SLUTRAPPORT

Projekttitel	Dnr
Hitta hotande hjärtinfarkter (MIMI)	160266

Projektledare
Johan Sundström

Innehåll:

1. Background and aims

Despite declining age-standardized rates, myocardial infarction is the leading and increasing cause of death globally.[1] Prevention of myocardial infarctions is highly prioritized,[2] but the targeting of primary preventive efforts is hampered by inefficient means to identify individuals at the highest risk of an imminent myocardial infarction. This could be partially explained by the inability of most risk prediction models to account for the highly dynamic nature of the period leading up to a myocardial infarction. For instance, traumatic events, such as cancer diagnosis or loss of a spouse, dramatically increase the risk of myocardial infarction,[3, 4] and the degree of stenosis in the culprit coronary artery lesion appears to increase in the months just before the myocardial infarction.[5] Nonetheless, to date, most biomarkers have been investigated over several years of follow-up because of a low number of individuals with first myocardial infarction shortly after baseline in the general population. Hence, a large population-based study focusing on identification of biomarkers of an imminent myocardial infarction is needed.

Primary prevention for asymptomatic risk factors over a long period of time is costly, and motivation among patients and providers is limited, even for secondary prevention.[6] Risk prediction in the short term, based on biomarkers of imminent myocardial infarction, might tilt the scales for prevention, as the knowledge of an increased risk of first myocardial infarction within the ensuing few months might motivate the patients and doctors to consider preventive strategies.

We hypothesized that circulating biomarkers of the dynamic biological processes that operate in the months preceding a myocardial infarction could be measured and used to assess risk. We tested this in a new nested case-cohort consortium and devised the first prediction model of an imminent first myocardial infarction. Thereafter, we have initiated follow-up studies in the consortium for in-depth studies of promising avenues of research.

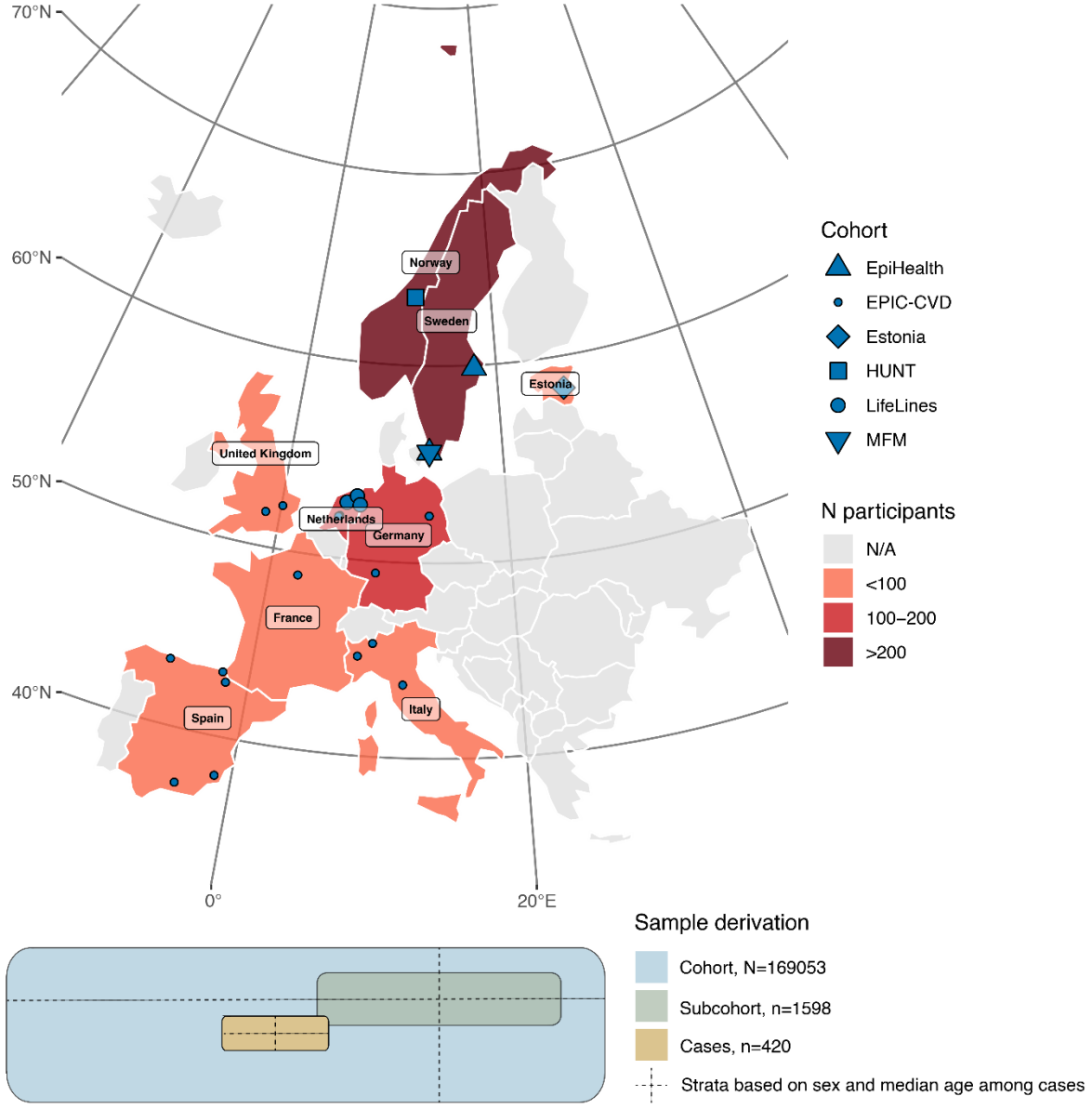
2. Methods

Study overview

We assembled a nested case-cohort study, the Markers of Imminent Myocardial Infarction study (MIMI; mimistudy.se). It includes initially cardiovascular disease-free individuals in the general population-based cohorts of the BBMRI-LPC collaboration,[7] who had developed a myocardial infarction within the first 6 months after baseline examination, with up to four cohort representatives

per case (Figure 1). The case-cohort design allows for time-to-event analyses, derivation of accurate prediction models, and is less prone to certain biases than the case-control design.[8]

Figure 1. Derivation of Sample Representing 169053 Individuals Without Previous Cardiovascular Disease from Six European Population-based Cohorts



Distribution of MIMI participants across Europe, with the participating countries and cohort centers indicated. Cases (n=420) were initially sampled, and center-specific strata based on sex and median age were constructed. From each cohort center, up to four subcohort representatives were drawn for each case from the same stratum. A subcohort (n=1598) was thus assembled, weighted to represent the total cohort (n=169053) based on the number of individuals in the age and sex strata in the total cohorts.

Briefly, more than 1800 proteins and metabolites were determined in biobanked blood samples from cohort baseline examinations of the MIMI study sample in a core laboratory. The study sample was divided into a discovery sample (EpiHealth[9], HUNT[10], and LifeLines[11]; 70% of the sample) and an external validation sample (EPIC-CVD[12, 13], Estonia[14, 15], and MFM[16]; 30% of the sample). Considering the limited sample size of the study, an internal validation was performed as an exploratory analysis by randomly splitting the study sample into 70/30 discovery/validation sample, repeated in 100 random draws. Associations of proteins, metabolites, and clinical variables with the risk of subsequent first myocardial infarction within 6 months of baseline in the discovery sample were investigated. Biomarkers that passed multiple-testing bounds were verified in the validation sample (this was done in the external and internal validation sets). Promising markers were thereafter

investigated in further models, and their associations with coronary calcium score at a cardiac computer tomography examination were examined in an external population-based cohort. Finally, the possibility to develop a clinical risk prediction algorithm in the discovery sample was investigated, and tested in the validation sample. Subsequent sub-projects were thereafter initiated to follow up on suitable research questions.

Study sample and outcome

The MIMI study sample draws biobanked blood and data from six Europeans cohorts as shown in Figure 1. After a sample size determination, we supplied each cohort with a standardized protocol in which all definitions were described in detail, and an R script for selection of cohort representatives to the subcohort.

Cohort participants with biobanked samples (at least 250 µL of plasma or serum), and who were free from previous clinical cardiovascular disease, were eligible for inclusion in the present study. Exclusion criteria were previous clinical cardiovascular disease, defined as presence at any time before baseline of any of the following: myocardial infarction, coronary procedure, heart failure, structural heart disease, tachyarrhythmias, stroke, thromboembolic disease, and peripheral vascular disease; or renal failure.

Individuals with acute myocardial infarction (ICD-10, I21; ICD-9, 410.0–410.6 and 410.8) as primary cause of hospitalization or death within 6 months after baseline were defined as imminent myocardial infarction (IMI) cases.

Up to four cohort representatives per available IMI case were randomly drawn from the full cohort to the subcohort in 50 strata based on sex, age (above/below median), and study center, in a stratified case–cohort design.[8] From the full cohort of 169,053 participants, of which 420 became IMI cases, all 420 IMI cases and 1598 subcohort representatives were drawn, as summarized in Figure 1.

Exposures

Clinical variables (age sex, height, weight, waist circumference, systolic and diastolic blood pressures, triglycerides, high- [HDL], non-HDL, low- [LDL] density lipoprotein and total cholesterol, glucose, diabetes status, highest education, smoking status, previous smoking exposure, alcohol intake, and physical activity) were harmonized between the cohorts. Non-HDL cholesterol was calculated as total cholesterol - HDL cholesterol. LDL levels were calculated using the extended Martin-Hopkins equation.[17]

All blood samples were randomized into appropriate measurement plates, stratified by cohort (i.e., with a similar number from each cohort is on every plate), and aliquoted into the plates. Quality controls are summarized below.

Protein measurements were done using the Olink proximity extension assay (Olink, Uppsala, Sweden), a highly specific 92-plex immunoassay. Overall, 829 proteins across 9 panels (Cardiometabolic, Cardiovascular II, Cardiovascular III, Development, Immune Response, Inflammation, Metabolism, Oncology II, and Organ Damage) were analyzed, including 804 unique proteins (considering overlap between panels). Relative protein values on a log2-scale are reported, with each protein value normalized by plate, by centering all plates at the same median, assuming random plate placement. Values below the lower limit of detection (LOD) of the assay were also included in the analyses.

Metabolites were analyzed using the Metabolon platform (Metabolon Inc, Durham, USA) of ultra-high performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) by four different methods: reversed-phase UPLC-MS/MS with positive-mode electrospray ionization (early and late phase); reversed-phase UPLC-MS/MS with negative-mode electrospray ionization; hydrophilic interaction liquid chromatography UPLC-MS/MS with negative-mode electrospray ionization. Overall, 1135 metabolites were captured, including 925 with known identity and 210 with unknown identity. Relative metabolite levels were determined, normalized by analysis day. Metabolite levels were log2-transformed and non-detectable levels (<LOD or metabolite not present in sample) were constant-value imputed to a value below the minimum value of the metabolite (minimum/sqrt[2]).

Samples that did not satisfy quality-control criteria were initially excluded: exclusion filters were applied separately for the proteomics and metabolomics analyses, and only samples passing quality control for both analyses were included in the analysis set. For the proteomics analysis, samples with more than 50% of panels failing for technical reasons were excluded (n-excluded=33). For the

metabolomics analysis, samples were excluded because of low volume, or detection of fewer metabolites than expected (n-excluded=4). Consequently, samples for 420 cases and 1598 subcohort representatives remained for analysis.

Next, biomarkers with an extremely high proportion of non-detectable/below LOD measurements were excluded, with the same exclusion filters for proteins and metabolites. Biomarkers had to be detected in all 6 cohorts with at least 30 detectable values across all cohorts (~1.5% of the MIMI samples), or were otherwise excluded. Consequently, 817 proteins (some duplicate) and 1025 metabolites were retained for analysis.

Statistical considerations

All analyses were done using R version 4.1.1[18] with the glmnet,[19] mice,[20] rms,[21] and survival[22] add-on packages. Clinical variables with high missingness (previous smoking exposure, alcohol intake, and physical activity) were not used in the analyses. Protein values below LOD were included in the analyses; non-detectable metabolite levels were replaced with a constant value, and a missing indicator was added, as described below. Remaining missing values in the covariates were multiple-imputed (n-imputations=20) using chained equations including the outcome, clinical covariates, and other variables correlated with the variable in the imputation model.[23] Regression results across imputed datasets were combined using Rubin's rules.[24]

3. Results

After exclusions, data for 2018 individuals were available for analysis (420 cases and 1598 subcohort representatives). Their characteristics at baseline are shown in Table 1.

Table 1. Baseline Characteristics

Variable	Missingness, % (cases/subcohort)	Cases of imminent myocardial infarction	Subcohort	Cohort
Number of individuals		420	1598	169,053
Age, years		64.1	63.0	52.5
Male, %		63.8	63.8	42.7
Body mass index, kg/m^2	0.7/0.6	27.6	27.4	26.1
Systolic blood pressure, mmHg	5.2/3.1	142.1	139.7	134.2
Diabetes, %	0.2/0.1	12.6	9.0	6.2
Glucose, mmol/L	6.2/4.9	6.0	5.8	5.4
LDL-cholesterol, mmol/L	5.2/4.5	3.9	3.8	3.4
Non-HDL cholesterol, mmol/L	5.2/4.4	4.7	4.5	4.0
Total cholesterol, mmol/L	4.8/4.3	6.0	5.8	5.4
Education, %	14.8/12.0			
Low		44.1	38.1	16.3
Medium		34.1	39.9	50.6
High		21.8	22.0	33.1
Smoking, %	3.8/3.8			
Never		33.4	37.8	46.6
Previous		33.9	37.8	28.7
Current		32.7	24.4	24.7

Baseline characteristics of the cases and weighted estimates of the full cohort of 169053 individuals from which the subcohort was drawn are shown. Numbers are percentages or arithmetic means, as indicated. LDL, low-density lipoprotein.

Sub-project 1. Etiology of an acute myocardial infarction

In the discovery sample, associations of all clinical variables (listed in Table 1), proteins, and metabolites with IMI were analyzed in separate weighted, stratified Cox proportional hazards regression models, adjusting for covariates, as described below. Inverse sampling probability weights (Borgan II) were applied to account for the case–cohort design in a stratified model, allowing a different shape of the baseline hazard for each MIMI cohort (six levels) and using a robust variance estimator (Huber–White). Non-linear relationships between continuous covariates (not including the biomarkers) and IMI were modelled using restricted cubic splines and all factor variables were considered unordered.

Associations with a false discovery rate (FDR) (Benjamini–Hochberg)<0.05 were taken forward to the validation sample, where directionally consistent results with p<0.05 were considered replicated.

Cox proportional hazards models adjusting for technical covariates (season, storage time, and plate) were initially applied. Replicating biomarkers from the model adjusting for technical covariates were investigated in a model further adjusting for age and sex. A model allowing for an interaction between the biomarker and sex was further tested. Replicating biomarkers in the model adjusted for age and sex were then subjected to causal assumptions, and a bias-minimized model for each biomarker was investigated, estimating the total effects (including the effects of mediators).

Interactions with sex were investigated by analyzing an interaction term for sex and each biomarker in models adjusting for the technical covariates, age, and sex. The interaction terms and all terms including the biomarker were tested using a multivariable chi^2 test with the same multiple testing correction as that described above, and requiring directionally consistent discovery and validation results

The following secondary sensitivity analyses were included: random-effects inverse variance weighted meta-analyses (DerSimonian–Laird) combining per-cohort results; investigation of the influence of single cohorts in leave-one-out analyses; and complete-case analyses not imputing missing values in the clinical covariates.

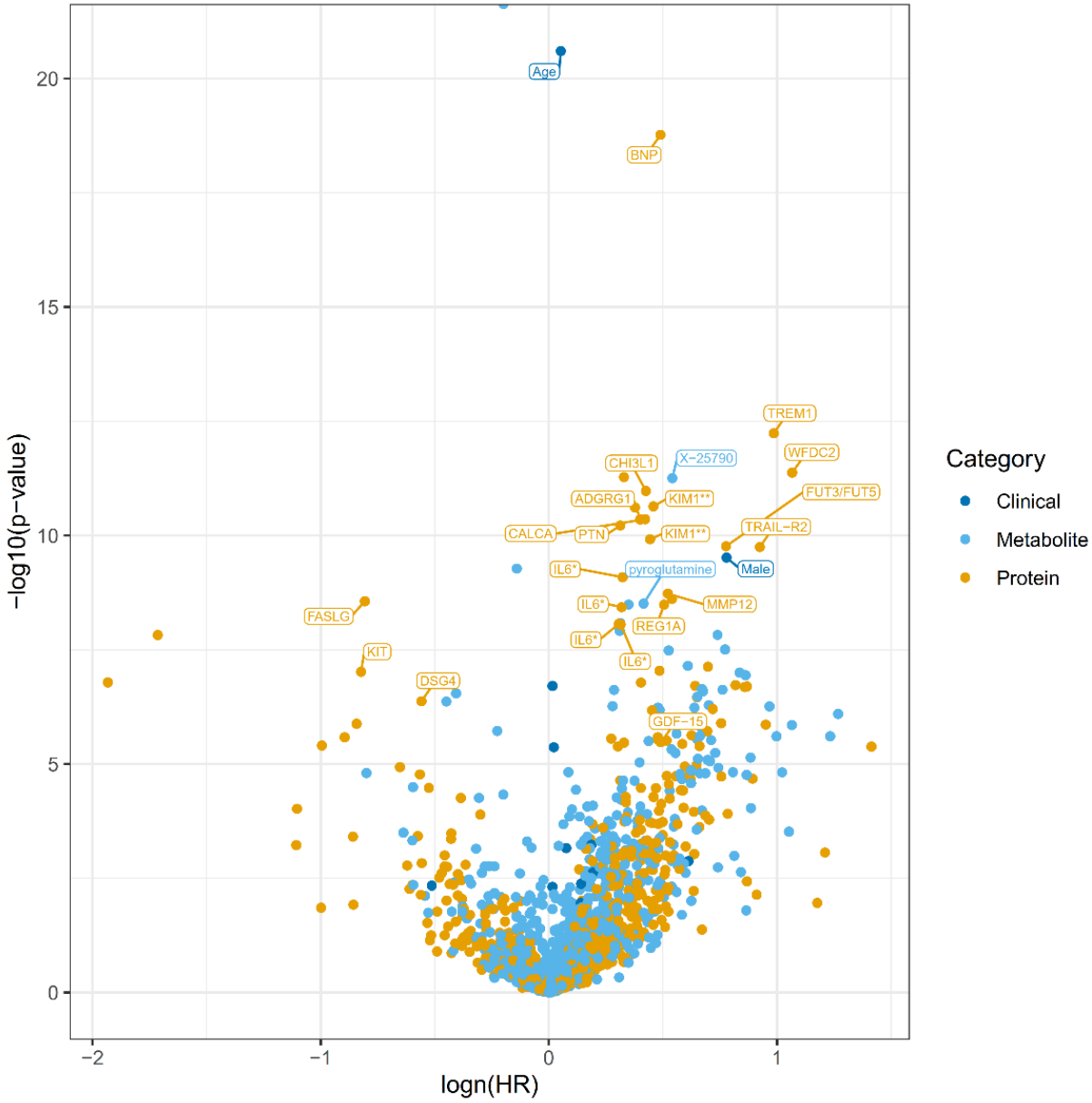
In one-by-one models adjusting for technical covariates (Figure 2), 48 proteins, 43 metabolites, and 3 clinical variables (age, sex, and systolic blood pressure) were found to be associated with IMI after the discovery-validation process (Figure 3**)**.

Among them, BNP was the only biomarker with a borderline significant association with IMI in models further adjusting for age and sex (hazard ratio, HR, per doubling of BNP (95% confidence interval, CI))=1.33(1.15,1.55), p=1.63e-04, FDR=0.11, in the discovery sample; and 1.40(1.00,1.94), p=0.049 in the validation sample) It was the only biomarker with a suggestive association in the internal validation, passing the formal replication criteria in 22 out of 100 random splits. By comparison, SCF and IL-6, biomarkers with a weaker support of an association, replicated in only 5 or 4 out of 100 random splits, respectively. For some of the 94 variables, we observed substantial between-cohort heterogeneity in the estimates when evaluated in a random-effects meta-analysis. The addition of interaction terms between sex and the biomarkers did not reveal any additional associations.

In a model investigating the total effect of the BNP-IMI association (with *a priori* selected confounders, not mediators) adjusting for age, sex, weight, height, creatinine, and systolic blood pressure, the association of BNP with IMI remained similar (HR[95% C.I.]=1.34[1.14,1.57], p=3.12e-04 in the discovery sample; and 1.51[1.05,2.18], p=0.028 in the validation sample; per doubling of BNP).

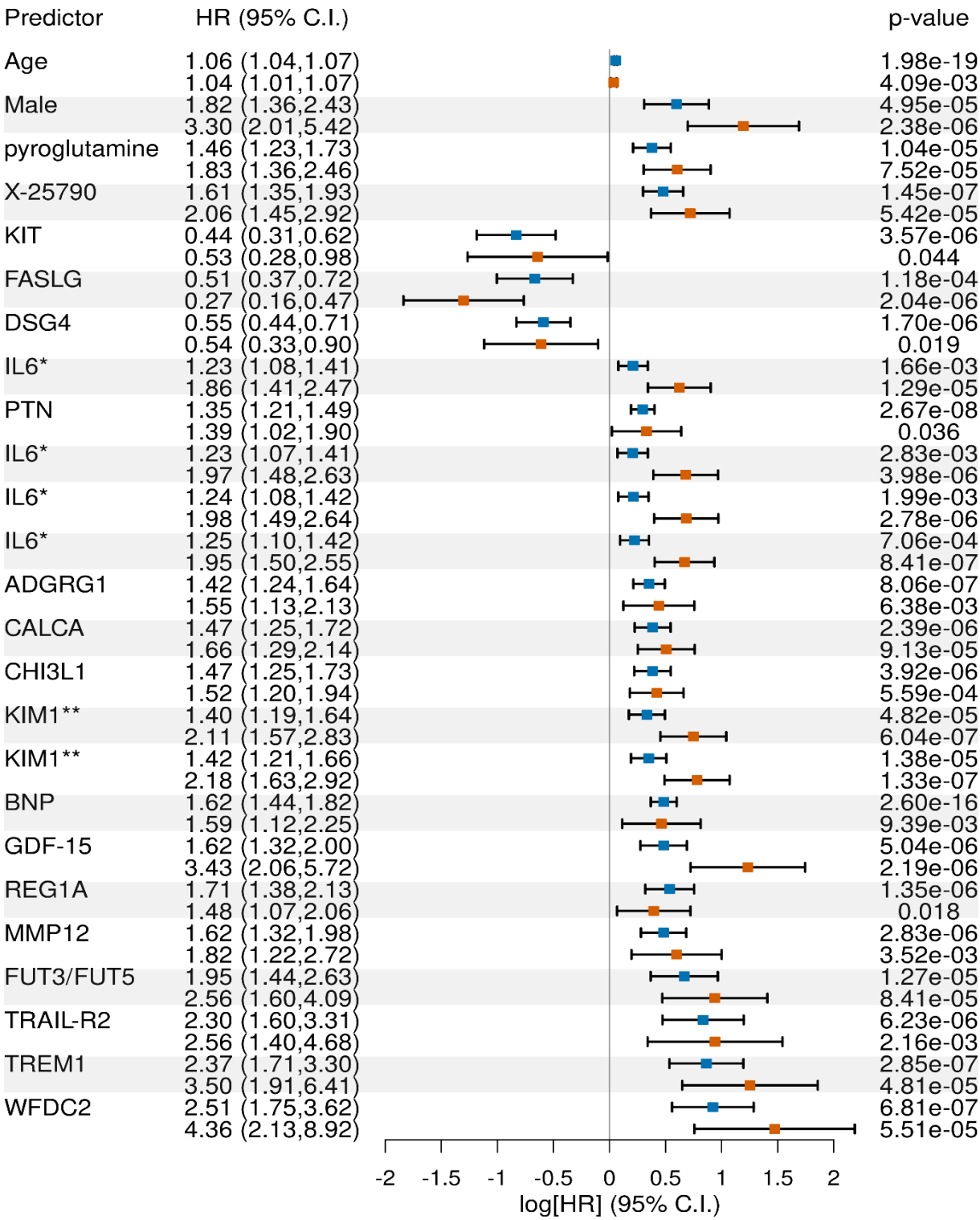
The associations of proteins detected using the Olink panels CVD2 and CVD3 with coronary artery calcium score (CACS) were available for testing in individuals free from cardiovascular disease (self-reported myocardial infarction, angina, coronary intervention, heart failure, atrial fibrillation, stroke, and peripheral artery disease) for 1586 participants at the Malmö or Uppsala centers of the Swedish CArdioPulmonary bioImage Study (SCAPIS).[25] Higher CACS reflects a higher myocardial infarction risk. Proteins replicated in the primary MIMI analysis (brain natriuretic peptide, BNP) were tested for an association with CACS using an ordinal regression model adjusting for age, sex, body mass index (BMI), systolic blood pressure, creatinine, center, Olink plate, analysis date, and season. A weak but directionally consistent association was found (ods ratio, OR [95% CI]=1.14 [0.91,1.42], p=0.25; per doubling of BNP) adjusting for the same covariates as in the total effects model.

Figure 2. Associations of Proteins, Metabolites, and Clinical Variables with Risk of an Imminent Myocardial Infarction



Associations of 817 proteins, 1025 metabolites, and 16 clinical variables with risk of a first myocardial infarction within 6 months in the discovery sample, adjusting for technical covariates, by each biomarker category (clinical, metabolite, and protein). Hazard ratio (HR) relates to a doubling of the concentration of protein and metabolite, and one-unit higher level of clinical biomarkers on their original scale (e.g., years, mmol/L). The top 25 biomarkers that passed external validation and ranked on how many internal validation splits the biomarker passed the replication criteria in the model adjusted for technical covariates in addition to the external validation are highlighted. IL6 (*) and KIM1 (**) were measured on multiple Olink panels and were tested in separate statistical tests.

Figure 3. Top Variables Associated with Risk of an Imminent Myocardial Infarction



Top 25 biomarkers that passed external validation and ranked on how many internal validation splits the biomarker passed the replication criteria in the model adjusted for technical covariates in addition to the external validation are shown. Each predictor is represented by two rows, with the discovery result (blue) first followed by the validation result (red). The results are sorted by type of predictor (clinical, metabolite, and protein) and effect size from the combined analysis of discovery and validation. P-value was calculated based on 2 d.f. Wald test for metabolites analyzed using the missing indicator method (biomarker and missing indicator), and 1 d.f. Wald test otherwise (biomarker only). The 95% confidence interval was calculated for the biomarker only, and might include 1 even if p < 0.05 from 2 d.f. (biomarker+indicator) Wald test. IL6 (*) and KIM1 (**) values were determined from multiple Olink panels and were tested in separate statistical tests.

In the current study, higher BNP levels in individuals without known cardiovascular disease were linked to a higher risk of a first myocardial infarction within 6 months in several models. Cardiomyocytes produce BNP in response to strain,[26] and N-terminal pro-BNP measurement is a pillar of clinical heart failure management[27] but is not used in the diagnosis of myocardial infarction.[28] Diastolic dysfunction is an early feature of myocardial ischemia, and higher BNP in this context is likely underpinned by diastolic dysfunction caused by subclinical ischemia[29] in individuals with some degree of coronary stenosis. This is supported by the weak association of BNP levels and CACS observed herein, although the association should be interpreted with care. The non-causal explanation is further supported by the non-causality suggested by Mendelian randomization studies (acknowledging that associations of genetically determined lifelong BNP levels with coronary disease may have limited relevance to a temporally boxed-in series of events): a genetic variant affecting the expression of the BNP gene (*NPPB*, rs198389) is not associated with cardiovascular endpoints[30] or coronary artery disease.[31] The influence of chance on the finding is low, as N-terminal pro-BNP was also significantly associated with imminent myocardial infarction in the discovery sample, with a borderline association in the validation sample.

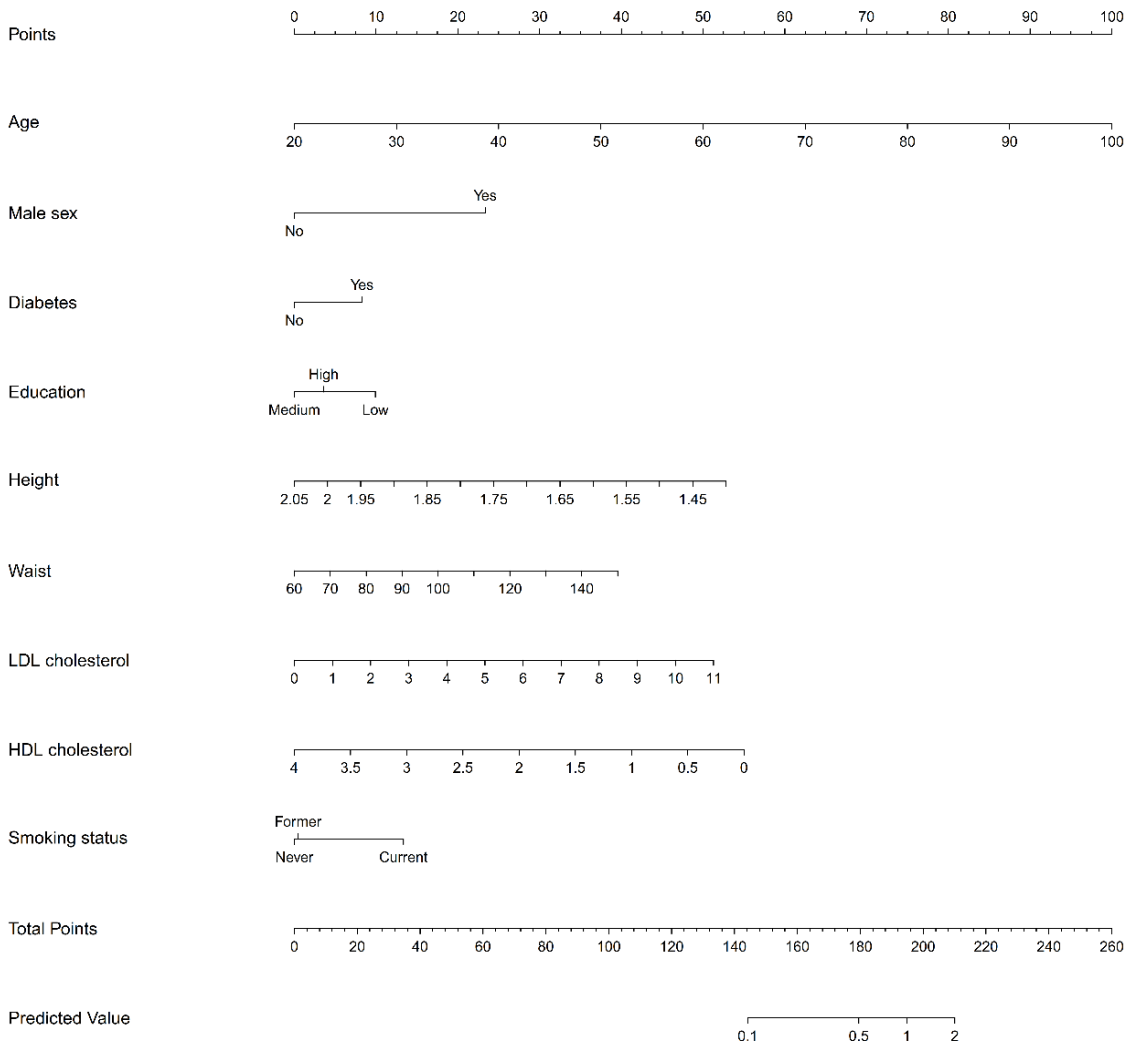
Several known biological pathways implicated in atherosclerosis and ischemia were represented among the other 94 biomarkers associated with an imminent myocardial infarction in both the discovery and validation samples after adjusting for technical covariates, including inflammation (interleukin 6 [IL6][32]), extracellular matrix metabolism (WAP four-disulfide core domain protein 2[WFDC2][33]), hypertrophy (adhesion G-protein coupled receptor G1 [AGRG1][34]), apoptosis (triggering receptor expressed on myeloid cells 1 [TREM1], tumor necrosis factor receptor superfamily member 10B [TRAIL-R2]), and cell adhesion (AGRG1). We also observed associations with markers representing mechanisms less often implicated in coronary disease, such as markers of kidney injury (kidney injury molecule 1 [KIM1]),[35] appetite regulation (growth differentiation factor 15 [GDF15]),[36] and an alpha amino acid found in dietary supplements and associated with paracetamol use (pyroglutamine).[37] While some associations may be causal, others, such as associations with levels of chitinase-3-like protein 1 (CHI3L1),[38] Pleiotrophin (PTN) or KIT, may more likely be responses to myocardial ischemia. These novel findings may accelerate further etiological studies of acute coronary events.

Sub-project 2. Prediction of an imminent myocardial infarction

As no prediction of this phenotype has been attempted before, we developed a new prediction model for IMI using age, sex, anthropometric variables (height, weight, and waist circumference), variables routinely collected in the laboratory (LDL- and HDL-cholesterol, creatinine, glucose, and triglycerides), systolic and diastolic blood pressure, smoking status (never, former, and current), and education level. Regression coefficients were estimated using a weighted penalized Cox model, which shrinks coefficients towards zero to accommodate overfitting. The strength of the penalty (lambda) was determined using 10-fold cross-validation over a grid of 250 values of lambda, repeated 100 times. The selection of lambda was repeated in each imputed dataset and the coefficients associated with the lambda giving the lowest cross-validated deviance were extracted. The final coefficient set was obtained by taking the median of the coefficients from each imputed data set. A single-imputed dataset was used for validation and calibration. The C-index, which indicates a model's ability to rank the risks, was determined using 100 repeats of 10-fold cross-validation. A calibration curve was constructed by dividing the predicted risk of IMI into intervals based on the deciles, as well as the 95th, 97.5th, and 99th percentiles. The fractions of events were then compared with the Kaplan–Meier estimate of 6-month risk of IMI in that interval using 100 repeats of 10-fold cross-validation. All modeling steps were repeated in each fold to provide an honest assessment of the calibration accuracy. The predictive value of BNP on top of the clinical variables was evaluated by building a second model where BNP was added to the predictors and the above steps were repeated. The model containing only clinical variables was then reduced by approximating the linear predictor from the full model via stepwise regression. Predictions from the full model were used in a linear model wherein variables were dropped sequentially until R^2>0.95. This yielded a highly parsimonious model incorporating the main drivers of predictions.

The full prediction model achieved a cross-validated C-index of 0.78, indicating good ability to discriminate between cases and non-cases of IMI, while the addition of BNP did not improve discrimination (C-index 0.79). A nomogram predicting IMI risk is shown in Figure 4 based on the reduced clinical model.

Figure 4. Nomogram of Model for Clinical Prediction of an Imminent Myocardial Infarction



Nomogram for predicting risk of an imminent myocardial infarction based on the reduced clinical model. Each value of a variable contributes points (ruler at the top of the figure) that are summed up and translated to the predicted risk of a myocardial infarction within 6 months (bottom two rulers). LDL, low-density lipoprotein, HDL, high-density lipoprotein.

We here developed the first-ever prediction model for imminent myocardial infarction in the general population. An imminent infarction is difficult to predict: signals are weak and we experienced power limitations. The model reached a good discriminative ability, with acceptable calibration in the lower risk range. In the validation sample, prediction centered around the cohort baseline hazard. It is possible to transpose to other settings by entering the base hazards and variable means of those settings. Given the increasing global burden of deaths from myocardial infarction, the importance of predicting them and increasing the individual motivation for preventing them may be significant; this can be tested in clinical trials.

Sub-project 3. Anticholinergic activity and imminent myocardial infarction risk

Tachycardia is an independent risk factor for cardiovascular disease[39] and can in part result from a dysregulated autonomic nervous system.[40, 41]. The function of the cardiac autonomic nervous system is most often assessed by measurement of the heart rate variability (HRV).[42] Low HRV has been coupled to mortality risk after a myocardial infarction and can also be an early sign of cardiac autonomic neuropathy in diabetes.[43-46] The origins of a low HRV are multifactorial, but one important factor is

certain pharmacological agents, most notably anticholinergic compounds.[47, 48] Assessments of serum anticholinergic activity is impractical but the anticholinergic burden of a patient can be assessed with rating scales [49]. The aim of this project was to investigate whether higher anticholinergic burden is associated with an increased risk of an imminent myocardial infarction. A secondary analysis investigated the association with anticholinergic activity on measures of HRV in SCAPIS.

Selected metabolites in MIMI were scored based on their anticholinergic activity[49] and the calculated score was used in a Cox regression model, adjusting for age, sex, body mass index, smoking status (never, former, current) and diabetes, stratified on cohort and weighted to reflect sub-cohort participation. The association between HRV and the anticholinergic score in SCAPIS was analyzed using ordered logistic regressions.

The regression model indicated a weak association between higher anticholinergic burden and imminent myocardial infarction (hazard ratio 1.13, 95% CI 0.90-1.43), as hypothesized. Notably, the effect was seen in vagal measures of HRV, further strengthening the association between anticholinergic burden and the regulation of the autonomic nervous system.

Our results point towards an independent negative effect of anticholinergic burden on the cardiac autonomic regulation, but more analyses are needed. Since dysregulation of the autonomic nervous system is associated with both cardiovascular disease and diabetes [43, 44], these findings call for further exploration and increased attention of anticholinergic compounds as a risk factor for cardiovascular disease.

Sub-project 4. Associations of circulating pain medication metabolites and imminent myocardial infarction risk

Patients with chronic pain have been shown to have a substantially increased risk for cardiovascular disease but the underlying mechanisms are incompletely understood. It has been suggested that common pain medications could be a causal factor that contributes to the progression of cardiovascular disease. Although randomized controlled trials are considered the gold standard in assessing the efficacy and safety of pharmacological treatments, most of the commonly used pain medications lack such safety data with regards to cardiovascular complications.

Previous pharmacoepidemiological studies on the subject may have been limited by misclassification due to unknown adherence to prescribed medications, limited validity on questionnaire-based data, or lack of data on over-the-counter medications. Metabolomics-based pharmacoepidemiology is a novel approach that could overcome some of these previous limitations. Therefore, to shed further insights to the role of pain medications in the development of cardiovascular disease we investigated the association between circulating metabolites of common pain medication and the risk of a subsequent myocardial infarction.

There was no significant association between any of the pain medication classes and incident myocardial infarction in multivariable cox regression analyses adjusting for age, sex, technical variables, education and established cardiovascular risk factors; systolic and diastolic blood pressure, body mass index, diabetes and creatinine (paracetamol HR per doubling of metabolite levels 0.77 (95% CI 0.55,1.07); NSAID HR 0.99 (0.73,1.33) and opioids HR 0.68 (0.21,2.14). There was however a notably higher risk of an imminent myocardial infarction in persons with detectable levels of two specific NSAIDs (ibuprofen and nimesulide), but these data should be interpreted with caution due to few events in these analyses (number of events 10 and 3, respectively) and that the multiple testing was not taken into account.

Our metabolomics-based pharmacoepidemiological approach did not provide support for common pain medication to be a major contributing factor for the detrimental interplay between chronic pain and the development of cardiovascular disease.

Sub-project 5. Protein biomarkers of imminent versus long-term risk of myocardial infarction

In recent studies using large-scale proteomics, a number of proteins have been associated with myocardial infarction that occur within several years from when the proteomics profile was measured[50] as well as for imminent myocardial infarction as shown in the MIMI study. To better understand the similarities and differences between the two outcomes, long-term and imminent myocardial infarction, we compared the protein profiles for these two different time courses of MI. The Uppsala Longitudinal Study of Adult Men (ULSAM) is a population-based cohort study initiated in 1970–1974 when all men in Uppsala City, Sweden, aged 50 years were invited to a health survey aiming to evaluate risk factors for cardiometabolic disease. A total of 2,322 men participated (82% of all men invited), and the study has been described in previous studies.[51] The present study sample derives from the re-examination at the age of 70 years. A set of 791 proteins were analyzed in plasma by the proximity extension assay (PEA, OLINK) in both the ULSAM study (852 men all aged 70 with a follow-up of >20 years, 125 incident cases) and the MIMI study. Regression results were compared for models adjusted for technical covariates, age, sex, systolic blood pressure, HDL-C, LDL-C, smoking, BMI, and diabetes.

Using a false discovery rate<0.05 for the age and sex-adjusted models and p<0.05 for the models also adjusted for traditional risk factors, 11 proteins were related to IMI, while 52 proteins were related to incident MI during long-term follow-up (Figure 5). Only two proteins were related to both IMI and MI at long-term follow-up; growth differentiation factor-15 (GDF-15) and kidney injury molecule (KIM-1). Pathway enrichment analysis highlighted interleukin/cytokine signaling and apoptosis for IMI, while TNF-binding to receptors and caspase activation were linked to incident MI during long-term follow-up.

The protein profiles signaling risk of imminent MI and long-term risk of MI are different, but with some shared proteins, reflecting that partly different pathophysiological mechanisms are likely to play a role in these two conditions.

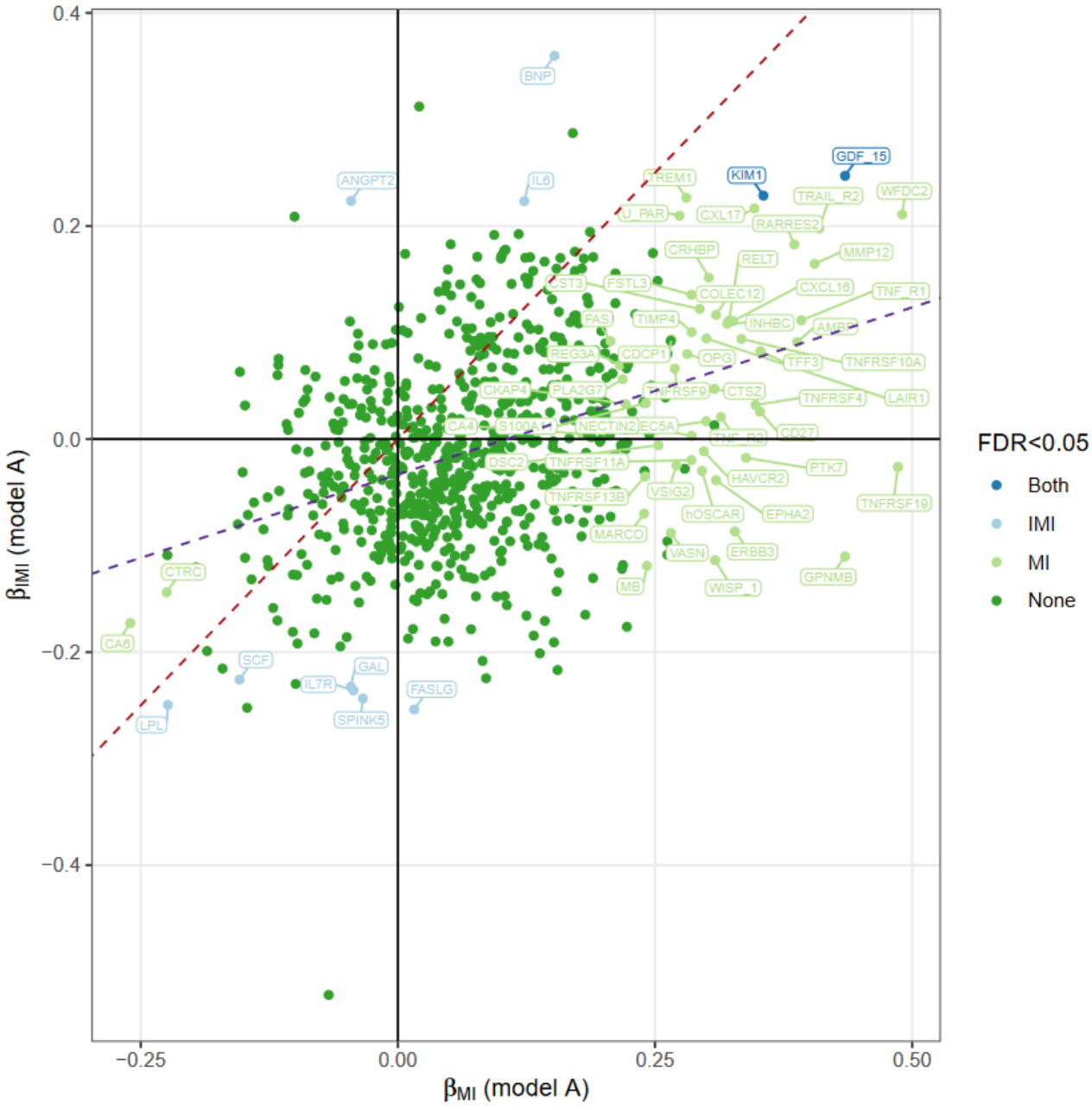
Sub-project 6. Physical activity and biomarkers of myocardial infarction risk

The underlying mechanisms by which physical activity prevents cardiovascular disease remain poorly understood. We aimed to investigate associations between long term physical activity and plasma proteins and as a second step evaluate associations between identified proteins of interest and imminent myocardial infarction.

We used data on self-reported physical activity from the ULSAM cohort (age 70 examination) together with the MIMI study. In age-adjusted Bonferroni-corrected linear regression, the level of physical activity during 20 years was associated with levels of 23 plasma proteins (Bonferroni p<0.000069). Of the 23 proteins associated with physical activity in ULSAM, 8 were also associated with imminent myocardial infarction risk in MIMI (p<0.05; Figure 6): TNF-related apoptosis-inducing ligand receptor 2 (TRAILR2), interleukin-6 (IL6), furin (FURIN), hepatocyte growth factor (HGF), WAP four-disulfide core domain protein 2 (WFDC2), growth differentiation factor 15 (GDF15), fibroblast growth factor 21 (FGF21), adhesion G-protein coupled receptor G1 (ADGRG1). After additional adjustment for known cardiovascular risk factors (education, smoking status, BMI, blood pressure, low-density lipoprotein, diabetes diagnosis, and hypertensive-, lipid- or diabetes-treatment), 5 proteins remained negatively associated with higher level of physical activity in the ULSAM cohort: leptin, fatty-acid-binding protein-4 (FABP4), fibroblast growth factor 21 (FGF21), interleukin-1 receptor antagonist (IL1RA) and CXADR-like membrane protein (CLMP). Of these proteins, FGF21 was also associated with imminent myocardial infarction risk in MIMI (p<0.05).

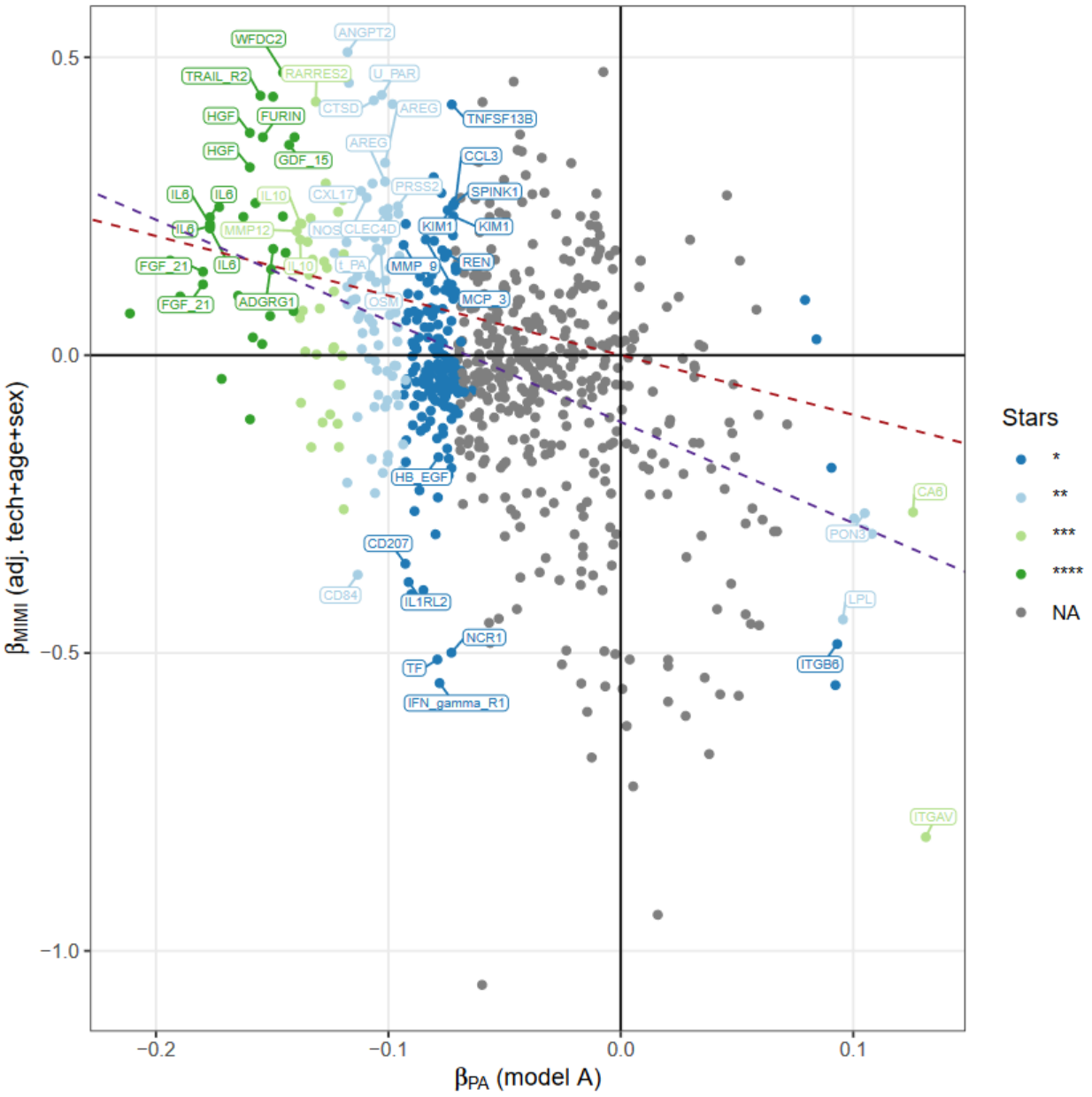
Multiple novel associations were found between long-term physical activity and plasma proteins. Several proteins were associated with imminent myocardial infarction in an independent cohort which could suggest that the cardioprotective effects of physical activity to some degree may be mediated via the circulating proteome. Our findings encourage additional studies in order to understand the underlying causal mechanisms of these associations.

Figure 5. Associations of regression coefficients for an imminent and long-term myocardial infarction



Regression coefficients from separate analyses of the association IMI ~ protein (y-axis) and MI ~ protein (x-axis). All analyses were adjusted for technical covariates as well as as age and sex. The colour of the dots corresponds to proteins reaching false discovery rate (FDR) < 0.05 in both, one, or neither analysis. Proteins reaching FDR < 0.05 in either analysis are labeled. The dashed red line is the identity line and the dashed purple line corresponds to a regression line fitted through the points of the figure.

Figure 6. Associations of regression coefficients for physical activity and imminent myocardial infarction risk



Regression coefficients from separate analyses of the association IMI ~ protein (y-axis) and protein ~ physical activity (x-axis). All analyses were adjusted for technical covariates as well as as age and sex. The colour of the dots corresponds to the significance level in ULSAM and all proteins reaching p-value < 0.05 in MIMI are highlighted with labels. The dashed red line is the identity line and the dashed purple line corresponds to a regression line fitted through the points of the figure.

Sub-project 7. Food-related metabolites and risk of imminent myocardial infarction

Research of individuals' diet is often based on self-reported data, where risk of bias is high. Analyzing biomarkers could be an objective way to identify how individuals are living, including their diet.

All metabolites on the Metabolon platform with a potential connection to dietary intake were considered in the project. This set of metabolites was further restricted to metabolites with a connection to dietary intake that was established in randomized clinical trials, after a systematic literature review. All regression models were at a minimum adjusted for technical covariates, age, sex, and smoking status.

At FDR <0.05, only taurine was inversely and significantly associated with risk of an imminent myocardial infarction. Taurine is found in several dietary products, including energy drinks, dairy, meat, and in especially high concentrations in shellfish and other types of seafood. It has previously been proposed to have a potentially protective effect against cardiovascular outcomes,[52, 53] and we could now establish that with adequate methods for the first time. There is a need for further research to determine the origin of taurine in specific food items or food categories in order to understand this association.

Sub-project 8. Metabolites and biomarkers in patients with diabetes versus no diabetes in a cohort with imminent myocardial infarction

It has been known for decades that the risk of myocardial infarction is increased for individuals with diabetes compared to patients without diabetes. In patients with type 2 diabetes (T2DM), cardiovascular disease is responsible for at least half of the mortality. It has been suggested that an altered hormonal gene regulation is associated with fat accumulation, with increased pro-atherogenic inflammatory markers (such as C-reactive protein [CRP], interleukin-6, monocyte chemotactic protein 1, and tumor necrosis factor-α), procoagulant factors (such as plasminogen activator inhibitor 1, fibrinogen, and factor VII), and disrupted metabolic equilibrium (increased free fatty acids and insulin resistance). A combination of these factors could be responsible for the metabolic changes and increased risk of arteriosclerosis mediated via hepatic lipid accumulation, with the liver being both the target of the resulting systemic abnormalities and a source of pro-atherogenic molecules that amplify the arterial damage and alter cardiac structure.[54] To be able to estimate prevalent disease or risk of disease progression, biomarkers easily measured in plasma is an appealing option in comparison to more costly or time-consuming alternatives, such as radiological evaluations. For example, proteomic signatures for identification of impaired glucose tolerance have been suggested as an additional screening method to detect individuals at an early stage, in order to prevent disease progression.[55] A proteomic risk score to predict cardiovascular events in patients with stable coronary heart disease has been suggested based on nine plasma proteins,[56] but this needs to be validated in many other populations and regions, and the applicability to persons without stable coronary heart disease is unknown. There are to our knowledge no studies of differences in associations of biomarkers with myocardial infarction risk in patients with versus without diabetes. Our aim was to identify biomarkers in participants with diabetes compared to non-diabetics that were present in relation to an imminent myocardial infarction within six months.

Due to the limited power for an interaction test in the MIMI study, the set of biomarkers considered in this projects was restricted to those with FDR < 0.25 (n=45) in a model adjusted for technical covariates, age, sex, systolic blood pressure, BMI, HDL-C, LDL-C, education level, smoking, and creatinine. For this subset of biomarkers with the strongest association with imminent myocardial infarction, an interaction term for diabetes was added to the regression model.

No biomarker had an interaction term with diabetes that was significant after adjustment for multiple testing. The only metabolites that were close of reaching the statistical significance threshold were a set of sphingomyelins. However, since they did not pass the formal cutoff for replication and since no replication study is available, these results should be interpreted with care. If the results would remain significant in a replication study, further research is required to investigate why the association between sphingomyelins and imminent myocardial infarctions differs between diabetics and non-diabetics.

Reflections

We here set out to identify and test novel biomarkers and prediction potential of an imminent first myocardial infarction using a novel case–cohort consortium of individuals without prior cardiovascular disease, with biobanked blood. From more than 1800 biomarkers, we identified 48 proteins, 43 metabolites, and 3 clinical variables as associated with the risk of an imminent first myocardial infarction independent of technical covariates. Further analyses revealed BNP as the only biomarker consistently associated with an imminent myocardial infarction risk. We also derived a prediction model to discriminate between subsequent cases and non-cases. This is the first time the imminent myocardial infarction phenotype was studied prospectively in the general population, and with such a wide panel of biomarkers. The findings may have implications for both clinical primary prevention studies and further etiological studies. We followed up with multiple sub-projects, of which some have a good chance of providing important results.

The current study has several limitations. First, the use of multiple cohorts introduced heterogeneity. We addressed this at the sampling, biomarker analysis, and statistical analysis stages, with the resulting limitation that the heterogeneity decreases statistical power. The strengths are the same as in other multicenter studies, that only biomarkers with a consistent importance in different settings are brought forward. Other limitations of the study are inherent to the uncertainty of rankings of top findings and the inability of one-by-one strategies to capture complex interrelationships. Complex interactions and/or non-linear associations could potentially be investigated by, e.g., random forest and boosting techniques. However, such methods are notoriously data hungry and require far larger data sets than classical modeling techniques.[57] While the studied markers are easily obtained by a simple blood test or clinical assessment, a limitation is that a blood sample will not always capture tissue specific pathways, and our study was limited to proteins and metabolites that remain stable in the freezer for many years. Further, causal assumptions are fundamentally difficult to make in a multimarker landscape where many of the causal pathways are unknown. Most markers could be potential mediators in pathways for known causes of myocardial infarction, including age and sex. Consequently, we provided both models adjusted for technical covariates only, and models with further biological covariate adjustment. Thus, some associations could be explained by confounding, by e.g., age and sex. Notably, also mediators of causal effects are important to identify, with implications for prediction and use as treatment targets.

In conclusion, we here identified novel biomarkers associated with the risk of an imminent first myocardial infarction, including BNP. Delineation of the distinct biological processes that operate in the months before the first myocardial infarction will be key to the discovery of new prevention targets. We developed and validated the first prediction model with fair ability to discriminate between persons with and without risk of an imminent first myocardial infarction. Risk prediction in the short term may enhance the motivation of patients and doctors for primary prevention.

4. Implementation

The absence of any striking novel findings in the etiological analyses in the present program have consequently not led to any attempts for independent external validation. The good ability to predict a first myocardial infarction within 6 months with the prediction model developed in this program motivates investigations of its usefulness in clinical practice. The fact that it only needs variables routinely collected in healthcare makes it attractive for further clinical studies. Validation of its predictive capacity in real-world samples is a first step (we are currently planning such a study). If successful, the next step would be randomized investigations of it. A suggestion would be to use a sample of individuals with high levels of single risk factors such as hypercholesterolemia, hypertension, or smoking, but without manifest atherosclerotic disease; to randomize them to exposure to this risk estimation or to a long-term prediction model such as the SCORE2,[58] WHO estimation,[59] or the pooled cohort equation;[60] and evaluate if knowledge of a risk of an imminent myocardial infarction leads to higher adherence to preventive drug therapy than knowledge of longer-term risk. We have developed digital tools to facilitate such a study,[61, 62] and are planning to conduct the study if the initial validation study proves successful.

5. Dissemination

Website mimistudy.se continuously updated. Multiple newsletters to participating cohorts and stakeholders sent out. Several oral presentations at Uppsala University and Uppsala University Hospital. Article in Upsala Nya Tidning 2019-09-02. Accepted for an oral presentation at the ESC Preventive Cardiology conference in Malaga in April 2023.

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