Polygenic Risk Scores for Prediction of Subclinical Coronary Artery Disease in Persons Living with HIV: The Swiss HIV Cohort Study

Isabella C. Schoepf[1-3]*, Christian W. Thorball[4,5]*, Helen Kovari[6], Bruno Ledergerber[6], Ronny R. Buechel[7], Alexandra Calmy[8], Rainer Weber[6], Philipp A. Kaufmann[7], René Nkoulou[9], Johannes M. Schwenke[3], Dominique L. Braun[6], Jacques Fellay[4,5], and Philip E. Tarr[3], for the Swiss HIV Cohort Study

** these authors contributed equally to the manuscript*

[1] Department of Infectious Diseases, Bern University Hospital, University of Bern, 3010 Bern, Switzerland;
[2] Hepatology, Department for Visceral Surgery and Medicine, Bern University Hospital, University of Bern, 3010 Bern, Switzerland
[3] University Department of Medicine and Infectious Diseases Service, Kantonsspital Baselland, University of Basel, 4101 Bruderholz, Switzerland
[4] Precision Medicine Unit, Lausanne University Hospital and University of Lausanne, 1011 Lausanne, Switzerland
[5] School of Life Sciences, Ecole Polytechnique Fédérale de Lausanne, 1015 Lausanne, Switzerland
[6] Division of Infectious Diseases and Hospital Epidemiology, University Hospital Zurich, University of Zurich, 8091 Zurich, Switzerland
[7]Department of Nuclear Medicine, Cardiac Imaging, University Hospital Zurich, Rämistr. 100, University of Zurich, 8091 Zurich, Switzerland
[8]Division of Infectious Disease, Geneva University Hospital, 1205 Geneva, Switzerland
[9]Division of Cardiology, University Hospital Geneva, University of Geneva, Rue Gabrielle-Perret-Gentil 4, 1205 Geneva, Switzerland

Corresponding author: Philip E. Tarr, MD, University Dept. of Medicine and Infectious Diseases Service, Kantonsspital Baselland, University of Basel, 4101 Bruderholz, Switzerland; philip.tarr@unibas.ch

Running head: Schoepf Subclinical CAD PRS HIV

Abstract

Background. In people living with HIV (PLWH), individual polygenic risk scores (PRSs) are associated with coronary artery disease (CAD) events. Whether PRSs are associated with subclinical CAD is unknown.

Methods. In Swiss HIV Cohort Study participants of European descent, we defined subclinical CAD as presence of soft, mixed, or high risk plaque (SMHRP) on coronary CT angiography, or as participants in the top tertile of the study population's coronary artery calcium (CAC) score, using non-contrast CT. We obtained uni-/multivariable odds ratios (OR) for subclinical CAD endpoints based on non-genetic risk factors, and validated genome-wide PRSs built from single nucleotide polymorphisms (SNPs) associated with CAD, carotid intima-media thickness (IMT), or longevity in the general population.

Results. We included 345 genotyped participants (median age 53 years, 89% male, 96% suppressed HIV RNA); 172 and 127 participants had SMHRP and CAC, respectively. CAD-associated PRS and IMT-associated PRS were associated with SMHRP and CAC (all $p<0.01$), but longevity-PRS was not. Participants with unfavorable CAD-PRS (top quintile) had adjusted SMHRP-OR=2.58 (95% confidence interval [CI], 1.18-5.67), and CAC-OR=3.95 (95% CI, 1.45-10.77), vs. bottom quintile. Unfavorable non-genetic risk (top vs. bottom quintile) was associated with adjusted SMHRP-OR=24.01 (95% CI, 9.75-59.11), and CAC-OR=65.07 (95% CI, 18.48-229.15). Area under the ROC curve increased when we added CAD-PRS to non-genetic risk factors (SMHRP: 0.75, 0.78, respectively; CAC: 0.80, 0.83, respectively).

Conclusions. In Swiss PLWH, subclinical CAD is independently associated with an individual CAD-associated PRS. Combining non-genetic and genetic cardiovascular risk factors provided the most powerful subclinical CAD prediction.

Keywords. HIV infection, subclinical coronary artery disease, polygenic risk score, aging, multivariable analysis.

Introduction

A major concern in people living with HIV (PLWH) includes the approximately 2-fold increased risk of coronary artery disease (CAD) events compared to the general population.[1,2] Considerable interest has been generated by the application of non-invasive cardiac imaging methods in PLWH for the early detection of asymptomatic (subclinical) CAD, which is associated with an increased risk of future acute CAD events.[3-5] Cardiac imaging in PLWH has typically relied on carotid ultrasound for measurement of carotid intima-media thickness (IMT),[6,7] and non-contrast CT for detection of coronary artery calcification (CAC).[8-11] More recently, in PLWH, we[10,11] and others[12-14] have applied coronary CT angiography (CCTA), which can detect not only calcified but also non-calcified plaque including high risk coronary plaque. CCTA is of particular interest in the setting of PLWH, because coronary plaque in younger persons typically is non-calcified [15] and the median age of many Western populations of PLWH is around 50 years.

CAD has a well recorded hereditary component.[16,17] Genome-wide association studies (GWAS) in the general population have now documented single nucleotide polymorphisms (SNPs) that are strongly associated with subclinical atherosclerosis.[5,18-21] Furthermore, by aggregating the effect of all SNPs included in these GWAS, it is possible to obtain a single measurement of the genetic risk conferred for this phenotype by all common SNPs in the form of a polygenic risk score (PRS). In PLWH, we have previously reported a 3.17-fold increased risk of clinical CAD events in Swiss PLWH with an unfavorable CAD-associated polygenic risk score (CAD-PRS) consisting of >600'000 SNPs.[22] Based on the concern that aging may be accelerated and/or accentuated in PLWH,[23,24] we had also assessed a PRS associated with longevity in the general population for its contribution to clinical CAD events in PLWH [22]: CAD event risk increased from 3.17-fold to 3.67-fold in PLWH with both unfavorable CAD-PRS and unfavorable longevity-PRS.[22] To our knowledge, and in contrast to *clinical* CAD events, there are no studies on the genetic prediction of *subclinical* CAD in PLWH. The aim of our present study therefore was to investigate validated, CAD-associated, IMT-associated and longevity-associated PRSs to subclinical coronary plaque in Swiss PLWH, in the context of traditional and HIV-related risk factors including adverse antiretroviral exposures.

Methods

Patient Consent Statement. Participants provided written informed consent including for genetic testing (**Supplementary Methods**).

Study Design, Participants. Participants were aged ≥45 years and were participants of the Metabolism and Aging (M+A) Core Project of the Swiss HIV Cohort Study (SHCS, www.shcs.ch).[25] In the M+A core project, we obtained baseline CAC/CCTA scans in 430 participants from 10/2013 to 7/2016 [10,11]. Enrollment criteria for the CAC/CCTA substudy included no documented angina, CAD or stroke, eGFR ≥50 mL/min, no allergy to iodinated contrast agent, and no history of atrial fibrillation or other irregular cardiac rhythm. Follow-up CAC/CCTA scans were performed 10/2015-04/2019, with a minimum 2-year interval between scans. Ineligibility for follow-up CAC/CCTA included coronary stenosis ≥50% at baseline CCTA, cardiovascular event in the interval, eGFR<50 mL/min, unwillingness to undergo repeat CAC/CCTA, death, or loss to follow-up. Because previous subclinical CAD-GWAS in the general population were conducted in populations of mostly European descent,[26] the present analysis was restricted to participants of European descent.

Subclinical coronary artery disease. As previously reported,[10,11] we investigated the presence of calcified, soft (non-calcified), mixed, or high risk coronary plaque by CCTA, and calculated the coronary artery calcium (CAC) score using non-contrast CT, based on the Agatston method.[27] Coronary arteries were subdivided into 16 segments,[28] with the intermediate artery defined as segment 16, if present. Segments with a diameter of ≥1.5mm at origin were included in the analysis. Atherosclerotic plaque was defined as a lesion ≥1mm^2 in orthogonal reconstructions within and/or adjacent to the vessel lumen, not belonging to surrounding tissue.[29] High risk plaque was defined[30] as plaque with remodeling index ≥1.1 and/or low attenuation plaque (≤30 Hounsfield units). For the present analyses, we investigated the association of different PRSs with 2 endpoints; i) a composite CCTA endpoint, with cases defined as having non-calcified, i.e. soft, mixed, or high risk plaque (SMHRP), present at the baseline and/or follow-up scan, and SMHRP-controls being all other participants, and ii) a CAC endpoint, with cases defined as participants in the top tertile of CAC score distribution at baseline and/or follow-up scan, and controls being those in the bottom tertile at each

scan; participants in the middle tertile were excluded from this analysis to better separate the phenotypes and thereby increase power to detect any genetic effects.[31]

Non-genetic subclinical-CAD risk factors. Co-variables included in the statistical models were as previously reported.[10,11] Briefly, traditional CAD risk factors included age, sex, smoking, cocaine use, family history of CAD, diabetes mellitus, hypertension, and dyslipidemia. HIV-related co-variables included HIV viremia at the baseline scan, CD4 nadir, and ART exposures (exposure to abacavir in past 6 months, and cumulative exposure [≥1 year] to lopinavir, indinavir, darunavir, or stavudine), cytomegalovirus and hepatitis C seropositivity, and intravenous drug use (IDU).

Genotyping. Genotyping was done using the Global Screening Array v2.0+MD (Illumina, San Diego, CA), or in the setting of previous SHCS genetic studies, using DNA samples obtained from stored peripheral blood mononuclear cells (PBMC). All quality control, imputation and filtering steps were performed separately for each batch of samples prior to merging as described (**Supplementary Methods).** Only SNPs with a minor allele frequency >1% and missingness <10% in the final merged dataset were retained for the PRS calculations.

Genome-wide Polygenic Risk Scores. The PRSs were calculated using PRSice (v2.3.3). As previously reported,[22] the CAD-PRS was built by directly applying the variant information from the subclinical CAD-PRS previously validated by Inouye,[32] with the Information on SNPs included in this score and their weights were downloaded from the PGS Catalog.[33] The longevity PRS was based on the genome-wide association summary statistics in the reference paper by Deelen,[24] and generated as described previously.[22] In addition, based on the summary statistics of the genome-wide association studies by Strawbridge,[21] we generated three carotid intimal media thickness (IMT)-associated PRSs, associated with the mean IMT, maximal IMT, and with the average of the 4 maximal values (2 segments for each of 2 sides), subsequently referred to as IMTmean-PRS, IMTmax-PRS, and IMTmean/max-PRS. For these, the variants were clumped using windows of 250kb and a linkage disequilibrium threshold of r^2=0.1, prior to p-value thresholding. In all IMT-associated PRS, the model including all SNPs (p = 1) were selected.

The CAD-PRS included 556,881 SNPs, the IMTmean-PRS, IMTmax-PRS, IMTmean/max-PRS, and

longevity-PRS included 102'679, 102'590, 102'377, and 4 SNPs after clumping, respectively. 51'567 SNPs in the IMTmean-PRS, and 4 SNPs in the longevity-PRS were also either included, or they were in linkage disequilibrium ($r^2 \geq 0.9$) with included SNPs in the CAD-PRS. There was evidence of a correlation between the CAD-PRS and the longevity-PRS (SpearmanPearson rho=-0.19, p<0.001).

Power calculation. To detect odds ratios of >1.9 with a power of 0.8 and alpha of 0.05 approximately 160 cases and 160 controls would be required.[34]

Statistical analyses. Categorical baseline characteristics of cases and controls were compared with Fisher's exact tests and continuous characteristics with Wilcoxon rank-sum tests. Univariable and multivariable logistic regression analyses were used to estimate associations of the different non-genetic and genetic risk factors. We decided *a priori* to stratify the genetic risk factors into quintiles for better visualization of their potentially non-linear associations with CAD events. Non-genetic variables were entered into the multivariable model if their association in the univariable model had p<0.2. Age (per 10 years older), sex and ART exposures (as defined above) were entered a priori. We combined all traditional and HIV-related risk factors into a single measure of non-genetic CAD event risk by creating quintiles of the individually predicted CAD event probabilities from the multivariable model with the non-genetic risk factors as described above. These non-genetic risk quintiles were then used to check for and visualize interactions with genetic risk factors. Model fit and interactions were analyzed using Akaike and Bayesian information criteria and likelihood ratio tests. CAD event variation explained by the different models with combinations of non-genetic and genetic risk factors were documented with Pseudo R^2 values and area under the receiver operating characteristic (AUROC) values. We used Stata/SE 17.0 (StataCorp, College Station, TX, USA).

Results

Participants. Analyses are based on 345 study participants, of which 172 participants had SMHRP and 127 participants had CAC. The disposition of participants is shown in **Figure 1** and clinical characteristics are shown in **Table 1**. Participants with SMHRP (vs. those without SMHRP) were more likely to be men, dyslipidemic, hypertensive, with CD4 nadir <50 cells/uL, with HIV RNA>50 copies/mL, HCV-antibody positive, and exposed to abacavir or stavudine. Participants with CAC (vs. those without CAC) were more likely to be men, diabetic, hypertensive, with HIV RNA>50 copies/mL, and exposed to abacavir. Among the participants undergoing both baseline and follow-up CCTA/CAC, soft/mixed plaque, high risk plaque, and coronary calcification were recorded in 108, 46, and 170 participants at the first scan, respectively, which were absent at the second scan in 16/108 (14.8%), 7/46 (15.2%), and 4/170 (2.4%), respectively.

Probability of Subclinical CAD: Univariable Analysis. SMHRP and CAC cases had higher non-genetic risk and higher genetic risk than controls, when applying either the CAD-PRS or the IMTmean-PRS (all p<0.01). Associations of SMHRP with other IMT-PRSs were weaker (**Supplementary Results**), therefore, we include only IMTmean-PRS in the subsequent analyses. There was no evidence for an association of longevity-PRS with SMHRP (p=0.45) or CAC (p=0.76). Higher non-genetic and genetic risk in cases vs controls is visualized in **Figure 2**, i.e. distribution of SMHRP cases was skewed towards the 5th (most unfavorable) vs. the other quintiles of non-genetic risk (**Figure 2A**), CAD-PRS (**Figure 2B**), and IMTmean-PRS (**Supplementary Figure 1**). The distribution of CAC cases was also skewed towards the 5th vs. the other quintiles of non-genetic risk (**Figure 2C**), CAD-PRS (**Figure 2D**), and IMTmean-PRS (**Supplementary Figure 1**).

Probability of Subclinical CAD According to Non-Genetic Risk Factors: Univariable Analysis. Compared to participants in the first quintile of *non-genetic* risk (i.e. traditional and HIV-related risk factors combined), participants in the second, third, fourth, and fifth quintiles had significantly increased odds ratio for both SMHRP and CAC (**Figure 3** and **Supplementary Table 3**). SMHRP and

CAC ORs separate for traditional and HIV-associated risk factors is shown in the **Supplementary Table 3**.

Probability of Subclinical CAD According to Polygenic Risk Scores: Univariable Analysis. Compared to the first (most favorable) CAD-PRS quintile, participants in the second, third, fourth, and fifth quintiles had SMHRP-OR=0.63 (0.32-1.23), 0.71 (0.37-1.38), 1.34 (0.69-2.60) and 2.06 (1.02-4.15), respectively, and CAC-OR=0.99 (0.44-2.24), 0.86 (0.38-1.93), 2.09 (0.93-4.68) and 2.99 (1.30-6.91), respectively. For comparison, dyslipidemia and recent abacavir exposure had univariable SMHRP-OR=2.08 (1.34–3.21) and 3.05 (1.70–5.46), respectively, and univariable CAC-OR=1.68 (1.01-2.79) and 2.25 (1.14-4.43), respectively. Increased SMHRP-ORs and CAC-ORs were similar when we applied quintiles of the IMTmean-PRS quintiles instead of CAD-PRS quintiles, but there was no evidence of any association of SMHRP or CAC with longevity-PRS (**Supplementary Table 2**).

Subclinical CAD Probability: Multivariable Analysis. Participants in the second, third, fourth, and fifth (vs. first) quintiles of *non-genetic* risk had significantly increased odds ratios for both SMHRP and CAC (**Figure 3** and **Supplementary Table 3**). Participants in the second, third, fourth, and fifth (vs. first) CAD-PRS quintiles had multivariable SMHRP-ORs=0.68 (0.32-1.44), 0.80 (0.38-1.69), 1.46 (0.69-3.11) and 2.58 (1.18-5.67), respectively, and CAC-OR=1.31 (0.49-3.51), 1.26 (0.47-3.41), 3.28 (1.19-9.01), and 3.95 (1.45-10.77), respectively (**Figure 3**). There was a trend for an association of IMTmean-PRS with SMHRP and CAC in multivariable analysis (**Supplementary Table 2).** SMHRP and CAC odds ratios separate for traditional and HIV-associated risk factors are shown in **Supplementary Table 3**.

Variability of Subclinical CAD Explained by Traditional Risk Factors, HIV-related Risk Factors, and PRSs. As shown in **Figure 4,** SMHRP variability explained by CAD-PRS was lower than that explained by traditional or HIV-related risk factors. SMHRP variability explained was highest, i.e. 18.6%, when we *combined* non-genetic and genetic risk factors. Similarly, CAC variability explained by CAD-PRS was lower than that explained by traditional risk factors or HIV-related risk factors, but CAC variability explained was highest (27.2%) when we *combined* non-genetic and genetic risk factors. We

obtained similar results in our recent study of CAD events in Swiss PLWH,[22] where CAD event variability explained was highest for the combination of traditional risk factors, HIV-related risk factors, plus CAD-PRS (35.3%; **Figure 4**). Results were similar when we applied the area under the receiver operating characteristic (AUROC). For SMHRP, AUROC for non-genetic risk factors alone, and combined with CAD-PRS was 0.75 and 0.78, respectively. For CAC, AUROC for non-genetic risk factors alone, and combined with CAD-PRS was 0.80 and 0.83, respectively. In comparison, for acute CAD events [22], AUROC for non-genetic risk factors alone, and combined with CAD-PRS, was 0.85 and 0.87, respectively.

Discussion

To our knowledge, this is the first study to report on genetic predisposition to subclinical atherosclerosis in PLWH, a population whose increased cardiovascular risk is now well recorded. Here we show that an unfavorable individual polygenic risk score independently is associated with a 2.6-fold to 4-fold increased risk of non-calcified (including high risk) plaque and calcified plaque in PLWH, respectively. Our findings in *subclinical* CAD are quantitatively similar to our previous report that an unfavorable PRS increases the risk of *clinical* CAD events 3.17 to 3.67-fold [22]. In addition, being in the highest quintile of the CAD-PRS is associated with a risk of non-calcified or calcified coronary plaque equivalent to that of dyslipidemia or current abacavir exposure.

While an unfavorable traditional and HIV-associated risk factor profile had a stronger impact on subclinical CAD than genetic background, the combination of traditional, HIV-related and genetic risk factors afforded the most powerful prediction. We were unable to document any association of the longevity-PRS with subclinical CAD, in contrast to our recent study in which the longevity-PRS had an independent association, although of limited effect size, with hard CAD events in PLWH.[22] Overall, our results suggest how genetic susceptibility to subclinical CAD and acute CAD events can be captured by a polygenic risk score in PLWH, which potentially could be assessed early during HIV infection, perhaps even before the in-depth evaluation of traditional risk factors. Knowledge of an unfavorable CAD-PRS may provide an early opportunity to investigate the presence of subclinical

CAD by targeted cardiac imaging studies and to implement early measures for cardiovascular prevention in PLWH at high risk for CAD.

Interestingly, CAD endpoint variability explained by both non-genetic and genetic factors increased when we moved from non-calcified plaque to calcified plaque (this report), and to hard CAD events (our previous report[22]). This might in part be explained by the precise definition, the irreversibility of hard clinical CAD endpoints, the longer exposure of patients with acute CAD events to non-genetic and genetic risk factors, and the larger study population. [22] Subclinical atherosclerosis endpoints were reversible in 2-15% of our study participants between baseline and follow-up scans, consistent with other longitudinal CCTA studies[30,35]. Even non-calcified plaque may occasionally undergo stable calcification rather than progressing to plaque rupture and acute CAD events. This is in part likely explained by effective statin or antihypertensive treatment.[35]

Strengths of our study include the exploitation of prospectively recorded information from well-characterized participants in the SHCS, which allowed us to quantify and compare the effects of relevant non-genetic and genetic risk factors for subclinical CAD. Our genetic results appear robust because we subjected the genetic data to rigorous quality control, i.e. we corrected for residual population stratification and excluded population outliers. In addition, only SNPs that have been validated in large reference GWAS in the general population were considered [21,24,32], and our genetic results are in line with findings in the general population regarding CAD events (with similar effect sizes) [32] and carotid intima media thickness [21]. Integration of all relevant CAD risk factors including individual PRS appears particularly relevant in the setting of PLWH who have an approximately 2-fold elevated CAD event risk, compared to the general population, even when HIV viremia is suppressed on successful antiretroviral therapy.[1,2]

In multivariable analyses, the CAD-PRS performed better than the different IMT-PRSs, for prediction of both non-calcified and calcified plaque. As expected, there is considerable overlap of SNPs associated with subclinical atherosclerosis and SNPs associated with CAD events, suggesting that some of the genetic determinants of subclinical atherosclerosis are also determinants of hard CAD

events.[36] A CAC and CIMT study in over 77'000 individuals from the CHARGE Consortium showed an association with known CAD loci such as at *CDKN2B* (the 9p21 locus), *PHACTR1*, *APOB*, and *APOE*.[5] These results were confirmed in >52'000 participants of the Multi-Ethnic Study of Atherosclerosis (MESA), where a CAD-PRS (including 157 genome-wide significant SNPs) strongly predicted CAC.[37] The three subclinical atherosclerosis endpoints investigated in the large GWAS by Strawbridge et al[21] (IMTmean, IMTmax, and IMTmean/max) are genetically highly correlated, the associated genetic loci partially overlap, and their clinical predictive value appears to differ only somewhat, with IMTmean being more associated with CAD and IMTmax more associated with stroke.[21,38]

Our study has limitations. Because most GWAS on subclinical CAD were performed in populations of European ancestry, we included only participants of European ancestry. Our study population was predominantly male and relatively young, therefore, results should be applied to females and elderly PLWH with caution. Knowledge on *how* individual genetic risk profiles contribute to HIV-related comorbidities remains limited. Due to the limited sample size, our study was not powered for detailed pathway analyses based on genetic information using the principle of Mendelian randomization.[39,40] The clinical value of genetic testing needs to be assessed in prospective trials, which was beyond the scope of our study.

In conclusion, here we document how PLWH may have a significantly increased subclinical CAD risk because of genetic and/or non-genetic risk factors. The integration of genetic, traditional, HIV-related, and antiretroviral CAD risk factors helps explain interindividual variation and provides the best prediction of individual risk of subclinical and clinical CAD in PWLH.

Notes

Author Contributions. Study design: ICS, CWT, BL, RRB, JF, PET. Data management, participant selection: BL. Data acquisition: HK, RRB, AC, RW. PAK, RN, DLB. Data analysis: ICS, CWT, BL, JF, PET. Drafting of the manuscript: ICS, CWT, BL, PET. Critical review and revision of the manuscript: All authors.

Acknowledgments. The authors acknowledge the effort and commitment of investigators, study nurses, laboratory personnel, and participants. We thank Rona Strawbridge (University of Glasgow, Scotland and Karolinska Institute Stockholm, Sweden) for data sharing and helpful discussions.

Members of the Swiss HIV Cohort Study. Abela I, Aebi-Popp K, Anagnostopoulos A, Battegay M, Bernasconi E, Braun DL, Bucher HC, Calmy A, Cavassini M, Ciuffi A, Dollenmaier G, Egger M, Elzi L, Fehr J, Fellay J, Furrer H, Fux CA, Günthard HF (President of the SHCS), Hachfeld A, Haerry D (deputy of "Positive Council"), Hasse B, Hirsch HH, Hoffmann M, Hösli I, Huber M, Kahlert CR (Chairman of the Mother & Child Substudy), Kaiser L, Keiser O, Klimkait T, Kouyos RD, Kovari H, Kusejko K (Head of Data Centre), Martinetti G, Martinez de Tejada B, Marzolini C, Metzner KJ, Müller N, Nemeth J, Nicca D, Paioni P, Pantaleo G, Perreau M, Rauch A (Chairman of the Scientific Board), Schmid P, Speck R, Stöckle M (Chairman of the Clinical and Laboratory Committee), Tarr P, Trkola A, Wandeler G, Yerly S.

Funding statement. This study has been financed within the framework of the Swiss HIV Cohort Study, supported by the Swiss National Science Foundation (grant 177499), by SHCS project 859 and by the SHCS research foundation. The data are gathered by the Five Swiss University Hospitals, two Cantonal Hospitals, 15 affiliated hospitals and 36 private physicians (listed in http://www.shcs.ch/180-health-care-providers). BL reports support for this work from Kantonsspital Baselland (Consultancy and statistical analyses).

Potential conflicts of interest statement. ICS's institution received a lecture fee from ViiV, outside the submitted work. PET's institution reports grants, advisory fees and support for educational activities from Gilead, ViiV, MSD and Sankyo Daiichi, outside the submitted work. BL received personal fees from Kantonsspital Baselland, Liestal, Switzerland, during the conduct of the study, and reports personal fees from Gilead (for lectures), and ViiV (for participation on Advisory Board),

outside the submitted work. RRB reports speaker honoraria from Pfizer (personal <2000 CHF), Sanofi-Aventis (personal 2000 CHF) and GE Healthcare (personal <2000 CHF); support for attending meetings from Pfizer for Registration EAMN Congress 2021; and unpaid roles as Chair of the Nuclear Certification Sub-committee of the European Association of Cardiovascular Imaging and Programme committee member for the annual congress of the European Association of Cardiology; and Research contract with Department of Nuclear Medicine, University Hospital Zurich, Switzerland from GE Healthcare. DLB reports honoraria for advisory boards, lectures and travel grants from AbbVie, Gilead, MSD and ViiV; and consulting fees from ViiV, MSD, Gilead, Pfizer, and AstraZeneka. AC reports educational grants from AbbVie, MSD, Gilead, and ViiV healthcare; and payment or honoraria for lectures, presentations, speakers bureaus, manuscript writing or education events from Gilead Sciences (unrestricted), MSD, and ViiV healthcare (educational grant to institution). RN reports payment or honoraria for lectures, presentations, speakers bureaus, manuscript writing or education events from Pfizer. PAK reports payment for Advisory Board meeting (Date 02/05/21; Focus: myocardial perfusion tracer) from GE Healthcare; unpaid role as Vice Chair of the Swiss Society of Nucleaer Medicine; and research contract with Department of Nuclear Medicine, University Hospital Zurich, Switzerland from GE Healthcare. All other authors report no potential conflicts of interest. All authors have prepared ICMJE forms for disclosure of potential conflicts of interest.

References

1. Shah ASV, Stelzle D, Lee KK, et al. Global Burden of Atherosclerotic Cardiovascular Disease in People Living With HIV. *Circulation*. **2018**;138(11):1100-1112.
2. Feinstein MJ, Hsue PY, Benjamin LA, et al. Characteristics, Prevention, and Management of Cardiovascular Disease in People Living with HIV: A Scientific Statement from the American Heart Association. *Circulation*. **2019**;140(2):e98-e124.3. Polonsky T, McClelland R, Jorgensen N, et al. Coronary artery calcium score and risk classification for coronary heart disease. *JAMA*. **2010**;303:1610–1616.
4. Yeboah J, Mcclelland RL, Polonsky TS, et al. Comparison of novel risk markers for improvement in cardiovascular risk assessment in intermediate-risk individuals. *JAMA*. **2012**;308(8):788-795.
5. Natarajan P, Bis JC, Bielak LF, Al. E. Multiethnic Exome-Wide Association Study of Subclinical Atherosclerosis. *Circ Cardiovasc Genet*. **2016**;9(6):511-520.
6. Hsue PY, Lo JC, Franklin A, et al. Progression of Atherosclerosis as Assessed by Carotid Intima-Media Thickness in Patients with HIV Infection. *Circulation*. **2004**;109(13):1603-1608.
7. Currier JS, Kendall MA, Zackin R, et al. Carotid artery intima-media thickness and HIV infection: Traditional risk factors overshadow impact of protease inhibitor exposure. *AIDS*. **2005**;19(9):927-933.
8. Kingsley LA, Cuervo-Rojas J, Muñoz A, et al. Subclinical coronary atherosclerosis, HIV infection and antiretroviral therapy: Multicenter AIDS Cohort Study. *AIDS*. **2008**;22(13):1589-1599.
9. Guaraldi G, Zona S, Alexopoulos N, et al. Coronary Aging in HIV-Infected Patients. *Clin Infect Dis*. **2009**;49(11):1756–1762.
10. Tarr PE, Ledergerber B, Calmy A, et al. Subclinical coronary artery disease in Swiss HIV-positive and HIV-negative persons. *Eur Heart J*. **2018**;39(23):2147-2154.
11. Tarr PE, Ledergerber B, Calmy A, et al. Longitudinal progression of subclinical coronary atherosclerosis in swiss HIV-positive compared with hiv-negative persons undergoing coronary calcium score scan and CT angiography. *Open Forum Infect Dis*. **2020**;7(10)
12. Post WS, Budoff M, Kingsley L, et al. Associations between HIV Infection and Subclinical Coronary Atherosclerosis: The Multicenter AIDS Cohort Study (MACS). *Ann Intern Med*. **2014**;160(7):458-467.
13. Lai H, Moore R, Celentano DD, et al. HIV infection itself may not be associated with subclinical coronary artery disease among African Americans without cardiovascular symptoms. *J Am Heart Assoc*.

2015;5(3):1-16.

14. Shaikh K, Bhondoekhan F, Haberlen S, et al. Coronary artery plaque progression and cardiovascular risk scores in men with and without HIV-infection. *Aids*. **2022**;36(2):215-224.

15. Ascenzo FD, Cerrato E, Calcagno A, et al. High prevalence at computed coronary tomography of non-calcified plaques in asymptomatic HIV patients treated with HAART: A meta-analysis. Atherosclerosis **2015**,240, 197–204.

16. Murabito JM, Pencina MJ, Nam BH, et al. Sibling cardiovascular disease as a risk factor for cardiovascular disease in middle-aged adults. *J Am Med Assoc*. **2005**;294(24):3117-3123.

17. Mangino M, Spector T. Understanding coronary artery disease using twin studies. *Heart*. **2013**;99(6):373-375.

18. Bis JC, White CC, Franceschini N, et al. Sequencing of 2 subclinical atherosclerosis candidate regions in 3669 individuals cohorts for heart and aging research in genomic epidemiology (charge) consortium targeted sequencing study. *Circ Cardiovasc Genet*. **2014**;7(3):359-364.

19. Vargas J. Common Genetic Variants and Subclinical Atherosclerosis: The Multi-Ethnic Study of Atherosclerosis (MESA). *Atherosclerosis*. **2016**;245:230-236.

20. Franceschini N, Giambartolomei C, de Vries PS, et al. GWAS and colocalization analyses implicate carotid intima-media thickness and carotid plaque loci in cardiovascular outcomes. *Nat Commun*. **2018**;9(1):1-14.

21. Strawbridge RJ, Ward J, Bailey MES, et al. Carotid intima-media thickness novel loci, sex-specific effects, and genetic correlations with obesity and glucometabolic traits in UK Biobank. *Arterioscler Thromb Vasc Biol*. **2020**;(February):446-461.

22. Schoepf IC, Thorball CW, Ledergerber B, et al. Coronary Artery Disease-associated and Longevity-associated Polygenic Risk Scores for Prediction of Coronary Artery Disease Events in Persons Living with HIV: The Swiss HIV Cohort Study. *Clin Infect Dis*. **2021**;ciab521.

23. Pilling LC, Kuo CL, Sicinski K, et al. Human longevity: 25 genetic loci associated in 389,166 UK biobank participants. *Aging (Albany NY)*. **2017**;9(12):2504-2520.

24. Deelen J, Evans DS, Arking DE, et al. A meta-analysis of genome-wide association studies identifies multiple longevity genes. *Nat Commun*. **2019**;10(1).

25. Scherrer AU, Traytel A, Braun DL, Al. E. Cohort Profile Update: The Swiss HIV Cohort Study (SHCS). *Int J*

Epidemiol. **2021**;dyab141.

26. Schunkert H, König IR, Kathiresan S, et al. Large-scale association analysis identifies 13 new susceptibility loci for coronary artery disease. *Nat Genet*. **2011**;43(4):333-340.

27. Agatston A, Janowitz W, Hildner F, Zusmer N, Viamonte MJ, Detrano R. Quantification of coronary artery calcium using ultrafast computed tomography. *J Am Coll Cardiol*. **1990**;15(4):827-832.

28. Austen W, Edwards J, Frye R, Al. E. A reporting system on patients evaluated for coronary artery disease. Report of the Ad Hoc Committee for Grading of Coronary Artery Disease, Council on Cardiovascular Surgery, American Heart Association. *Circulation*. **1975**;51:5-40.

29. Buechel R, Husmann L, Herzog B, Al. E. Low-dose computed tomography coronary angiography with prospective electrocardiogram triggering: feasibility in a large population. *J Am Coll Cardiol*. **2011**;57:332–336.

30. Motoyama S, Ito H, Sarai M, et al. Plaque characterization by coronary computed tomography angiography and the likelihood of acute coronary events in mid-term follow-up. *J Am Coll Cardiol*. **2015**;66(4):337-346.

31. Dietrich LG, Barcelo C., Thorball C, et al. Contribution of genetic background and clinical D:A:D risk score to chronic kidney disease in Swiss HIV-positive persons with normal baseline estimated glomerular filtration rate. *Clinical Infectious Diseases*. **2019**;, 70:(), 890–897.

32. Inouye M, Abraham G, Nelson CP, et al. Genomic Risk Prediction of Coronary Artery Disease in 480,000 Adults: Implications for Primary Prevention. *J Am Coll Cardiol*. **2018**;72(16):1883-1893.

33. Lambert SA, Gil L, Jupp S, et al. The Polygenic Score Catalog: an open database for reproducibility and systematic evaluation. *medRxiv*. Published online January 1, 2020:2020.05.20.20108217.

34. Fleiss JL, Levin B, Paik MC. Statistical Methods for Rates and Proportions. New York: Wiley. Agresti, A. **2013**. Categorical Data Analysis. 3rd ed. Hoboken, NJ: Wiley.

35. Iwasaki K, Matsumoto T. Dynamic change of high-risk plaque detected by coronary computed tomographic angiography in patients with subclinical coronary artery disease. *Int J Cardiovasc Imaging*. **2016**;32(11):1667-1673.

36. Aragam KG, Natarajan P. Polygenic Scores to Assess Atherosclerotic Cardiovascular Disease Risk: Clinical Perspectives and Basic Implications. *Circ Res*. Published online 2020:1159-1177.

37. Severance L, Contijoch F, Carter H, Al. E. Using a genetic risk score to calculate the optimal age for an

individual to undergo coronary artery calcium screening. *J Cardiovasc Comput Tomogr*. **2019**;13:203–210.

38. Katakami N, Matsuoka T, Shimomura I. Clinical utility of carotid ultrasonography: application for the management of patients with diabetes. *J Diabetes Investig*. **2019**;10:883–898.

39. Nordestgaard BG, Palmer TM, Benn M, et al. The effect of elevated body mass index on ischemic heart disease risk: Causal estimates from a mendelian randomisation approach. *PLoS Med*. **2012**;9(5).

40. Erdmann J, Kessler T, Munoz Venegas L, Schunkert HA. Decade of genome-wide association studies for coronary artery disease: The challenges ahead. *Cardiovascular Research*. **2018**;114:1241–1257.

1 **Table 1: Characteristics of Study Participants**

		Soft, Mixed, or High Risk Plaque			Coronary Artery Calcification		
		Cases (n=172)	**Controls** (n=173)	**p-value**	**Cases** (n=127)	**Controls** (n=124)	**p-value**
Male sex, n (%)		161 (93.6)	147 (85)	0.01 [a]	121 (95.3)	104 (83.8)	<0.01 [a]
Age (years), median (IQR)		54 (50-60)	52 (49-56)	<0.01 [b]	55 (50-62)	50.5 (47-54)	<0.01 [b]
HIV acquisition mode, n (%)				0.54 [a]			0.94 [a]
	heterosexual	35 (20.4)	45 (26)		31 (24.4)	34 (27.4)	
	MSM	110 (64)	108 (62.4)		80 (63)	76 (61.3)	
	IDU	23 (13.4)	17 (9.8)		13 (10.2)	12 (9.7)	
	other	4 (2.3)	3 (1.8)		3 (2.4)	2 (1.6)	
Smoking, n (%)				0.11 [a]			0.07 [a]
	current	74 (43)	64 (37)		54 (42.5)	46 (37.1)	
	past	47 (27.3)	39 (22.5)		39 (30.7)	28 (22.6)	
	never	51 (29.7)	70 (40.5)		34 (26.8)	50 (40.3)	
Cocaine use, n (%)	Recent*	8 (4.7)	9 (5.2)		8 (6.3)	5 (4)	
	Ever	26 (15.1)	23 (13.3)	0.76 [a]	20 (15.7)	14 (11.3)	0.39 [a]
Family History of CAD, n (%)		20 (11.6)	17 (9.8)	0.61 [a]	14 (11)	11 (8.9)	0.68 [a]
Diabetes mellitus, n (%)		11 (6.4)	4 (2.3)	0.07 [a]	10 (7.9)	2 (1.6)	0.03 [a]
Hypertension, n (%)		60 (34.9)	45 (26)	0.08 [a]	47 (37)	25 (20.2)	<0.01 [a]
Dyslipidemia, n (%)		88 (51.2)	57 (33)	<0.01 [a]	61 (48)	44 (35.5)	0.06 [a]
On ART, n (%)		170 (98.8)	172 (98.8)	0.62 [a]	125 (98.4)	124 (100)	0.50 [a]
On ART, HIV RNA <50 copies/mL (undetectable), n (%)		160 (93)	169 (97.7)	0.02 [a]	117 (92.1)	123 (99.2)	0.02 [a]
Total years on ART, median (IQR)		14 (6-18)	10 (5-17)	<0.01 [b]	13 (7-18)	9 (5-17)	<0.01 [b]

Currently on Abacavir, n (%)		47 (27.3)	19 (11)	<0.01 [a]	30 (23.6)	15 (12.1)	0.02 [a]
Lopinavir, exposure ≥1 year, n (%)		46 (26.7)	44 (25.4)	0.81 [a]	33 (26)	36 (29)	0.67 [a]
Indinavir, exposure ≥1 year, n (%)		28 (16.3)	25 (14.5)	0.66 [a]	19 (15)	17 (13.7)	0.86 [a]
Darunavir, exposure ≥1 year, n (%)		50 (29.1)	52 (30.1)	0.91 [a]	35 (27.6)	40 (32.3)	0.49 [a]
Stavudine, exposure ≥1 year, n (%)		61 (35.5)	38 (22)	<0.01 [a]	39 (30.8)	26 (21)	0.09 [a]
CD4 median (IQR), cells/µL		592 (433-739)	602 (489-759)	0.25 [b]	623 (440-786)	589 (493-749)	0.94 [b]
CD4 nadir (cells/µL), median (IQR)		184 (91-266)	220 (130-293)	<0.01 [b]	199 (104-303)	220 (122-287)	0.57 [b]
CD4 nadir <50 cells/µL, n (%)		32 (18.6)	13 (7.5)	<0.01 [a]	18 (14.2)	11 (8.9)	0.24 [a]
Hepatitis C Seropositivity, n (%)		32 (18.6)	16 (9.3)	0.01 [a]	17 (13.4)	12 (9.7)	0.43 [a]
CMV Seropositivity, n (%)		143 (83.1)	155 (89.6)	0.09 [a]	110 (86.6)	104 (83.9)	0.60 [a]

Note. All data shown apply to the date of the baseline CCTA/CAC scans, unless otherwise indicated. Shown are number (%) of participants, unless otherwise indicated.

[a] Fisher exact test.

[b] Wilcoxon rank-sum test.

Abbreviations. ART, antiretroviral therapy; CAD, coronary artery disease; CMV, cytomegalovirus; IDU, intravenous drug use; IQR, interquartile range; MSM, men who have sex with men.

FIGURE LEGENDS

Figure 1. Study flowchart.

Figure 2, A-B: Distribution of non-genetic risk factors and coronary artery disease polygenic risk score (CAD-PRS) in controls without non-calcified, i.e. soft, mixed, or high risk plaque (SMHRP; white bars) and in cases with SMHRP (gray bars). C-D: Distribution of non-genetic risk factors and CAD-PRS in controls in the lower tertile of coronary calcium distribution (CAC; white bars) and in cases in the upper tertile of CAC distribution (gray bars).

Abbreviations: CAD, coronary artery disease; CI, confidence interval; PRS, polygenic risk score.

We divided study participants into 5 quintiles according to their non-genetic risk and polygenic risk scores and show here the number, percentage and 95% confidence intervals of participants in each quintile.

A: Distribution of soft and/or mixed and/or high risk plaque (SMHRP) cases and controls according to quintiles of non-genetic risk. There were 17 (25%) cases vs. 51 (75%) controls in the 1st (most favorable) quintile, 22 (31.9%) vs. 47 (68.1%) in the 2nd quintile, 29 (42%) vs. 40 (58%) in the 3rd quintile, 46 (66.7%) vs. 23 (33.3%) in the 4th quintile, and 172 (50%) vs. 172 (50%) in the 5th (most unfavorable) quintile.

B: Distribution of SMHRP cases and controls according to quintiles of CAD-PRS. There were 35 (49.3%) cases vs. 36 (50.7%) controls in the 1st quintile, 27 (38%) vs. 44 (62%) in the 2nd quintile, 29 (40.9%) vs. 42 (59.2%) in the 3rd quintile, 39 (56.5%) vs. 30 (43.5%) in the 4th quintile, and 42 (66.7%) vs. 21 (33.3%) in the 5th quintile.

C: Distribution of coronary calcium (CAC) cases and controls according to quintiles of non-genetic risk. There were 10 (18.9%) cases vs. 43 (81.1%) controls in the 1st quintile, 20 (39.2%) vs. 31 (60.8%) in the 2nd quintile, 17 (34.7%) vs. 32 (65.3%) in the 3rd quintile, 38 (74.5%) vs. 13 (25.5%) in the 4th quintile, and 42 (89.4%) vs. 5 (10.6%) in the 5th quintile.

D: Distribution of CAC cases and controls according to quintiles of CAD-PRS. There were 19 (42.2%) cases vs. 26 (57.8%) controls in the 1st quintile, 21 (42%) vs. 29 (58%) in the 2nd quintile, 20 (38.5%) vs. 32 (61.5%) in the 3rd quintile, 32 (60.4%) vs. 21 (39.6%) in the 4th quintile, and 35 (68.6%) vs. 16 (31.4%) in the 5th quintile.

Figure 3: Subclinical Coronary Artery Disease (CAD) Odds Ratios (OR) according to Non-Genetic Risk Factors and CAD Polygenic Risk Score (CAD-PRS).

Uni- and multivariable conditional logistic regression of associations with **A.)** *SMHRP* Results involve 172 participants with SMHRP and 173 participants without SMHRP; and **B.)** *CAC* Results involve 127 participants in the top tertile of the CAC score and 124 participants in the bottom tertile of the CAC score. Compared to the first (most favorable) quintile of non-genetic risk factors, participants in the second, third, fourth, and fifth (most unfavorable) quintile had multivariable *SMHRP* and *CAC* ORs that remained similar, after adjustment for CAD-PRS (**left column A and B).** After adjustment for non-genetic risk factors, the CAD-PRS remained significantly associated with *SMHRP* and *CAC* (**right column A and B**). **Abbreviations**: CAC, coronary artery calcium; CAD, coronary artery disease; CI, confidence interval; PRS, polygenic risk score; SMHRP, soft, mixed, high risk plaque;

Figure 4. Subclinical Coronary Artery Disease (CAD) and acute CAD Event Variability Explained by Traditional, HIV-related, Combined Non-Genetic Risk Factors and CAD Polygenic Risk Score (PRS).

Soft, mixed, or high risk plaque (SMHRP), Coronary Artery Calcium (CAC) and, as previously reported [22], acute coronary artery disease (CAD) event variability that is explained by traditional risk factors (white bars), HIV-related risk factors (light grey bars), combined non-genetic risk factors (i.e. traditional and HIV-related factors; grey bars), CAD Polygenic risk score (PRS; dark grey bars), and combined non-genetic risk factors and CAD-PRS (black bars). The bars represent variability based on pseudo-*R*2 test.

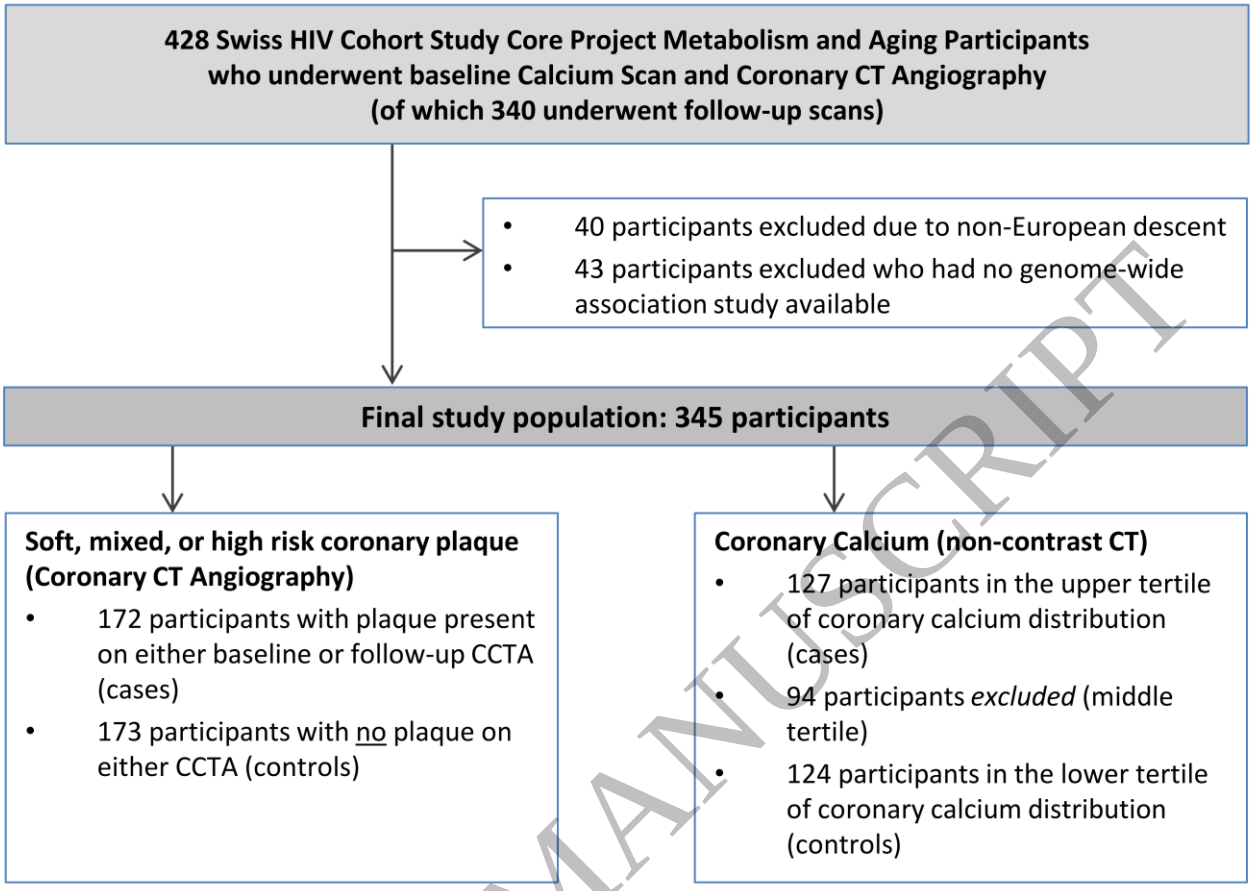


Figure 1
170x121 mm (x DPI)

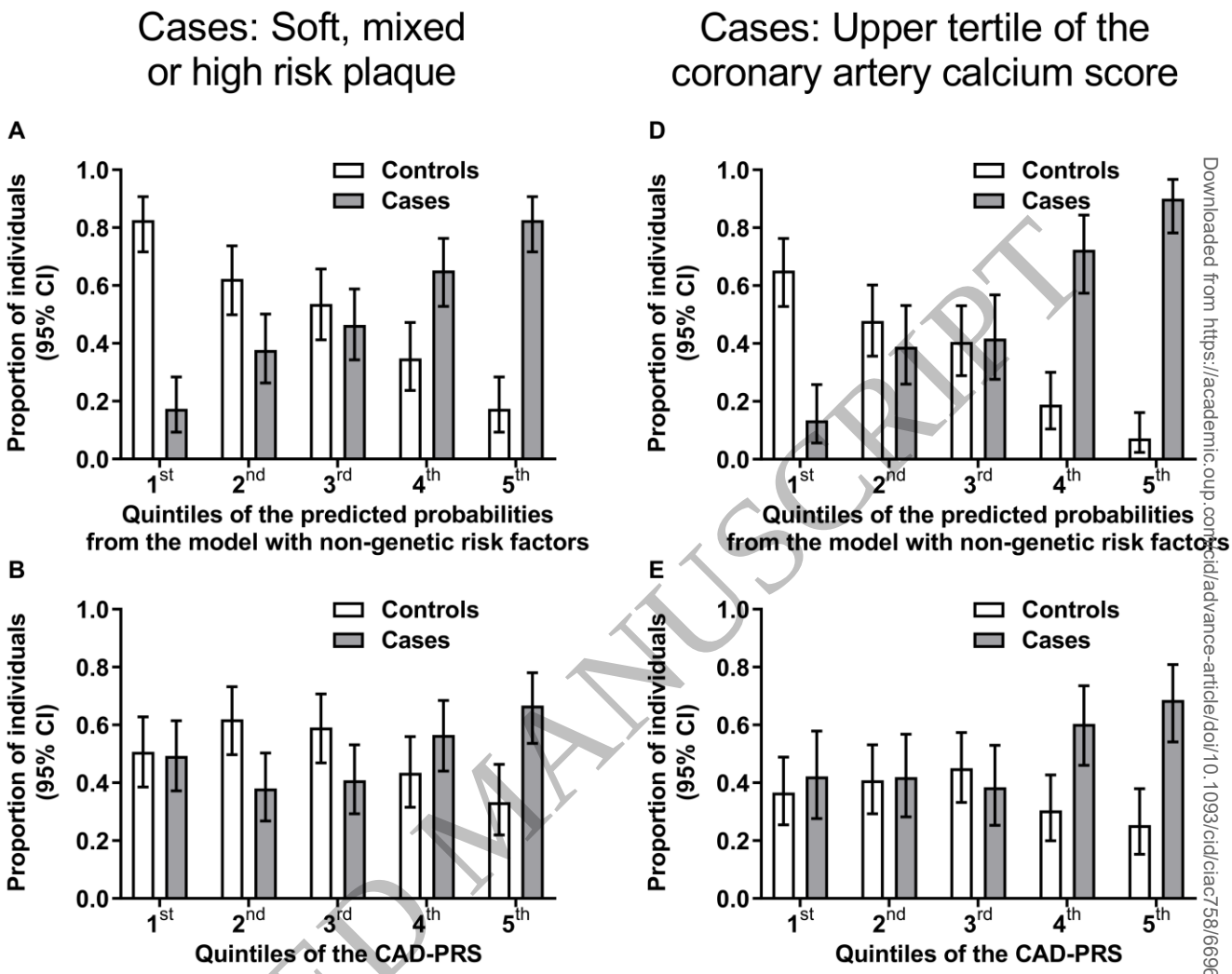


Figure 2
190x147 mm (x DPI)

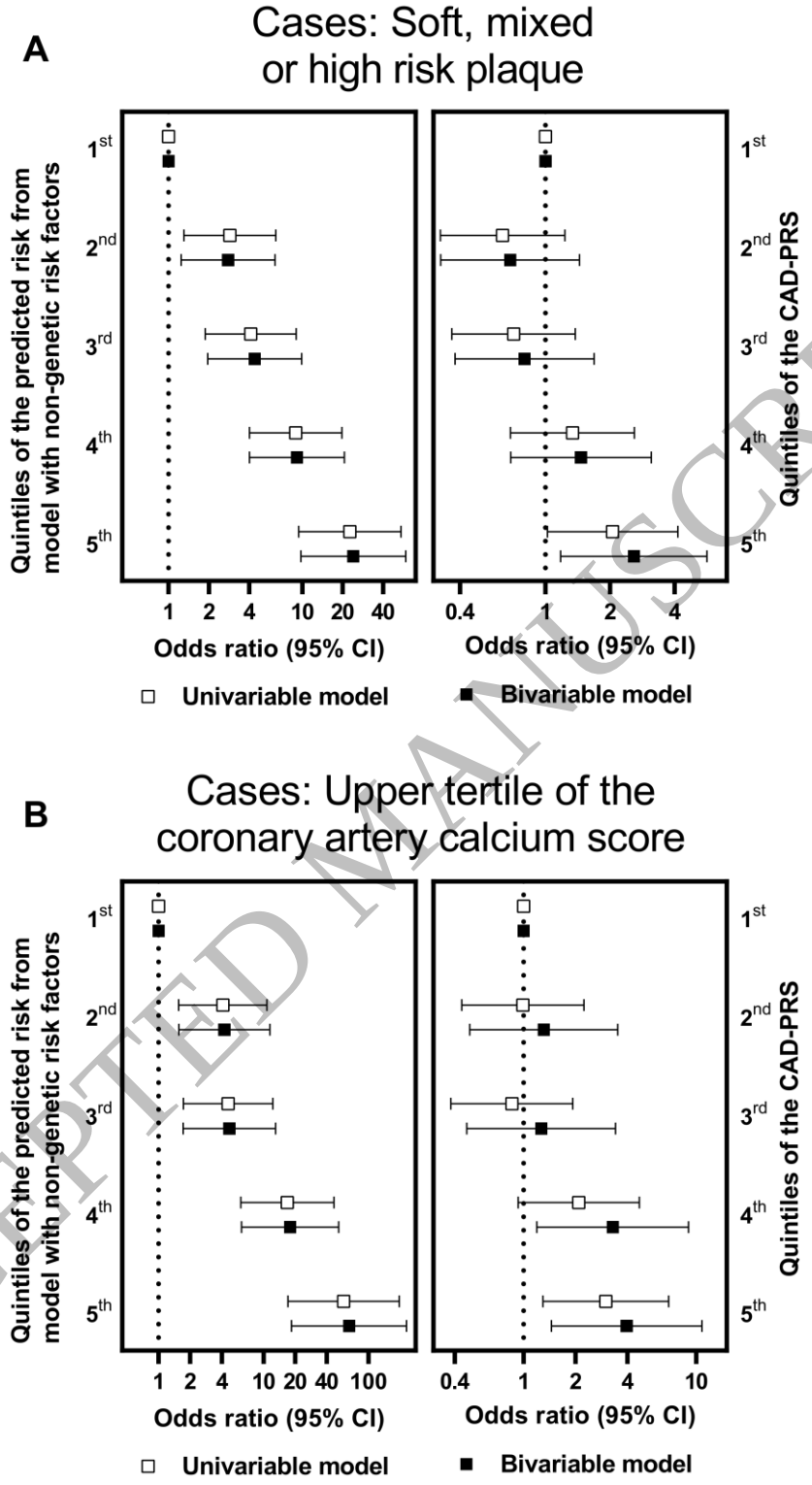
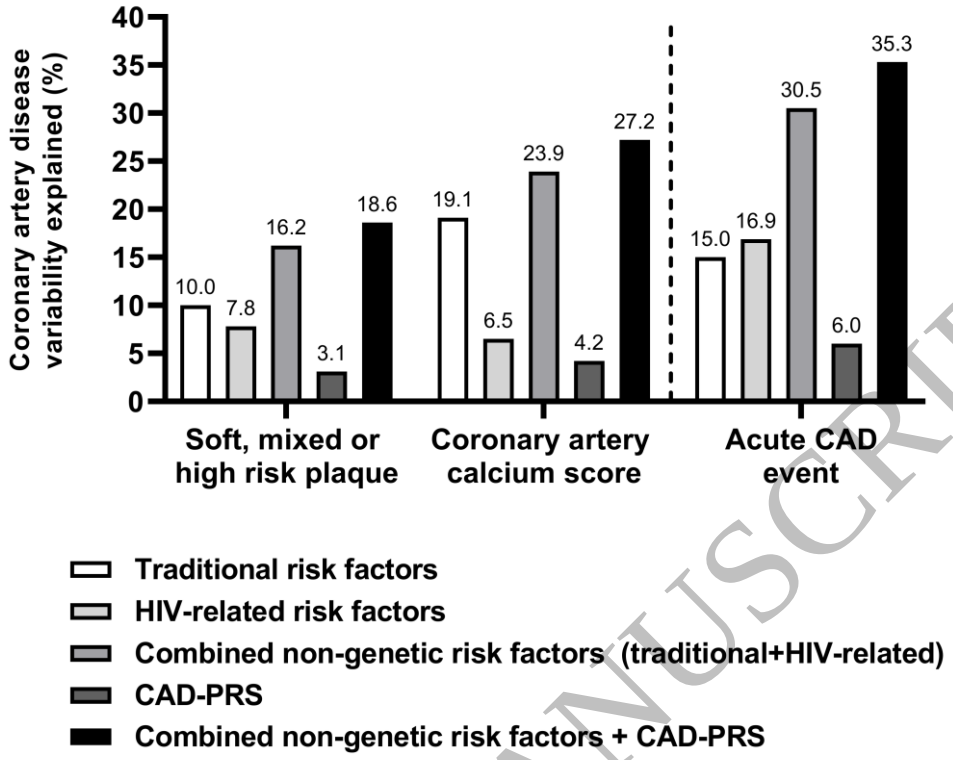


Figure 3
122x212 mm (x DPI)



1

2 **Figure 4**

3 **137x108 mm (x DPI)**