Gut microbiota-derived imidazole propionate predicts cardiometabolic risk in patients with coronary artery disease

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Abstract

Background and Aims: The gut microbiota is a modulator of cardiometabolic disease. Circulating imidazole propionate (ImP) is a microbiota-derived proatherogenic amino acid metabolite modulating the inflammatory response of myeloid cells, endothelial function and glucose metabolism. This study examined the prognostic value of ImP in patients with coronary artery disease (CAD).

Methods: Circulating ImP levels were measured in independent prospective cohorts of patients with acute coronary syndrome (ACS, Switzerland *n*=4937, Germany *n*=1497) and chronic coronary syndrome (CCS, Germany *n*=701). Major adverse cardiovascular events (MACE), defined as the first occurrence of a composite of death, nonfatal myocardial infarction, or nonfatal stroke after admission, were the primary endpoint. Cox models, accounting for established risk factors including the gut-derived cardiovascular risk factor trimethylamine N-oxide (TMAO), were used to evaluate the predictive value of ImP.

Results: Circulating ImP was associated with more advanced CAD and with cardiometabolic characteristics including diabetes and elevated high-sensitivity C-reactive protein. High ImP was an independent predictor of MACE (Swiss ACS cohort: hazard ratio [HR] per log2 increase 1.22, 95% confidence interval [CI] 1.10–1.35, *P*<0.001; German ACS cohort: HR 2.34, 95% CI 1.46–3.76, *P*<0.001; German CCS cohort: HR 1.32, 95% CI 1.13–1.53, *P*<0.001) and of mortality (Swiss ACS cohort: HR 1.34, 95% CI 1.17–1.54, *P*<0.001; German ACS cohort: HR 2.38, 95% CI 1.48–3.82, *P*<0.001; German CCS cohort: HR 1.50, 95% CI 1.14–1.98, *P*=0.004) after adjustment for established risk factors. ImP provided predictive value beyond TMAO (Swiss ACS cohort: HR 1.30, 95% CI 1.05–1.61, *P*=0.014; German CCS cohort: HR 1.31, 95% CI 1.11–1.53, *P*=0.001).

Conclusions: Gut microbiota-derived ImP predicted MACE in patients with CAD independently of traditional risk factors and holds promise as a therapeutic target. ImP may refine risk stratification for personalised secondary prevention strategies.

Keywords: Imidazole Propionate – Gut Microbiota – Cardiometabolic Risk – Coronary Artery Disease

Introduction

Despite substantial advances in the control of traditional drivers of atherosclerosis, coronary artery disease (CAD) remains the leading cause of death in adults worldwide.[1,2] With the evolution of risk factor profiles, attention has shifted increasingly to non-traditional cardiovascular risk factors.[3,4]

Imidazole propionate (ImP) is a gut microbiota-derived proatherogenic histidine metabolite[5-7] that impairs endothelial function[8] and glucose metabolism.[5,9] At a molecular level, ImP induces inflammatory activation of myeloid and endothelial cells, hampers endothelial repair, and promotes atherogenesis. Circulating levels of ImP are primarily determined by the composition of the gut microbiota.[5,6] Indeed, ImP is microbially produced by gut bacteria that are abundant in patients with type 2 diabetes[9,10] and in individuals with subclinical coronary atherosclerosis.[11]

ImP may thus constitute a novel therapeutic target for modulating cardiometabolic risk[12] and holds potential to refine cardiovascular risk stratification. Clinical studies suggest that circulating ImP is associated with systemic inflammation[10], diabetes[10], heart failure,[13] and subclinical atherosclerosis in asymptomatic volunteers.[7] A recent study[8] reported elevated ImP levels in subjects with CAD yet extensive clinical investigations are lacking.

Here, we aim to assess the relationship between ImP and clinical outcomes in patients with CAD including acute coronary syndromes (ACS) and chronic coronary syndromes (CCS).

Methods

Study design and participants

We aimed to encompass the full spectrum of CAD including patients presenting with acute and stable clinical conditions. We used prospective ACS and CCS cohorts from Switzerland and Germany (***Supplementary data online, Figures S1 and S2***).

In the investigator-initiated prospective multicentre Swiss ACS cohort (SPUM-ACS Biomarker Study, ClinicalTrials.gov Identifier: NCT01000701), a total of 4787 patients with ACS were recruited from December 8, 2009 until December 31, 2017.[14-23] Diagnoses of ACS were independently confirmed by expert cardiologists at local study facilities.[14,17] Participants received guideline-directed therapy regimens, as reported previously.[17] To investigate the coronary microcirculation, we analysed patients with ACS, enrolled in the Controlled Level EVERolimus in Acute Coronary Syndromes (CLEVER-ACS; ClinicalTrials.gov Identifier: NCT01529554) study in Switzerland from November 26, 2014 to October 29, 2021 undergoing cardiac magnetic resonance (CMR) imaging at 30-day follow-up (Swiss CMR cohort; $n = 60$; 8 of whom were co-enrolled in SPUM-ACS).

The prospective German ACS cohort (Heidelberg II-ACS study) includes 1428 patients with ACS presenting to Heidelberg University Hospital, Heidelberg, Germany between September 14, 2010, and July 21, 2014. Patients in this cohort were analysed using a case-cohort design, permitting unbiased assessment of exposure–outcome relationships, while reducing measurement costs. The design of case-cohort analyses has been reported previously[24,25] and a detailed description is provided in the ***Suppl. material***. In brief, we selected a random sample of patients (i.e., the subcohort) from the pool of eligible participants. We then also selected all individuals

with incident events who were not part of the subcohort. The final selection of patients (n = 250) consists of the subcohort and participants with incident events outside the subcohort.

The prospective German CCS cohort (LipidCardio Study, German Clinical Trial Register Identifier: DRKS00020915) included 701 consecutive patients with CSS undergoing elective diagnostic cardiac catheterisation at the Department of Cardiology, Campus Benjamin Franklin, Charité-Universitätsmedizin, Berlin, Germany between October 27, 2016 and March 8, 2018. Chronic coronary syndrome diagnoses were confirmed by angiographic evidence of obstructive CAD (≥50% stenosis).

The cohort profiles and detailed inclusion and exclusion criteria of the involved cohorts have been reported previously[26] and are summarised in the ***Suppl. material***.

Imidazole propionate measurements

Blood samples were obtained at the time of presentation without a dedicated fasting interval and stored at -80°C. ImP and its precursor molecules, histidine and trans-urocanate, were measured centrally using ultra-high performance liquid chromatography coupled to tandem mass spectrometry. Samples were prepared and analysed at the University of Gothenburg, Gothenburg, Sweden, using an established method with minor modifications.[10] In brief, 25 µL of sample volume were extracted with 6 volumes of acetonitrile containing internal standards (100 nmol/L ImP-$^{13}C_3$ and urocanate-$^{13}C_3$, AstraZeneca, Cambridge, United Kingdom and 1 µmol/L L-histidine D_5, $^{15}N_3$, Cambridge Isotope Laboratories, Inc. Andover, MA, USA) before drying under a nitrogen stream. The samples were then reconstituted in 5% HCl (37%) in 1-butanol for n-butyl ester derivatization at 70°C for 40 min dried and finally reconstituted in 150 µL water:acetonitrile (9:1). ImP, urocanate, and histidine levels were quantified using multiple reaction monitoring of the transitions 197.2/81.2, 195.2/93.0, 213.2/110.1 and internal standards of the transitions

200.2/82.0, 198.2/95.0 and 220.3/118.1. Investigators performing the measurements were blinded to sample allocation and patient data other than the deidentified barcode label.

Cardiac magnetic resonance imaging

In Switzerland, patients with ACS included in the CLEVER-ACS study underwent CMR imaging at 30-day follow-up according to a pre-specified study protocol. In brief, within 24 h after onset of chest pain, patients with proximal occlusion of a coronary artery underwent primary percutaneous coronary intervention (PCI) with placement of a drug-eluting stent in the culprit lesion.[27] The CMR imaging protocol at 30-day follow-up included T1- and T2-weighted sequences for tissue characterisation and calculation of ventricular volumes, function, and mass.[27] Fifteen minutes after administration of a gadolinium-chelate (0.20 mmol/kg of body weight), an inversion recovery fast-gradient echo imaging sequence was used to visualise scar tissue.[27] The extent of coronary microvascular obstruction was quantified by contouring the dark core areas manually in the core of the necrotic zone.[27] Imaging data analysis was performed in blinded fashion by expert physicians in the CMR Core Laboratory at the University Hospital Zurich, Zurich, Switzerland, employing dedicated heart imaging software (GTVolume, Gyrotools Ltd).

Study endpoints, follow-up, and outcome adjudication

Major adverse cardiovascular events (MACE), defined as the first occurrence of a composite of death, nonfatal myocardial infarction, or nonfatal stroke after admission, were the primary endpoint. Mortality was analysed as an additional endpoint. An event horizon of 1 year (365 days) for the ACS cohorts and of 3 years (1095 days) for the German CCS cohort was used.

In Switzerland, patients with ACS were followed up at hospital discharge, at 30 days (via phone call) and at 1 year (clinical visit). Baseline and event data were collected by study personnel at the respective study centres via a centralised web-based data entry system

(CARDIOBASE, Clinical Trial Unit and Department of Cardiology, University Hospital Bern, Bern, Switzerland, and Webspirit Systems GmbH, Ulm, Germany).[14] Events were assessed based on pre-specified adjudication forms by an independent clinical endpoint committee composed of three certified experienced cardiologists, who were blinded to patients' baseline characteristics .[14] At each study site, a committee of expert cardiologists supervised data collection. In the German ACS cohort, patients were followed up via telephone call and/or a questionnaire forwarded by post or e-mail, as previously described.[28] All deaths (retrieved via local resident registry records) and nonfatal adverse cardiovascular events were recorded.[28] In the German CCS cohort, patients were followed up via phone call at 3 years after study enrolment[26] to assess nonfatal adverse cardiovascular events.[26] Mortality was retrieved from the obligatory registry office.[26]

Statistical analyses

Continuous variables are presented as median and interquartile range (IQR).[14] Categorical data are reported as counts and valid percentages.[14] Continuous variables were compared with the Mann-Whitney U test for comparisons across two groups and the Kruskal-Wallis test for comparisons across three or more groups. Trends across independent groups were tested using the Jonckheere-Terpstra test. We used partial Spearman rank-correlation to examine the association between pairs of continuous variables while adjusting for additional variables.[29]

The association of ImP with cardiometabolic characteristics was examined in the pooled CAD patient population (data from the Swiss ACS cohort and the German CCS cohort) to increase the number of patients in the analyses, as well as stratified by ACS and CCS status. Given the different clinical implications, risk modelling was stratified according to presentation with ACS and presentation with CCS. The association of circulating ImP with clinical outcomes was studied in complete case Cox proportional hazards regression models,[17,28,30] using log 2-

transformed ImP levels[14,31,32], i.e., relative effect estimates correspond to a doubling in ImP levels. In addition, patients were divided according to ImP quartile (Q) and biomarker levels analysed on a categorical scale (***Supplementary data online, Table S20***).[10,31] Quartile cut-offs were derived from the largest cohort and applied to all cohorts (Q1: 9.4 nM; Q2: 9.4–14.3 nM; Q3: 14.3–24.4 nM; Q4: > 24.4 nM; ***Supplementary data online***). In a sensitivity analysis, cohort-specific ImP cut-offs were explored (***Supplementary data online, Table S19***).

Cumulative incidence curves were plotted and differences between curves were examined using log-rank statistics. We constructed smoothed hazard ratio (HR) plots with three knots across the ImP distribution. Multivariable analyses were conducted using a stepwise approach adjusting for predefined covariables, based on sample size and clinical considerations (***Supplementary data online***). ImP levels were analysed in (i) crude, (ii) sex-, age- and diabetes-adjusted, and (iii) fully adjusted models. In the fully adjusted model, covariables included age, sex, diabetes, high-sensitivity troponin T (hs-TnT), high-sensitivity C-reactive protein (hs-CRP), revascularisation, ACS type (if applicable), body mass index and estimated glomerular filtration rate. Analyses on mortality in patients with ACS were additionally adjusted for the GRACE 2.0 score for 1-year mortality.[33] We further evaluated the predictive value of circulating ImP after additionally adjusting for its precursor molecules histidine and urocanate (***Supplementary data online, Tables S15 and S16***). Additionally, we accounted for ethnicity (only available in German CCS cohort), lipoprotein(a) [Lp(a)] levels, history of chronic liver disease, and leukocyte count in the Swiss ACS and in the German CCS cohorts (***Supplementary data online, Tables S15 and S16***). The proportional hazards assumption was checked in the fully adjusted model using Schoenfeld residuals (***Supplementary data online***). In a subgroup of patients from the Swiss ACS cohort and the German CCS cohort with available TMAO levels,[13,34] the incremental predictive value of ImP beyond TMAO was assessed by additionally adjusting for TMAO. Moreover, we analysed joint

groups of high (≥ Q4 cut-off) *vs.* low (< Q4 cut-off) levels of ImP and TMAO yielding four strata: Low ImP/Low TMAO, Low ImP/High TMAO, High ImP/Low TMAO, and High ImP/High TMAO. Patients with CMR imaging at 30-day follow-up were stratified into two groups according to baseline ImP levels (i.e., Q4 vs. Q1–3) and coronary microvascular obstruction was compared between groups using analysis of covariance adjusted for age, sex, diabetes, hypertension, low-density lipoprotein cholesterol, glucose, and preprocedural coronary Thrombolysis in Myocardial Infarction (TIMI) flow grade. We conducted several subgroup analyses to assess the consistency of the results with respect to different population characteristics including sex, age, diabetes, proton pump inhibitor use, and dietary habits (only available in German CCS cohort). In addition, rehospitalisation rates were examined in an exploratory analysis (***Supplementary data online, Figure S14***). P-values and confidence intervals (CI) are two-sided. The study was conducted in accordance with the principles of the STROBE statement.[35] Statistical analyses were performed with R software version 4.3 or later and Stata version 16.1 or later. A detailed description of the statistical analyses is provided in the ***Supplementary data online***.

Results

Circulating imidazole propionate is linked to the extent of coronary artery disease

A total of 7066 patients were enrolled in Switzerland (ACS *n* = 4937*)* and Germany (ACS *n* = 1428, CCS *n*= 701) (***Supplementary data online, Figures S1***). Baseline and treatment characteristics are summarised in ***Tables 1 and 2*** and in the ***Supplementary data online*** (***Tables S1-S12***). Circulating ImP levels were significantly elevated in patients with CAD (***Figure 1A***). Moreover, high ImP was associated with more advanced atherosclerotic disease, as determined

by a higher number of affected coronary arteries, lower left ventricular ejection fraction, and more severe symptoms of heart failure (***Figure 1A***). These findings were consistent across clinical presentation with ACS and CCS (***Supplementary data online, Figures S7 and S9***) but absent or less pronounced for the precursor molecules histidine and urocanate (***Supplementary data online, Figures S6, S8, S10, Table S13***). ImP levels were comparable across different times of the day (***Supplementary data online, Figures S3 and S4***).

High imidazole propionate associates with cardiometabolic characteristics

Given the established effects of ImP on glucose metabolism, we studied its relationship to cardiometabolic characteristics of patients with CAD. High ImP was associated with high body mass index, systemic inflammation, impaired glucose metabolism, and hypertension (***Figure 1B; Supplementary data online, Figure S11***). These results were similarly observed in patients with ACS and in patients with CCS (***Supplementary data online, Figures S7 and S9***) but were absent or less pronounced for the precursor molecules histidine and urocanate (***Supplementary data online, Figures S8 and S10***).

High imidazole propionate is associated with coronary microvascular obstruction

Considering the intricate involvement of ImP in central processes of myocardial microcirculation in preclinical experiments including endothelial dysfunction, regulation of endothelium-dependent vascular tone, leucocyte function and adhaesion, and osmotic regulation, we performed an exploratory analysis to examine a potential association of ImP with CMR-assessed microvascular obstruction. High ImP levels were associated with increased coronary microvascular obstruction at 30 days (***Figure 2, Supplementary data online, Table S14***). These results remained consistent on multivariable adjustment but were not observed for the precursor molecules histidine and urocanate (***Supplementary data online, Figure S12***).

High imidazole propionate predicts cardiovascular outcomes beyond established risk factors and GRACE 2.0

Across all cohorts, there were a total of 595 MACE and 270 deaths. Cumulative incidence curves showed a substantial increase in MACE and mortality in patients with high ImP with HR plots indicating a dose-response relationship (***Figure 3***). After multivariable adjustment, high ImP levels were associated with increased risk of MACE (Swiss ACS cohort: fully adjusted HR per log2 increase, 1.22, 95% CI 1.10–1.35, $P < 0.001$, fully adjusted HR Q4 vs. Q1–3, 1.33, 95% CI 1.03–1.72, $P = 0.032$; German ACS cohort: fully adjusted HR per log2 increase, 2.34, 95% CI 1.46–3.76 $P < 0.001$, fully adjusted HR Q4 vs. Q1–3, 7.97, 95% CI 2.70–23.52, $P < 0.001$; German CCS cohort: fully adjusted HR per log2 increase, 1.32, 95% CI 1.13–1.53, $P < 0.001$, fully adjusted HR Q4 vs. Q1–3, 1.83 95% CI 1.22–2.74, $P = 0.004$; ***Supplementary data online, Table S15*****).** Likewise, high plasma ImP emerged as a strong independent predictor of all-cause mortality (Swiss ACS cohort: fully adjusted + GRACE 2.0 HR per log2 increase, 1.24, 95% CI 1.06–1.46, $P = 0.007$, fully adjusted HR + GRACE 2.0 Q4 vs. Q1–3, 1.47, 95% CI 0.96–2.26, $P = 0.076$; German ACS cohort: fully adjusted + GRACE 2.0 HR per log2 increase, 2.32, 95% CI 1.46–3.41, $P < 0.001$, fully adjusted HR + GRACE 2.0 Q4 vs. Q1–3, 4.84, 95% CI 1.42–16.47, $P = 0.012$; German CCS cohort: fully adjusted HR per log2 increase, 1.50, 95% CI 1.14–1.98, $P = 0.004$, fully adjusted HR Q4 vs. Q1–3, 2.01, 95% CI 0.96–4.19, $P = 0.063$; ***Supplementary data online, Table S16***). These findings were consistent across patient subgroups (***Supplementary data online, Tables S17 and S18, Figure S5***). Similar results were obtained when additionally adjusting for Lp(a), liver disease, leukocyte count and for the precursor molecules of ImP (***Supplementary data online, Tables S15 and S16***).

Imidazole propionate adds to microbiota-related risk beyond TMAO

ImP provided incremental predictive value for MACE beyond TMAO (Swiss ACS cohort: fully adjusted + TMAO HR per log2 increase: 1.30, 95% CI 1.05–1.61, *P* = 0.014; German CCS cohort: fully adjusted + TMAO HR per log2 increase: 1.31, 95% CI 1.12 – 1.53, *P* = 0.001; ***Supplementary data online, Table S21***). When stratifying patients into joint groups according to both ImP and TMAO level, individuals with high levels of both markers exhibited excessive risk of MACE (***Figure 4A***). After multivariable adjustment, joint elevation of ImP and TMAO conferred a more than 2-fold increased risk of MACE in patients with ACS and CCS (Swiss ACS cohort: fully adjusted HR: 2.28, 95% CI 1.14–4.56, *P* = 0.020; German CCS cohort: fully adjusted HR: 4.30, 95% CI 2.53–7.32, P < 0.001; ***Figure 4B***). Circulating ImP ranked among the strongest predictors of MACE (***Figure 4C***). These findings were similarly observed for mortality (***Supplementary data online, Figure S13 and Table S22***).

Discussion

Here, we show for the first time, that the gut microbiota-derived amino-acid metabolite ImP is linked to cardiometabolic characteristics and independently predicts adverse outcomes in patients with ACS and CCS beyond established risk markers (Structured Graphical Abstract).

Despite advancements in the management of patients with CAD over the past decades, there is considerable residual cardiovascular risk attributed to non-traditional risk factors such as inflammation, disturbed sleep, air pollution, physical inactivity, environmental stress, and microbial dysbiosis. ImP is produced from urocanate, a metabolite of the essential amino-acid histidine, by the microbial enzyme urocanate reductase.[6] Previous studies have shown that circulating ImP levels are primarily determined by the composition and metabolism of gut microbiota, rather than histidine intake.[10]

At the cellular level, ImP induces imidazoline-1 receptor (I1R) signalling[7] and inhibits insulin-receptor signalling,[5,9] in turn leading to activation of inflammation-associated pathways in macrophages, fibroblasts and endothelial cells,[8] impaired endothelial cell regeneration after vascular injury, reduced endothelium-dependent arterial relaxation, impaired glucose metabolism[5,6] and increased atherogenesis.[8] Notably, ImP promotes the expression of leucocyte adhesion molecules for leucocyte-endothelial cell interactions leading to an inflammatory milieu at the endothelial interface and increased immune cell infiltration, a hallmark of atherosclerosis.[3] [4] The atherogenic effects of ImP involve different molecular pathways compared to TMAO.[36-38]

In line with previous mechanistic studies, our results demonstrate that ImP is associated with signs of cardiometabolic dysregulation and more extensive coronary atherosclerosis in patients with CAD. An exploratory analysis of a small cohort of patients with ACS undergoing primary PCI followed up with CMR, indicated that patients with high ImP also had more extensive coronary microvascular obstruction. These findings align well with preclinical studies showing that ImP can influence key processes related to the microcirculation including endothelial cell regeneration, regulation of the endothelium-dependent vascular tone,[8] leucocyte adhesion, and osmotic regulation. This suggests the possibility that ImP may contribute to the individual susceptibility to microvascular dysfunction in response to ischaemia-reperfusion injury,[39] as encountered in patients with ACS undergoing primary PCI. In contrast, no such associations were observed for the precursor molecules histidine and urocanate.

Our study affirms that interindividual heterogeneity in the gut microbiome has important implications for clinical risk stratification. Circulating ImP was strongly associated with both MACE and all-cause mortality. These findings were consistent across patients with ACS and CCS, geographies, subgroups, and follow-up periods. Notably, high ImP conferred incremental

gut microbiota-related risk above and beyond TMAO, an established gut-derived cardiovascular risk factor. Patients with high levels of both metabolites had more than double the risk of MACE after accounting for established risk factors. Indeed, elevation of both ImP and TMAO may characterise a novel gut microbiota-related signature associated with increased cardiovascular risk. Compared to established risk factors, ImP ranked among the strongest predictors of MACE holding promise to refine risk stratification for personalised secondary prevention strategies. Moreover, the absence of these findings for precursor molecules of ImP supports a potential causal contribution of ImP to adverse outcomes. Currently, no cut-off value for elevated ImP is established in the general population or in patients with CAD. In this study, we observed particularly high risk of adverse events in patients in the highest ImP quartile (i.e., > 24 nM).

The biological actions of ImP and its complex interplay with cardiometabolic disease are the subject of ongoing investigations. While more studies are needed to decipher its precise role in atherogenesis and identify thresholds for optimal risk stratification, future treatment approaches[8] may target microbial ImP production by inhibiting the microbial enzyme urocanate reductase to modulate cardiometabolic risk.[12] The management of traditional cardiovascular risk factors has significantly improved outcomes in the past decades, yet the current drug armamentarium lacks strategies directed at modulating the microbiome and its metabolites. The potential integration of the gut-heart axis[40] in future treatment approaches holds promise for more personalised and effective secondary prevention.

In conclusion, our results identify circulating ImP as a novel biomarker of cardiometabolic risk in patients with CAD and a potential therapeutic target for secondary prevention.

Strengths and limitations

This study has several strengths. First, the rigorous multinational design including external validation of the results in independent, well-characterised cohorts from different countries confirmed the external validity of the findings and accounts for regional differences in treatment practices, patient characteristics, and gut microbiota composition. Second, the SPUM-ACS Biomarker Study comprises one of the largest and best characterised cohorts of patients with ACS worldwide. Its prospective multicentre design together with a contemporary patient population undergoing guideline-based treatment approaches grants for high quality of the collected data in SPUM-ACS. Moreover, the central assessment of established biomarkers including hs-TnT and hs-CRP in the core laboratory at the University Hospital Zurich, Zurich, Switzerland, using a validated assay minimises the impact of analytical variability. Third, assessment bias is prevented by external adjudication of clinical outcomes during follow-up by an independent clinical event adjudication committee. Further, centralised blinded measurement of ImP according to established protocols enhancing the technical validity of the measurements and the comparability of the results. Finally, we also measured the precursor molecules of ImP to inform hypothesis generation regarding potential causal relationships.

Our study also has limitations. First, there was a limited number of events at 1 year in the validation cohort. However, the results were consistent across endpoints and at 3 years considering a higher number of events. Second, for external validation of our results in patients with ACS, we did not measure ImP in all patients of the German ACS cohort due to the use of a case-cohort design. Nonetheless, the results of the case-cohort analysis are statistically robust and provide valid approximations of full-cohort analyses. Third, a limited number of female patients (less than a third across all cohorts) was included, and ethnicity was only available in the German

CCS cohort. Of note, we considered these variables in specific subgroup analyses and adjusted models. Nevertheless, further studies are required to validate our findings in more ethnically diverse cohorts, accounting for differences in dietary patterns across geographies and ethnicities. In addition, due to limited availability of TMAO, analyses including this variable could only be performed in a subcohort of patients. These analyses should therefore be interpreted with great caution yet are consistent with the main results. Further, CMR data were only available in a small subset of patients with ACS, precluding any definitive conclusions and should be interpreted as hypothesis-generating. Next, the pooled analyses in ***Figure 1*** do not account for cohort-specific data properties. However, similar results were observed in the individual cohorts. Finally, drug dosages were not routinely recorded, and there was limited availability of gastrointestinal, hepatic and host immune system-related parameters in the included cohorts.

Conclusions

Circulating levels of ImP are linked to cardiometabolic traits and independently predict adverse outcomes in patients with ACS and CCS. ImP represents a novel biomarker of cardiometabolic risk and a potential therapeutic target in patients with CAD. Our findings highlight the increasing importance of non-traditional risk factors and may open new avenues for targeting the gut-heart axis to address residual cardiovascular risk.

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Author contributions

F.A.W., A.H., U.L., F.B., and T.F.L. lead the conceptualisation of the study. Data curation was carried out by F.A.W., P.W., F.K., K.R.B., B.E.S., and A.H.. F.A.W. and P.W. were involved in the formal analysis of the data. F.A.W., P.W., and M.A.S were involved in the visualisation of the results. F.A.W. wrote the original draft of the manuscript. F.A.W., A.H., U.L., F.B., and T.F.L were primarily involved in funding acquisition. F.A.W. was primarily concerned with the administration and coordination of the project. K.R.B. and F.B. supervised the measurements of gut microbiota-derived metabolites. A.v.E. supervised the biomarker measurements in the SPUM-ACS core laboratory. R.M. directed the analysis of the CMR images. T.F.L. supervised the patient recruitment in SPUM-ACS and CLEVER-ACS in Switzerland. A.H. and U.L. supervised the patient recruitment at Charité University Hospital, Germany. E.G. supervised the patient recruitment at University Hospital Heidelberg, Germany. All the authors vouch for the data and analyses reported. All authors revisited the work critically for important intellectual content, were involved in reviewing and editing of the manuscript, approved the submission for publication and agreed to be accountable for all aspects of the work.

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Legends

Figure 1: Circulating imidazole propionate is associated with extent of coronary artery disease and metabolic traits. Circulating imidazole propionate (ImP) levels according to extent of coronary artery disease (A) and metabolic traits (B). Data are represented as boxplots and correlation plots. Medians with interquartile ranges are displayed on top of each box. Spearman partial correlation coefficients and P-values were calculated using partial correlation adjusted for Model 1: age and sex, Model 2: Model 1 plus diabetes status, Model 3: Model 2 plus hypercholesterolaemia. False discovery rate (FDR)-adjusted using Benjamini–Hochberg correction $*P < 0.05$, $**P < 0.01$. Values in the group with 0 affected vessels refer to the reference population without coronary artery disease. Results are based on pooled data from the Swiss ACS cohort and the German CCS cohort. See also ***Supplementary data online, Table S13***.

Figure 2: Imidazole propionate associates with coronary microvascular obstruction. (A) Coronary microvascular obstruction (MVO) in patients undergoing primary percutaneous coronary intervention (PCI) according to ImP (Q1: 0–9.4 nM; Q2: 9.4–14.3 nM; Q3: 14.3–24.4 nM; Q4: > 24.4 nM). Bars are mean values. (B) Correlation of ImP and coronary MVO. Spearman partial correlation coefficients and P-values were calculated using partial correlations adjusted for Model 1: age, sex, and diabetes status, Model 2: Model 1 plus hypertension, low density lipoprotein cholesterol, and glucose, Model 3: Model 2 plus preprocedural coronary flow based on Thrombolysis in Myocardial Infarction (TIMI) classification and culprit vessel. False discovery rate (FDR)-adjusted using Benjamini–Hochberg correction $*P < 0.05$, $**P < 0.01$. Results in all figure panels are based on data from the Swiss CMR cohort. See also ***Supplementary data online, Table S14 and Figure S12***.

Figure 3: Imidazole propionate predicts cardiovascular outcomes in patients with acute and chronic coronary syndrome. (A) Cumulative event curves for major adverse cardiovascular events (MACE), defined as a composite of death, nonfatal myocardial infarction, and nonfatal stroke, of patients presenting with acute (left) and chronic (right) coronary syndrome stratified by circulating imidazole propionate (ImP) level (Q1: 0–9.4 nM; Q2: 9.4–14.3 nM; Q3: 14.3–24.4 nM; Q4: > 24.4 nM). (B) Restricted cubic spline plot showing the adjusted hazard ratio (HR) for MACE at 1 year and at 3 years according to ImP level in patients with acute (left; n=3966) and

chronic (right; n=385) coronary syndromes, respectively. The solid line indicates the predicted HR with the colour band signifying the corresponding 95% CI. Three knots were fixed at the 10th, 50th, and 90th percentile of the ImP distribution. Note that HRs are shown in log scale (left Y axis) and as untransformed values (right Y axis). The dashed horizontal line indicates the reference. Dashed vertical lines mark quartile cut-offs (Q1: 0–9.4 nM; Q2: 9.4–14.3 nM; Q3: 14.3–24.4 nM; Q4: > 24.4 nM). (C) Crude and adjusted HRs for MACE at 1 year and at 3 years in patients with acute (left) and chronic (right) coronary syndromes, respectively. Cox proportional hazards regression models were controlled for established risk factors and precursor molecules of ImP in a stepwise fashion. Squares indicate point estimates of the HR per log2 increase and line lengths equal corresponding 95% CIs. Plots in A) and B) are based on data from the Swiss ACS cohort and the German CCS cohort. Patient numbers and event counts for C) are summarised in the ***Supplementary data online, Table S15***.

Figure 4: Imidazole propionate predicts adverse cardiovascular outcomes beyond trimethylamine N-oxide.

(A) Cumulative event curves for major adverse cardiovascular events (MACE), defined as a composite of death, nonfatal myocardial infarction, and nonfatal stroke in patients presenting with acute coronary syndrome (ACS) and chronic coronary syndromes (CCS) according to joint groups of high (≥ 75th percentile; Q4: > 24.4 nM) *vs.* low (< 75th percentile; Q1-Q3: 0–24.4 nM) levels of imidazole propionate (ImP) and trimethylamine N-oxide (TMAO). (B) Multivariable-adjusted hazard ratio (HR) and 95% confidence interval (CI) for MACE at 1 year in patients with ACS and at 3 years in patients with CCS, according to ImP quartile (Q1: 0–9.4 nM; Q2: 9.4–14.3 nM; Q3: 14.3–24.4 nM; Q4: > 24.4 nM). Squares indicate point estimates and line lengths equal corresponding 95% CIs. (C) Feature importance for the prediction of MACE measured by partial Wald χ^2 minus the degrees of freedom. Results are based on 1281 patients from the Swiss ACS cohort and 385 patients from the German CCS cohort in the fully adjusted model.

1

	Swiss ACS cohort (*n* = 4318)	German ACS cohort (*n* = 1428)	German CCS cohort (*n* = 577)
Age (years)	63 (54–73)	68 (57–76)	76 (67–81)
Female	881/4318 (20.4%)	440/1428 (30.8%)	124/577 (21.5%)
BMI (kg/m²)	26.6 (24.2–29.4)	27.1 (25.0–30.5)*	26.9 (24.5–30.0)
Body surface area (m²)	1.9 (1.8–2.1)	..	2.0 (1.8–2.1)
Heart rate (bpm)	75 (66–86)	74 (65–85)	..
Systolic blood pressure (mm Hg)	128 (112–143)	150 (137–166)	134 (121–149)
Signs and symptoms of heart failure (ACS: Killip class, CCS: NYHA class)			
I	3682/4204 (87.6%)	1365/1428 (95.6%)	93/555 (16.8%)
II	343/4204 (8.2%)	50/1428 (3.5%)	294/555 (53.0%)
III	85/4204 (2.0%)	11/1428 (0.8%)	166/555 (29.9%)
IV	94/4204 (2.2%)	2/1428 (0.1%)	2/555 (0.4%)
Smoking	1649/4254 (38.8%)	449/1371 (32.8%)	98/577 (17.0%)
Medical history			
Diabetes	753/4318 (17.4%)	319/1414 (22.6%)	178/577 (30.8%)
Prediabetes[#]	630/2153 (29.3%)	194/670 (29.0%)	168/577 (29.1%)
Hypertension	2413/4316 (55.9%)	1099/1417 (77.6%)	477/577 (82.7%)
Hyperlipoproteinemia	2757/4317 (63.9%)	838/1369 (61.2%)	378/577 (65.5%)
Myocardial infarction	524/4310 (12.2%)	344/1402 (24.5%)	405/576 (70.3%)
Family history of CAD	1056/4259 (24.8%)	491/1352 (36.3%)	161/478 (33.7%)
Prior PCI	644/4317 (14.9%)	498/1400 (35.6%)	..
Prior CABG	169/4318 (3.9%)	157/1399 (11.2%)	62/577 (10.7%)
Cerebrovascular disease	160/4318 (3.7%)	..	74/577 (12.8%)
Peripheral artery disease	251/4318 (5.8%)	..	30/577 (5.2%)
Heart failure	54/4316 (1.3%)	294/1348 (21.8%)	..
Chronic liver disease	25/4318 (0.6%)	3/139 (2.2%)*	12/577 (2.1%)
Dialysis	21/4318 (0.5%)	..	..
Clinical chemistry and haematology			
Leukocyte count (G/L)	9.6 (7.4–12.2)	7.6 (5.6–13.3)*	7.4 (6.0–8.9)
hs-CRP (mg/L)	3 (1–7)	4 (2–12)	2 (1–7)
Total cholesterol (mg/dL)	190 (159–220)	189 (172–206)*	153 (129–187)
LDL-C (mg/dL)	120 (92–149)	91 (79–106)*	85 (65–115)
HDL-C (mg/dL)	44 (36–53)	47 (43–50)*	46 (38–57)
Lipoprotein (a) (mg/dL)	6.4 (2.5–21.8)	..	8.3 (2.0–34.9)
Triglycerides (mg/dL)	88 (60–136)	148 (109–185)*	121 (91–176)

HbA1c (%)	5.8 (5.5–6.3)	5.8 (5.5–6.3)	5.8 (5.5–6.3)
Glucose (mmol/L)	6.4 (5.6–7.9)	6.6 (5.7–8.2)	5.9 (5.2–7.7)
NT-proBNP (ng/L)	334 (108–1158)	586 (164–2440)	427 (134–1238)
hs-TnT (ng/L)	196 (59–642)	46 (9–220)	15 (9–30)
Creatinine (mg/dL)	0.9 (0.7–1.0)	0.9 (0.8–1.1)*	1.0 (0.8–1.2)
eGFR (mL/min/1.73m^2)	93 (77–103)	86 (76–96)	73 (59–90)
ImP metabolism			
ImP (nM)	14 (9–24)	13 (9–20)*	13 (8–26)
Urocanate (nM)	44 (30–66)	128 (81–203)*	47 (33–84)
Histidine (µM)	66 (57–76)	88 (68–103)*	62 (53–70)
Baseline medication			
Aspirin	1230/2779 (44.3%)	33/139 (23.7%)*	381/577 (66.0%)
P2Y12 inhibitor	321/1915 (16.8%)	..	192/577 (33.3%)
Beta-blocker	978/2779 (35.2%)	22/139 (15.8%)*	371/577 (64.3%)
ACE inhibitor/ARB	1475/2759 (53.5%)	100/139 (71.9%)*	447/577 (77.5%)
Vitamin K antagonist/DOAC	171/2777 (6.2%)	19/139 (13.7%)*	148/577 (25.6%)
Diuretic	674/2773 (24.3%)	50/139 (36.0%)*	247/577 (42.8%)
Insulin	214/2777 (7.7%)	35/139 (25.2%)*	57/577 (9.9%)
Oral antidiabetics	503/2777 (18.1%)	4/139 (2.9%)*	108/577 (18.7%)
Immunosuppressive drugs	119/2777 (4.3%)	..	..
PPI	643/2777 (23.2%)	32/139 (23.0%)*	247/577 (42.8%)

Table 1. Baseline characteristics of patients with acute or chronic coronary syndromes
Categorical data are shown as numbers and percentages (%). Continuous data are presented as median and interquartile range (IQR). ACE, angiotensin-converting enzyme; ACS, acute coronary syndrome; ARB, angiotensin II receptor blocker; BMI, body mass index; CABG, coronary artery bypass grafting; CAD, coronary artery disease; CCS, chronic coronary syndrome; DOAC, direct oral anticoagulant; eGFR, estimated glomerular filtration rate; HbA1c, hemoglobin A1c; HDL-C, high-density lipoprotein cholesterol; hs-CRP, high-sensitivity C-reactive protein; hs-TnT, high-sensitivity troponin T; ImP, imidazole propionate; LDL-C, low-density lipoprotein cholesterol; NT-proBNP, N-terminal pro B-type natriuretic peptide; NYHA, New York Heart Association; PCI, percutaneous coronary intervention; PPI, proton pump inhibitor; STEMI, ST-segment elevation myocardial infarction. *Values refer to the subcohort #defined as HbA1c of 5.7–6.4% in individuals without a history of diabetes.

	Swiss ACS cohort (*n* = 4318)	German ACS cohort (*n* = 1428)	German CCS cohort (*n* = 577)
Revascularization strategy during baseline visit			
PCI	3949/4318 (91.5%)	843/1283 (65.7%)	350/577 (60.7%)
CABG	127/4318 (2.9%)	..	..
Symptom-to-door time (minutes)	180 (105–420)	480 (120–2160)	..
No. of affected vessels†			
One	3371/4095 (82.3%)	209/1283 (16.3%)	135/577 (23.4%)
Two	563/4095 (13.7%)	237/1283 (18.5%)	194/577 (33.6%)
Three	161/4095 (3.9%)	724/1283 (56.4%)	248/577 (43.0%)
Left ventricular ejection fraction (%)			
> 50	1380/2725 (50.6%)	420/1361 (30.9%)	437/577 (75.7%)
50–40	858/2725 (31.5%)	418/1361 (30.7%)	81/577 (14.0%)
40–30	314/2725 (11.5%)	349/1361 (25.6%)	38/577 (6.6%)
< 30	173/2725 (6.3%)	174/1361 (12.8%)	21/577 (3.6%)
Discharge medication			
Aspirin	4233/4273 (99.1%)	1259/1384 (91.0%)	..
P2Y12 inhibitor	4070/4163 (97.8%)	989/1393 (71.0%)	..
Beta-blocker	3339/4269 (78.2%)	1227/1393 (88.1%)	..
ACE inhibitor/ARB	3749/4271 (87.8%)	1218/1393 (87.4%)	..
Vitamin K antagonist/DOAC	303/4318 (7.0%)	147/1391 (10.6%)	..
Diuretic	997/4272 (23.3%)	..	..
Insulin	277/4270 (6.5%)	108/1392 (7.8%)	..
Oral antidiabetics	503/4270 (11.8%)	175/1392 (12.6%)	..
Immunosuppressive drugs	112/4272 (2.6%)	..	..
PPI	1258/4272 (29.4%)	40/139 (15.8%)*	..
Outcomes§			
MACE	314/4318 (7.3%)	117/1428 (8.2%)	164/577 (28.42%)
Mortality	148/4318 (3.4%)	75/1428 (5.3%)	47/577 (8.15%)

Table 2. Treatment characteristics of patients with acute or chronic coronary syndromes

Categorical data are shown as numbers and percentages (%). Continuous data are presented as median and interquartile range (IQR). ACE, angiotensin-converting enzyme; ACS, acute coronary syndrome; ARB, angiotensin II receptor blocker; CABG, coronary artery bypass grafting; CCS, chronic coronary syndrome; DOAC, direct oral anticoagulant; MACE, major adverse cardiovascular events; PCI, percutaneous coronary intervention; PPI, proton pump inhibitor. †Refers to vessels with significant coronary stenosis in Heidelberg II-ACS and LipidCardio. §Refers to 1-year outcomes for ACS and to 3-year outcomes for CCS included in the analyses. *Values refer to the subcohort

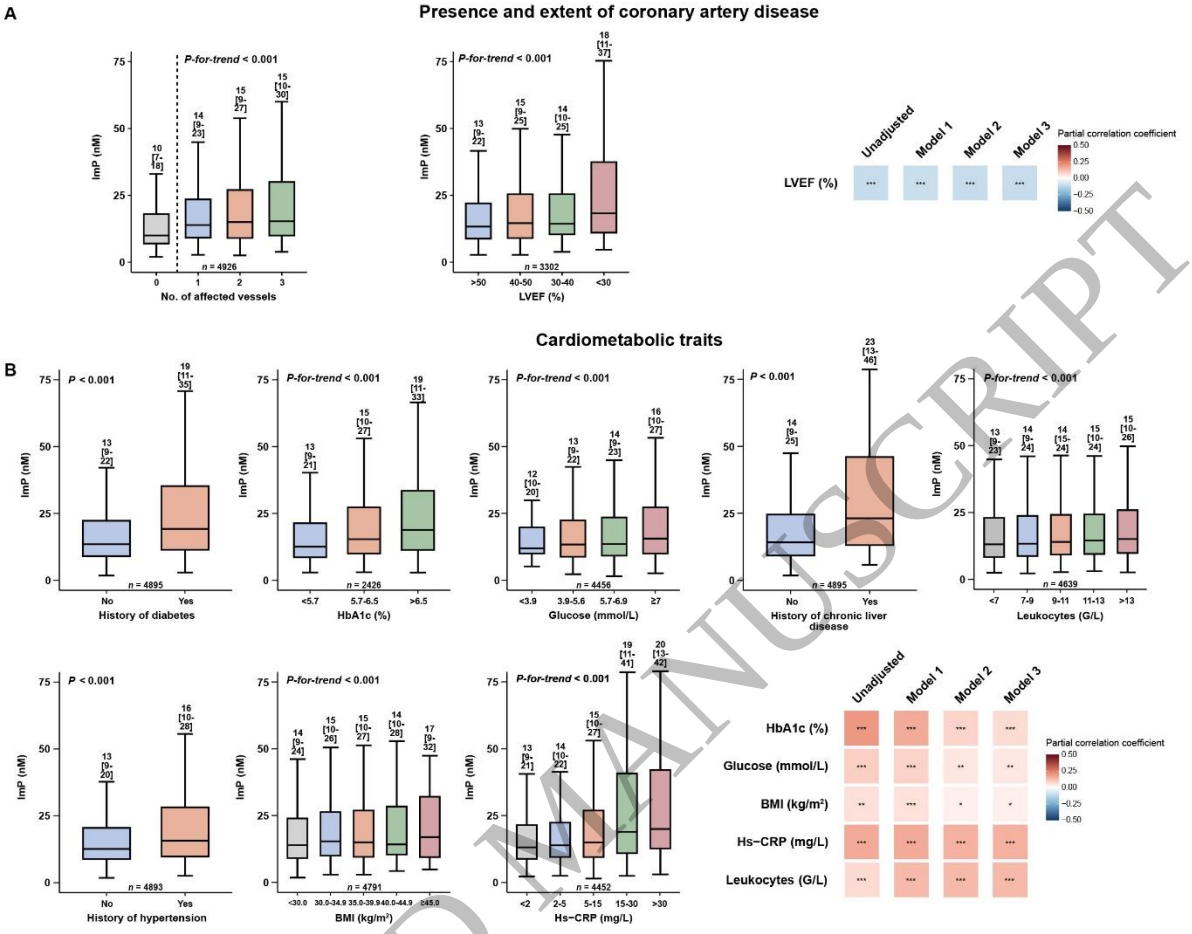

Figure 1
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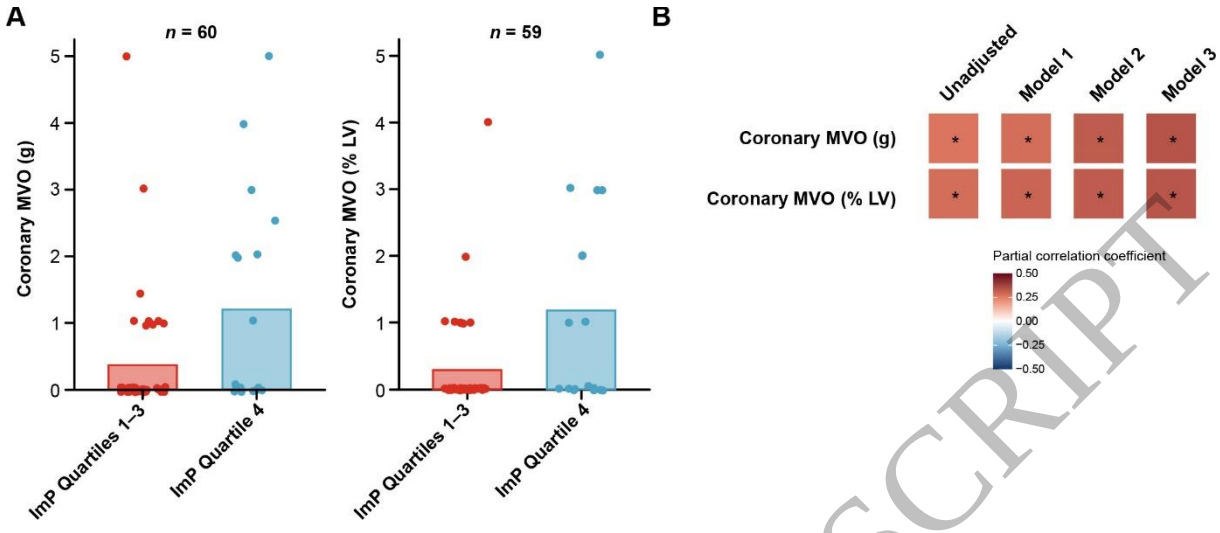

Figure 2
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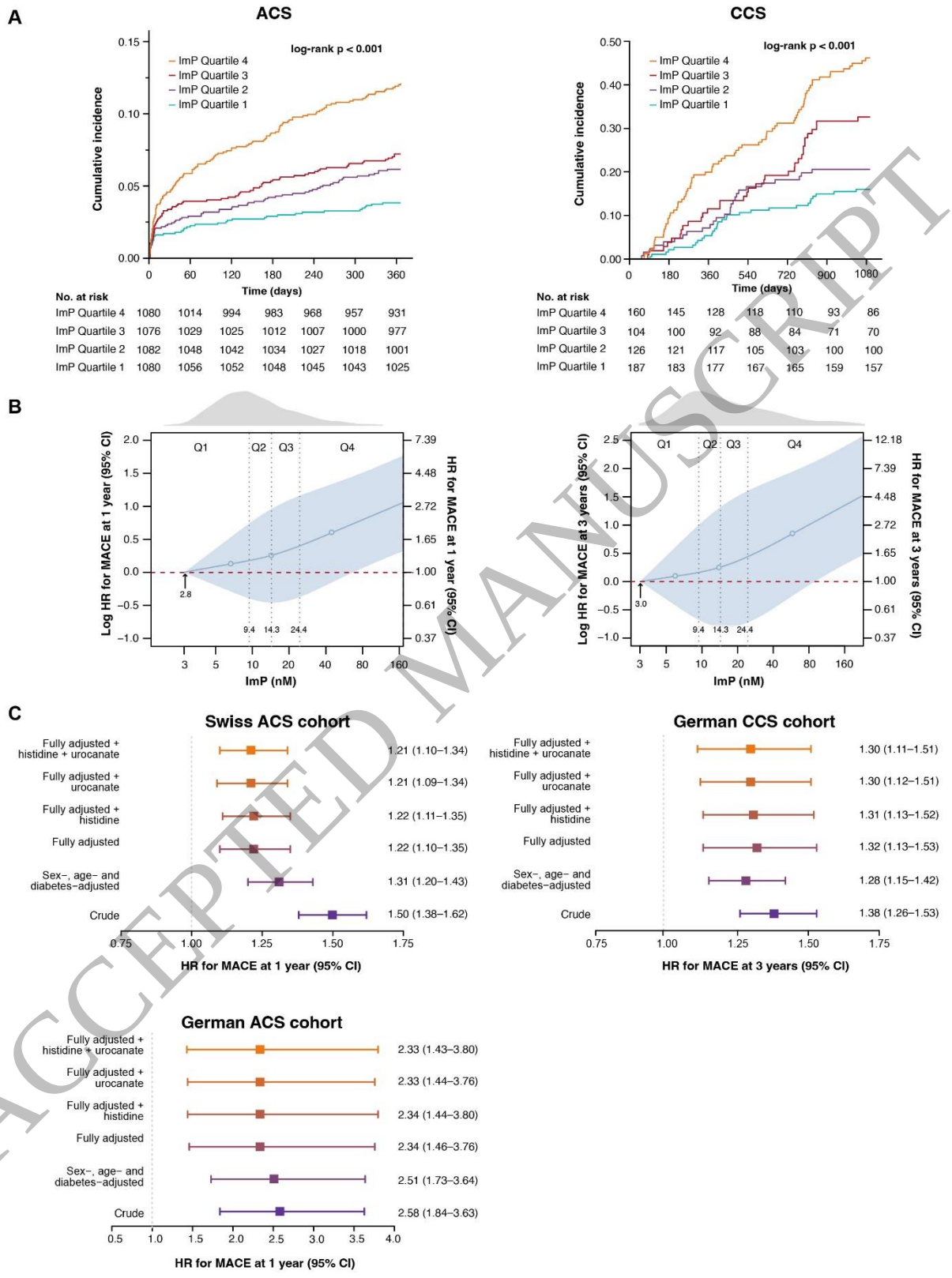

Figure 3
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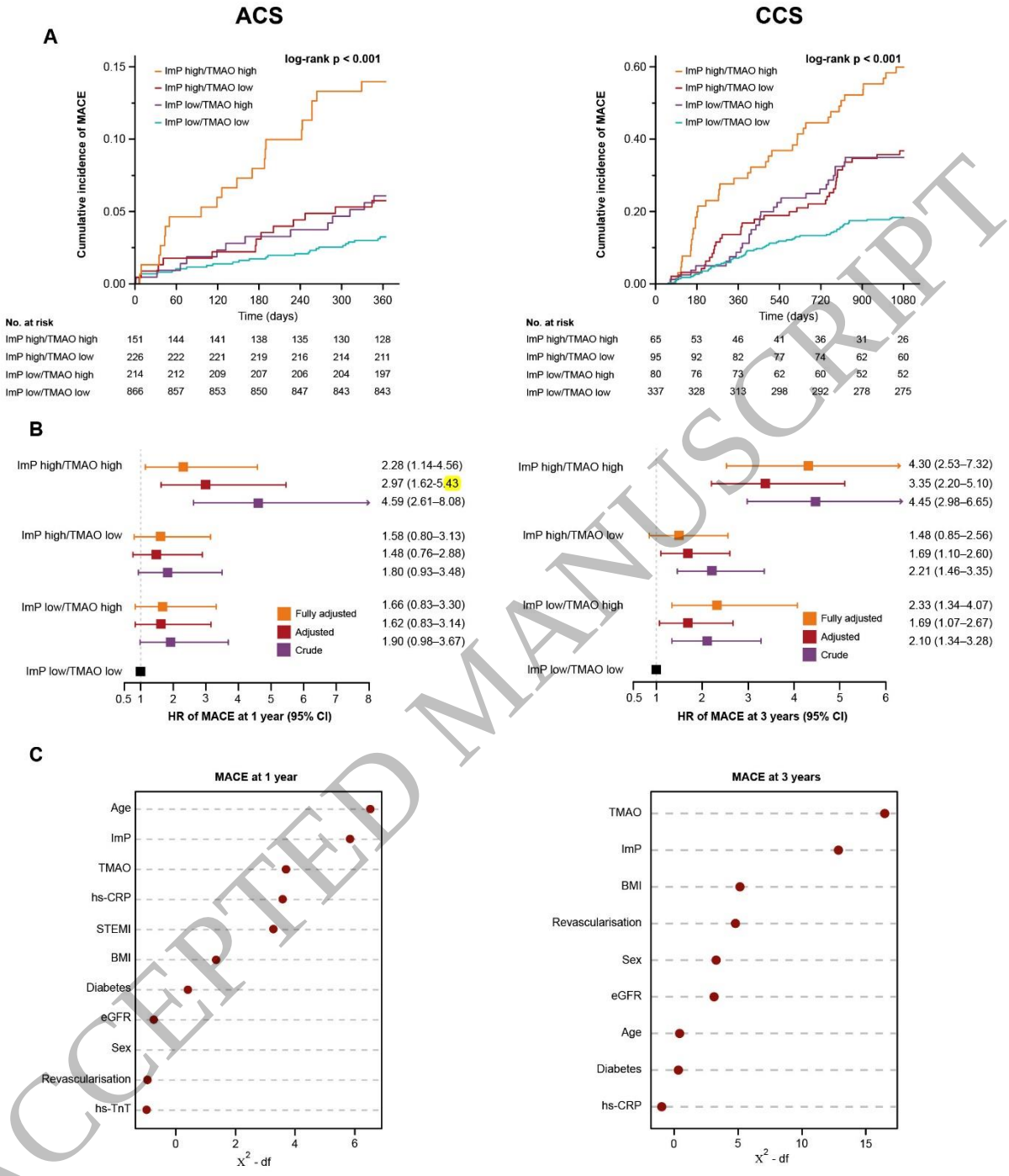


Figure 4
160x185 mm (x DPI)

Structured Graphical Abstract

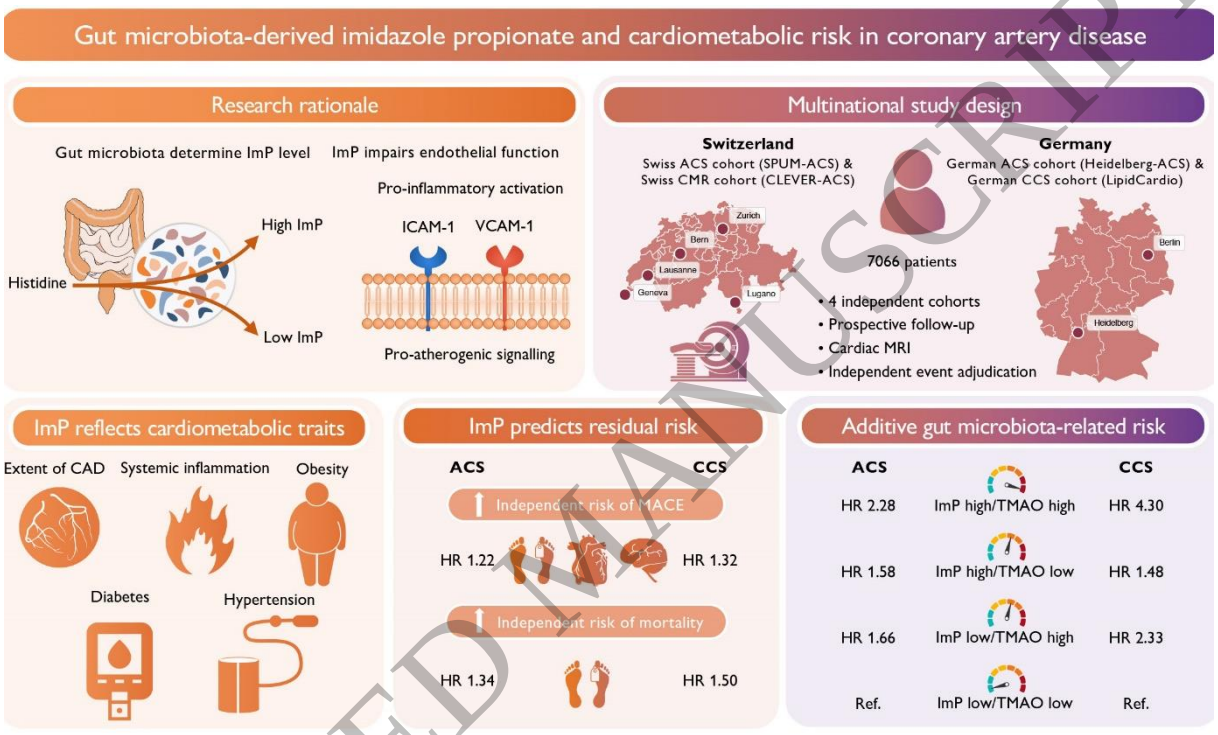
Key Question: Experimental evidence shows that gut microbiota-derived imidazole propionate (ImP) is mechanistically linked to atherosclerotic plaque formation and abnormal glucose metabolism. This study investigated the relationship between ImP and adverse outcomes in patients with coronary artery disease (CAD).

Key Finding: Circulating levels of ImP were linked to cardiometabolic characteristics and independently predicted major adverse cardiovascular events both in patients with acute coronary syndrome and in patients with chronic coronary syndrome.

Take Home Message: Gut microbiota-derived ImP is a novel marker of cardiometabolic risk in patients with CAD and represents a potential therapeutic target.

Legend: Gut microbiota-derived imidazole propionate (ImP) was identified as a novel marker of cardiometabolic risk in patients with coronary artery disease (CAD). Circulating ImP levels were measured in 4 independent cohorts of patients with acute (ACS) and chronic coronary syndrome (CCS) from Switzerland and Germany. ImP results from microbial histidine metabolism and has been mechanistically linked to inflammatory activation of myeloid and endothelial cells driving atherogenesis. Circulating levels of ImP were associated with cardiometabolic traits and independently predicted major adverse cardiovascular events (MACE) in patients with ACS and CCS. ImP added to the gut microbiota-related risk beyond the established microbiota-derived metabolite trimethylamine N-oxide (TMAO). Joint groups of high (≥ 75th percentile; ImP ≥ 24.4 nM) *vs.* low (< 75th percentile; ImP < 24.4 nM) circulating levels of ImP and TMAO were analysed. Values refer to hazard ratios (HR) in the fully adjusted model.

CMR, cardiac magnetic resonance; ICAM-1, intercellular adhesion molecule 1; MRI, magnetic resonance imaging; VCAM-1, vascular cell adhesion molecule 1.



Graphical Abstract

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