**SUPPLEMENTARY MATERIALS**

**Von Willebrand Factor and ADAMTS13 in relation to atherosclerosis in the General Population: The Rotterdam Study**

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**Method S1:** Covariates Assessment

**Table S1 :** Characteristics of the study population at CT by sex.

**Table S2:** Multivariable partial proportional odds ordinal logistic regression analysis of the associations between von willebrand factor antigen, ADAMTS13 activity, their ratio, and levels of calcification in different arteries.

**Table S3:** Multivariable linear regression analyses of the association between von willebrand factor antigen, ADAMTS13 activity, their ratio, with each quantiles of calcification in different arteries.

**Method S1:** Covariates assessment

Systolic and diastolic blood pressure were measured twice at the right arm using a random-zero sphygmomanometer, and the average of measurements was used. Hypertension was defined as having a systolic blood pressure higher than 160 mmHg or a diastolic blood pressure higher than 100 mmHg or taking blood pressure medication. Diabetes was defined as the use of anti-diabetic medications or a fasting glucose level greater than 7.1 mmol/l. Smoking behavior was categorized as current smoking and non-smoking. Waist circumference was measured midway between the lower rib margin and the iliac crest with participants in a standing position without heavy outer garments and with emptied pockets, breathing out gently. Hip circumference was recorded as the maximum circumference over the buttocks. Waist-to-hip ratio (WHR) was consequently calculated as the ratio of waist circumference over hip circumference.

History of prevalent CVD was defined as a history of myocardial infarction, percutaneous transluminal coronary angioplasty (PCI), coronary artery bypass graft (CABG), or stroke. This information was collected at baseline in 1990-1993 and also during follow-up visits in 2000-2001, as described previously (1).

Information on medication use, including statins, vitamin K antagonists, and bisphosphonates was gathered from prescription records in fully computerized, connected community pharmacies in the research area, which stored data on a common network. Detailed descriptions of the data collection methods are available elsewhere (1). For the current study, we used dispensing data from inception (May 1, 1991), until the dates of the CT scan. Following this, each study participant was classified as an 'ever' user if they had at least one prescription related to these medications and as a 'never' user if they had none.

**Table S1 :** Characteristics of the study population at CT by sex.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | | Male  1076 (48.4 %) | Female  1148 (51.6%) | p-value |
| Age | Mean (SD)  Median [Min, Max] | 69.0 (6.54)  68.0 [59.0, 98.0] | 68.9 (6.89)  67.0 [59.0, 94.0] | 0.786 |
| Current smoking | N (%) | 195 (18.1%) | 155 (13.5%) | 0.003 |
| Diabetes Mellitus | N (%) | 157 (14.6%) | 136 (11.8%) | 0.064 |
| Hypertension | N (%) | 554 (51.5%) | 567 (49.4%) | 0.344 |
| Prevalent CVD | N (%) | 106 (9.9%) | 40 (3.5%) | <0.001 |
| Statins use | N (%) | 256 (23.8%) | 291 (25.3%) | 0.422 |
| Bisphosphonates use | N (%) | 47 (4.4%) | 159 (13.9%) | <0.001 |
| Vitamin K antagonists use | N (%) | 127 (11.8%) | 108 (9.4%) | 0.077 |
| Waist to hip ratio | Mean (SD)  Median [Min, Max] | 1.0 (0.07)  0.97 [0.8, 1.2] | 0.8 (0.07)  0.8 [0.6, 1.1] | <0.001 |
| HDL-cholesterol (mmol/l) | Mean (SD)  Median [Min, Max] | 1.3 (0.3)  1.3 [0.7, 2.9] | 1.6 (0.4)  1.5 [0.7, 3.6] | <0.001 |
| Non-HDL-cholesterol (mmol/l) | Mean (SD)  Median [Min, Max] | 4.11 (0.9)  4.13 [1.2, 7.5] | 4.36 (1.0)  4.29 [1.7, 7.8] | <0.001 |
| Fibrinogen (g/L) | Mean (SD)  Median [Min, Max] | 3.7(0.88)  3.5 [1.8, 9.5] | 3.90 (0.82)  3.80 [1.4, 8.4] | <0.001 |
| Blood collection to CT scan (years) | Mean (SD)  Median [Min, Max] | 4.59 (0.5)  4.58 [1.6, 7.9] | 4.59 (0.6)  4.58 [1.4, 6.8] | 0.777 |
| VWF antigen (IU/dL) | Mean (SD)  Median [Min, Max] | 123 (47.5)  114 [36.0, 327] | 122 (49.1)  111 [29.0, 345] | 0.530 |
| ADAMTS13 activity (%) | Mean (SD)  Median [Min, Max] | 89.8 (16.8)  89.6 [37.4, 172] | 96.9 (17.1)  96.4 [5.0, 175] | <0.001 |
| VWF antigen levels | ≥50 (IU/dL) , N (%) | 1065 (99.0%) | 1131 (98.5%) | 0.454 |
| 30-50 (IU/dL), N (%) | 11 (1.0%) | 16 (1.4%) |
| ≤30 (IU/dL), N (%) | 0 (0%) | 1 (0.1%) |
| ADAMTS13 activity levels | ≥20% , N (%) | 1076 (100%) | 1147 (99.9%) | 0.805 |
| 10-20%, N (%) | 0 (0%) | 0 (0%) |
| ≤10%, N (%) | 0 (0%) | 1 (0.1%) |
| Presence of CAC | N (%) | 967 (89.9%) | 854 (74.4%) | <0.001 |
| Presence of AOC | N (%) | 1002 (93.1%) | 1056 (92.0%) | 0.348 |
| Presence of ECAC | N (%) | 850 (79.0%) | 773 (67.3%) | <0.001 |
| Presence of ICAC | N (%) | 889 (82.6%) | 940 (81.9%) | 0.689 |
| Volume of CAC (mm3) | Mean (SD)  Median [Min, Max] | 403 (675)  132 [0, 6920] | 138 (322)  16.6 [0, 2850] | <0.001 |
| Volume of AAC (mm3) | Mean (SD)  Median [Min, Max] | 798 (1350)  279 [0, 11900] | 663 (1130)  222 [0, 11900] | 0.011 |
| Volume of ECAC (mm3) | Mean (SD)  Median [Min, Max] | 134 (247)  40.5 [0, 2830] | 78.4 (163)  12.8 [0, 2030] | <0.001 |
| Volume of ICAC (mm3) | Mean (SD)  Median [Min, Max] | 137 (214)  49.0 [0, 1450] | 96.8 (156)  36.8 [0, 1130] | <0.001 |

Diabetes was defined as a fasting glucose level ≥7 mmol/L and/or the use of anti-diabetic medication. Hypertension was defined as a systolic blood pressure ≥160 mmHg and/or a diastolic blood pressure ≥100 mmHg, and/or the use of blood pressure-lowering medication.

CVD: Cardiovascular diseases, defined as a history of heart failure, myocardial infarction, or stroke; VWF: Von Willebrand factor; CT: Computed tomography; Blood collection to CT scan interval: The time between blood collection (for measuring VWF and ADAMTS13 levels) and the CT scan; HDL: High-Density Lipoprotein; LDL: Low-Density Lipoprotein; CAC: Coronary artery calcification; AAC: Aortic arch calcification; ECAC: Extracranial internal carotid artery calcification; ICAC: Intracranial internal carotid artery calcification. P-values are from t-tests or Mann-Whitney U test for continuous variables and chi-squared tests for categorical variables

**Table S2:** Multivariable partial proportional odds ordinal logistic regression analysis of the associations between von Willebrand factor antigen, ADAMTS13 activity, their ratio, and levels of calcification in different arteries

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | | **A: VWF:Ag** | **B: ADAMTS13** | **C: ADAMTS13:VWF ratio** |
| **Model 2, quartiles of CAC** | | | | |
| Β(95%CI) | | 0.06 (-0.02, 0.14) | -0.10 (-0.18, -0.02) | -0.28 (-0.48, -0.07) |
| Intercepts  Β(SE) | Q1 [-1.45, -1.01] | Q2 (-1.0, 0.17] | 7.22 (0.82) | 7.27 (0.81) | 6.85 (0.84) |
| Q2 (-1.01, 0.17] | Q3 (0.17, 0.83] | 8.54 (0.83) | 8.59 (0.82) | 8.17 (0.84) |
| Q3 (0.17, 0.83] | Q4 (0.83, 2.14] | 9.91 (0.84) | 9.96 (0.83) | 9.55 (0.85) |
| **Model 2, quartiles of AAC** | | | | |
| Β(95%CI) | | 0.08 (-0.00, 0.16) | -0.13 (-0.21, -0.04) | -0.45 (-0.66, -0.25) |
| Intercepts  Β(SE) | Q1 [-2.17 ,-0.54] | Q2 (-0.54, 0.22] | 10.05 (0.83) | 10.16 (0.82) | 9.45 (0.85) |
| Q2 (-0.54, 0.22] | Q3 (0.22, 0.73] | 11.38 (0.84) | 11.49 (0.83) | 10.78 (0.85) |
| Q3 (0.22, 0.73] | Q4 (0.73, 1.88] | 12.76 (0.85) | 12.87 (0.84) | 12.16 (0.86) |
| **Model 2, quartiles of ECAC** | | | | |
| Β(95%CI) | | 0.03 (-0.05, 0.11) | -0.11 (-0.20, -0.03) | -0.18 (-0.38, 0.01) |
| Intercepts  Β(SE) | Q1 (-1.27] | Q2 (-1.27, 0.14] | 7.48 (0.81) | 7.47 (0.80) | 7.20 (0.83) |
| Q2 (-1.27, 0.14] | Q3 (0.14, 0.86] | 8.64 (0.82) | 8.63 (0.81) | 8.36 (0.84) |
| Q3 (0.14, 0.86] | Q4 (0.86, 2.28] | 9.95 (0.83) | 9.94 (0.82) | 9.67 (0.84) |
| **Model 2, quartiles of ICAC** | | | | |
| Β(95%CI) | | 0.02 (-0.05, 0.10) | -0.08 (-0.16, -0.00) | -0.19 (-0.39, 0.01) |
| Intercepts  Β(SE) | Q1 [-1.67,-0.63] | Q2 (-0.63,0.19] | 8.12 (0.82) | 8.12 (0.81) | 7.83 (0.83) |
| Q2 (-0.63,0.19] | Q3 (0.19,0.77] | 9.38 (0.82) | 9.38 (0.82) | 9.09 (0.84) |
| Q3 (0.19,0.77] | Q4 (0.769,1.92] | 10.70 (0.83) | 10.70 (0.82) | 10.41 (0.85) |

Each quartile (Q1 to Q4) contains approximately 25% of the total data points with the first quartile (Q1) representing the lowest level and the fourth quartile representing the highest level (Q4). The number of participants per quartile are as follows: for CAC, Q1 = 558, Q2 = 554, Q3 = 556, Q4 = 556; for AAC, Q1 to Q4 = 556 each; for extracranial carotid calcification, Q1 = 601, Q2 = 512, Q3 = 557, Q4 = 554; and for intracranial carotid calcification, Q1 to Q4 = 556 each.

Single coefficients (for VWF, ADAMTS13, or their ratio) indicate that the proportional odds assumption holds for these variables, meaning their association with the outcome is consistent across all levels. Intercepts represent the log odds of being in a certain quartile versus the lower quartiles. Model 2 was adjusted for sex, age, current smoking, diabetes mellitus, HDL cholesterol, LDL cholesterol, hypertension, vitamin K antagonist use, statin use, bisphosphonate use, and prevalent cardiovascular diseases.

**Abbreviations:** VWF: Von Willebrand factor; N: number of individuals; CAC: coronary artery calcification; AAC: aortic arch calcification; ECAC: extracranial internal carotid artery calcification; ICAC: intracranial internal carotid artery calcification; B: beta coefficient; CI: confidence interval; SE: Standard Error.

**Table S3:** Multivariable linear regression analyses of the association between von willebrand factor antigen, ADAMTS13 activity, their ratio, with each quantiles of calcification in different arteries.

|  |  |  |  |
| --- | --- | --- | --- |
|  | **A: VWF:Ag**  **Β (95%CI)** | **B: ADAMTS13**  **Β (95%CI)** | **C: ADAMTS13:VWF ratio**  **Β (95%CI)** |
| **Model 2, quartiles of CAC** | | | |
| Q1 [-1.45, -1.01], (N= 555) | 0.00 (-0.01 0.01) | 0.01 (-0.01, 0.02) | -0.01 (-0.03, 0.02) |
| Q2 (-1.0, 0.17], (N= 552) | 0.01 (-0.02, 0.04) | -0.01 (-0.04, 0.02) | -0.02 (-0.09, 0.05) |
| Q3 (0.17, 0.83], (N=555) | -0.00 (-0.02, 0.01) | -0.00 (-0.02, 0.02) | 0.01 (-0.03, 0.05) |
| Q4 (0.83, 2.14], (N=553) | -0.00 (-0.02, 0.02) | 0.00 (-0.02, 0.02) | 0.02 (-0.05, 0.09) |
| **Model 2, quartiles of AAC** | | | |
| Q1 [-2.17 ,-0.54], (N=555) | -0.02 (-0.08, 0.03) | -0.06 (-0.12, -0.01) | -0.01 (-0.12, 0.11) |
| Q2 (-0.54, 0.22], (N= 555) | 0.00 (-0.02, 0.02) | 0.00, (-0.02, 0.02) | 0.00 (-0.04, 0.05) |
| Q3 (0.22, 0.73], (N=555) | 0.00 (-0.01, 0.01) | -0.01 (-0.02, 0.05) | 0.01 (-0.03, 0.04) |
| Q4 (0.73, 1.88], (N=555) | -0.02 (-0.04, 0.00) | -0.01 (-0.03, 0.01) | 0.03 (-0.02, 0.09) |
| **Model 2, quartiles of ECAC** | | | |
| Q1 (-1.27], (N=601) | -0.00 (-0.00, -0.00) | 0.00 (-0.00, 0.00) | 0.00 (-0.00, 0.00) |
| Q2 (-1.27, 0.14], (N=512) | 0.01 (-0.03, 0.05) | 0.04 (0.00, 0.07) | 0.02 (-0.08, 0.12) |
| Q3 (0.14, 0.86], (N=557) | 0.00 (-0.01, 0.02) | -0.01 (-0.03, 0.01) | -0.02 (-0.07, 0.02) |
| Q4 (0.86, 2.28], (N=554) | 0.01 (-0.01, 0.04) | -0.02 (-0.04, 0.01) | -0.04 (-0.11, 0.02) |
| **Model 2, quartiles of ICAC** | | | |
| Q1 [-1.67,-0.63], (N=556) | -0.003 (-0.04, 0.03) | -0.005 (-0.04, 0.03) | -0.00 (-0.07, 0.07) |
| Q2 (-0.63,0.19], (N=556) | 0.01 (-0.01, 0.03) | -0.00 (-0.02, 0.02) | -0.02 (-0.07, 0.03) |
| Q3 (0.19,0.77], (N=556) | 0.00 (-0.01, 0.02) | -0.01(-0.02, 0.01) | -0.015 (-0.05, 0.02) |
| Q4 (0.769,1.92], (N=556) | 0.00 (-0.02, 0.02) | -0.01 (-0.03, 0.02) | -0.00 (-0.07, 0.06) |

Each quartile (Q1 to Q4) contains approximately 25% of the total data points with the first quartile (Q1) representing the lowest level and the fourth quartile representing the highest level (Q4). Model 2 was adjusted for sex, age, for current smoking, diabetes mellitus, HDL- cholesterol, LDL-cholesterol, hypertension, vitamin K antagonist use, statin use, bisphosphonates use, and prevalent cardiovascular diseases.

**Abbreviations:** VWF: Von Willebrand factor; N: number of individuals; CAC: coronary artery calcification; AAC: aortic arch calcification; ECAC: extracranial internal carotid artery calcification; ICAC: intracranial internal carotid artery calcification; B: beta coefficient; CI: confidence interval.

1. Ikram MA, Brusselle G, Ghanbari M, Goedegebure A, Ikram MK, Kavousi M, et al. Objectives, design and main findings until 2020 from the Rotterdam Study. European journal of epidemiology. 2020;35:483-517.