk factors:
- Diabetes: 5.6% (53) 9.3% (275) 16.8% (158)
- Hypertension: 41.3% (389) 51.3% (1526) 61.7% (580)
- Dyslipidemia: 38.2% (360) 44.7% (1327) 52.5% (494)
- Obesity: 16.3% (154) 23.9% (712) 33.2% (312)
- Smoking: 21.3% (200) 19.4% (574) 17.0% (160)
- FH of MI/stroke: 22.3% (210) 23.6% (702) 24.4% (228)

Abbreviations: BMI, body mass index; CVD, cardiovascular disease; FH, family history; MI, myocardial infarction; vWF, von Willebrand factor.
### TABLE 2 Sample characteristics according to percentiles of vWF activity

| Variable                        | <20% N = 991 | 20%-80% N = 2985 | >80% N = 994 |
|---------------------------------|--------------|------------------|--------------|
| Sex (women)                     | 47.9% (475)  | 48.5% (1449)     | 51.8% (515)  |
| Age, y                          | 51.2 ± 10.2  | 55.4 ± 10.8      | 59.9 ± 10.3  |
| BMI, kg/m²                      | 25.6 (23.3/28.5) | 26.5 (24.0/29.7) | 27.4 (24.5/31.3) |
| Blood-type (0-type)             | 72.6% (618)  | 36.2% (900)      | 13.2% (108)  |
| Prevalent CVD                   | 7.5% (74)    | 12.0% (357)      | 20.3% (200)  |

#### Prevalence of cardiovascular risk factors

- Diabetes: 6.0% (59)
- Hypertension: 43.3% (429)
- Dyslipidemia: 39.3% (389)
- Obesity: 17.7% (175)
- Smoking: 20.9% (207)
- Family history of MI/stroke: 21.8% (216)

| Prevalence of cardiovascular risk factors |
|------------------------------------------|
| Diabetes                                | 6.0% (59) |
| Hypertension                            | 43.3% (429) |
| Dyslipidemia                            | 39.3% (389) |
| Obesity                                 | 17.7% (175) |
| Smoking                                 | 20.9% (207) |
| Family history of MI/stroke             | 21.8% (216) |

**Abbreviations:** BMI, body mass index; CVD, cardiovascular disease; FH, family history; MI, myocardial infarction; vWF, von Willebrand factor.

### TABLE 3 Distribution of vWF antigen and vWF activity

| Variable        | Minimum | Mean (SD) | Median (Q1/Q3) | 5th percentile | 20th percentile | 95th percentile | Maximum |
|-----------------|---------|-----------|---------------|---------------|----------------|----------------|---------|
| vWF antigen (%) | 28.0    | 122 (52.6) | 112 (88.0/141)| 61.2          | 83.0           | 236            | 369     |
| vWF activity (%)| 22.8    | 112 (39.5) | 107 (81.6/138)| 56.4          | 76.2           | 179            | 300     |

**Abbreviations:** Q1, quartile 1; Q3, quartile 3; SD, standard deviation; vWF, von Willebrand factor.

### TABLE 4 Multiple robust Poisson regression analysis of the incidence of CVD and vWF antigen and activity below the 20th percentile of the total study population

| Variable                        | Model 1a | Model 2b | Model 3c |
|---------------------------------|----------|----------|----------|
|                                 | RR (95% CI) | p-value | RR (95% CI) | p-value | RR (95% CI) | p-value |
| vWF antigen <20th percentile    | 0.72 (0.46–1.11) | 0.14    | 0.77 (0.49–1.19) | 0.24    | 0.79 (0.50–1.26) | 0.33    |
| of the total study population   |          |         |          |         |           |         |
| vWF activity <20th percentile   | 0.57 (0.35–0.90) | **0.018** | 0.59 (0.37–0.95) | **0.029** | 0.60 (0.36–0.99) | **0.046** |
| of the total study population   |          |         |          |         |           |         |

**Note:** All models were excluding subjects with prevalent CVD. N cases low vWF = 19, N noncases low vWF = 972. N cases total study population = 170, N noncases total study population = 3809.

**Abbreviations:** CI, confidence interval; RR, relative risk; vWF, von Willebrand factor.

- Model 1 was adjusted for age and sex.
- Model 2 was additionally adjusted for the traditional cardiovascular risk factors.
- Model 3 was additionally adjusted for levels of F-VIII.

### 3.4 Cumulative incidence plots

As presented in Figure 1, vWF antigen below the 20th percentile was significantly correlated with decreased death as a result of cardiovascular events (p = 0.00011 for trend). Furthermore, the vWF activity below and within the 20th percentile of the reference group was associated with a reduced mortality because of cardiovascular events (p = 0.0027 for trend).
3.5 | VWF and mortality

The analysis of the low levels of vWF antigen and vWF activity (i.e., below the 20th percentile of the reference group) and risk of all-cause and cardiovascular mortality is shown in Table 5. During a follow-up period until June 2020 (median follow-up of 10.1 years), a total of 351 deaths were registered of which 81 deaths were registered as a result of cardiovascular events. VWF antigen below the 20th percentile (i.e., vWF antigen levels below 83%) was significantly associated with lower risk of all-cause mortality (HR: 0.60, 95% CI: 0.41–0.88), adjusted for age and sex. After adjusting for the traditional cardiovascular risk factors and a history of CVD, the association remained significant (HR: 0.61, 95% CI: 0.41–0.91). However, after further adjusting for F-VIII (%), the association was lost (HR: 0.81, 95% CI: 0.53–1.23). Furthermore, vWF activity below the 20th percentile (i.e., vWF activity levels below 76.2%) was associated with a reduced risk for all-cause mortality (HR: 0.69, 95% CI: 0.49–0.99) in a model corrected for age, sex, and traditional cardiovascular risk factors. However, after additionally adjusting for pre-existing CVD and F-VIII (%), the association did not persist (HR: 0.99, 95% CI: 0.67–1.47).

In addition, subjects with vWF antigen levels below the 20th percentile showed a tendency for diminished risk of cardiac cause-related death (HR: 0.40, 95% CI: 0.14–1.10). After additionally adjusting for F-VIII levels, the association was lost (HR: 0.38, 95% CI: 0.12–1.23).

4 | DISCUSSION

One of the main findings of this study was that vWF activity below 76% (i.e., below the 20th percentile of the reference group) was associated with a 40% decreased risk for CVD, independent of age, sex, cardiovascular risk factors, and previous CVD. Furthermore, the current study showed that having vWF antigen levels below 83% (i.e., below the 20th percentile of the reference group) correlated with a 40% decreased risk of all-cause mortality, which was attenuated after adjusting for F-VIII (%).

In addition to this, the cumulative incidence plots showed that vWF antigen below 83% and vWF activity below 76.2% were significantly associated with fewer deaths related to CVD.

To the best of our knowledge, this is the first study that demonstrated an association of lower risk of incident CVD in individuals with physiologically low levels of vWF activity and increased survival when having physiologically low levels of vWF antigen. Previously, Seaman and colleagues conducted a study to compare the incidence of CVD in a vWF-deficient and vWF-nondeficient group. However, the definition of vWF deficiency was limited to vWF antigen (<0.5 IU/dl).19

VWF affects hemostasis by mediating platelet adhesion to the damaged vascular wall on the one hand, as well as by binding to F-VIII, thereby limiting its degradation on the other hand.25 Hemostasis also plays a pivotal role in the pathogenesis of atherosclerosis, which

FIGURE 1 (A) Cumulative incidence plots demonstrating the cumulative incidence of cardiovascular mortality during a follow-up period of 10 years, in individuals with vWF antigen levels below the 20th percentile (green line), between 20th and 40th percentile (turquoise line), between 40th and 60th percentile (blue line), between 60th and 80th percentile (red line), and above the 80th percentile (purple line). (A) p value of 0.00011 for the difference between the percentiles. The plots are corrected for competing risks (death by noncardiovascular causes). (B) Cumulative incidence plots demonstrating the cumulative incidence of cardiovascular mortality during a follow-up period of 10 years, in individuals with vWF activity levels below the 20th percentile (green line), between 20th and 40th percentile (turquoise line), between 40th and 60th percentile (blue line), between 60th and 80th percentile (red line), and above the 80th percentile (purple line). (B) p-value of 0.0027 for the difference between the percentiles. The plots are corrected for competing risks (death by noncardiovascular causes).
studies that were investigating the prevalence of atherosclerotic VWD type 3 patients, meaning patients have a full quantified.29,30

Sramek and coworkers could not find differences in prevalence of atherosclerotic plaque in type 3 VWD patients as compared with the reference sample.27,28 However, these reports were from small-scale studies that were investigating the prevalence of atherosclerotic plaque rather than occurrence of CVD events. Besides, the studies included VWD type 3 patients, meaning patients have a full quantitative deficiency of vWF.

The link between blood group ABO and quantity and quality of vWF along with the relation with CVD has long been recognized.29,30 Emerging recent data suggested that there is an enhanced clearance of vWF in subjects with blood group O, rather than a decreased synthesis.31 It has been demonstrated that blood group O has 25% lower plasma levels of vWF compared to non-O blood group. Furthermore, in individuals with blood group O, VWF demonstrated enhanced susceptibility to ADAMTS13 proteolysis. Preliminary findings also suggested that the interaction of vWF in blood group O with platelets may also be reduced.31 In addition to the relation with vWF quantitative and qualitative properties, in the same study population, we have previously shown that individuals with blood group O have lower FVIII:c. Adjusted for sex and age, ABO-blood group accounted for 18.3% of FVIII:c variation.32 Therefore, ABO blood group was omitted as confounder in the analysis. Furthermore, when the analysis was adjusted for levels of F-VIII, the association persisted, illustrating that the association was independent of levels of F-VIII. Folsom et al. previously demonstrated that the incidence of CVD was affected by both vWF and F-VIII, independently.33 However, based on the results it could not be determined whether the effect was mediated through one another or whether vWF and F-VIII are intertwined. The present analysis may suggest that the observed association is predominantly determined by levels of vWF rather than F-VIII. Holm and colleagues previously reported a decreased CVD-related mortality in hospitalized VWD patients. However, the analysis was conducted within a patient population, rather than assessing the individual vWF antigen and vWF activity test outcomes.34 Our findings demonstrate that CVD-related and all-cause mortality is decreased in individuals with physiologically low vWF antigen levels, in addition to VWD patients.

As in accordance with previous studies, this analysis demonstrated increasing levels of vWF with age.35,36 The mechanism underlying the relation between age and vWF levels may be manifold, as described by a recent, extensive review by Alavi and colleagues. A pivotal underlying process is the age-related vascular dysfunction inflammation resulting from chronic endothelial stress. Indeed, vWF is a known marker of endothelial dysfunction. Moreover, there may be increased constitutive secretion of vWF through factors known to affect vWF levels like thrombin, histamine, and vasopressin. It has also been postulated that vWF increases with age due to a decrease of ADAMTS-13, resulting in the persistent circulation of ultralarge vWF multimers.37

| TABLE 5 Cox regression models exploring the correlation between vWF antigen and activity levels below the 20th percentile of the total study population and mortality |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|
|                                 | Model 1<sup>a</sup> | Model 2<sup>b</sup> | Model 3<sup>c</sup> | Model 4<sup>d</sup> |
|                                 | HR (95% CI)        | HR (95% CI)        | HR (95% CI)        | HR (95% CI)        |
| All-cause mortality             |                  |                  |                  |                  |
| vWF antigen <20th percentile    | 0.60 (0.41–0.88)  | 0.62 (0.42–0.92)  | 0.61 (0.41–0.91)  | 0.81 (0.53–1.23)  |
| of the total study population   |                  |                  |                  |                  |
| vWF activity <20th percentile   | 0.69 (0.49–0.99)  | 0.72 (0.51–1.03)  | 0.73 (0.50–1.05)  | 0.99 (0.67–1.47)  |
| of the total study population   |                  |                  |                  |                  |
| Cardiovascular mortality        |                  |                  |                  |                  |
| vWF antigen <20th percentile    | 0.40 (0.14–1.10)  | 0.41 (0.15–1.13)  | 0.33 (0.10–1.058) | 0.38 (0.12–1.23)  |
| of the total study population   |                  |                  |                  |                  |
| vWF activity <20th percentile   | 0.82 (0.39–1.71)  | 0.82 (0.39–1.71)  | 0.77 (0.35–1.70)  | 0.91 (0.41–2.04)  |
| of the total study population   |                  |                  |                  |                  |

Note: N cases low vWF = 19, N noncases low vWF = 972. N cases total study population = 170, N noncases total study population = 3809.
Abbreviations: CI, confidence interval; CVD, cardiovascular disease; F, factor; HR, hazard ratio; vWF, von Willebrand factor.
<sup>a</sup>Model 1 was adjusted for age and sex.
<sup>b</sup>Model 2 was additionally adjusted for traditional cardiovascular risk factors.
<sup>c</sup>Model 3 was additionally adjusted for CVD.
<sup>d</sup>Model 4 was additionally adjusted for levels of F-VIII.
Limitations of the study were as follows: (1) The composite study population could have caused potential bias because of prevalent cardiovascular risk factors and diseases. (2) There were a total number of 741 participants lost to follow-up. As provided in Table S2, the rate of prevalent CVD was in this group higher than the overall study population (20.8% vs. 11.4%). This should be considered as it could cause potential bias. (3) There may have been unknown confounders (e.g., social deprivation) that were not adjusted for, as these were not measured in the GHS. The major strength of this large-scale study is the prospective design.

To summarize, this study revealed associations between low levels of vWF antigen and activity and a diminished risk of (CVD-related) mortality. Importantly, these individuals were not identified as VWD patients. Thus, there was no bleeding tendency in these individuals.

Overall, this may shine new light on vWF as a potential target for novel therapeutic prevention of CVD; however, its implementation is questionable. On one side, reducing levels of vWF will also diminish F-VIII levels, thereby inducing a bleeding tendency. On the other side, reducing F-VIII levels to approximately 50% in those with levels above 100% and high CVD risk could be beneficial without apparent bleeding risk.

AUTHOR CONTRIBUTIONS
P.C.S. van Paridon performed research, analyzed and interpreted data, and wrote the paper; M. Panova-Noeva interpreted data and contributed to writing the paper; R. van Oerle performed research and contributed to writing the paper; A. Schulz performed the statistical analysis; N. Arnold and J.H. Prochaska contributed to discussion of the results and to critical review; I. Schmidtmann, M. Beutel, and N. Pfeiffer performed research; T. Münzel, K.J. Lackner, and H. ten Cate contributed to writing the paper and to critical review; P. Wild and H.M.H. Spronk designed and performed research, interpreted data and contributed to writing the paper.

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**SUPPORTING INFORMATION**

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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