**SUPPLEMENTARY MATERIALS**

**Statin Use Is Associated With Increased Arterial Calcification in the General Population: The Rotterdam Study**

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**Content:**

* **eMethod-1**: Rotterdam Study; Information on ethical and WBO approval and ethical guidelines
* **eFigure-1:** Flow diagram of study participants at baseline and follow-up
* **eMethods-2:** Scanning modalities, Scanning protocols, Calcium identification threshold, and Scoring methods
* **eMethods-3:** Assessment of statin use and cumulative duration
* **eMethods-4:** Measurement of covariates and cardiovascular risk factors
* **eMethods-5:** Genotyping
* **eMethods-6:** Phenotyping
* **eTable-1:** Star alleles of the SLCO1B1 gene and corresponding variants
* **eFigure-2:** Procedure for Phenotype assignment (Normal, Decreased, or Poor function)
* **eTable-2:** Follow-up population characteristics by statin use
* **eTable-3:** The baseline analysis
* **eTable-4:** The follow-up analysis
* **eTable-5:** Distribution of active statin use across OATP1B1 transporter function levels
* **eTable-6:** Effect measure modification by OATP1B1 transporter function
* **eTable-7:** Linear trend analysis across transporter function levels using orthogonal polynomial contrasts
* **eFigure-3:** The negative control population results

**eMethods-1:** Rotterdam Study; Information on ethical and WBO approval and ethical guidelines

Rotterdam Study ethical approval (MEC 02.1015) is granted by Erasmus MC, and WBO approval (1071272-159521-PG) from the Dutch Ministry of Health, Welfare, and Sport. It adheres to the Declaration of Helsinki, is registered with the Netherlands National Trial Registry Platform (NTR6831), and has participants' written informed consent, allowing access to all medical data and pharmacy records.

**eFigure-1:** Flow diagram of study participants at baseline and follow-up

Total population of 3,229 were invited for a non-contrast MDCT scan



Not participated

N= 775

Completed scans

N= 2,524 (78% response)

Scans that had artifacts and lacked prescription information were excluded; N= 111 and N= 14



**Baseline population of the study**

**N= 2,399**

Unavailable population

N=814

Participants were invited for a follow-up scan

N= 1,599



Not participated

N=648

Completed scans

N= 951 (59.5% response)

Scans that had artifacts and lacked prescription information were excluded; N=89 and N=47

N=648



Foot note: Participants included those invited to the RS research center for a non-contrast multidetector computer tomography (CT) scan. Initially, 3,229 were invited, with 2,524 successfully completing the scan from June 2003 to February 2006 (78% response). Exclusions involved CTs with artifacts, coronary stents, or pacemakers, leaving 2,413 usable scans. Among these, 2,399 had prescription information, constituting the ‘baseline population’ of the study. Subsequently, between May 2018 and December 2019, 1,599 baseline CT participants were invited for a follow-up scan, of whom 951 underwent it (59.5% response). After excluding unusable scans, 815 participants with prescription information remained and constracted the follow-up population for this study.

**Follow-up population of the study**

**N= 815**

**eMethod-2:** Scanning modalities, Scanning protocols, Calcium identification threshold, and Scoring methods

At baseline, we used 16-slice or 64-slice MDCT scanners (Somatom Sensation 16 or 64; Siemens, Forchheim, Germany). For the follow-up examination, a 128-slice dual-source CT scanner was used (Somatom Drive, Siemens, Forchheim, Germany). The scan protocol has been described elsewhere (1). Calcification was identified at a threshold of 130 Hounsfield units and measured in cubic millimeters using a dedicated software (Syngo Calcium Scoring, Siemens, Forchheim, Germany). For ICAC, a semi-automated scoring method was used that allowed for the manual segmentation of calcification in each consecutive CT slice. A detailed description of quantification methods is provided elsewhere (2, 3). Two experienced physicians quantified calcification independently with very good to excellent intra-rater and inter-rater reliabilities for the scoring method. The evaluation method has been described in more depth previously (1-3).

**eMethods-3: Assessment of statin use and cumulative duration**

Prescription episode calculated by dividing the total number of dispensed tablets by the prescribed daily number. Additionally, for dosage comparisons, we calculated the average daily dose for each participant, expressed in the standardized ‘defined daily dose’ (DDD) in accordance with World Health Organization guidelines (4).

**eMethod-4: Measurement of covariates and cardiovascular risk factors**

Information on cardiovascular risk factors was obtained through standardized home interviews, physical examination, and blood sampling. 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. Non-high-density lipoprotein (non-HDL) cholesterol level was calculated as total cholesterol minus high-density lipoprotein (HDL) cholesterol. History of prevalent CVD was defined as a history of myocardial infarction, percutaneous transluminal coronary angioplasty (PCI), coronary artery bypass graft (CABG), or stroke. These information was collected at inception in 1990-1993 or during the first follow-up visits until 2001, as described previously (5)

**eMethod-5: Genotyping**

Genotyping was performed with the 550 or 610K Illumina microarrays. Quality control criteria for genotyping involved a call rate of <95%, Hardy-Weinberg equilibrium with a threshold of P < 1.0 × 10-6, and a minor allele frequency of <1%. Single Nucleotide Polymorphism (SNP) imputation was performed using the Haplotype Reference Consortium 1.1 (6). Genetic variants with poor imputation quality scores (R2 < 0.3) were removed.

**eMethod-6: Phenotyping**

These star alleles were \*1 and \*37, linked to normal function, and \*5, \*15, and \*17, linked to decreased function. They were included based on clinical functionality with 'strong' evidence, according to the SLCO1B1 allele functionality table (7). The combination of star alleles was used to determine each patient's diplotype, providing insight into their phenotypes. Individuals carrying two decreased function alleles (e.g., SLCO1B1 \*5/\*15) were categorized as having a "poor function" phenotype. Those with one decreased function allele and one normal function allele (e.g., SLCO1B1\*5/\*1) were categorized as having an intermediate activity and a “decreased function” phenotype. Individuals with only normal function alleles (e.g., SLCO1B1\*1/\*1) were characterized as having a “normal function” phenotype. The frequency of identified haplotypes, such as \*5 (1%) and \*15 (13%), was generally consistent with previously published prevalence rates in the Caucasian population (2% and 16%) (8). Missing values related to SNPs and their associated haplotypes were not imputed and thus were excluded from the analyses.

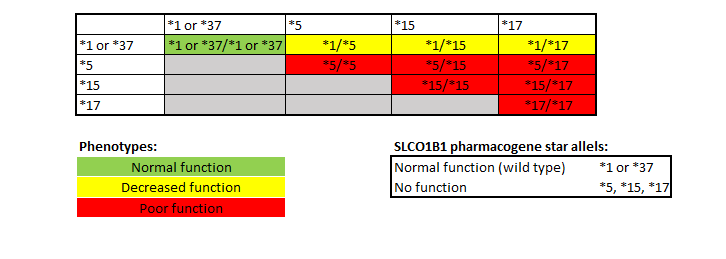
**eTable-1:** Star alleles of the SLCO1B1 gene and corresponding variants

|  |  |  |  |
| --- | --- | --- | --- |
|  | rs4149015 | rs2306283 | rs4149056 |
| \*1 | G | A | T |
| \*5 | G | A | ***C*** |
| \*15 | G | ***G*** | ***C*** |
| \*17 | ***A*** | ***G*** | ***C*** |

The rows represent the star alleles (haplotypes). The columns represent single-nucleotide polymorphisms (SNPs). The cells represent nucleotides on the corresponding SNPs, defining the haplotypes. Bolded nucleotides represent the minor alleles. The haplotype "\*1" represents the wild type.

A proxy SNP (rs4149040) from European ancestry was replaced with rs2306283, which was missing from the Rotterdam Study genetic data (full linkage disequilibrium, r2=1.0).

**eFigure-2:** Procedure for Phenotype assignment (Normal, Decreased, or Poor function)



\* The procedure illustrated in the figure followe the diplotype-to-phenotype map

**eTable-2:** Follow-up population characteristics by statin use

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Total (N=815)** | | | **Ever- users**  **N=385** | **Ever users;**  **Stratified as initiator or prevalent users (N=380\*)** | | **Never-users**  **N=430** | **Total**  **N=815** |
| Initiator  N=224 | Prevalent  N=156 |
| Age, (years) | | Mean (SD)  Median [Min, Max] | 79.3 (4.20)  79.0 [73.0, 98.0] | 79.2 (4.09)  79.0 [73.0, 96.0] | 79.4 (4.37)  79.0 [73.0, 98.0] | 79.5 (4.26)  79.0 [73.0, 100] | 79.4 (4.23)  79.0 [73.0, 100] |
| Female | | N (%) | 198 (51.4%) | 108 (48.2%) | 86 (55.1%) | 238 (55.3%) | 458 (53.5%) |
| Cumulative duration of statin use since inception (years) | | Mean (SD)  Median [Min, Max] | 7.95 (5.04)  8.05 [0.0219, 16.4] | 5.41 (4.10)  5.27 [0.0219, 14.8] | 11.6 (3.91)  13.3 [0.247, 16.4] | N/A | 7.95 (5.04)  8.05 [0.0219, 16.4] |
| Average daily defined dose of statins since inception | | Mean (SD)  Median [Min, Max] | 0.74 (0.74)  0.67 [0, 4.00] | 0.67 (0.70)  0.67 [0, 4.0] | 0.86 (0.79)  0.67 [0, 4.0] | N/A | 0.35 (0.63)  0 [0, 4.0] |
| Interval between inception and second CT scan (years) | | Mean (SD)  Median [Min, Max] | 19.2 (2.64)  18.0 [17.0, 26.0] | 19.2 (2.65)  18.0 [17.0, 26.0] | 19.2 (2.62)  18.0 [17.0, 26.0] | 19.5 (2.89)  18.0 [16.0, 28.0] | 19.4 (2.78)  18.0 [16.0, 28.0] |
| Interval between baseline and follow-up CT scans (years) | | Mean (SD)  Median [Min, Max] | 13.6 (0.50)  14.0 [13.0, 15.0] | 13.6 (0.50)  14.0 [13.0, 15.0] | 13.6 (0.51)  14.0 [13.0, 15.0] | 13.6 (0.52)  14.0 [13.0, 15.0] | 13.6 (0.51)  14.0 [13.0, 15.0] |
| Calcification volume | CAC (mm3) | Mean (SD),  Median [Min, Max] | 716 (859)  351 [0, 4360] | 618 (781)  289 [0, 3820] | 879 (946)  486 [0, 4360] | 316 (515)  120 [0, 4390] | 509 (729)  210 [0, 4390] |
| AAC (mm3) | Mean (SD),  Median [Min, Max] | 2060 (2990)  1080 [0, 27900] | 1810 (2700)  918 [0, 23500] | 2450 (3370)  1440 [0, 27900] | 1280 (1700)  695 [0, 14400] | 1660 (2440)  866 [0, 27900] |
| ECAC (mm3) | Mean (SD),  Median [Min, Max] | 336 (641)  158 [0, 8610] | 322 (745)  137 [0, 8610] | 363 (467)  203 [0, 2630] | 172 (247)  83.7 [0, 2210] | 250 (483)  111 [0, 8610] |
| ICAC (mm3) | Mean (SD),  Median [Min, Max] | 261 (322)  134 [0, 1850] | 240 (295)  126 [0, 1850] | 292 (353)  167 [0, 1820] | 164 (245)  74.6 [0, 1890] | 210 (290)  99.3 [0, 1890] |

The follow-up population consisted of individuals from the baseline population who had usable follow-up CT scans and available prescription information. The table shows the follow up charachtristics including statin use, age, and arterial calcification, assessed at follow-up CT. Continuous variables are presented as mean, standard deviation (SD), median, minimum, and maximum [Min, Max], while categorical variables are presented as absolute numbers (percentage).

Ever use of statins is defined as having at least one statin prescription from inception until the time of the second CT scan. Participants were categorized as 'initiators' if they were newly prescribed statins between the two CT scan dates, or 'prevalent' if they had been prescribed statins before the first scan and continuously between scans.

\*: Five individuals received statins before the first scan but not between the scans, so they were not classified into either the prevalent or initiator groups.

**Abbreviations:**

CAC: coronary artery calcification, AAC: aortic arch calcification, ECAC: extracranial internal carotid artery calcification, ICAC: intracranial internal carotid artery calcification, N/A: not applicable.

**eTable 3. The baseline analysis**

Multivariable linear regression assessing the association between ever statin use compared to never use with calcification volume measured at baseline.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | CAC  β (95%CI) | AAC  β (95%CI) | ECAC  β (95%CI) | ICAC  β (95%CI) |
| **Model 1** | | | | |
| Never use, (N=1811) | REFERENCE | REFERENCE | REFERENCE | REFERENCE |
| Ever use, (N=588) | 0.54 (0.44, 0.63) | 0.42 (0.32, 0.52) | 0.52 (0.42, 0.62) | 0.41 (0.30, 0.50) |
| **Model 2** | | | | |
| Never use, (N=1811) | REFERENCE | REFERENCE | REFERENCE | REFERENCE |
| Ever use, (N=588) | 0.21 (0.05, 0.38) | 0.24 (0.06, 0.42) | 0.36 (0.17, 0.55) | 0.28 (0.07,0.44) |

Model 1 was adjusted for age and sex. Model 2 further adjusted for diabetes mellitus, hypertension, waist-to-hip ratio, smoking, prevalent cardiovascular diseases, vitamin K antagonists, bisphosphonates use.

**Abbreviations:** CAC, Coronary Artery Calcification; AAC, Aortic Arch Calcification; ECAC, Extracranial Internal Carotid Artery Calcification; ICAC, Intracranial Internal Carotid Artery Calcification; β, Beta-coefficient; CI, Confidence Interval.

**eTable 4. The follow-up analysis**

Multivariable mixed linear regression assessing the association between ever statin use and each exposure category (prevalent users and initiators) with calcification volume measured at both baseline and follow-up as a repeated outcome, each compared separately to never users. Comparative effectiveness analyses were conducted between prevalent users and initiators.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | | CAC  β (95%CI) | AAC  β (95%CI) | ECAC  β (95%CI) | ICAC  β (95%CI) |
| **Mixed Model 2** | | | | | |
| Never use, (N=430) | | REFERENCE | REFERENCE | REFERENCE | REFERENCE |
| Ever use, (N=380) | | 0.36 (0.27, 0.46) | 0.29 (0.19, 38) | 0.28 (0.18, 0.38) | 0.33 (0.23, 0.43) |
| **Mixed Model 2: Initiator (N=156), prevalent (N=224)** | | | | | |
| Never use, (N=430) | | REFERENCE | REFERENCE | REFERENCE | REFERENCE |
| Ever use | Prevalent | 0.50 (0.35, 0.65) | 0.33 (0.18, 0.48) | 0.40 (0.23, 0.54) | 0.35 (0.19, 0.51) |
| Initiators | 0.27 (0.15, 0.40) | 0.26 (0.13, 0.37) | 0.20 (0.07, 0.32) | 0.32 (0.19, 0.45) |
| **Comparative effectiveness**  Mixed Model 2 | | | | | |
| Prevalent, (N=156) | | REFERENCE | REFERENCE | REFERENCE | REFERENCE |
| Initiator, (N=224) | | -0.14 (-0.32, 0.04) | -0.03 (-0.20, 0.12) | -0.06 (-0.25, 0.14) | 0.04 (-0.15, 0.22) |

A total of 810 individuals were included in these analyses. Statin use was categorized as ever use, prevalent use, and initiation. Each exposure category was compared separately to never use, which served as the consistent reference group across analyses. Initiators had newly started using statins between the baseline and follow-up scans, while prevalent users had used statins both before baseline and between baseline and the follow-up scans.

Model 2 was a mixed linear model with a random intercept. Fixed effects included age, sex, time (since inception), diabetes, hypertension, waist-to-hip ratio, smoking, prevalent cardiovascular disease, use of vitamin K antagonists, use of bisphosphonates. Comparative effectiveness models were further adjusted for daily dose and cumulative duration of statin use.

**Abbreviations:**

CAC: Coronary Artery Calcification, AAC: Aortic Arch Calcification, ECAC: Extracranial Internal Carotid Artery Calcification, ICAC: Intracranial Internal Carotid Artery Calcification, β: Beta-coefficient, CI: Confidence Interval, N = Number of unique individuals

**eTable 5:** Distribution of active statin use across OATP1B1 transporter function levels

|  |  |  |  |
| --- | --- | --- | --- |
|  |  | Active statin use  N=473 | Never use  N= 1811 |
| OATP1B1 transporter function | Normal, N(%) | 137 (29.0%) | 520 (28.7%) |
| Decreased, N(%) | 178 (37.6%) | 714 (39.4%) |
| Poor, N(%) | 58 (12.3%) | 247 (13.6%) |
| Missing, (N%) | 100 (21.1%) | 330 (18.2%) |

The proportion of active statin use was also similar across strata of transporter function.

**eTable 6:** Effect measure modification by OATP1B1 transporter function

Multivariable linear regression of the association between active statin use and baseline arterial calcification volume, estimated separately within each transporter function group using never users as the constant reference

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | | CAC  β (95%CI) | AAC  β (95%CI) | ECAC  β (95%CI) | ICAC  β (95%CI) |
| **Model 2** | | | | | |
| Never use, (N= 1811) | | REFERENCE | REFERENCE | REFERENCE | REFERENCE |
| Normal function level | Active statin use,  (N=137) | 0.21 (-0.21, 0.45) | 0.23 (0.07, 0.39) | 0.23 (-0.01, 0.47) | 0.26 (0.02, 0.51) |
| **Model 2** | | | | | |
| Never use, (N= 1811) | | REFERENCE | REFERENCE | REFERENCE | REFERENCE |
| Decreased function level | Active statin use, (N=178) | 0.35 (0.15, 0.55) | 0.38 (0.17, 0.58) | 0.41 (0.27, 0.56) | 0.38 (0.17, 0.59) |
| **Model 2** | | | | | |
| Never use, (N= 1811) | | REFERENCE | REFERENCE | REFERENCE | REFERENCE |
| Poor function level | Active statin use, (N=58) | 0.50 (0.16, 0.85) | 0.39 (0.02, 0.75) | 0.51 (0.19, 0.86) | 0.40 (0.13, 0.68) |

OATP1B1 transporter function was categorized as normal, decreased, or poor. Active statin use was defined as concurrent current and long-term statin use at baseline. A total of 473 active statin users and 1,811 never users were included in the analysis.

Model 2 was adjusted for age, sex, diabetes mellitus, hypertension, waist-to-hip ratio, current smoking, prevalent cardiovascular diseases, use of vitamin K antagonists, and bisphosphonates.

**Abbreviations:**

OATP1B1: Organic Anion-Transporting Polypeptide 1B, CAC: Coronary Artery Calcification, AAC: Aortic Arch Calcification, ECAC: Extracranial Internal Carotid Artery Calcification, ICAC: Intracranial Internal Carotid Artery Calcification, β: Beta-coefficient, CI: Confidence Interval, N = Number of individuals

**eTable 7:** Linear trend analysis across transporter function levels using orthogonal polynomial contrasts, with baseline calcification volume as the outcome, presented separately for active statin users and never users

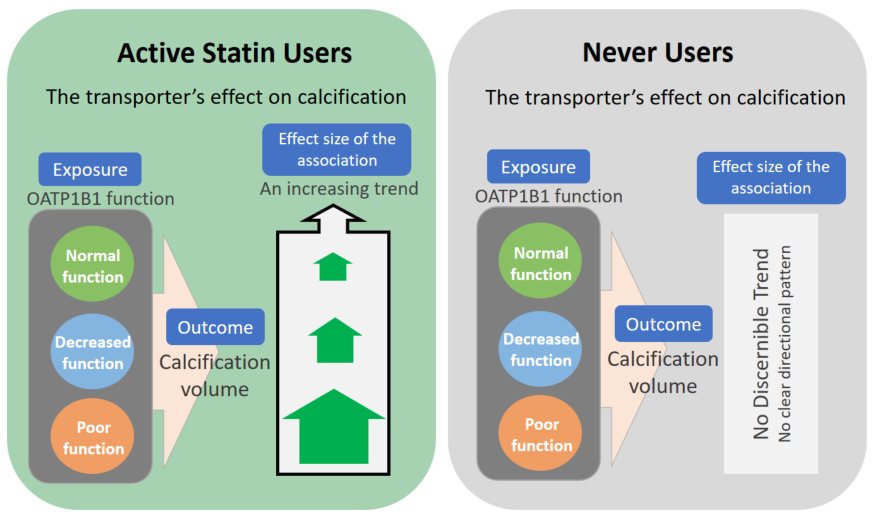
|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | CAC  β (95%CI) | AAC  β (95%CI) | ECAC  β (95%CI) | ICAC  β (95%CI) |
| Active statin users | 0.12 (0.10, 0.13)\* | 0.05 (-0.02, 0.07) | 0.08 (0.07, 0.09)\* | 0.05 (-0.1, 0.08) |
| Never users | -0.02 (-0.09, 0.05) | -0.00 (-0.10, 0.09) | -0.00 (-0.09, 0.10) | -0.00 (-0.11, 0.10) |

**\***Pvalue= 0.003 and 0.007 indicate statistically significant linear trends.

**Abbreviations:**

**CAC: Coronary Artery Calcification, AAC: Aortic Arch Calcification, ECAC: Extracranial Internal Carotid Artery Calcification, ICAC: Intracranial Internal Carotid Artery Calcification, β: Beta-coefficient, CI: Confidence IntervaleFigure 3. The negative control population results**

The association between OATP1B1 transporter (a statin’s plasma concentration modifier) and calcification volume with and without active statin use. Among active statin users, lower transporter function was associated with higher calcification volume. This trend was observed across all arterial beds.



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