JOURNAL OF THE AMERICAN COLLEGE OF CARDIOLOGY
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AMERICAN COLLEGE OF CARDIOLOGY FOUNDATION

VOL. 72, NO. 10, 2018

Identifying Pathophysiological Mechanisms in Heart Failure With Reduced Versus Preserved Ejection Fraction

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ABSTRACT

BACKGROUND Information on the pathophysiological differences between heart failure with reduced ejection fraction (HFrEF) versus heart failure with preserved ejection fraction (HFpEF) is needed

OBJECTIVES The purpose of this study was to establish biological pathways specifically related to HFrEF and HFpEF.

METHODS The authors performed a network analysis to identify unique biomarker correlations in HFrEF and HFpEF using 92 biomarkers from different pathophysiological domains in a cohort of 1,544 heart failure (HF) patients. Data were independently validated in 804 patients with HF. Networks were enriched with existing knowledge on protein–protein interactions and translated into biological pathways uniquely related to HFrEF, HF with a midrange ejection fraction, and HFpEF.

RESULTS In the index cohort (mean age 74 years; 34% female), 718 (47%) patients had HFrEF (left ventricular ejection fraction [LVEF] <40%) and 431 (27%) patients had HFpEF (LVEF ≥50%). A total of 8 (12%) correlations were unique for HFrEF and 6 (9%) were unique to HFpEF. Central proteins in HFrEF were N-terminal B-type natriuretic peptide, growth differentiation factor-15, interleukin-1 receptor type 1, and activating transcription factor 2, while central proteins in HFpEF were integrin subunit beta-2 and catenin beta-1. Biological pathways in HFrEF were related to DNA binding transcription factor activity, cellular protein metabolism, and regulation of nitric oxide biosynthesis. Unique pathways in patients with HFpEF were related to cytokine response, extracellular matrix organization, and inflammation. Biological pathways of patients with HF with a midrange ejection fraction were in between HFrEF and HFpEF.

CONCLUSIONS Network analysis showed that biomarker profiles specific for HFrEF are related to cellular proliferation and metabolism, whereas biomarker profiles specific for HFpEF are related to inflammation and extracellular matrix reorganization. (The BIOlogy Study to TAilored Treatment in Chronic Heart Failure [BIOSTAT-CHF]; EudraCT 2010-020808-29) (J Am Coll Cardiol 2018;72:1081–90) © 2018 Published by Elsevier on behalf of the American College of Cardiology Foundation.

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ISSN 0735-1097/$36.00 https://doi.org/10.1016/j.jacc.2018.06.050

ABBREVIATIONS AND ACRONYMS

GDF = growth differentiation factor

HFmrEF = heart failure with a mid-range ejection fraction

HFpEF = heart failure with a preserved ejection fraction

HFrEF = heart failure with a reduced ejection fraction

IL1RL1 = interleukin-1 receptor-like type 1

ITGB2 = integrin subunit beta 2

NT-proBNP = N-terminal pro-B-type natriuretic peptide

Heart failure with reduced ejection fraction (HFrEF) and heart failure with preserved ejection fraction (HFpEF) were originally considered to be 2 extremes of the same disease. However, where angiotensin-converting enzyme inhibitors (ACEis), angiotensin receptor blockers (ARBs), and mineralocorticoid receptor antagonists are associated with improved clinical outcome in patients with HFrEF (1–3), no such benefit was seen in patients with HFpEF (4–6). The underlying pathophysiology is currently considered to be different between HFrEF and HFpEF (7–11).

The current paradigm on the underlying pathophysiology of HFpEF suggests that a proinflammatory state is responsible for stiffening of the heart muscle and increased filling pressures (7). Indeed, Paulus et al. (7) suggested that the plethora of comorbidities that usually affect patients with HFpEF cause low-level inflammation, which affects the coronary vascular endothelium and reduces nitric oxide bioavailability. Their hypothesis suggests that this directly affects the cardiomyocytes and causes cellular hypertrophy as well as cardiac stiffening (7,12).

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Network analysis is a tool to gain novel insights in disease pathways and pathophysiology by studying protein-protein (biomarker-biomarker) correlations (9,10,13). By enriching experimentally found protein biomarker networks with knowledge-based protein-protein interactions, empirically found correlations can be placed in the context of known pathways (14,15). We therefore performed a network analysis enriched by knowledge-based interactions to uncover biological mechanisms that are unique for patients with HFrEF and HFpEF.

METHODS

PATIENT POPULATION. We studied patients from the BIOSTAT-CHF (BIOlogy Study to TAilored Treatment in Chronic Heart Failure) project, which is described elsewhere (16–20). In brief, BIOSTAT-CHF includes 2 cohorts of patients with heart failure (HF) included in Scotland and Europe. The aim of the BIOSTAT-CHF study was to characterize biological pathways related to response/no-response to guideline-recommended pharmacological therapy for HF. Therefore, patients had to be suboptimally treated at inclusion. We used the Scottish cohort of the BIOSTAT-CHF study as our primary study cohort and the European cohort of the BIOSTAT-CHF study as our validation cohort because this was a less-selected population. The Scottish cohort consisted of 1,738 patients from 6 centers in Scotland, United Kingdom. Patients were required to be ≥18 years of age, diagnosed with HF, and previously admitted with HF requiring diuretic treatment. Biomarkers were measured in 1,707 of the total of 1,738 patients. From these patients, echocardiography was available in 1,544 patients. We validated our findings in the European cohort of the BIOSTAT-CHF study, which originally consisted of 2,516 patients with HF from 69 centers in 11 European countries. Inclusion criteria for the European cohort include: >18 years of age and having symptoms of new-onset or worsening HF confirmed either by a LVEF of ≤40% or B-type natriuretic peptide and/or N-terminal pro-B-type natriuretic peptide (NT-proBNP) plasma levels >400 or >2,000 ng/l, respectively. Because of this difference in inclusion criteria for patients with LVEF >40%, we excluded all patients with HFrEF and an NT-proBNP

Support was also provided by the Netherlands Cardiovascular Research Initiative: an initiative with support of the Dutch Heart Foundation, CVON2014-11 RECONNECT. Dr. Westenbrink has received consulting and speaker fees from Boehringer Ingelheim and Bayer; and has received a travel grant from Novartis. Dr. van Veldhuisen has received board membership fees or travel expenses from Novartis, Johnson & Johnson, and Vifor. Dr. Metra has received consulting honoraria from Amgen, AstraZeneca, Bayer, Novartis, Relypsa, Servier, Stealth Therapeutics, and Trevena; and speaker fees from Abbott Vascular, Novartis, and Servier. Dr. Anker has received grants from Vifor and Abbott Vascular; and fees for consultancy or speaking from Vifor, V-Wave, Impulse Dynamics, Bayer, Boehringer Ingelheim, Brahms, Janssen, Novartis, Servier, Stealth Peptides, and ASTRA. Dr. Cleland has served on the advisory board for Amgen, Novartis, and Medtronic; and has received research grants from Amgen, Medtronic, Novartis, Philips, Stealth Biotherapeutics, and Bristol-Myers Squibb. Dr. Filippatos has received committee fees and/or research grants from Novartis, Bayer, Medtronic, Vifor, and Servier; has received a research grant from the European Union; and is a member of the steering committee of trials sponsored by Novartis and Servier. Dr. Lang has received consultancy fees and/or research grants from Amgen, AstraZeneca, Merck Sharp & Dohme, Novartis, and Servier. Dr. Voors has received consultancy fees and/or research grants from Alere, Amgen, Bayer, Boehringer Ingelheim, Cardio3Biosciences, Celladon, GlaxoSmithKline, Merck/Merck Sharp & Dohme, Novartis, Servier, Stealth Peptides, Singulex, Sphingotec, Trevena, Vifor, and ZS Pharma. All other authors have reported that they have no relationships relevant to the contents of this paper to disclose.

Manuscript received May 3, 2018; revised manuscript received June 15, 2018, accepted June 18, 2018.

TABLE 1 Baseline Characteristics of the Scottish Cohort Across LVEF Categories

	HFrEF (n = 718)	HFmrEF (n = 395)	HFpEF (n = 431)	p Value-$_{trend}$
Demographics				
Age, yrs	72.0 ± 10.9	74.9 ± 10.0	76.2 ± 9.9	**<0.001**
Female	188 (26.2)	137 (34.7)	187 (43.4)	**<0.001**
BMI, kg/m^2	28.2 ± 6.0	28.9 ± 5.9	30.0 ± 6.8	**<0.001**
SBP, mm Hg	122.7 ± 21.3	127.3 ± 22.3	129.9 ± 23.3	**<0.001**
DBP, mm Hg	69.8 ± 12.3	68.5 ± 13.1	68.0 ± 13.7	0.006
NYHA functional class				
I	6 (0.8)	5 (1.3)	4 (0.9)	**<0.001**
II	337 (46.9)	160 (40.6)	136 (31.6)	
III	300 (41.8)	176 (44.7)	206 (47.8)	
IV	75 (10.4)	53 (13.5)	85 (19.7)	
LVEF, %	30.1 ± 7.3	43.7 ± 2.8	57.3 ± 6.0	**<0.001**
Heart rate, beats/min	73.9 ± 16.5	72.3 ± 16.4	75.0 ± 15.8	0.172
Comorbidities				
Anemia	316 (44.4)	142 (36.0)	199 (46.4)	0.001
Diabetes mellitus	212 (29.6)	133 (34.0)	158 (36.9)	0.009
COPD	110 (15.5)	61 (15.6)	110 (25.6)	**<0.001**
Hypertension	363 (50.8)	249 (63.2)	293 (68.0)	**<0.001**
PVD	144 (20.5)	88 (23.0)	116 (27.7)	0.007
Stroke	117 (16.5)	84 (21.5)	84 (19.6)	0.138
Atrial fibrillation on ECG	199 (27.7)	136 (34.4)	162 (37.6)	<0.001
PCI	132 (18.5)	80 (20.5)	74 (17.3)	0.713
CABG	137 (19.1)	86 (21.8)	62 (14.4)	0.089
Laboratory				
NT-proBNP, ng/l	1,672 (667–4,615)	1,209.5 (428.0–2,942.0)	1,062 (392–2,820)	**<0.001**
eGFR, ml/min/1.73 m^2	59.8 (43.3–77.4)	59.7 (42.1–76.6)	58.4 (42.0–76.0)	0.310
Urea, mmol/l	8.6 (6.7–12.3)	8.6 (6.6–11.2)	8.6 (6.4–11.7)	0.289
Hemoglobin, g/dl	13.6 (4.9)	13.5 (6.6)	13.1 (7.6)	<0.001
Medication				
ACEi/ARB	538 (74.9)	274 (69.4)	268 (62.2)	**<0.001**
Beta-blocker	570 (79.4)	293 (74.2)	257 (59.6)	**<0.001**
MRA	295 (41.1)	109 (27.6)	85 (19.7)	**<0.001**
Diuretic agents	712 (99.2)	391 (99.0)	425 (98.6)	0.375

Values are mean ± SD, n (%), or median (interquartile range). **Bold** indicates $p < 0.05$.

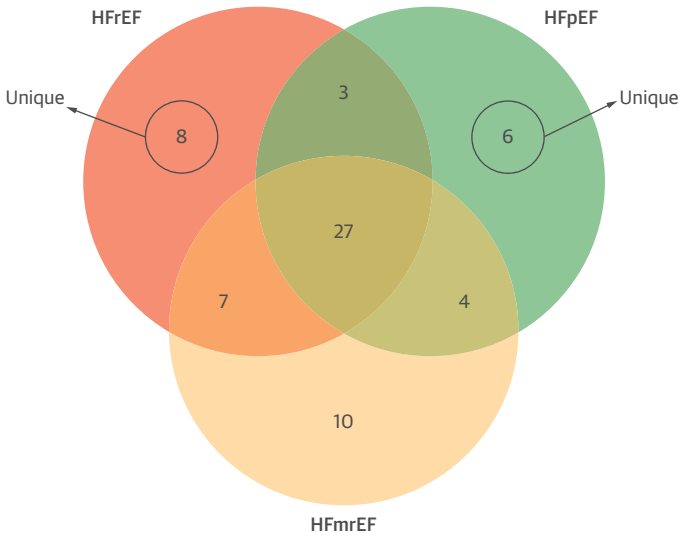
ACEi = angiotensin-converting enzyme inhibitor; ARB = angiotensin-receptor blocker; BMI = body mass index; CABG = coronary artery bypass grafting; COPD = chronic obstructive pulmonary disease; DBP = diastolic blood pressure; ECG = electrocardiography; eGFR = estimated glomerular filtration rate; LBBB = left bundle branch block; LVEF = left ventricular ejection fraction; LVH = left ventricular hypertrophy; MRA = mineralocorticoid receptor antagonist; NYHA = New York Heart Association; NT-proBNP = N-terminal pro-B-type natriuretic peptide; PCI = percutaneous coronary intervention; PVD = peripheral vascular disease; SBP = systolic blood pressure.

level of <2,000 ng/l or patients with HFrEF and no available NT-proBNP levels (Online Figure 1). In total, the European cohort consisted of 808 patients with HF with biomarkers available in all patients. All patients needed to be treated with loop diuretics but had not been previously treated with an ACEi/ARB and/or beta-blocker, or they were receiving ≤50% of the target doses of these drugs at the time of inclusion and anticipated initiation or up-titration of ACEi/ARBs and beta-blockers.

Patients in both cohorts were suboptimally treated with ACEi/ARBs and/or beta-blockers, and anticipated initiation or up-titration of ACEi/ARBs and beta-blockers to ESC-recommended target doses (21). Furthermore, all patients were enrolled with worsening signs and symptoms of HF as in patients or from outpatient clinics (16). To adequately characterize biomarker profiles in patients with HFrEF and HFpEF, we investigated biomarker profiles unique to patients with HFrEF and HFpEF, which showed no overlap with HFmrEF. HFrEF was defined as having an LVEF of <40%, HFmrEF was defined as having an LVEF of 40% to 49%, and HFpEF was defined as having an LVEF of ≥50%.

CLINICAL AND BIOMARKER MEASUREMENTS. Medical history, current use of medications, and a physical examination were all recorded at baseline. Standard echocardiography was strongly recommended, but not mandatory for study inclusion. In the combined

FIGURE 1 Venn Diagram With Unique Protein-Protein Correlations in HFrEF, HFmrEF, and HFpEF



In total, 6 unique protein–protein correlations were identified in HFpEF, while 8 unique protein-protein correlations were identified in HFrEF. HFmrEF = heart failure with a midrange ejection fraction; HFpEF = heart failure with a preserved ejection fraction; HFrEF = heart failure with a reduced ejection fraction.

cohorts, more than 80% of echocardiography were performed within 1 year before inclusion, with more than 70% of echocardiographies performed within 3 months. The timing of echocardiography was similar across HFrEF and HFpEF in both the Scottish and European cohorts.

A large biomarker panel with 92 biomarkers from a wide range of pathophysiological domains were measured in the Scottish and European cohorts. An overview of biomarkers and their pathophysiological function are presented in Online Table 1. Assay characteristics are presented in Online Table 2. Ninety-two proteins were measured using a high-throughput technique using the Olink Proseek Multiplex CVD III^{96X96} kit (Olink Proteomics, Uppsala, Sweden), which measures cardiovascular-related proteins simultaneously in 1-μl plasma samples (22). The kit uses proximity extension assay technology, where 92 oligonucleotide-labeled antibody probe pairs are allowed to bind to their respective target present in the sample. Proximity extension assay is a homogeneous assay that uses pairs of antibodies equipped with DNA reporter molecules. When binding to their correct targets, they give rise to new DNA amplicons, each ID-barcoding its respective antigen. The amplicons are subsequently quantified using a Fluidigm BioMark HD real-time PCR platform. Four internal controls and 2 external controls (in triplicate) are included in the assay. The laboratory operators were blinded to all information regarding the study population.

STATISTICAL ANALYSIS. A test for trend was performed to investigate trends in baseline characteristics across HFrEF, HFmrEF, and HFpEF. An in-depth description of the methods used for network analysis can be found in the Online Appendix. In brief, we performed network analysis using unique pairwise correlations between proteins (biomarkers) within HFrEF, HFmrEF, and HFpEF. We retained only those biomarkers that passed the p value cutoff point following multiple comparisons correction. The p value cutoff point was based on the number of principal components following principal component analyses, which determined >95% of the variance among the biomarkers in the separate cohorts (10). A total of 51 PCs, of which the eigenvalues cumulatively explained >95% of the variation observed in the discovery dataset, were found. To correct for multiple comparison for interbiomarker correlations, $0.05/([PC \times PC - 1]/2)$ was used for the adjusted p cutoff value, where PC is the number of principal components found. This procedure was repeated for the independent European cohort. Here, 50 PCs explained >95% of the variance in the biomarkers. Following, only pairwise correlations were retained that occurred in both the discovery as well as validating cohort. In sensitivity analyses, we tested whether biomarker-biomarker correlations were dependent on NT-proBNP levels by performing separate analyses in patients with NT-proBNP levels above and below 2,000 ng/l in the Scottish cohort. Furthermore, in additional sensitivity analyses, we tested whether biomarker-biomarker correlations were similar between patients with HFrEF from the European cohort and patients with HFrEF who were excluded based on missing NT-proBNP values or NT-proBNP values below the 2,000 ng/l cutoff point in our European cohort. Last, as an additional sensitivity analysis, we repeated our analyses in patients with HFrEF, HFmrEF, or HFpEF included from the outpatient and inpatient settings alone. To explore whether performing correlation analyses was suitable for our network analyses, we compared the R^2 values to mutual information values according to Steuer et al. (23). Due to the difference in n of HFrEF, HFmrEF, and HFpEF, correlations retained after a p value cutoff point had a lower mean R^2 compared to correlations retained in HFmrEF and HFpEF (Online Figure 1). To make the correlation networks comparable, an additional cutoff was applied, based on the

correlation strength (R^2). To tune the cutoff parameter, the lowest cutoff was chosen that reduced the relation between sample size and R^2, while still retaining a reasonable number of correlations. Online Figure 2 shows the relation between number of correlations and sample size for 6 different R^2 cutoffs. Based on these observations, a cutoff of R^2 >0.2 was chosen. Following, we identified unique correlations between biomarkers for HFrEF and HFpEF, which showed no overlap with HFmrEF and enriched these using knowledge-based protein interactions from a comprehensive list of sources (Online Table 3). We then performed pathway over-representation analysis to examine over-represented pathways in HFrEF and HFpEF.

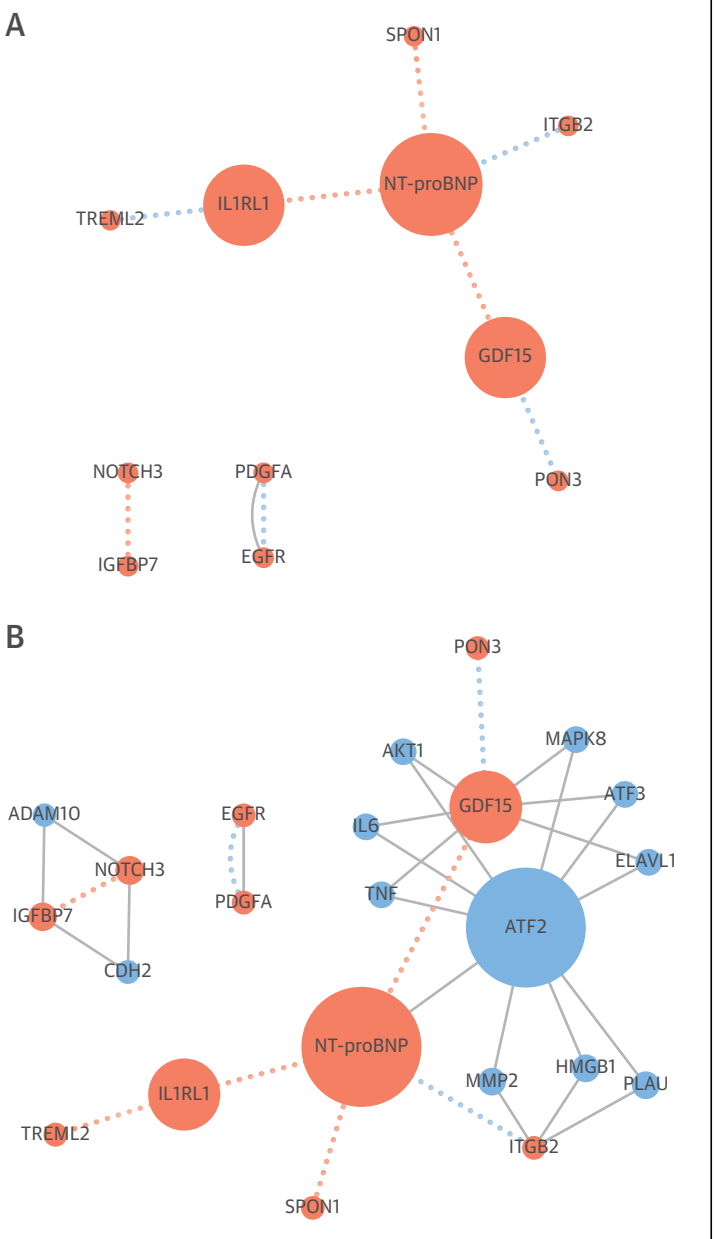
RESULTS

BASELINE CHARACTERISTICS. Baseline characteristics are presented in **Table 1**. Overall, patients had a mean age of 73.7 ± 10.7 years, and 34.2% were women. Of a total of 1,544 patients, 718 (47%) had HFrEF, 395 (26%) had HFmrEF, and 431 (28%) had HFpEF. With increasing LVEF, patients were older; were more often female; had higher rates of diabetes, chronic obstructive pulmonary disease, hypertension, and atrial fibrillation on electrocardiography; were less often on ACEi/ARBs and mineralocorticoid receptor antagonists; and had lower levels of NT-proBNP.

Patients from the European cohort had higher NT-proBNP levels (5,122 ng/l vs. 1,334 ng/l); other characteristics were generally comparable (Online Table 4). Differences between patients according to LVEF strata in the European cohort are presented in Online Table 5.

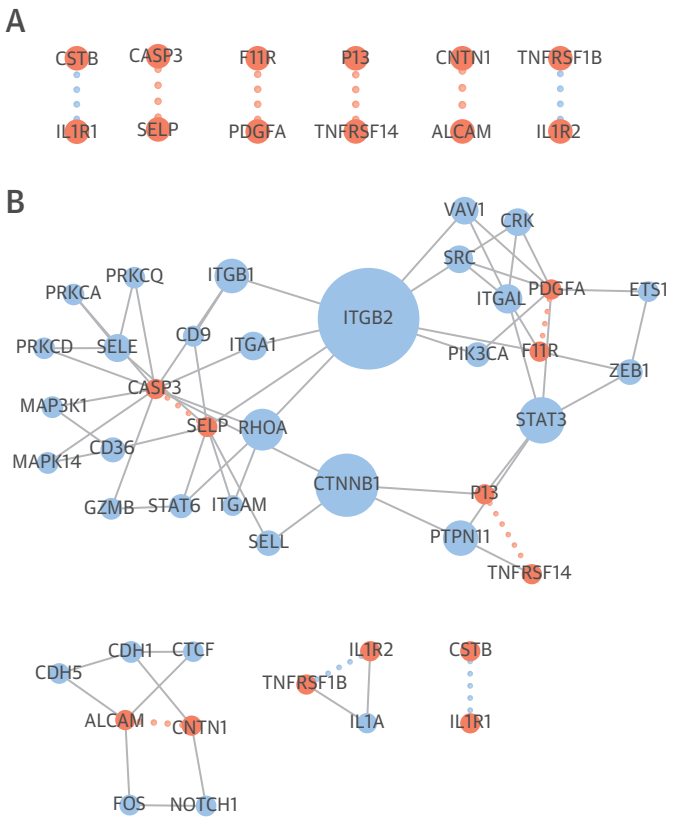
NETWORK ANALYSIS. To investigate differences in biomarker profiles between HFrEF and HFpEF, pairwise correlations were extracted that passed a p value cutoff point corrected for multiple comparisons. We found no high R^2 values with low mutual information values, which suggests that Pearson correlation analyses is suitable (Online Figure 3). We studied unique correlation for HFrEF and HFpEF, which showed no overlap with HFmrEF. These pairwise comparisons reflect potential interacting proteins within HFrEF and HFpEF. In total, 65 biomarker correlations passed the p value cutoff point in HFrEF, HFmrEF, and HFpEF in both the Scottish and European cohorts (**Figure 1**). Of these, 45 biomarker correlations passed the p value cutoff point in HFrEF and could be

FIGURE 2 Network Analysis Depicting Unique Protein–Protein Correlations in HFrEF With Knowledge-Based Interactions



(A) Unique protein–protein correlations in heart failure with a reduced ejection fraction (HFrEF) with **(B)** knowledge-based interactions. **Orange nodes** are derived from data, and **blue nodes** are knowledge-based correlations. The size of the node corresponds to the betweenness-centrality, which signified the importance of the node in the network. The larger the node, the more important it is to the network. The edges **(dotted lines)** between the nodes represent the correlation coefficient, which is either positive **(orange)** or negative **(blue)** for empirically derived correlations **(orange nodes)**. In case of knowledge-based nodes **(blue)**, the **line** signifies a protein–protein interaction.

FIGURE 3 Network Analysis Depicting Unique Protein-Protein Correlations in HFpEF With Knowledge-Based Interactions



(A) Unique protein-protein correlations in heart failure with a preserved ejection fraction (HFpEF) with **(B)** knowledge-based interactions. **Orange nodes** are derived from data, and **blue nodes** are knowledge-based correlations. The size of the node corresponds to the betweenness-centrality, which signified the importance of the node in the network. The larger the node, the more important it is to the network. The edges **(dotted lines)** between the nodes represent the correlation coefficient which is either positive **(orange)** or negative **(blue)** for empirically derived correlations **(orange nodes)**. In case of knowledge-based nodes **(blue)**, the line signifies a protein-protein interaction.

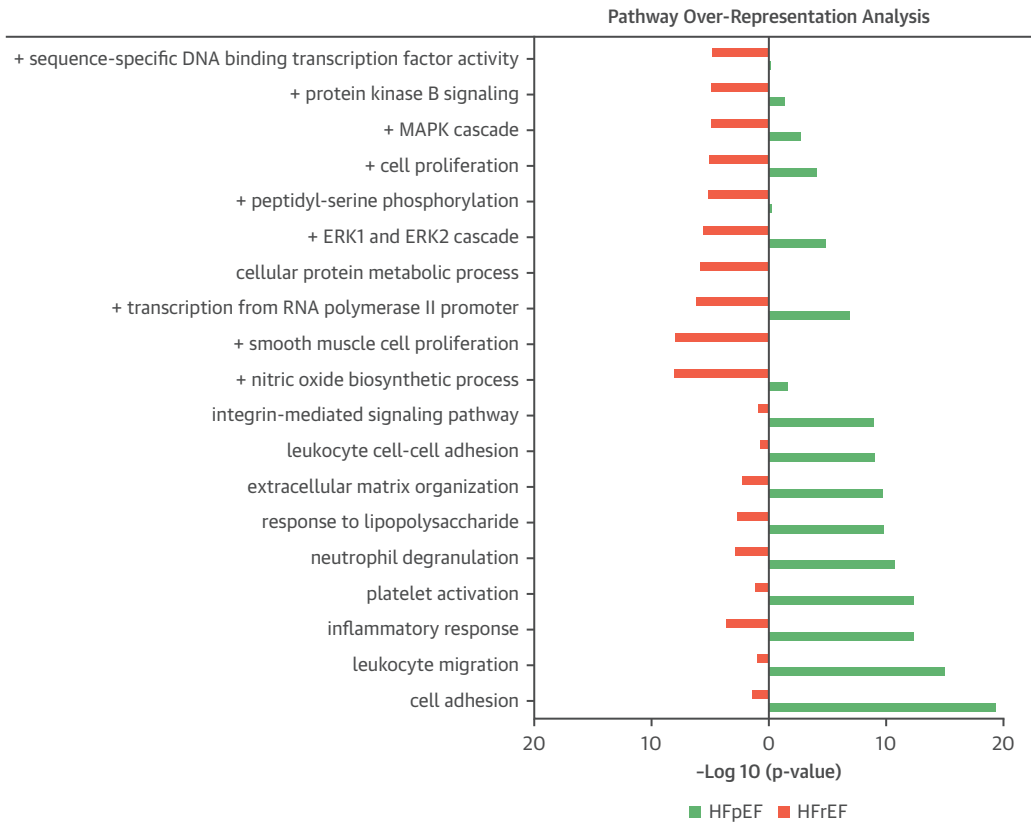
successfully validated in the European cohort. Of these 45 significant correlations, 8 were unique to HFrEF alone (Figure 1). Patients with HFpEF showed 40 significant correlations that could be successfully validated; of the total 40 correlations, 6 were exclusive to HFpEF (Figure 1). There was considerable overlap between HFrEF, HFmrEF, and HFpEF with a total of 27 significant correlations that were shared. In sensitivity analyses, biomarker-biomarker correlations were independent of timing of echocardiography and were similar in both patients with NT-proBNP levels below and above 2,000 ng/l. Furthermore, we found that biomarker-biomarker correlations were similar in patients with HFrEF who were excluded in the European cohort because of missing NT-proBNP values or NT-proBNP values below 2,000 ng/l compared with included HFrEF patients. Last, sensitivity analyses restricted to inpatients or outpatients did not affect our results.

Results of the network analyses for HFrEF and HFpEF are presented in Figures 2 and 3. The size of the node (hub) is related to the centrality and importance of the hub in the particular network. In other words, biomarkers that form large hubs within a network can be considered biologically more important compared with biomarkers that are smaller hubs. Network analysis showed that main hubs in HFrEF were NT-proBNP, growth differentiation factor (GDF)-15, and interleukin-1 receptor-like type 1 (IL1RL1) (Figure 2A). In HFpEF, no clear hubs were observed among the unique correlations between the measured biomarkers (Figure 3A).

KNOWLEDGE-BASED ENRICHMENT OF NETWORK ANALYSIS. We enriched the experimentally found networks with protein-protein associated based on various independent databases as described in the Online Appendix. By including knowledge-based data analysis, the cyclic AMP-dependent transcription factor activating transcription factor 2 (ATF2) became an additional hub in HFrEF (Figure 2B). When adding knowledge-based interactions to the biomarker networks in HFpEF, integrin subunit beta 2 (ITGB2) and catenin beta-1 became prominent hubs in HFpEF (Figure 3B). In the enriched networks of HFmrEF, we found that plasminogen urokinase receptor, signal transducer and activator of transcription 1, Transcription factor AP-1, and IL-1B were possible hubs (Online Figure 4).

TRANSLATION INTO BIOLOGICAL PATHWAYS. The proteins found in our network analysis, which was enriched by existing knowledge on biomarker interactions, were translated into biological pathways that were typically related to HFrEF and HFpEF (Figure 4). The top 10 over-represented pathways in HFrEF were characterized by processes relating to DNA binding transcription factor activity, phosphorylation of peptidyl-serine, cellular protein metabolic processes, as well as the regulation in nitric oxide biosynthetic processes. In contrast, the top 10 over-represented pathways in patients with HFpEF were characterized by inflammatory processes, including cytokine response, extracellular matrix organization, as well as response to lipopolysaccharides and inflammation. In HFmrEF, the top 10 up-regulated pathways were related to neutrophil degranulation, leucocyte migration, and DNA-binding transcription factor activity (Online Figure 5).

FIGURE 4 **Pathway Over-Representation Analysis Showing Biological Processes Unique to HFrEF and HFpEF**



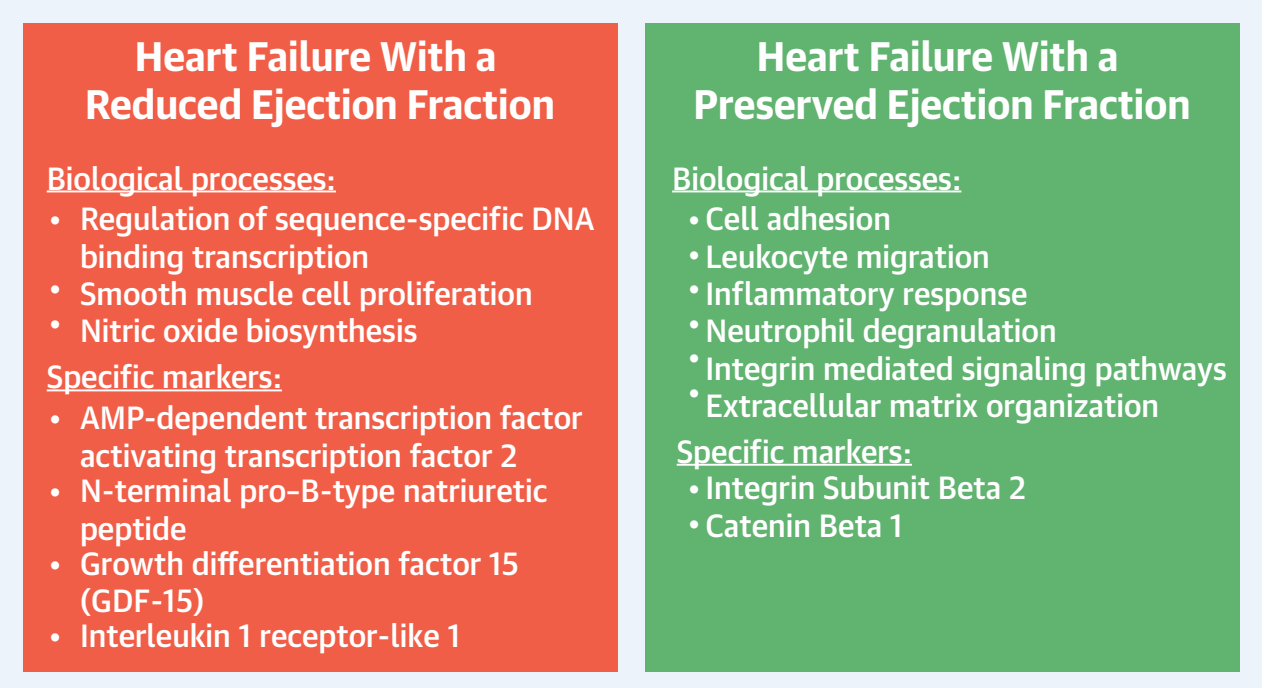
Orange indicates HFrEF and **green** indicates HFpEF. The y-axis signifies the top 10 over-represented biological processes in HFrEF and HFpEF. + stands for "positive regulation of" in a name of a given GO term. The x-axis is the −log10 of the p value; the larger the bar, the more significant is the over-representation of that particular biological process. Abbreviations as in **Figure 1**.

DISCUSSION

To our knowledge, this is the first study using a comprehensive knowledge-based network analysis approach to characterize differences in circulating biomarker signatures among patients with HFrEF, HFmrEF, and HFpEF. Overall, there was an important overlap between protein-protein correlations in HFrEF, HFmrEF, and HFpEF. This suggests that a large proportion of these protein-protein correlations belong to common pathways related to HF. However, we also found distinct differences, which are summarized in the **Central Illustration**. Our findings show that pathways specifically up-regulated in patients with HFrEF were related to cellular growth and metabolism. Pathways that were specifically up-regulated in patients with HFpEF were related to inflammation and extracellular matrix reorganization.

Network analysis of unique biomarker correlations in HFrEF showed that NT-proBNP, GDF-15, and IL1RL1 were central hubs. NT-proBNP is associated with cardiac stretch and was previously found to be a specific hub in network analyses in HFrEF in 2 independent studies (9,10). GDF-15 was previously found to be associated with more adverse outcomes in HFrEF (24,25). Furthermore, the results of our study show that IL1RL1 is a potential hub in patients with HFrEF. In patients with HFmrEF, IL1-B was a hub, suggesting that IL1 inhibition in these patients might be worth investigating. Network analysis in HFpEF showed a more diffuse combination of biomarker correlations with no specific central hubs. This is in line with earlier studies, which suggested that HFpEF might be more heterogenous than HFrEF (26,27). The majority of biomarkers found in HFpEF were related to inflammation, which is a hallmark of the underlying

CENTRAL ILLUSTRATION **Biomarkers in Heart Failure With a Reduced Versus Preserved Ejection Fraction**



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pathophysiology of HFpEF (7). After adding knowledge-based protein-protein interactions to our experimentally found networks, we observed that ATF2 was an important additional hub in HFrEF. ATF2 is a protein involved in cardiac hypertrophy triggered by TGF-β. A previous experimental study found that suppression of ATF2 attenuated left ventricular hypertrophic response (28). In HFpEF, we observed that ITGB2 and catenin-beta were important hubs. Previous studies show that ITBG2 is involved in chronic inflammatory processes and endothelial dysfunction (29). In addition, an experimental study showed that catenin-β levels were increased in dahl salt-sensitive rats when they developed an HFpEF phenotype (30). This suggests that catenin-β in particular could be a protein of interest in HFpEF. The knowledge-based enrichment of our networks was performed using combined data from various publicly available bioinformatic repositories, which together provide a comprehensive data source on all known protein-protein interactions. The combination of these resources reduced overall bias in our enrichment. Yet, without knowledge-based enrichment, HFpEF did not show meaningful hubs. This suggests that the overall pathophysiology of HFpEF is more heterogenous compared with HFrEF.

The last step in our analysis was to perform pathway over-representation analysis of the proteins found in our knowledge-enriched networks. Results showed that in HFrEF, biological processes were related to sequence-specific DNA binding, phosphorylation of peptidyl-serine, and proliferation of smooth muscle cells. Taken together, these processes are all related to cell proliferation. Furthermore, biological pathways related to protein kinase B signaling and MAPK cascade were also enriched. Both protein kinase B signaling and MAPK are related to cell proliferation and an increase in metabolism (31,32,33). In contrast, biological processes in HFpEF related to inflammation, integrin signaling, and extracellular matrix organization (33). These data confirm earlier findings regarding HFpEF, but also allow future studies to focus on protein-protein interaction within certain existing pathways, such as integrin-mediated signaling and extracellular matrix organization (7). Biological pathways that were upregulated in patients with HFmrEF were in between patients with HFrEF and HFpEF. This is in line with a

previous study that suggested that biomarker profiles of patients with HFmrEF are in between patients with HFrEF and HFpEF (34). Our approach might be used to identify HFmrEF patients with an HFrEF-like biomarker profile who could derive more benefit from guideline-directed treatment.

This study has several clinical implications. First, the results of this study provide biological context for the presence of clearly distinct syndromes, which may potentially explain the divergent response to HF therapy. Second, processes of cardiac stress response and cell proliferation are enriched in patients with HFrEF, whereas processes related to inflammation are enriched in HFpEF. Particularly, ATF2 could be a potential novel treatment target in HFrEF, whereas ITGB2 and catenin-beta could be novel treatment targets for HFpEF; this possibility deserves further study.

STUDY LIMITATIONS. First, echocardiography was not performed at inclusion. Nevertheless, sensitivity analysis showed that the timing of echocardiography did not influence biomarker levels across HFrEF and HFpEF. Furthermore, we were able to validate our findings in an independent cohort, significantly reducing the potential effect of this limitation. Unfortunately, there were missing values for NT-proBNP in our validation cohort. This might have introduced a potential bias in our European cohort, because these patients had to be excluded. In contrast to our Scottish cohort, our European cohort had patients with both HFrEF and HFpEF with an NT-proBNP value >2,000 ng/l. This is a limitation, because it might inflate the type II error. However, this is also a particular strength of this study because protein-protein correlations as well as differences in biomarker levels found for HFrEF and HFpEF in this study are relatively stable throughout the disease severity spectrum. Last, patients in BIOSTAT-CHF were suboptimally treated, which might introduce potential bias.

CONCLUSIONS

Biological pathways unique to HFrEF are associated with increased metabolism and cellular hypertrophy. A potential novel target for HFrEF is ATF2. Biological pathways unique to HFpEF are related to inflammation, neutrophil degranulation, and integrin signaling. Potential novel treatment targets in HFpEF are IGTB2 and catenin-beta. These profound dissimilarities in the underlying biological processes emphasizes the need for distinct drug development programs in HFrEF and HFpEF.

ACKNOWLEDGMENT The authors thank Mr. M.F.G.A Hoes from the University Medical Centre Groningen for his help with the concept figure.

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PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: Key differences in pathophysiological processes distinguish HFrEF and HFpEF. Patients with HFrEF exhibit DNA-binding transcription factor activity, cellular protein metabolism, and regulation of nitric oxide, whereas HFpEF is associated with cytokine response, extracellular matrix organization, and inflammation.

TRANSLATIONAL OUTLOOK: Treatment of patients with HFpEF should focus on inflammation as a central pathophysiological mechanism, and future biomarker-based network analyses might identify additional disease mechanisms.

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KEY WORDS biomarkers, HFpEF, HFrEF, network analysis, pathophysiology

APPENDIX For an expanded Methods section as well as supplemental figures and tables, please see the online version of this paper.