Combining Polygenic and Proteomic Risk Scores With Clinical Risk Factors to Improve Performance for Diagnosing Absence of Coronary Artery Disease in Patients With De Novo Chest Pain

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Combining Polygenic and Proteomic Risk Scores with Clinical Risk Factors to Improve Performance for Diagnosing Absence of Coronary Artery Disease in Patients with de novo Chest Pain

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Peter Loof Møller, MSc[1,2]; Palle Duun Rohde, MSc, PhD[2]; Jonathan Nørtoft Dahl, MD[3,4]; Laust Dupont Rasmussen, MD, PhD[3,4]; Samuel Emil Schmidt, MSc, PhD[2]; Louise Nissen, MD, PhD[3,4], Victoria McGilligan, PhD[5]; Jacob F. Bentzon, MD, PhD[4,6]; Daniel F. Gudbjartsson, BSc, PhD[7,8]; Kari Stefansson, MD, PhD[7,9]; Hilma Holm, MD[7]; Simon Winther, MD, PhD[3,4]; Morten Bøttcher, MD, PhD[3,4]; Mette Nyegaard, MSc, PhD[1,2]

[1]Dept of Biomedicine, [4]Dept of Clinical Medicine, Aarhus Univ, Aarhus; [2]Dept of Health Science and Technology, Aalborg Univ, Aalborg; [3]Dept of Cardiology, Gødstrup Hospital, Herning, Denmark; [5]Personalized Medicine Centre, Ulster Univ, Derry, Northern Ireland; [6]Centro Nacional de Investigaciones Cardiovasculares, Madrid, Spain; [7]deCODE genetics/Amgen, Inc.; [8]School of Engineering & Natural Sciences, [9]Faculty of Medicine, Univ of Iceland, Reykjavik, Iceland

Correspondence:

Mette Nyegaard

Department of Health Science and Technology

Aalborg University

Fredrik Bajers vej 5

DK-9210 Aalborg East

Denmark

E-mail**:** nyegaard@hst.aau.dk

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Abstract:

Background - Patients with de novo chest pain, referred for evaluation of possible coronary artery disease (CAD), frequently have absence of CAD resulting in millions of tests not having any clinical impact. The objective of this study was to investigate whether polygenic risk scores and targeted proteomics improve prediction of absence of CAD in patients with suspected CAD, when added to the Prospective Multicenter Imaging Study for Evaluation of Chest Pain (PROMISE) minimal risk score (PMRS).

Methods - Genotyping and targeted plasma proteomics (N=368 proteins) were performed in 1440 patients with symptoms suspected to be caused by CAD undergoing coronary computed tomography angiography. Based on individual genotypes, a polygenic risk score for CAD (PRS_{CAD}) was calculated. Prediction was performed using combinations of PRS_{CAD}, proteins, and PMRS as features in models using stability selection and machine learning.

Results - Prediction of absence of CAD yielded an area under the curve (AUC) of: PRS_{CAD}-model 0.64 ± 0.03, proteomic-model 0.58 ± 0.03, and PMRS-model 0.76 ± 0.02. No significant correlation was found between the genetic and proteomic risk scores (Pearson's correlation-coefficient = -0.04, P=0.13). Optimal predictive ability was achieved by the full model (PRS_{CAD} + protein + PMRS) yielding an AUC of 0.80 ± 0.02 for absence of CAD, significantly better than the PMRS model alone (P<0.001). For re-classification purpose, the full model enabled down-classification of 49% (324 of 661) of the 5-15% pre-test probability (PTP) patients and 18% (113 of 611) of >15% PTP patients.

Conclusions - For patients with chest pain and low-intermediate CAD risk, incorporating targeted proteomics and polygenic risk-scores into the risk assessment substantially improved the ability to predict absence of CAD. Genetics and proteomics seem to add complementary information to the clinical risk factors and improve risk stratification in this large patient group.

Clinical Trial Registration - https://clinicaltrials.gov/ct2/show/NCT02264717, unique identifier: NCT02264717

Nonstandard Abbreviations and Acronyms
CAD: Coronary artery disease
CACS: Coronary artery calcium score
CCTA: Coronary computed tomography angiography
Dan-NICAD 1: Danish study of non-invasive testing in coronary artery disease
NPX: Normalized protein expression
PMRS: PROMISE minimal risk score
PROMISE: Prospective Multicenter Imaging Study for Evaluation of Chest Pain
PRS: Polygenic risk score
PTP: Pre-test probability
SNP: Single nucleotide polymorphism

Introduction

Symptoms of coronary artery disease (CAD) are often vague or uncharacteristic and millions of individuals worldwide therefore undergo examinations to diagnose CAD, while only a minority require revascularization or changed medical management. To guide decisions on test referral/deferrals, guidelines for diagnosing CAD recommend the use of a pre-test probability (PTP) score when patients present with symptoms suspected to be caused by CAD.[1,2] Classically, the PTP estimation is based on sex, age, and angina typicality.[2] Downstream of the PTP, diagnostic testing for CAD includes e.g., coronary computed tomography angiography (CCTA) followed, if positive, by either non-invasive or invasive methods to determine the functional significance of a suspected stenosis. Both the 2019 European[2] and 2021 American guidelines[1] on chronic coronary syndrome recommend diagnostic testing in patients with higher PTPs of CAD (PTP >15%). However, the guidelines are ambiguous regarding testing in patients with 5-15% PTP of obstructive CAD.[3] Because most patients in this group do not have CAD, referral of this group for downstream investigations results in many tests not having any clinical consequences.

Improved tools for determining CAD absence in this intermediate PTP group (5-15%) could therefore be of substantial clinical value.

One recently developed tool for predicting the absence of CAD is the Prospective Multicenter Imaging Study for Evaluation of Chest Pain (PROMISE) minimal risk score (PMRS).[4] The PMRS relies on traditional CAD risk factors, and a recent study showed that the PMRS could correctly down-classify one-third of patients with chest pain and intermediate PTP (5-15%).[5] Similarly, in an attempt to assess the potential value of proteomic data for detecting absence of CAD in symptomatic patients, a study performed targeted proteomic measurements in 196 patients with chest pain, including 26 without CAD and found that a signature of 35 plasma proteins predicted absence of CAD.[6] Finally, recent studies have shown that polygenic risk scores can improve CAD prediction when used in combination with clinical risk factors.[7] For instance, a study predicting CAD in the UK Biobank found a 3-percentage point improvement in predictive ability, when adding the PRS to a clinical risk score.[8] Whether a combination of targeted proteomics and genetic information could have a clinical role and improve prediction of absence of CAD in symptomatic patients when added to clinical risk factors has not been systematically tested.

The aim of this study was therefore to examine the incremental value of adding a PRS_{CAD} and proteins to the PMRS model, and secondly test the performance of this model to correctly down classify patients with a PTP >5% of obstructive CAD.

Methods

The study was approved by the Central Denmark Regional Committee on Health Research Ethics (record number: 1-10-72-190-14) and the Danish Data Protection Agency (record number:

1-16-02-345-14). Informed written consent was obtained from all patients. The trial was registered at https://www.clinicaltrials.gov (unique identifier: NCT02264717). The data that support the findings of this study are available from the corresponding author upon reasonable request. The complete methods are available in the supplementary.

Results

Study population

Of the initial 1675 patients in the Dan-NICAD 1 cohort, 1440 patient were eligible for statistical analyses, as they had complete data for all 307 variables (PMRS, PRS_{CAD}, and 305 proteins) and CCTA images revealing CAD status (Supplementary Figure VI). Based on the CCTA results, 675/1440 patients (47%) had absence of CAD (no stenosis and a calcium score of zero), and 765/1440 patients (53%) had some level of CAD, including 141/1440 (10%) patients with obstructive CAD (Figure 1). The PTP was associated with CAD presence (OR=1.08; $P<0.001$), histograms of the PTP distribution can be seen in Supplementary Figure VII. Patient characteristics of both groups are illustrated in Table 1. The baseline characteristics of the 235 patients with incomplete data are available in Supplementary Table I.

Single protein associations

In a single protein analysis, 20 proteins showed a nominally significant ($P<0.01$) association to absence of CAD (Figure 2). Of these, only one protein had a positive odds ratio (CLEC4C, OR = 1.17). The discriminatory value of the significant proteins ranged from an AUC of 0.54 ± 0.03 to 0.56 ± 0.03. Adding any of the individual proteins to the PMRS did not increase the predictive performance significantly above the PMRS baseline (Figure 3). An interactive browser https://dannicad.com/shiny/protein_browser/ allows look-ups of single proteins including their

ability to predict absence of CAD, the improvement in AUC when added to the PMRS model, the effect of age and sex on protein levels, the pQTLs, and the variance explained by genome-wide significant variants.

Discriminatory value of the genetic and proteomic models

Predictions of absence of CAD resulted in AUCs of 0.64 ± 0.03 for the PRS_{CAD} model and 0.58 ± 0.03 for the multi-protein model. Seven proteins were included in this model. Their odds ratios are shown in Figure 4. Among the 7 proteins in the protein model, CLEC4C was the most protective protein and renin was the most detrimental. The PRS_{CAD} model performed significantly better than the protein model (P<0.001). We found that the outputs of the two models were uncorrelated (Pearson's correlation coefficient: -0.04; P=0.13, Supplementary Figure VIII), suggesting that PRS_{CAD} and proteins capture somewhat different aspects of CAD risk. Finally, we tested reduced protein models, by gradually removing proteins from the 7-protein model. AUCs of each model can be seen in Supplementary Figure IX. The AUCs became significantly worse when only 2 proteins remained in the model (P=0.02).

Discriminatory value of the combined models

The PMRS model, developed specifically to predict absence of CAD, resulted in an AUC of 0.76 ± 0.02. This value was consistent with the values reported in the initial study by Fordyce et al. (2017) (c-statistic: 0.71)[4] and in the external validation study by Adamson et al. (2018) (c-statistic: 0.79).[9]

Compared to the PMRS model, a model using only PRS_{CAD} and protein performed significantly worse (AUC: 0.66 ± 0.03, P<0.001). Adding the 7-protein model to the PMRS did not significantly increase the AUC (0.77 ± 0.02, P=0.06). The modest AUC increase could be explained by the low predictive ability of the proteins and correlation between the outputs of the

protein and PMRS models (Pearson's correlation coefficient: 0.09, $P<0.001$, Supplementary Figure VIII). Adding the PRS_{CAD} to the PMRS resulted in a significantly better AUC of 0.79 ± 0.02 ($P<0.001$) as seen in Figure 5. The outputs of the PRS_{CAD} and PMRS models did not correlate (Pearson's correlation coefficient: -0.01, $P=0.57$, Supplementary Figure VIII), which is consistent with other studies of clinical risk factors and various cardiometabolic PRSs (including CAD) reviewed by O'sullivan et al. (2023)[10] A full model (PMRS + PRS_{CAD} + Protein) resulted in an AUC of 0.80 ± 0.02, a significant improvement of 3.86 percentage points, compared to the PMRS model ($P<0.001$; Figure 5) but not significantly better than the PMRS + PRS_{CAD} model ($P=0.28$).

Density plots for each basic model and the full model, stratified by CAD status, are shown in Supplementary Figure X. The full model showed comparable predictive ability for males and females (AUC: 0.78 [95% CI: 0.74-0.81] vs. 0.80 [95% CI: 0.77-0.83], $P=0.20$) and in patients under and over 55 years of age (AUC: 0.78 [95% CI: 0.75-0.82] vs. 0.78 [95% CI: 0.74-0.81], $P=0.63$), which suggested that the model could be applied to both genders and in all ages.

Reclassification potential

To reduce the need for diagnostic testing we assessed whether prediction of absence of CAD could have a clinical application. For each model in Table 2 we performed down-classification of patients with suspected CAD, while maintaining a prevalence of obstructive CAD below 5% in the new low-risk group.

For each model the output score was converted to a binary variable using a model specific threshold and subsequently used to reclassify patients. The full model outperformed all other included models. Using the full model enabled successful down-classification of 324 out of 661 patients (49%) in the 5-15% PTP group and 113 out of 611 patients (18%) in the >15% PTP

group, while 138 out of 168 patients (82.1%) in the ≤5% PTP group remained low-risk, resulting in a new low-risk group containing 575 patients with an obstructive CAD prevalence of 2.3% (Figure 6). Prior to down-classification the obstructive CAD prevalence in the <5% PTP group was 3.0%. Reclassification performance of all models can be seen in Table 2.

Discussion

In this study, we combined genetic information, proteomics, and a clinical prediction model (PMRS) to improve prediction of absence of CAD in a large consecutive population of patients with suspected coronary artery disease. The two main findings were 1) Combining PRS_{CAD} with the PMRS significantly increased the ability to predict absence of CAD in patients with chest pain, with no further improvement by the tested proteins; and 2) From a re-classification perspective, it seems to be most beneficial to use a model including clinical risk factors, genetics and proteomics. To our knowledge, this study is the first to add both the PRS_{CAD} and proteins to a clinical risk model in patients with symptoms of CAD.

Prediction improvement

The AUC improvement of 0.03 from PRS_{CAD} when added to the PMRS was consistent with other studies combining PRS with various clinical risk scores (pooled cohort equation and QRISK3), as seen in e.g., UK Biobank[8,11] and the Malmö Diet and Cancer cohort.[12] Despite the modest increase in predictive ability when adding the PRS_{CAD} and proteins to the PMRS, we were able to further improve reclassification of low-intermediate (5-15% PTP) risk patients from 31% to 49%. Our overall improvement was achieved because of the relatively low initial discriminative ability of the ESC PTP (AUC: 0.71), which allowed the PMRS (AUC: 0.76) to immediately down-classify 31% of the low-intermediate risk patients (Figure 6A), similar to

what was found by Rasmussen et al.[5] The PRS_{CAD} and proteins provided another 4 percentage points to the PMRS AUC, resulting in a total reclassification of 49% of low-intermediate risk patients relative to the initial ESC PTP classification.

Protein associations

Our protein model included seven proteins. Of these proteins, four (MMP12, REN, OPG, and GDF-15) were also identified among the most predictive proteins for plaque absence in a previous study by Bom et al. (2019)[6] Our findings are remarkably consistent with theirs, although they studied a smaller cohort with a larger fraction of patients with more extensive disease. This means that despite differences in sample collection, patient characteristics, the different machine learning techniques used, and having only 3 out of 4 overlapping panels between the studies, we replicated the top proteins. Of note, five proteins (GDF-15, MMP12, OPG, CHI3L1, and KIM1) were also chosen in a previous study as part of a proteomic profile predicting acute myocardial infarction.[13] Also, there is an overlap in proteins (KIM1, GDF-15, REN, OPG) between our model and studies that identified proteins for predicting recurrent atherosclerotic cardiovascular disease[14] and predicting all-cause mortality.[15] Overall, these overlaps suggest that these proteins are markers of pathological processes that are shared among many conditions. Interestingly, our study included the Immune Response panel, which was not included in any of the other studies. In this panel we identified CLEC4C as a marker of absence of CAD; thus, high CLEC4C levels were associated with absence of CAD. CLEC4C (also known as CD303/BDCA-2) is a marker of plasmacytoid dendritic cells, which may play a role in CAD.[16] Consistent with our observation is the fact that the level of plasmacytoid dendritic cells in blood has been shown to be reduced in patients with CAD.[17] CLEC4C might represent a new player in cardiovascular disease, but this hypothesis must be validated in other cohorts.

The relatively weak proteomic signal found in this study can in part be explained by normalizing for sex and age, which are both strong predictors of CAD absence. The non-significant increase in predictive performance achieved by adding proteins may also be explained by the fact that we investigated targeted panels of proteins which have previously been linked to various aspects of CAD, e.g., renin leading to hypertension, which is already included in the PMRS. It is likely that future studies including much larger and more hypothesis-free proteomics panels will yield better predictive signatures for the absence of CAD.

Clinical implications

A direct clinical approach of utilizing a combined proteomics and PRS panel to assess risk in chest pain patients is not available for large scale implementation at present. However, based on our findings, such approach seems feasible and at very low cost when PRS information is readily available from a central database. Such databases are being established in several societies and only require a one-time sampling.

Similarly, the area of proteomics is under rapid development and will likely expand in the number of proteins analyzed and it would also be expected to be obtainable at a much lower price and much faster than today. Most likely a more comprehensive array of proteins will be readily available but whether this will improve the ability to discriminate the absence of CAD remains to be seen.

Strengths and limitations

The main strengths of our study are the large sample size of consecutively enrolled patients with chest pain and the detailed data on findings at downstream CCTA. In addition, a referral bias was unlikely, because the Danish healthcare system is without direct payment for all citizens, and CCTA is the recommended first-line diagnostic test for patients with a low-intermediate PTP of

obstructive CAD. The main study limitation is that we tested our results internally. The optimal solution would be a validation in an independent external cohort. To avoid overfitting, we used stability selection prior to training and retained a subset of data for testing; finally, the results were given as the mean of 100 iterations. Some overfitting may still remain in our own models relative to PMRS and PRS_{CAD} as their weights were predetermined.

Similarly, all re-classification thresholds were based on the intra-cohort prevalence of obstructive CAD, meaning that our re-classification results may be somewhat inflated. This limitation however applies to all models, meaning that their relative performances can still be compared.

This study used targeted proteomics from four Olink® panels, measuring proteins previously implicated in immune response, inflammation, and cardiovascular conditions. This approach was both a strength and a limitation. The strength was that we could compare our results to relevant studies that used the same panels, however this approach may have caused an overlap between the underlying information captured by both clinical risk factors and proteins. Future research should include larger, more hypothesis-free proteome panels to facilitate potential new discoveries and further improvements in risk classifications.

Finally, it is known that a PRS developed in a European population will perform less well in individuals of non-European ancestry.[18] This phenomenon is far less studied in protein risk scores, and it remains to be determined whether our results can be directly extrapolated to other populations.

Conclusions

In conclusion, combining clinical and genetic risk scores with circulating plasma proteins as a supplementary test to the pre-test probability assessment can improve down-classification of

intermediate risk (5-15% PTP) patients, where European and American guidelines differ. This study therefore demonstrated that genetics and proteomics provide additional, complementary information to clinical risk factors. This combinatory approach has the potential to substantially reduce the large number of CCTA scans and likely also other diagnostic tests like e.g., perfusion scans and invasive angiographies showing no CAD.

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Supplemental Materials
Supplemental Methods
Supplemental Tables I
Supplemental Figures I-X
References [19-42]

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Table 1. Baseline characteristics of patients with symptoms suggestive of CAD.

Demographics	Overall (n = 1440)	Absence of CAD (n = 675)	CAD (n = 765)
Age, years	57 ± 9	54 ± 8	60 ± 8
Males	692 (48%)	249 (37%)	443 (58%)
Risk factors, n (%)			
Family history	525 (36%)	227 (34%)	298 (39%)
Current smoker	226 (16%)	93 (14%)	133 (17%)
Body mass index, kg/m^2 *	27 ± 4	27 ± 4	27 ± 4
Type 2 diabetes	88 (6%)	26 (4%)	62 (8%)
Hypertension	517 (36%)	187 (28%)	330 (43%)
Dyslipidemia †	352 (24%)	111 (16%)	241 (32%)
Type of chest pain, n (%)			
Typical angina	385 (27%)	164 (24%)	221 (29%)
Atypical angina	493 (34%)	244 (36%)	249 (33%)
Non-specific chest discomfort	266 (18%)	145 (21%)	121 (16%)
Dyspnea	296 (21%)	122 (18%)	174 (23%)
Laboratory tests			
Total cholesterol, mmol/L *	5.4 ± 1.1	5.3 ± 1.0	5.4 ± 1.2
LDL cholesterol, mmol/L *	3.3 ± 1.0	3.2 ± 0.9	3.3 ± 1.0
HDL cholesterol, mmol/L	1.5 ± 0.4	1.5 ± 0.5	1.5 ± 0.4
Triglyceride, mmol/L *	1.3 [1.0 - 2.0]	1.3 [0.9 - 1.9]	1.4 [1.0 - 2.1]
Troponin, ng/L ‡	6.0 [4.0 - 11.0]	6.0 [4.0 - 12.0]	6.0 [4.0 - 10.0]
Creatinine, µmol/L	75.3 ± 14	73.3 ± 13	77.0 ± 14.6
eGFR <60 mL/min, n (%)	66 (5%)	25 (4%)	41 (5%)
CRP ≥2.5 mg/L, n (%) ‡	379 (26%)	166 (25%)	213 (28%)
Other			
Systolic blood pressure, mm Hg *	139 ± 19	136 ± 18	141 ± 19
Diastolic blood pressure, mm Hg *	83 ± 11	82 ± 11	84 ± 11
PROMISE minimal risk score	0.20 [0.10 - 0.34]	0.30 [0.17 - 0.44]	0.13 [0.07 - 0.23]
Pre-test probability of CAD	0.12 [0.06 - 0.22]	0.10 [0.06 - 0.16]	0.17 [0.11 – 0.26]

Values are the mean ± standard deviation (for normally distributed data), the number (%), or the median [interquartile range], as indicated. * Missing values were observed in: BMI n=8, total cholesterol n=6, LDL cholesterol n=7, triglyceride n=10, systolic and diastolic blood pressures n=3; † Dyslipidemia was defined as cholesterol treatment at arrival; ‡ Values below the LOD were observed in troponin n=741 and CRP n=34. Abbreviations: CAD, coronary artery disease; LDL, low density lipoprotein; HDL, high density lipoprotein; eGFR, estimated glomerular filtration rate; CRP, C-reactive protein; PROMISE, Prospective Multicenter Imaging Study for Evaluation of chest pain; LOD, limit of detection.

Table 2. Re-classification performance

Model	Threshold	≤5% PTP patients remaining low-risk	5-15% PTP patients down-classified	>15% PTP patients down-classified	Patients in new low-risk group	Patients with obstructive CAD in new low-risk group
PRS_{CAD}	<30	12 (7.1%)	47 (7.1%)	45 (7.4%)	104	1 (1.0%)
Protein	>85	2 (1.2%)	12 (1.8%)	13 (2.1%)	27	0 (0.0%)
Protein + PRS_{CAD}	>65	69 (41.1%)	221 (33.4%)	210 (34.4%)	500	16 (3.2%)
PMRS	>60	123 (73.2%)	206 (31.2%)	24 (3.9%)	353	11 (3.1%)
PMRS + protein	>70	69 (41.1%)	109 (16.5%)	9 (1.5%)	187	3 (1.6%)
PMRS + PRS_{CAD}	>55	140 (83.3%)	325 (49.2%)	85 (13.9%)	550	14 (2.5%)
Protein + PMRS + PRS_{CAD}	>55	138 (82.1%)	324 (49.0%)	113 (18.5%)	575	13 (2.3%)

CAD, coronary artery disease; PRS_{CAD}, polygenic risk score for CAD; PMRS, PROMISE minimal risk score; PTP, Pre-test probability of CAD. For each model, the score was divided in bins of 5, setting the threshold at the lowest bin, which maintained an obstructive CAD prevalence ≤5%.

Figure Legends:

Figure 1. Overview of the Dan-NICAD 1 cohort. 1440 patients in total, of which 675 had no coronary artery disease (CAD) and 765 had some level of CAD. Patients with symptoms suggestive of obstructive CAD (n=350) were referred for invasive coronary angiography (ICA) with fractional flow reserve (FFR), where 141 patients were found to have obstructive disease. CACS: Coronary artery calcium score; CTA: Computed tomography angiography.

Figure 2. Volcano plots with odds ratio (OR) for absence of CAD on the x-axis and -log10(*P* value) on the y-axis for each protein stratified by Olink protein panel. Nominally significant proteins ($P<0.01$) are shown in either red (OR < 1, predicting disease in the coronary arteries) or blue (OR > 1, predicting absence of CAD).

Figure 3. Area under the curve (AUC) for each nominally significantly ($P<0.01$) associated single protein (white dots) and added to the PROMISE minimal risk score (PMRS) (black dots). The vertical dashed line at 0.76 indicates the AUC of the PMRS without any proteins. Error bars indicated 95% confidence interval.

Figure 4. Protein model odds ratio for absence of coronary artery disease (CAD). Average odds ratios (OR) for each protein in the protein model across 100 iterations of training data are shown. Error bars indicate standard deviation. Proteins with an OR > 1 predict absence of CAD and are shown in blue, proteins with an OR < 1 predict disease in the coronary arteries and are shown in red.

Figure 5. Diagnostic performance of prediction models in test sets. Areas under the curve (AUCs) of the polygenic risk score (PRS_{CAD}) model, the protein model, the PROMISE minimal risk score (PMRS) and four combined models. Error bars indicate standard deviation. The vertical dashed line indicates the AUC of the PMRS model (0.76), which was used as the baseline for comparisons. ns: non-significant; *** $P<0.001$

Figure 6. Re-classification of all patients using the PMRS model (A) and full model (B). A) In the ≤5% PTP group 123 patients (73.2%) remained low-risk, in the >5-15% PTP group 206 patients (31%) were down-classified and in the >15% PTP group 24 patients (4%) were down-classified to low-risk. B) In the ≤5% PTP group 138 patients (82.1%) remained low-risk, in the >5-15% PTP group (N = 661) 324 patients (49%) were down-classified and in the >15% PTP group (N = 611) 113 patients (18%) were down-classified to low-risk.