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RESEARCH LETTER

Biomarker Correlates of Coronary Microvascular Dysfunction in Heart Failure With Preserved Ejection Fraction

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Reduced coronary flow reserve (CFR), reflecting coronary microvascular dysfunction, is common in heart failure with preserved ejection fraction (HFpEF)[1] and can be caused by a number of factors, including extrinsic compression resulting from myocardial interstitial fibrosis, myocardial hypertrophy with insufficient microvascular supply, diffuse atherosclerosis, coronary microvascular inflammation, and elevated left ventricular (LV) filling pressures. Circulating biomarker correlates of CFR may provide important insights into underlying mechanisms of coronary microvascular dysfunction in HFpEF.

Our aim was to uncover a biomarker profile specific to reduced CFR in HFpEF that is independent of myocardial hypertrophy, elevated cardiac filling pressures, atrial fibrillation, and epicardial coronary artery disease. Among 192 with blood samples available of the original 202 patients with a validated diagnosis of HFpEF (LV ejection fraction ≥40% without unrevascularized epicardial coronary artery disease) in the prospective multinational PROMIS-HFpEF study (Prevalence of Microvascular Dysfunction in HFpEF),[1] we measured 265 biomarkers using high-throughput proximity extension assays (Olink Proseek Multiplex CVD II and III, and inflammation 96×96 kits). We excluded 23 biomarkers that had >15% of values below the detection limit, leaving us with a total of 242 unique biomarkers for further analyses. CFR was measured with adenosine stress transthoracic Doppler echocardiography, and coronary microvascular dysfunction was defined as CFR <2.5. To assess the association between CFR and biomarkers, we used lasso penalized regression analyses including all biomarkers, age, sex, body mass index, creatinine, and study site. Multivariable regression analyses were performed to determine whether associations between biomarkers and CFR (as a continuous variable) were independent of smoking, LV mass index (cardiac hypertrophy), E/e' (elevated LV filling pressures), and history of atrial fibrillation, revascularized coronary artery disease, and hypertension. Protein–protein interaction networks were generated with the Genemania plug-in in Cytoscape version 3.6.1. Pathway overrepresentation analyses used data from the Gene Ontology network with a false discovery rate–corrected value of $P<0.05$ and the 242 biomarkers plus additional markers identified by Genemania as background. All analyses were performed with R version 3.4.0. A 2-sided value of $P<0.05$ was considered statistically significant, with correction for multiple testing by the Benjamini–Hochberg method. All study participants gave written informed consent, and the institutional review board at each of the participating sites approved the study. The PROMIS-HFpEF study complies with the Declaration of Helsinki.

In total, 13 biomarkers were selected for their association with CFR after lasso regression analysis (penalized $\beta>0$; Figure [A]), of which growth differentiation factor 15, osteoprotegerin, pulmonary surfactant-associated protein, angiotensin-converting enzyme 2, and insulin growth factor binding protein 1 showed

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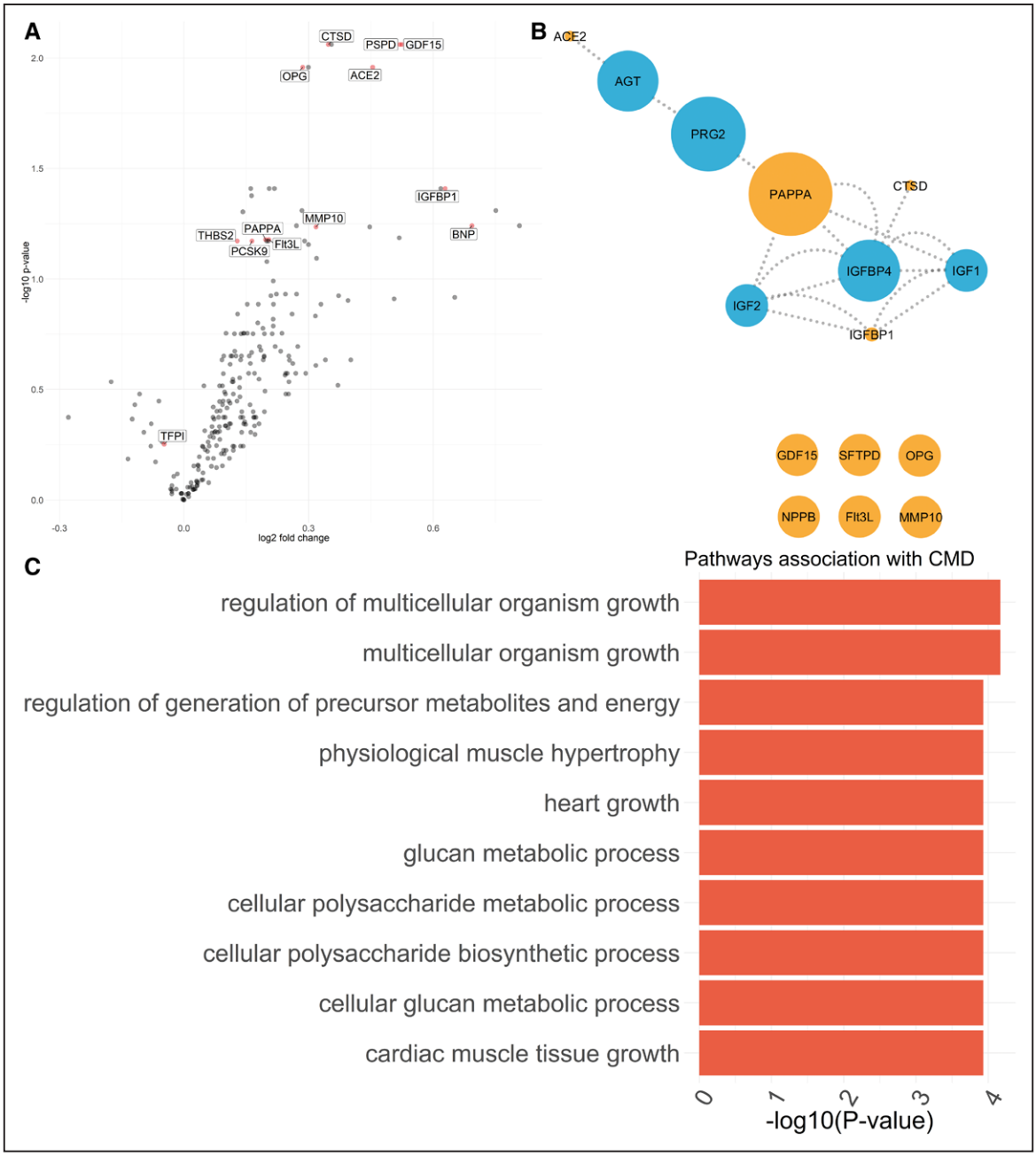

Figure. Biomarkers associated with coronary microvascular dysfunction in PROMIS.
A, Volcano plot showing biomarkers selected by lasso penalized regression analyses (red). The *y* axis shows the –log10 of the false discovery rate–corrected *P* value for the association of each individual biomarker with coronary microvascular dysfunction (coronary flow reserve <2.5) vs no coronary microvascular dysfunction. The *x* axis shows the log2 of the fold change of biomarker difference between patients with and without coronary microvascular dysfunction. **B**, Results of network analysis for the biomarkers independently associated with coronary microvascular flow reserve (orange) and those predicted in the network (blue). The size of the node reflects the edge betweenness of each biomarker. **C**, Bar graph depicting the top 10 overrepresented pathways with the –log10 of the false discovery rate–corrected *P* value on the *x* axis. ACE2 indicates angiotensin-converting enzyme 2; AGT, angiotensinogen; BNP, brain natriuretic protein; CMD, coronary microvascular dysfunction; CTSD, cathepsin D; Flt3L, *FMS-like tyrosine kinase 3* ligand; GDF15, growth differentiation factor 15; IGF, insulin growth factor; IGFP, insulin growth factor binding protein; MMP10, matrix metalloproteinase 10; NPPB, natriuretic peptide precursor B; OPG, osteoprotegerin; PAPP-A, pregnancy-associated plasma protein A; PCSK9, proprotein convertase subtilisin/kexin type 9; PRG2, *proteoglycan 2;* PSPD, pulmonary surfactant-associated protein; SFTPC, surfactant protein D; THBS2, thrombospondin 2; and TFPI, tissue factor pathway inhibitor.

the strongest associations. Two of these, tissue factor pathway inhibitor (*P*=0.18) and proprotein convertase subtilisin/kexin type 9 (*P*=0.20), lost significance in multivariable regression analyses. Of the 11 remaining biomarkers, 5 (in orange) were hits in our network (Figure [B]), which was then enriched with additional protein-protein interactions (blue). Pregnancy-associated plasma protein A (PAPP-A) formed an important hub in the network, suggesting greater biological importance, with similar results for PAPP-A when the analyses were restricted to patients with LV ejection fraction ≥50%. When the network is translated into biological

pathways, those relating to cellular metabolism and physiological muscle hypertrophy were overrepresented (Figure [C]). Results were unchanged when we excluded biomarkers with either 10% or 20% of measurements below the limit of detection.

PAPP-A emerged as a novel key hub in the network associated with reduced CFR in HFpEF, and it has been shown to be elevated in patients with unstable atherosclerosis and to be a predictor of cardiovascular events in patients with acute coronary syndrome through extracellular matrix degradation and interaction with insulin growth factor-1.[2] Our findings extend the current paradigm of coronary microvascular dysfunction in HFpEF by suggesting that beyond microvascular inflammation,[3] subclinical atherosclerosis may also play an important role. Limitations of this study include possible selection bias with regard to proteins measured and lack of data on how circulating proteins relate to proteins at the tissue level.

In HFpEF, circulating biomarker profiles suggest that coronary microvascular dysfunction is related to subclinical atherosclerosis (via the PAPP-A pathway), potentially leading to cardiac hypertrophy and metabolic abnormalities.

ARTICLE INFORMATION

Data availability: The data, analytical methods, and study materials will not be made available to other researchers for purposes of reproducing the results or replicating the procedure.

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