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von Willebrand factor and coronary heart disease

Prospective study and meta-analysis

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Aims To determine whether circulating von Willebrand factor concentrations are prospectively related to risk of coronary heart disease in the general population.

Methods and Results We measured baseline von Willebrand factor values in the stored serum samples of 625 men with major coronary events and in 1266 controls 'nested' in a prospective study of 5661 men aged 40–59 years, recruited from general practices in 18 British towns in 1978–1980 and followed up for 16 years for fatal coronary heart disease and non-fatal myocardial infarction. We conducted a meta-analysis of previous relevant studies to place our results in context. Men in the top third of baseline von Willebrand factor values (tertile cutoff >126 IU . dl^{-1}) had an odds ratio for coronary heart disease of 1·83 (95% confidence interval 1·43–2·35; $2P<0·0001$) compared with those in the bottom third (tertile cutoff <90 IU . dl^{-1}), after adjustments for age and town. The odds ratio was little changed after further adjustment for risk factors (1·82, 95% CI 1·37–2·41), and was not significantly different in an analysis restricted to the 404 cases and 1007 controls without baseline evidence of coronary heart disease (odds ratio 1·53, 95% CI 1·10–2·12). A meta-analysis of all relevant population-based prospective studies (including the present study) yielded a combined odds ratio of 1·5 (95% CI 1·1–2·0). von Willebrand factor values were strongly correlated with *Helicobacter pylori* seropositivity and circulating concentrations of C-reactive protein ($2P<0·0001$ for each), but not with smoking, blood lipids, or most other measured risk factors.

Conclusion Though circulating von Willebrand factor concentrations may be associated with incident coronary heart disease, further studies are needed to determine the extent to which this is causal.

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Introduction

von Willebrand factor is a large glycoprotein produced mainly by vascular endothelial cells and also contained within platelets[1,2]. It enhances haemostasis and thrombosis as an important cofactor in platelet adhesion and aggregation, and acts as the carrier protein for coagulation factor VIII[1,2]. People with inherited deficiencies of von Willebrand factor may suffer a bleeding disorder, von Willebrand disease, while substantially increased levels are associated with thrombotic disorders[3]. The physiological functions of the protein have led to suggestions that increased circulating concentrations of von Willebrand factor might be relevant to coronary heart disease, but epidemiological studies have been inconclusive. By mid-2001, five long-term prospective studies of von Willebrand factor in general populations involved a total of 899 cases of fatal coronary heart disease or non-fatal myocardial infarction[4–8]. A combined analysis of them yielded an odds ratio for coronary heart disease of 1·2 (95% CI 0·8–1·9) in individuals with baseline values in the top third compared with those in

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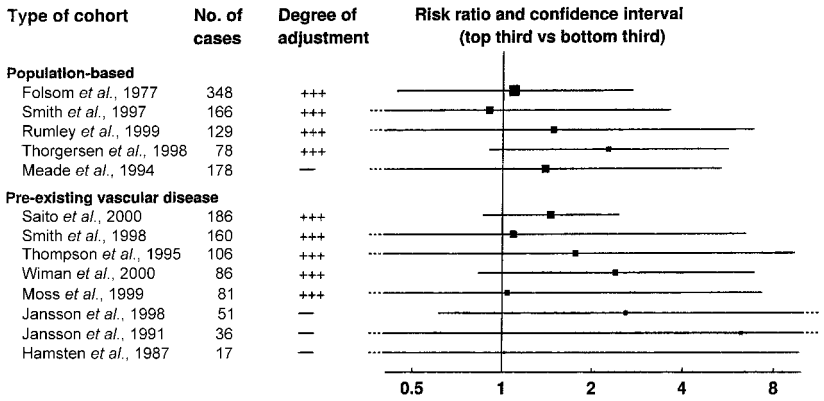

Figure 1 **Prospective studies of von Willebrand factor and coronary heart disease published before mid-2001. Risk ratios compare top and bottom thirds of baseline measurements. Black squares indicate the risk ratio in each study, with the square size proportional to the number of cases and the horizontal lines representing 99% confidence intervals. ——, no adjustment reported for possible confounders; +, adjustment for age and sex only; ++ for these plus smoking; +++, for these plus some other classical vascular risk factors.**

the bottom third of the population (Fig. 1). Even collectively, therefore, available studies cannot reliably confirm or refute a 50% excess coronary heart disease risk. To help clarify the association, we report the largest and most prolonged prospective study thus far of circulating von Willebrand factor values and coronary heart disease, with a meta-analysis of previous relevant studies to place our findings in context.

Methods

In 1978–1980, 7735 males aged 40–59 were randomly selected from general practice registers in each of 24 British towns and invited to take part in the British Regional Heart Study (response rate 78%). Nurses administered questionnaires, made physical measurements, recorded an ECG and collected non-fasting venous blood samples; in 5661 men in 18 of the towns, serum was stored at −20 °C for subsequent analysis[9]. All men have been monitored subsequently for all-cause mortality and for cardiovascular morbidity, with a follow up loss of <1% to date[10]. Fatal cases were ascertained through National Health Service Central Registers on the basis of a death certificate with ICD-9 codes 410-414; the diagnosis of non-fatal myocardial infarction was based on reports from general practitioners, supplemented by regular reviews of general practice records, and diagnosed in accordance with World Health Organisation criteria[11]. We conducted a nested case-control study based on all 643 coronary heart disease cases (279 coronary heart disease deaths and 364 cases of non-fatal myocardial infarction) occurring between 1978 and 1996 (comprising cases reported in two previous studies[12,13]), and a total of 1278 controls 'frequency matched' to cases on town of residence and age in 5-year bands was randomly selected from among men who had survived to the end of the study period free from incident coronary heart disease. Serum samples were available for 625 of these cases and 1266 controls for von Willebrand factor measurements to be carried out during the year 2000. The validity of von Willebrand factor measurement in frozen samples stored for up to ten years has previously been reported[6,14].

Laboratory workers, who were blind to the case-control status of participants, measured serum concentrations of von Willebrand factor using an enzyme immunoassay, as in a previous study[6]. Serum concentrations of IgG antibodies to *Helicobacter pylori* and *Chlamydia pneumoniae* were measured with a commercial ELISA method[13] and by time-resolved fluorimetry using the whole organism respectively[15]. C reactive protein and serum amyloid A concentrations were determined by sensitive enzyme immunoassays[16] and homocysteine measured using a modified automated reverse phase HPLC method[17]. To provide comparison with previous studies which had measured von Willebrand factor in plasma, we assayed von Willebrand factor in paired plasma and serum samples from 56 healthy individuals. Close agreement was observed between plasma and serum values in these individuals (correlation coefficient=0·94), as in an earlier report[18]. Because of fluctuations of von Willebrand factor values within individuals over time, case-control comparisons of measured baseline values tend to underestimate any association with coronary heart disease risk[19–21], von Willebrand factor measurements were therefore made in pairs of samples collected at an interval of 5 years apart in 1009 controls in a separate study[5], yielding a self-correlation coefficient of 0·63 (GDO Lowe *et al.*, unpublished data). These data were used to estimate the magnitude of regression dilution and to allow for it, by providing adjusted estimates of the mean values for the thirds of the von Willebrand factor distribution, but without altering the odds ratios (see Results)[20,21].

Table 1 Baseline characteristics of men with coronary heart disease and of age- and town-matched male controls. Values indicate mean ± standard deviation or numbers (%) unless otherwise indicated

Characteristic	Cases (n=625)	Controls (n=1266)	*P* value
Questionnaire			
Age (years)	52·6 ± 5·3	52·5 ± 5·3	matched
Current smoker	333 (52)	540 (42)	<0·0001
Evidence of coronary disease*	230 (36)	259 (20)	<0·00001
Treated diabetic	18 (3)	21 (2)	0·09
>2 drinks alcohol/day	128 (20)	288 (23)	0·18
Non-manual occupation	143 (22)	355 (28)	0·009
Homeowner**	344 (63)	823 (69)	0·02
Physical measurements			
Body mass index ($kg . m^{-2}$)	26·0 ± 3·4	25·4 ± 3·3	0·0006
Systolic blood pressure (mmHg)	152 ± 22	146 ± 21	<0·00001
Diastolic blood pressure (mmHg)	86 ± 14	83 ± 13	<0·00001
FEV_1 ($l . min^{-1}$)	308 ± 72	323 ± 78	<0·00001
Blood sample			
Total cholesterol ($mmol . l^{-1}$)	6·62 ± 1·07	6·19 ± 0·99	<0·00001
HDL cholesterol ($mmol . l^{-1}$)	1·09 ± 0·48	1·16 ± 0·29	<0·00001
Triglyceride ($mmol . l^{-1}$)	2·25 ± 1·32	1·95 ± 1·18	<0·00001
von Willebrand factor ($IU . dl^{-1}$)	121 ± 43	113 ± 44	0·0003

*Evidence of ischaemia on baseline electrocardiogram or reported history of angina or myocardial infarction.
**Information on home ownership was available for only 548 cases and 1200 controls.

We pre-specified case-control analyses by thirds of von Willebrand factor values in controls, involving unmatched stratified logistic regression fitted by unconditional maximum likelihood (STATA Corporation). For associations between von Willebrand factor and a variety of known and suspected risk factors, emphasis was mainly given to differences more extreme than 2·6 SD ($2P \approx 0·01$) to make some allowance for multiple comparisons. Using methods previously described, a meta-analysis was conducted of available prospective studies of von Willebrand factor and coronary heart disease published before mid-2001 with greater than 1 year of follow-up[21]. Cases were compared only with controls within the same study to avoid potential biases. To minimize biases further, previous studies of approximately general population samples[4–8] were considered separately from studies of cohorts defined on the basis of pre-existing vascular disease[22–29]. Several smaller studies could not be included because they did not report separately on non-fatal myocardial infarction or coronary heart disease death, but in total they involved fewer than 10% of the cases included in the present meta-analysis[30–36].

Results

There were highly significant differences between cases and controls with respect to various known vascular risk factors and von Willebrand factor (Table 1). Baseline von Willebrand factor values in the control population were highly significantly associated with age ($P<0·00001$), *H. pylori* seropositivity, C-reactive protein concentration, and globulin ($P<0·001$ for each), even after adjustments, whereas the association between von Willebrand factor and smoking became non-significant after adjustment (Table 2). No strong associations were observed of von Willebrand factor values with a variety of other classical and suspected risk factors, including blood glucose concentration (Table 2), nor with crude markers of renal function or indicators of childhood socioeconomic status (data not shown). In a comparison of men in the top third compared with those in the bottom third of baseline von Willebrand factor values (tertile cutoffs, >126 vs <90 $IU . dl^{-1}$), the odds ratio for coronary heart disease was 1·83 (95% CI 1·43–2·35; $2P<0·0001$) after adjustments for age and town (Table 3). The mean observed von Willebrand factor values in the top and bottom thirds were 164 and 76 $IU . dl^{-1}$; the corresponding mean usual von Willebrand factor values in these groups (after adjustment for within-individual variation) were 140 and 80 $IU . dl^{-1}$[20]. The odds ratio was little changed after further adjustment for smoking and other risk factors (1·82, 1·37–2·41), and was not significantly different in an analysis restricted to the 404 cases and 1007 controls without baseline evidence of coronary heart disease (1·53, 1·10–2·12; $\chi^2_1=0·6$; $P>0·1$). The findings were unaffected by varying the pre-specified cut-off level of von Willebrand factor for analysis.

Discussion

The present community based study is larger (625 vs 348 cases) and more prolonged (16 vs 5 years of

Table 2 Comparisons of the levels of risk factors and other characteristics by thirds of von Willebrand factor values in controls. Values indicate mean ± standard deviation or numbers (%) unless otherwise indicated

Numbers	High >126 IU . dl^{-1} 430	Middle 90–126 IU . dl^{-1} 415	Low <90 IU . dl^{-1} 421	t+	t++
Classical risk factors					
Age (years)	53·3 ± 5·1	52·7 ± 5·0	51·3 ± 5·5	6·2	5·3****
Current smoker	199 (46)	179 (43)	157 (37)	2·6	1·6
Total cholesterol (mmol . l^{-1})	6·17 ± 1·00	6·21 ± 1·07	6·21 ± 0·91	0·2	0·5
HDL cholesterol (mmol . l^{-1})	1·17 ± 0·32	1·15 ± 0·27	1·15 ± 0·26	0·4	0·1
Triglyceride (mmol . l^{-1})	1·90 ± 1·18	1·93 ± 1·18	2·03 ± 1·21	1·4	1·6
Body mass index (kg . m^{-2})	25·4 ± 3·6	25·2 ± 3·3	25·6 ± 2·9	1·1	0·2
Systolic blood pressure (mmHg)	150 ± 22	145 ± 20	146 ± 20	2·0	3·0*
Diastolic blood pressure (mmHg)	84 ± 13	81 ± 13	83 ± 14	0·2	1·0
>2 drinks alcohol/day	112 (26)	74 (18)	97 (23)	0·5	0·0
FEV_1 (l)	309 ± 78	324 ± 77	337 ± 74	2·8	1·7
Evidence of CHD at entry	95 (22)	83 (20)	79 (19)	0·3	0·6
Markers of infection or inflammation					
Helicobacter pylori seropositivity	286 (81)	235 (72)	214 (64)	4·3	3·4**
Chlamydia pneumoniae titres (FC × 10^6)	185·8 ± 61·7	180·8 ± 55·7	176·2 ± 62·4	1·5	1·1
Log_{10} C-reactive protein (mg . l^{-1})	0·27 ± 0·53	0·13 ± 0·54	0·04 ± 0·50	4·5	3·3**
Log_{10} serum amyloid A protein (mg . l^{-1})	0·86 ± 0·33	0·84 ± 0·30	0·82 ± 0·27	1·6	1·3
White cell count (× 10^9 . l^{-1})	7·3 ± 1·8	7·2 ± 1·7	7·1 ± 1·8	1·6	0·3
Albumin (g . l^{-1})	44·1 ± 2·6	44·4 ± 2·4	45·0 ± 2·3	3·7	2·5
Globulin (g . l^{-1})	29·7 ± 3·3	28·9 ± 2·9	28·6 ± 3·0	5·9	4·9****
Other suspected risk factors					
Homocysteine (μmol . l^{-1})	16 ± 12	15 ± 9	13 ± 4	2·3	1·6
Haematocrit (%)	42·6 ± 9·6	42·3 ± 9·1	42·2 ± 9·1	0·5	0·7
Insulin (mU . l^{-1})	17 ± 18	16 ± 17	17 ± 14	0·4	0·8
Glucose (mmol . l^{-1})	5·63 ± 1·26	5·66 ± 1·61	5·58 ± 1·19	0·4	0·6
Socioeconomic factors					
Non-manual occupation	105 (24)	116 (28)	130 (31)	0·9	0·6
Homeowner	247 (63)	265 (68)	303 (75)	3·1	2·7*
Married	387 (90)	363 (87)	371 (88)	0·6	0·7
Car owner	284 (72)	290 (74)	339 (84)	3·4	3·1*

+t tests derived from regression of von Willebrand factor values on each characteristic separately adjusting for age and town only.
++t tests derived from regression of von Willebrand factor values adjusting for age, town, smoking, body mass index, and markers of socioeconomic status (including height).
Adjustments for social class were omitted in the regressions involving markers of socioeconomic status.*P<0·01, **P<0·001, ****P<0·00001. FC=fluorescent count.

follow-up) than the previous largest study of circulating von Willebrand factor concentrations and subsequent coronary heart disease[4]. It suggests that even after adjustment for baseline values of classical risk factors, a moderately strong association persists between von Willebrand factor and coronary heart disease. Despite the approximately 20 year period of sample storage before measurement, the validity of our von Willebrand factor values is supported by the consistency with previous results, by their expected increase with age and by the similarity of results from the Caerphilly Study using the same assay method[6,14].

Meta-analysis of previous prospective studies

Five previous prospective studies of von Willebrand factor in approximately general populations have involved a total of 899 coronary heart disease cases and 18 552 controls, with a mean weighted age at entry of 56 and mean weighted follow-up of 7 years[4–8] (Fig. 1). All but one[8] used enzyme-linked immunoassays (the validity of the immunoassay in serum, as used in the present study, was confirmed by our own comparative study — see Methods). All previous studies reported adjustment for smoking and other classical coronary risk factors, and there was no significant heterogeneity among their separate results (χ^2_4=3·3; P>0·1). A combined analysis of them yielded an odds ratio of 1·2 (95% CI 0·8–1·9) in individuals with baseline von Willebrand factor values in the top third compared with those in the bottom third. Although this combined odds ratio is somewhat less extreme than the odds ratio in the present study of 1·8 (95% CI 1·4–2·4: Fig. 2), it is only marginally significantly different (χ^2_1=4·8; P=0·03) as the present study may have somewhat overestimated (and previous studies somewhat underestimated) the true risk due to the play of chance: a combined analysis of them should provide a more reliable estimate.

Overall, the studies involved 1524 coronary heart disease cases and 19 830 controls, yielding a combined

Table 3 Odds of coronary heart disease in men who had values of von Willebrand factor in the top third of the distribution of controls relative to those who had values in the bottom third of this distribution

vWF values (IU . dl^{-1})	Cases	Controls	Odds ratio (95% CI) adjusted for:			
			Age and town only	Age, town and smoking	Age, town, smoking and risk factors*	Age, town, smoking, risk factors and SES**
All 625 cases and 1266 controls						
>126	267	430	1·83 (1·43–2·35)	1·78 (1·39–2·29)	1·85 (1·42–2·41)	1·82 (1·37–2·41)
90 to 126	214	415	1·53 (1·19–1·98)	1·49 (1·16–1·93)	1·54 (1·18–2·02)	1·51 (1·13–2·01)
<90	144	421	1·00	1·00	1·00	1·00
404 cases and 1007 controls without baseline evidence of coronary heart disease						
>126	159	334	1·55 (1·16–2·09)	1·52 (1·13–2·04)	1·59 (1·17–2·18)	1·53 (1·10–2·12)
90 to 126	137	332	1·32 (0·98–1·78)	1·25 (0·93–1·70)	1·30 (0·95–1·79)	1·29 (0·92–1·80)
<90	108	341	1·00	1·00	1·00	1·00

SES=socioeconomic status.
*Blood pressure, total cholesterol, HDL cholesterol, triglycerides, body mass index.
**Information on occupation, housing tenure, marital status, and car ownership and childhood socioeconomic factors(i.e. father's social class, family car ownership, bathroom in house, hot water tap in house, bedroom sharing, and height).

odds ratio of 1·5 (95% CI 1·1–2·0). Eight other published prospective studies of von Willebrand factor in cohorts defined on the basis of previous disease (including diabetes[22], peripheral vascular disease[23], angina[24], and previous myocardial infarction[25–29]) have included an additional 723 coronary heart disease cases and 5720 controls. They give a similar combined odds ratio for coronary heart disease of 1·6 (1·0–2·5), but, again, the confidence interval is wide (Fig. 2).

Biological relevance of associations

The relevance of von Willebrand factor coronary heart disease remains uncertain. As well as having roles in haemostasis and thrombosis[1,2], circulating von Willebrand factor values can increase markedly during acute phase responses to systemic and/or local inflammation and to endothelial injury[1,2,37,38]. Thus, it might be that von Willebrand factor is a marker of systemic inflammatory processes (such as chronic infection)[39], or a marker of factors that might produce local endothelial damage (such as elevated circulating concentrations of homocysteine)[40], or just a marker of the extent of subclinical atherosclerosis (which is partly 'inflammatory')[41]. To help distinguish between these possibilities, we related serum von Willebrand factor values to serological evidence of chronic infection with *C. pneumoniae* and *H. pylori*, circulating concentrations of several acute phase reactants and of homocysteine, and several other known or suspected vascular risk factors in 1266 controls. Baseline von Willebrand factor concentrations were strongly correlated with circulating values of C-reactive protein and globulin and, perhaps, with low serum albumin (all three of which are indicators of systemic inflammation[19]), but not with baseline evidence of coronary heart disease. Although we observed a significant association between von Willebrand factor values and *H. pylori* seropositivity (a chronic gastric infection weakly associated with coronary heart disease in previous studies[42]), a previous placebo-controlled randomized trial of *H. pylori* eradication treatment involving a few hundred patients with non-ulcer dyspepsia[43] found no significant difference in von Willebrand factor values 1 year after randomization (G. D. O. Lowe, K McColl *et al.*, unpublished data), suggesting that any influence of *H. pylori* infection on von Willebrand factor levels is not rapidly reversible. It has recently been suggested, on the basis of a small non-randomized trial[44], that aspirin use may lower circulating concentrations of von Willebrand factor. However, in the Caerphilly Study, von Willebrand factor levels were not related to the use of aspirin or of other prescribed medication[14]. In the present study, the prevalence of aspirin use was not recorded at entry but as its regular use in the general population in the late 1970s was relatively uncommon, it is unlikely that aspirin use could materially influence the association between von Willebrand factor and coronary heart disease.

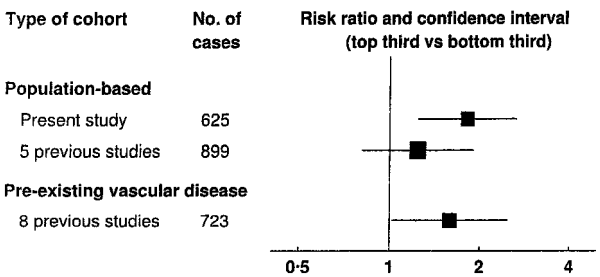

Figure 2 **Prospective studies of von Willebrand factor and coronary heart disease including the present study. Risk ratios compare top and bottom thirds of baseline measurements. Black squares indicate the risk ratio in each study, with the square size proportional to the number of cases and the horizontal lines representing 95% confidence intervals.**

Conclusions

The present study, together with a synthesis of previous relevant studies, suggests the existence of an association between von Willebrand factor levels and risk of future coronary heart disease. Further studies are needed to determine whether this association is causal.

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