

Pathophysiological pathways in patients with heart failure and atrial fibrillation

Bernadet T. Santema, MD^a, Vicente Artola Arita, MD^a, Iziah E. Sama, PhD^a,
Mariëlle Kloosterman, MD^a, Maarten P. van den Berg, MD, PhD^a,
Hans L.A. Nienhuis, MD, PhD^b, Isabelle C. Van Gelder, MD, PhD^a,
Peter van der Meer, MD, PhD^a, Faiez Zannad, MD, PhD^c, Marco Metra, MD^d,
Jozine M. Ter Maaten, MD, PhD^a, John G. Cleland, MD^{e,f}, Leong L. Ng, MD^{g,h},
Stefan D. Anker, MD, PhDⁱ, Chim C. Lang, MD^j, Nilesh J. Samani, MD^{g,h},
Kenneth Dickstein, MD, PhD^{k,l}, Gerasimos Filippatos, MD, PhD^m,
Dirk J. van Veldhuisen, MD, PhD^a, Carolyn S.P. Lam, MD, PhD^{a,n},
Michiel Rienstra, MD, PhD^a, Adriaan A. Voors, MD, PhD^a

Brief title: Pathways in heart failure and atrial fibrillation

Word count text: 6186 (including abstract, manuscript text, references and figure legends)

Abstract word count: 268

Category manuscript: Original Article

Affiliations:

a. Department of Cardiology, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands.

b. Department of Internal Medicine, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands.

c. INSERM, Centre d'Investigations Cliniques Plurithématique 1433, INSERM U1116, Université de Lorraine, CHRU de Nancy, F-CRIN INI-CRCT, Nancy, France

d. Institute of Cardiology, Department of Medical and Surgical Specialties, Radiological Sciences and Public Health, University of Brescia, Brescia, Italy

e. National Heart & Lung Institute, Royal Brompton & Harefield Hospitals, Imperial College, London, UK

f. Robertson Institute of Biostatistics and Clinical Trials Unit, University of Glasgow, Glasgow, UK

g. Department of Cardiovascular Sciences, University of Leicester, Leicester, UK

h. NIHR Leicester Biomedical Research Centre, Glenfield Hospital, Leicester, UK

i. Department of Cardiology (CVK); and Berlin Institute of Health Center for Regenerative Therapies (BCRT); German Centre for Cardiovascular Research (DZHK) partner site Berlin; Charité Universitätsmedizin Berlin, Germany

j. School of Medicine Centre for Cardiovascular and Lung Biology, Division of Molecular and Clinical Medicine, University of Dundee, Ninewells Hospital & Medical School, Dundee, UK

k. University of Bergen, Bergen, Norway

l. Stavanger University Hospital, Stavanger, Norway

m. National and Kapodistrian University of Athens, Attikon University Hospital, Athens, Greece

n. National Heart Centre Singapore and Duke-National University of Singapore, Singapore

Address for correspondence:

Prof. Dr. A.A. Voors

Department of Cardiology

University Medical Center Groningen

Hanzeplein 1, 9713 GZ, Groningen, The Netherlands

Tel: +31 (0)50 3612355

Fax: +31 (0)50 3618062

E-mail: a.a.voors@umcg.nl

Abstract.

Aims. Atrial fibrillation (AF) and heart failure (HF) are two growing epidemics that frequently co-exist. We aimed to gain insights into underlying pathophysiological pathways in HF patients with AF by comparing circulating biomarkers using pathway overrepresentation analyses.

Methods and Results. From a panel of 92 biomarkers from different pathophysiological domains available in 1,620 patients with HF, we first tested which biomarkers were dysregulated in patients with HF and AF (n=648) compared with patients in sinus rhythm (n=972). Secondly, pathway overrepresentation analyses were performed to identify biological pathways linked to higher plasma concentrations of biomarkers in patients who had HF and AF. Findings were validated in an independent HF cohort (n=1,219, 38% with AF). Patient with AF and HF were older, less often women, and less often had a history of coronary artery disease compared with those in sinus rhythm. In the index cohort, 24 biomarkers were upregulated in patients with AF and HF. In the validation cohort, 8 biomarkers were upregulated, which all overlapped with the 24 biomarkers found in the index cohort. The strongest up-regulated biomarkers in patients with AF were spondin-1 (fold change 1.18, $p=1.33 \times 10^{-12}$), insulin-like growth factor-binding protein-1 (fold change 1.32, $p=1.08 \times 10^{-8}$), and insulin-like growth factor-binding protein-7 (fold change 1.33, $p=1.35 \times 10^{-18}$). Pathway overrepresentation analyses revealed that the presence of AF was associated with activation amyloid-beta metabolic processes, amyloid-beta formation, and amyloid precursor protein catabolic processes with a remarkable consistency observed in the validation cohort.

Conclusion. In two independent cohorts of patients with HF, the presence of AF was associated with activation of three pathways related to amyloid-beta. These hypothesis-generating results warrant confirmation in future studies.

Keywords. Heart failure – Atrial fibrillation – Pathway analysis – Amyloid-beta

1 **Translational Perspective** (89 words, max. 100)

2 Using an unbiased approach, we identified and validated dysregulation of three amyloid-beta
3 related pathways in patients who had heart failure (HF) with concomitant atrial fibrillation
4 (AF). Amyloid-beta depositions are a hallmark of Alzheimer's disease, but might also play a
5 role in pathophysiological processes outside the central nervous system. Biopsy studies are
6 needed to confirm the pathophysiological role of amyloid-beta in patients with AF and HF.
7 Diagnostic and therapeutic implications should be investigated in the light of potential
8 pathophysiological overlap between the three aging-related epidemics: Alzheimer's disease,
9 AF and HF.

1 Introduction

2 Atrial fibrillation (AF) is the most common arrhythmia in heart failure (HF) with a prevalence
3 between 20-60% depending on the type and severity of HF.¹⁻³ Both AF and HF are strongly
4 associated with ageing, share many other clinical risk factors such as obesity and
5 hypertension, and can trigger each other.^{2,3} Distinct differences are observed when comparing
6 patients with HF with and without AF. We recently showed that patients with AF and HF are
7 older, less often have an ischemic aetiology of HF, and have a distinct biomarker profile as
8 compared with HF patients in sinus rhythm.^{4,5} Moreover, patients with AF and HF have a
9 poorer quality of life, and worse outcome as compared with those without AF.^{4,6} Pooled
10 individual-patient data revealed that in contrast to the beneficial effects observed in patients
11 with heart failure with a reduced ejection fraction (HFrEF) in sinus rhythm, β -blockers did not
12 improve clinical outcomes in patients with AF and HFrEF.⁷ The potential lack of β -blocker
13 efficacy suggests differences in pathophysiology between HF patients with and without AF,
14 but the exact mechanisms remain poorly understood and understudied.⁷
15 Unravelling the underlying pathophysiology of AF in HF is important since this population
16 might respond to different therapies than HF patients without AF.^{8,9} Underlying
17 pathophysiological mechanisms can be studied by performing pathway overrepresentation
18 analyses, a method that can identify associated pathways based on circulating biomarker
19 profiles in specific subgroups.^{10,11} Therefore, we compared patients who had HF with and
20 without AF, studied their biomarker profiles and associated pathophysiological pathways,
21 which might help discover new treatment targets for patients with HF and AF.

Methods

Patient population

We performed a *post-hoc* study of patients enrolled in A Systems Biology Study to Tailored Treatment in Chronic Heart Failure (BIOSTAT-CHF), of which the design and primary results have been published previously.^{12, 13} In brief, BIOSTAT-CHF was a prospective, observational, multinational, European HF study, in which a total of 2,516 patients were included. Patients were eligible with either a left ventricular ejection fraction (LVEF) $\leq 40\%$, or plasma concentrations of N-terminal pro-B-type natriuretic peptide (NT-proBNP) > 2000 ng/L. Another 1,738 patients with HF were included in an independent cohort from six centers in Scotland, which we have used as our validation cohort. Patients were enrolled into the validation cohort when they were diagnosed with HF and had a previous documented admission with HF requiring diuretic treatment.¹² This study complies with the Declaration of Helsinki, and medical ethics committees of participating centers approved the study. All patients provided written informed consent.

Definitions

Patients were classified as AF when they met the following criteria: 1) a documented history of AF, and 2) AF registered on the standard 12-lead electrocardiogram (ECG) at baseline of the study. Patients were classified as having sinus rhythm when they met the following criteria: 1) no documented history of AF, and 2) sinus rhythm on the baseline ECG. Patients with a pacemaker rhythm (n=320) or unknown rhythm (n=58) were excluded from our analyses. Patients with prior episodes of AF but who were in sinus rhythm at baseline (n=197), and those without a history of AF but with AF on the baseline ECG (n=82) were excluded from our analyses, since these patients could interfere with the contrast in underlying pathophysiological pathways between the AF and sinus rhythm groups under study. We did include these patients in a previous biomarker study on AF in BIOSTAT-CHF,

but since subsequent analyses revealed that many biomarkers tend to fluctuate with paroxysmal episodes of AF, we chose to include HF patients with ‘permanent’ AF as compared with those who never had any previously documented episode of AF in the current analyses.^{4, 14} A flow chart of the selected patients is displayed in *Supplementary Figure 1*.

Biomarkers

The Olink Cardiovascular III (CVD III) panel includes 92 biomarkers from different pathophysiological domains. The Proseek Multiplex 96*96 kit of Olink Bioscience (Uppsala, Sweden) analysis service was used, which measured the 92 biomarkers in 1 µl plasma samples. The reagents are based on the Proximity Extension Assay (PEA) technology, which binds 92 oligonucleotide-labeled antibody probe pairs to the target biomarker.¹⁵ For further quantification, real-time PCR was performed. Olink wizard and GenEx software were used for further data analysis. Proseek® data are presented as arbitrary units (AU) on a log2 scale (*Supplementary Table 1 and 2*). Complete biomarker data was available in 87% of the patients under study.

Statistical analyses

Normally distributed continuous variables were displayed as mean ± standard deviation, non-normally distributed variables as median with the first and third quartile (Q1-Q3). Categorical variables were presented as numbers with percentages. Group comparisons were tested using Student’s t tests, Mann-Whitney U tests, or Chi-square tests where appropriate. Differences in expression of the 92 biomarkers between patients with AF versus sinus rhythm were tested using Linear Models for Microarray data analysis (Limma) software (version 3.34.9), using a log2 fold change cutoff of 0.2, and a false discovery rate <0.05 according to the Benjamini-Hochberg method. The biomarkers that were upregulated in patients with AF compared to those in sinus rhythm were further studied by using pathway overrepresentation analysis. Pathway overrepresentation analysis was performed with knowledge from established

pathways in publicly available databases: Gene Ontology (GO), Reactome, and the Kyoto Encyclopedia of Genes and Genomes (KEGG), using Cytoscape (version 3.7.1) and plugin ClueGO (version 2.5.4).^{10, 16, 17} Multivariable logistic regression was performed to study the association between the biomarkers within pathways and AF status, adjusting for age, sex, body mass index (BMI), heart rate, a history of coronary artery disease, and renal disease. Since we were interested in underlying pathophysiological differences between patients with heart failure with a reduced/mid-range/preserved ejection fraction HFrEF/HFmrEF/HFpEF, we performed the same analyses in these subgroups. Unfortunately, these subgroups were too small to gain results with pathway overrepresentation analyses. This was still the case when we analyzed only two groups: LVEF <45% versus LVEF \geq 45%. We therefore tested for interactions to determine whether the association of biomarkers and AF status was present in patients with HFrEF/HFmrEF/HFpEF by adding the interaction term to the logistic regression model, and also tested for an interaction with LVEF on a continuous scale. A separate network analysis focusing on pathophysiological differences between patients with HFpEF and HFrEF has been published previously.¹⁸ A p-value smaller than 0.1 was considered statistically significant for testing interactions. All other tests were performed two-sided, and a p-value of less than 0.05 was considered statistically significant. Statistical analyses were conducted using R, A Language and Environment for Statistical Computing, version 3.5.3 (R Foundation for Statistical Computing, Vienna, Austria).

Results

Index cohort

Clinical characteristics

Of 1,620 patients with HF enrolled, 648 (40%) had AF and 972 (60%) were in sinus rhythm. Patients with AF were older, less often women, had a higher BMI and a higher heart rate

(Table 1). Fewer patients with AF had a history of coronary artery disease, but more often a history of renal disease as compared with those in sinus rhythm. Patients with AF had larger left atrial diameters, and greater interventricular and posterior wall thickness on echocardiography.

Biomarker concentrations

In the index cohort, 24 biomarkers were upregulated and three biomarkers were downregulated in patients with AF as compared to those in sinus rhythm (Figure 1). A volcano plot with the up- and downregulated biomarkers is presented in Figure 2A. The three biomarkers that were most significantly differentially expressed in patients with AF as compared with those in sinus rhythm were neurogenic locus notch homolog protein 3 (NOTCH3, fold change 1.30, $p=6.40 \times 10^{-24}$), insulin-like growth factor binding protein-7 (IGFBP7, fold change 1.33, $p=1.35 \times 10^{-18}$), and interleukin-1 receptor-like 1 (IL1RL1, fold change 1.35, $p=1.75 \times 10^{-16}$) (Supplementary Table 1).

Pathway overrepresentation analyses of upregulated biomarkers

Pathway overrepresentation analyses of the 24 upregulated biomarkers in the index cohort revealed seven pathways that were dysregulated specifically in patients with AF: 1) amyloid-beta metabolic process, 2) amyloid-beta formation, 3) amyloid precursor protein catabolic process, 4) regulation of insulin-like growth factor (IGF) transport and uptake by IGF binding proteins, 5) embryo implantation, 6) membrane protein ectodomain proteolysis, and 7) regulation of neuroinflammatory response (Figure 3).

Validation cohort

Clinical characteristics

The baseline characteristics of patients included in the smaller validation cohort are presented in Table 2. In general, patients enrolled in the validation cohort were older, had a higher

LVEF, and lower plasma concentrations of NT-proBNP as compared with patients included in the index cohort. However, similar trends were observed in patients with AF compared to those in sinus rhythm, in which patients with AF were older, less often women, had higher heart rates, and less often a history of coronary artery disease.

Biomarker concentrations

In the validation cohort, eight biomarkers were significantly upregulated in patients with AF, all of which overlapped with the 24 biomarkers that were found to be significantly upregulated in the index cohort (*Figure 1*). The eight biomarkers that were upregulated in AF patients in both HF cohorts included IGFBP7 (fold change 1.30, $p=5.13 \times 10^{-18}$), NOTCH3 (fold change 1.25, $p=1.44 \times 10^{-17}$), spondin 1 (SPON1, fold change 1.18, $p=1.29 \times 10^{-12}$), IL1RL1 (fold change 1.31, $p=3.44 \times 10^{-11}$), natriuretic peptide B (fold change 1.49, $p=3.78 \times 10^{-11}$), matrix metalloproteinase 2 (MMP2, fold change 1.18, $p=1.25 \times 10^{-9}$), IGFBP1 (fold change 1.26, $p=6.68 \times 10^{-5}$), and growth differentiation factor 15 (GDF15, fold change 1.16, $p=1.07 \times 10^{-4}$) (*Supplementary Table 2*). Low-density lipoprotein receptor (LDLR) and paraoxonase 3 (PON3) were significantly downregulated in patients with AF in both the index and the validation cohort.

Pathway overrepresentation analyses of upregulated biomarkers

Pathway overrepresentation analysis of the eight upregulated biomarkers in patients with AF in the validation cohort revealed three activated pathways: 1) amyloid-beta metabolic process, 2) amyloid-beta formation, and 3) amyloid precursor protein catabolic process (*Figure 3*).

Overlap index and validation cohort

The three amyloid-beta-related pathways were found in both the index and validation cohort, and were related to three upregulated biomarkers in patients with AF: SPON1 (fold change 1.18, $p=1.33 \times 10^{-12}$), IGFBP1 (fold change 1.32, $p=1.08 \times 10^{-8}$), and IGFBP7 (fold change 1.33,

p=1.35x10⁻¹⁸). After adjusting for the clinical covariates age, sex, body mass index, heart rate, a history of coronary artery disease, and renal disease, the concentrations of SPON-1, IGFBP-1, and IGFBP-7 remained associated with the presence of AF (all p<0.001), in both the index and the validation cohort. These associations were similar among HF phenotypes (HFrEF/HFmrEF/HFpEF; p for interaction = 0.42) and across LVEF as a continuous variable (p for interaction = 0.96).

Discussion

We sought to identify pathophysiological pathways in HF patients with AF using pathway overrepresentation analyses. In two independent HF cohorts we found that the presence of AF was associated with amyloid-beta metabolic processes, amyloid-beta formation, and amyloid precursor protein catabolic processes. These three pathophysiological pathways were found based on higher levels of spondin-1, IGFBP1, and IGFBP7 in those with AF. In the larger index cohort, four more pathophysiological pathways were found, which were not observed in the independent validation cohort. Previous studies investigating specific phenotypes or subgroups in HF (e.g. diabetes, ischemic HF, old vs. young, and HFpEF vs. HFrEF), have not revealed any amyloid-beta-related pathways despite using the same methodology, which supports that the current findings might be specific to the presence of AF in patients with HF.^{10, 11, 19}

Individual biomarkers

In the present study, the concentrations of SPON-1, IGFBP-1 and IGFBP-7 were closely linked to the three amyloid-beta-related pathways. Although IGFBP-1 and IGFBP-7 are linked to a wide range of biological processes associated with inhibition and stimulation of cell growth, the current knowledge indicates a more specific role for SPON-1.²⁰ SPON-1 is an extracellular matrix cell adhesion glycoprotein that is expressed in multiple organs including

the heart and brain.²⁰ The SPON-1 protein binds to the extracellular domain of amyloid precursor protein and inhibits beta-secretase cleavage of this amyloid precursor protein, a process that is strongly related to the formation of amyloid-beta depositions.²¹ In a large genome wide association study (GWAS) investigating the rate of cognitive decline in patients with Alzheimer's disease, the most interesting candidate gene identified was *SPON-1*, since it was strongly associated with the rate of cognitive decline in two independent cohorts.²² Recent studies showed that increased levels of IGFBP-7, a marker of ageing and cellular senescence, were strongly associated with increased left atrial size, and the presence of AF in patients with HF.²³⁻²⁵ In Framingham Heart Study participants without HF, increased levels of IGFBP1 were strongly associated with incident AF.²⁶ In the present study, NOTCH3 was strongly upregulated in patients with AF in both cohorts. The NOTCH system communicates in multiple tissues and systems, including cell proliferation, differentiation, and apoptosis.²⁷ In the heart specifically, NOTCH signaling has been suggested to be associated with repair of infarcted and overloaded myocardium, but this has only been investigated in a pre-clinical setting.²⁷ The role of the NOTCH system in patients with AF and HF is yet to be elucidated.

Amyloid-beta in heart failure and atrial fibrillation

Cardiac amyloidosis has been reported to be associated with a high prevalence of AF in several previous studies;²⁸⁻³³ however prior work focused on isolated atrial amyloidosis (IAA) and transthyretin-derived amyloidosis (ATTR) – the most commonly described forms of cardiac amyloidosis in elderly patients. Our results concerned amyloid-beta depositions, which are generally acknowledged as a hallmark of Alzheimer's disease, in which abnormal cleavage of the amyloid precursor protein leads to pathological amyloid-beta fragments, protein aggregation, and formation of extracellular plaques that can lead to degradation of neurons.^{34, 35} Even though Alzheimer's disease has been traditionally considered as a brain-specific disease, recent discoveries suggest that other organs might also be involved in the

pathophysiology, suggesting that Alzheimer's disease might be a focal manifestation of a systemic disorder.^{34, 36} The epidemiological link between AF and Alzheimer's disease was first described in 1977, followed by studies showing that younger patients with AF had an increased risk of developing all-cause dementia which could not be explained by the increased incidence of stroke alone.³⁷⁻³⁹ Since then, contradictory results have been reported, but neuropathological analyses of autopsies did reveal a higher incidence of amyloid-beta plaques and amyloid angiopathy in the brains of patients with permanent AF.⁴⁰ Suggesting a unifying pathogenesis, Troncone et al. performed a cross-sectional study investigating cardiac involvement of patients with a primary diagnosis of Alzheimer's disease as compared to age-matched controls. Indeed, those with Alzheimer's disease had increased left ventricular septal and inferolateral wall thickness on echocardiography, and expression of amyloid-beta plaques in both the heart and the brain.³⁶

Clinical relevance

Even though the current study revealed pathways related to amyloid-beta specifically, considerable overlap can be observed with more commonly recognized protein-misfolding diseases that are known to affect the heart. As recently reviewed, cardiac involvement has predominantly been reported in IAA, light chain (AL) and ATTR amyloidosis, but may occur in other types of amyloidosis, with cardiac arrhythmias, especially atrial fibrillation, as common presenting clinical features.⁴¹ The consistency of cardiac clinical presentations among the various types of amyloidosis, despite differences in involved proteins (e.g. ANP versus AL versus ATTR), suggest common cardiac effects which may also plausibly apply to amyloid-beta deposition.⁴² Notably, the emergence of promising new treatment options for ATTR amyloidosis has raised awareness of the importance of screening for amyloidosis in patients with suggestive clinical features.⁴³⁻⁴⁵ Whether similar mechanistic approaches can be of use in patients with cardiac amyloid-beta depositions warrants further study.

Strengths and Limitations

To our knowledge, there have been no previous studies investigating underlying pathophysiological processes using pathway overrepresentation in patients with AF and HF. Therefore, our study adds to the limited understanding of the underlying pathophysiological mechanisms of AF in patients with HF. The greatest strength of the current study is that we were able to validate our results in an independent HF cohort with clear definitions, and that the pathway overrepresentation analysis was based on a large number of measured plasma biomarkers.

A limitation of this study is that the findings are based on *post-hoc* analyses. Unfortunately, we did not have direct biopsy evidence of cardiac/atrial amyloid-beta involvement which are pivotal to confirm the cardiac amyloid-beta hypothesis. Future research in cases (patients with AF+HF) and controls (patients in sinus rhythm with and without HF) with markers derived from atrial tissue will provide more direct insights into our hypothesis.⁴⁶ Another limitation concerns the selected biomarker panel, which did cover many pathophysiological domains, but was primarily a cardiovascular disease-related biomarker panel. The number of significantly upregulated biomarkers was higher in the index cohort than in the validation cohort, which resulted in more associated pathophysiological processes in the index cohort than in the validation cohort. This could be the consequence of the larger number of patients included in the index cohort, the different inclusion criteria of patients that were used for the two independent cohorts, or the different regions of inclusion of the study participants (11 European countries versus six centers in Scotland). The use of amiodarone was higher in the index cohort compared with the validation cohort. However, despite these differences between the two cohorts, all upregulated biomarkers and pathways found in the validation cohort overlapped with those found in the index cohort. The HFrEF, HFmrEF and HFpEF subgroups were unfortunately too small to yield results with pathway overrepresentation

analyses. There was, however, no interaction between the amyloid-beta-related biomarkers and the HF subgroups, suggesting a pathophysiological role of amyloid-beta in HF patients across the full LVEF spectrum. Not all (combinations of) biomarkers were annotated by the publicly available databases even though these biomarkers were deemed to be significantly up- or downregulated in our analyses (e.g. NOTCH3 and IL1RL1), since the content of these databases is based on what is currently known about these biomarkers. Therefore, the results of the current pathway overrepresentation analyses might change over time, when the knowledge on the (combination of) biomarkers has increased. The current findings might reflect underlying pathophysiological processes specific to elderly patients with AF and HF, since the mean age of these patients was 72 and 75 years in the two cohorts respectively. The number of women included in the cohorts was limited (n=462 [index] and n=439 [validation], 32%) and mainly comprised postmenopausal women. Even though we have attempted to define the AF and sinus rhythm group mutually exclusive, it is possible that patients with asymptomatic paroxysmal AF were misclassified. Based on the current definition, we may have predominantly included patients with persistent/permanent AF and less patients with paroxysmal AF. Moreover, we do not have data on the duration of AF since no continuous rhythm monitoring was incorporated in the study protocol, which might have influenced the biomarker concentrations. Unfortunately, we also do not have information on cognitive function, other neurologic diseases, nor information on systemic or cardiac amyloidosis of patients enrolled in BIoSTAT-CHF, which could have strengthened our hypothesis linking AF – HF to Alzheimer’s disease and/or amyloidosis. As with all cross-sectional studies, we cannot prove causality.

Conclusion

In two independent cohorts of patients with HF, the presence of AF was associated with amyloid-beta metabolic processes, amyloid-beta formation, and amyloid precursor protein

- 1 catabolic processes, based on higher levels of spondin-1, IGFBP1, and IGFBP7 in those with
- 2 AF. These hypothesis-generating results warrant confirmation in future studies.

3

Funding

This work was supported by the European Commission [FP7-242209-BIOSTAT-CHF].

Author Contribution Statement

AAV, NJS, LLN, CCL, PVDM, JMTM, KDI, JGC, FZ, SDA, MM, DJVV, GF conceived and designed the research. BTS, VAA, and IES have performed statistical analyses. AAV, CSPL, DJVV handled funding and supervision. AAV, NJS, LLN, CCL, PVDM, JMTM, KDI, JGC, FZ, SDA, MM, GF, DJVV acquired the data. BTS drafted the manuscript. VAA, IES, MK, MPVDB, HLAN, ICVG, PVDM, FZ, MM, JMTM, JGC, LLN, SDA, CCL, NJS, KD, GF, DJVV, CSPL, MR, AAV have provided critical revisions of the manuscript.

Conflict of Interest

AAV and/or his institution received grants and/or consultancy reimbursements from Amgen, AstraZeneca, Bayer AG, Boehringer, Cytokinetics, Merck, Myokardia, Novartis, Roche, Novo Nordisk. BTS received funding from the Dutch Heart Foundation (2019T094). ICVG reports support from the Netherlands Cardiovascular Research Initiative, an initiative with support of the Dutch heart Foundation (CVON 2014-9, CVON 2018-28) and support from Medtronic to the institution. FZ reports personal fees from Amgen, Applied Therapeutics , AstraZeneca, Bayer, Boehringer, Boston Scientific, Cardior, Cardiorenal, Cereno pharmaceutical , Cirijs, CVCT, CVRx, Janssen, Novartis, Merck, and Vifor Fresenius. PVDM received consultancy fees and/or grants from Novartis, Servier, Vifor, Pharma, Astra Zeneca, Pfizer and Ionis. CSPL is supported by a Clinician Scientist Award from the National Medical Research Council of Singapore; has received research support from Boston Scientific, Bayer, Roche Diagnostics, AstraZeneca, Medtronic, and Vifor Pharma; has served as consultant or on the Advisory Board/ Steering Committee/ Executive Committee for Boston Scientific, Bayer, Roche Diagnostics, AstraZeneca, Medtronic, Vifor Pharma,

Novartis, Amgen, Merck, Janssen Research & Development LLC, Menarini, Boehringer
Ingelheim, Novo Nordisk, Abbott Diagnostics, Corvia, Stealth BioTherapeutics, JanaCare,
Biofourmis, Darma, Applied Therapeutics, MyoKardia, Cytokinetics, WebMD Global LLC,
Radcliffe Group Ltd and Corpus; and serves as co-founder & non-executive director of
eKo.ai. GF is committee member in trials and/or registries sponsored by Medtronic, Novartis,
BI, Vifor, Servier, outside the submitted work. SDA reports receiving fees from Abbott
Vascular, Bayer, Boehringer Ingelheim, Cardiac Dimension, Impulse Dynamics, Novartis,
Servier, and Vifor Pharma, and grant support from Abbott Vascular and Vifor Pharma. VAA
received funding from the European Union's Horizon 2020 research and innovation
programme under the Marie Skłodowska-Curie grant (754425). None of the funders had any
role in the trial design, the collection or analysis of the data, or the writing of the manuscript.
All other authors declare no conflict of interest.

Data availability

The data underlying this article will be shared on reasonable request to the corresponding
author.

References

1. Maisel WH, Stevenson LW. Atrial fibrillation in heart failure: epidemiology, pathophysiology, and rationale for therapy. *The American Journal of Cardiology* 2003;**91**:2-8.
2. Kotecha D, Lam CS, Van Veldhuisen DJ, Van Gelder IC, Voors AA, Rienstra M. Heart Failure With Preserved Ejection Fraction and Atrial Fibrillation: Vicious Twins. *J Am Coll Cardiol* 2016;**68**:2217-2228.
3. Kloosterman M, Santema BT, Roselli C, Nelson CP, Koekemoer A, Romaine SPR, Van Gelder IC, Lam CSP, Artola VA, Lang CC, Ng LL, Metra M, Anker S, Filippatos G, Dickstein K, Ponikowski P, van der Harst P, van der Meer P, van Veldhuisen DJ, Benjamin EJ, Voors AA, Samani NJ, Rienstra M. Genetic risk and atrial fibrillation in patients with heart failure. *Eur J Heart Fail* 2020;.
4. Santema BT, Kloosterman M, Van Gelder IC, Mordi I, Lang CC, Lam CSP, Anker SD, Cleland JG, Dickstein K, Filippatos G, Van der Harst P, Hillege HL, Ter Maaten JM, Metra M, Ng LL, Ponikowski P, Samani NJ, Van Veldhuisen DJ, Zwinderman AH, Zannad F, Damman K, Van der Meer P, Rienstra M, Voors AA. Comparing biomarker profiles of patients with heart failure: atrial fibrillation vs. sinus rhythm and reduced vs. preserved ejection fraction. *Eur Heart J* 2018;**39**:3867-3875.
5. Santema BT, Chan MMY, Tromp J, Dokter M, van der Wal, H H, Emmens JE, Takens J, Samani NJ, Ng LL, Lang CC, van der Meer P, Ter Maaten JM, Damman K, Dickstein K, Cleland JG, Zannad F, Anker SD, Metra M, van der Harst P, de Boer RA, van Veldhuisen DJ, Rienstra M, Lam CSP, Voors AA. The influence of atrial fibrillation on the levels of NT-proBNP versus GDF-15 in patients with heart failure. *Clin Res Cardiol* 2019;.
6. Lam CS, Rienstra M, Tay WT, Liu LC, Hummel YM, van der Meer P, de Boer RA, Van Gelder IC, van Veldhuisen DJ, Voors AA, Hoendermis ES. Atrial Fibrillation in Heart Failure With Preserved Ejection Fraction: Association With Exercise Capacity, Left Ventricular Filling Pressures, Natriuretic Peptides, and Left Atrial Volume. *JACC Heart Fail* 2017;**5**:92-98.
7. Kotecha D, Holmes J, Krum H, Altman DG, Manzano L, Cleland JG, Lip GY, Coats AJ, Andersson B, Kirchhof P, von Lueder TG, Wedel H, Rosano G, Shibata MC, Rigby A, Flather MD, Beta-Blockers in Heart Failure Collaborative Group. Efficacy of beta blockers in patients with heart failure plus atrial fibrillation: an individual-patient data meta-analysis. *Lancet* 2014;**384**:2235-2243.
8. Verma A, Kalman JM, Callans DJ. Treatment of Patients With Atrial Fibrillation and Heart Failure With Reduced Ejection Fraction. *Circulation* 2017;**135**:1547-1563.
9. Rienstra M, Hobbelt AH, Alings M, Tijssen JGP, Smit MD, Bruggemann J, Geelhoed B, Tieleman RG, Hillege HL, Tukkier R, Van Veldhuisen DJ, Crijns, H J G M, Van Gelder IC, RACE 3 Investigators. Targeted therapy of underlying conditions improves sinus rhythm maintenance in patients with persistent atrial fibrillation: results of the RACE 3 trial. *Eur Heart J* 2018;**39**:2987-2996.
10. Tromp J, Voors AA, Sharma A, Ferreira JP, Ouwerkerk W, Hillege HL, Arevelo Gomez K, Lang CC, Ng LL, van der Harst P, van Veldhuisen DJ, van der Meer P, Lam CSP, Zannad F, Sama IE. Distinct Pathological Pathways in Heart Failure Patients with Diabetes. *JACC Heart Failure*.
11. Tromp J, Westenbrink BD, Ouwerkerk W, van Veldhuisen DJ, Samani NJ, Ponikowski P, Metra M, Anker SD, Cleland JG, Dickstein K, Filippatos G, van der Harst P, Lang CC, Ng LL, Zannad F, Zwinderman AH, Hillege HL, van der Meer P, Voors AA. Identifying Pathophysiological Mechanisms in Heart Failure With Reduced Versus Preserved Ejection Fraction. *J Am Coll Cardiol* 2018;**72**:1081-1090.

12. Voors AA, Anker SD, Cleland JG, Dickstein K, Filippatos G, van der Harst P, Hillege HL, Lang CC, Ter Maaten JM, Ng L, Ponikowski P, Samani NJ, van Veldhuisen DJ, Zannad F, Zwinderman AH, Metra M. A systems BIOlogy Study to TAIlored Treatment in Chronic Heart Failure: rationale, design, and baseline characteristics of BIOSTAT-CHF. *Eur J Heart Fail* 2016;**18**:716-726.
13. Ouwerkerk W, Voors AA, Anker SD, Cleland JG, Dickstein K, Filippatos G, van der Harst P, Hillege HL, Lang CC, Ter Maaten JM, Ng LL, Ponikowski P, Samani NJ, van Veldhuisen DJ, Zannad F, Metra M, Zwinderman AH. Determinants and clinical outcome of uptitration of ACE-inhibitors and beta-blockers in patients with heart failure: a prospective European study. *Eur Heart J* 2017;**38**:1883-1890.
14. Santema BT, Chan MMY, Tromp J, Dokter M, van der Wal, H H, Emmens JE, Takens J, Samani NJ, Ng LL, Lang CC, van der Meer P, Ter Maaten JM, Damman K, Dickstein K, Cleland JG, Zannad F, Anker SD, Metra M, van der Harst P, de Boer RA, van Veldhuisen DJ, Rienstra M, Lam CSP, Voors AA. The influence of atrial fibrillation on the levels of NT-proBNP versus GDF-15 in patients with heart failure. *Clin Res Cardiol* 2019;.
15. Assarsson E, Lundberg M, Holmquist G, Björkstén J, Thorsen SB, Ekman D, Eriksson A, Rennefick E, Ohlsson S, Edfeldt G, Andersson A, Lindstedt P, Stenvang J, Gullberg M, Fredriksson S. Homogenous 96-plex PEA immunoassay exhibiting high sensitivity, specificity, and excellent scalability. *PLoS One* 2014;**9**:e95192.
16. Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, Amin N, Schwikowski B, Ideker T. Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome Res* 2003;**13**:2498-2504.
17. Bindea G, Mlecnik B, Hackl H, Charoentong P, Tosolini M, Kirilovsky A, Fridman WH, Pages F, Trajanoski Z, Galon J. ClueGO: a Cytoscape plug-in to decipher functionally grouped gene ontology and pathway annotation networks. *Bioinformatics* 2009;**25**:1091-1093.
18. Tromp J, Westenbrink BD, Ouwerkerk W, van Veldhuisen DJ, Samani NJ, Ponikowski P, Metra M, Anker SD, Cleland JG, Dickstein K, Filippatos G, van der Harst P, Lang CC, Ng LL, Zannad F, Zwinderman AH, Hillege HL, van der Meer P, Voors AA. Identifying Pathophysiological Mechanisms in Heart Failure With Reduced Versus Preserved Ejection Fraction. *J Am Coll Cardiol* 2018;**72**:1081-1090.
19. Sama IE, Woolley RJ, Nauta JF, Romaine SPR, Tromp J, Ter Maaten JM, van der Meer P, Lam CSP, Samani NJ, Ng LL, Metra M, Dickstein K, Anker SD, Zannad F, Lang CC, Cleland JGF, van Veldhuisen DJ, Hillege HL, Voors AA. A network analysis to identify pathophysiological pathways distinguishing ischaemic from non-ischaemic heart failure. *Eur J Heart Fail* 2020;.
20. UniProt Consortium. UniProt: a worldwide hub of protein knowledge. *Nucleic Acids Res* 2019;**47**:D506-D515.
21. Ho A, Sudhof TC. Binding of F-spondin to amyloid-beta precursor protein: a candidate amyloid-beta precursor protein ligand that modulates amyloid-beta precursor protein cleavage. *Proc Natl Acad Sci U S A* 2004;**101**:2548-2553.
22. Sherva R, Tripodis Y, Bennett DA, Chibnik LB, Crane PK, de Jager PL, Farrer LA, Saykin AJ, Shulman JM, Naj A, Green RC, GENAROAD Consortium, Alzheimer's Disease Neuroimaging Initiative, Alzheimer's Disease Genetics Consortium. Genome-wide association study of the rate of cognitive decline in Alzheimer's disease. *Alzheimers Dement* 2014;**10**:45-52.

23. Januzzi JL, Packer M, Claggett B, Liu J, Shah AM, Zile MR, Pieske B, Voors A, Gandhi PU, Prescott MF, Shi V, Lefkowitz MP, McMurray JJV, Solomon SD. IGFBP7 (Insulin-Like Growth Factor-Binding Protein-7) and Neprilysin Inhibition in Patients With Heart Failure. *Circ Heart Fail* 2018;**11**:e005133.
24. Gandhi PU, Gaggin HK, Redfield MM, Chen HH, Stevens SR, Anstrom KJ, Semigran MJ, Liu P, Januzzi JL. Insulin-Like Growth Factor-Binding Protein-7 as a Biomarker of Diastolic Dysfunction and Functional Capacity in Heart Failure With Preserved Ejection Fraction: Results From the RELAX Trial. *JACC Heart Fail* 2016;**4**:860-869.
25. Gandhi PU, Chow SL, Rector TS, Krum H, Gaggin HK, McMurray JJ, Zile MR, Komajda M, McKelvie RS, Carson PE, Januzzi JL, Anand IS. Prognostic Value of Insulin-Like Growth Factor-Binding Protein 7 in Patients with Heart Failure and Preserved Ejection Fraction. *J Card Fail* 2017;**23**:20-28.
26. Staerk L, Preis SR, Lin H, Lubitz SA, Ellinor PT, Levy D, Benjamin EJ, Trinquart L. Protein Biomarkers and Risk of Atrial Fibrillation: The FHS. *Circ Arrhythm Electrophysiol* 2020;**13**:e007607.
27. Ferrari R, Rizzo P. The Notch pathway: a novel target for myocardial remodelling therapy? *Eur Heart J* 2014;**35**:2140-2145.
28. van den Berg, M P, Mulder BA, Klaassen SHC, Maass AH, van Veldhuisen DJ, van der Meer P, Nienhuis HLA, Hazenberg BPC, Rienstra M. Heart failure with preserved ejection fraction, atrial fibrillation, and the role of senile amyloidosis. *Eur Heart J* 2019;**40**:1287-1293.
29. Rapezzi C, Merlini G, Quarta CC, Riva L, Longhi S, Leone O, Salvi F, Ciliberti P, Pastorelli F, Biagini E, Coccolo F, Cooke RM, Bacchi-Reggiani L, Sangiorgi D, Ferlini A, Cavo M, Zamagni E, Fonte ML, Palladini G, Salinaro F, Musca F, Obici L, Branzi A, Perlini S. Systemic cardiac amyloidoses: disease profiles and clinical courses of the 3 main types. *Circulation* 2009;**120**:1203-1212.
30. Longhi S, Quarta CC, Milandri A, Lorenzini M, Gagliardi C, Manuzzi L, Bacchi-Reggiani ML, Leone O, Ferlini A, Russo A, Gallelli I, Rapezzi C. Atrial fibrillation in amyloidotic cardiomyopathy: prevalence, incidence, risk factors and prognostic role. *Amyloid* 2015;**22**:147-155.
31. Pinney JH, Whelan CJ, Petrie A, Dungu J, Banyersad SM, Sattianayagam P, Wechalekar A, Gibbs SD, Venner CP, Wassef N, McCarthy CA, Gilbertson JA, Rowczenio D, Hawkins PN, Gillmore JD, Lachmann HJ. Senile systemic amyloidosis: clinical features at presentation and outcome. *J Am Heart Assoc* 2013;**2**:e000098.
32. Grogan M, Scott CG, Kyle RA, Zeldenrust SR, Gertz MA, Lin G, Klarich KW, Miller WL, Maleszewski JJ, Dispenzieri A. Natural History of Wild-Type Transthyretin Cardiac Amyloidosis and Risk Stratification Using a Novel Staging System. *J Am Coll Cardiol* 2016;**68**:1014-1020.
33. Rocken C, Peters B, Juenemann G, Saeger W, Klein HU, Huth C, Roessner A, Goette A. Atrial amyloidosis: an arrhythmogenic substrate for persistent atrial fibrillation. *Circulation* 2002;**106**:2091-2097.
34. Wang J, Gu BJ, Masters CL, Wang YJ. A systemic view of Alzheimer disease - insights from amyloid-beta metabolism beyond the brain. *Nat Rev Neurol* 2017;**13**:612-623.
35. Murphy MP, LeVine H. Alzheimer's disease and the amyloid-beta peptide. *J Alzheimers Dis* 2010;**19**:311-323.

36. Troncone L, Luciani M, Coggins M, Wilker EH, Ho CY, Codispoti KE, Frosch MP, Kayed R, Del Monte F. Abeta Amyloid Pathology Affects the Hearts of Patients With Alzheimer's Disease: Mind the Heart. *J Am Coll Cardiol* 2016;**68**:2395-2407.
37. Cardiogenic Dementia. *Lancet* 1977;**1**:27-28.
38. Ott A, Breteler MM, de Bruyne MC, van Harskamp F, Grobbee DE, Hofman A. Atrial fibrillation and dementia in a population-based study. The Rotterdam Study. *Stroke* 1997;**28**:316-321.
39. Ihara M, Washida K. Linking Atrial Fibrillation with Alzheimer's Disease: Epidemiological, Pathological, and Mechanistic Evidence. *J Alzheimers Dis* 2018;**62**:61-72.
40. Dublin S, Anderson ML, Heckbert SR, Hubbard RA, Sonnen JA, Crane PK, Montine TJ, Larson EB. Neuropathologic changes associated with atrial fibrillation in a population-based autopsy cohort. *J Gerontol A Biol Sci Med Sci* 2014;**69**:609-615.
41. Ihne S, Morbach C, Obici L, Palladini G, Stork S. Amyloidosis in Heart Failure. *Curr Heart Fail Rep* 2019;**16**:285-303.
42. Goette A, Kalman JM, Aguinaga L, Akar J, Cabrera JA, Chen SA, Chugh SS, Corradi D, D'Avila A, Dobrev D, Fenelon G, Gonzalez M, Hatem SN, Helm R, Hindricks G, Ho SY, Hoit B, Jalife J, Kim YH, Lip GY, Ma CS, Marcus GM, Murray K, Nogami A, Sanders P, Uribe W, Van Wagoner DR, Nattel S, Document Reviewers: EHRA/HRS/APHRS/SOLAECE expert consensus on atrial cardiomyopathies: definition, characterization, and clinical implication. *Europace* 2016;**18**:1455-1490.
43. Solomon SD, Adams D, Kristen A, Grogan M, Gonzalez-Duarte A, Maurer MS, Merlini G, Damy T, Slama MS, Brannagan TH, Dispenzieri A, Berk JL, Shah AM, Garg P, Vaishnav A, Karsten V, Chen J, Gollob J, Vest J, Suhr O. Effects of Patisiran, an RNA Interference Therapeutic, on Cardiac Parameters in Patients With Hereditary Transthyretin-Mediated Amyloidosis. *Circulation* 2019;**139**:431-443.
44. Minamisawa M, Claggett B, Adams D, Kristen AV, Merlini G, Slama MS, Dispenzieri A, Shah AM, Falk RH, Karsten V, Sweetser MT, Chen J, Riese R, Vest J, Solomon SD. Association of Patisiran, an RNA Interference Therapeutic, With Regional Left Ventricular Myocardial Strain in Hereditary Transthyretin Amyloidosis: The APOLLO Study. *JAMA Cardiol* 2019;**4**:466-472.
45. Maurer MS, Schwartz JH, Gundapaneni B, Elliott PM, Merlini G, Waddington-Cruz M, Kristen AV, Grogan M, Witteles R, Damy T, Drachman BM, Shah SJ, Hanna M, Judge DP, Barsdorf AI, Huber P, Patterson TA, Riley S, Schumacher J, Stewart M, Sultan MB, Rapezzi C, ATTR-ACT Study Investigators. Tafamidis Treatment for Patients with Transthyretin Amyloid Cardiomyopathy. *N Engl J Med* 2018;**379**:1007-1016.
46. Molina CE, Abu-Taha IH, Wang Q, Rosello-Diez E, Kamler M, Nattel S, Ravens U, Wehrens XHT, Hove-Madsen L, Heijman J, Dobrev D. Profibrotic, Electrical, and Calcium-Handling Remodeling of the Atria in Heart Failure Patients With and Without Atrial Fibrillation. *Front Physiol* 2018;**9**:1383.

Figure Legends.

Figure 1. Venn diagram displaying the number of significantly upregulated and downregulated biomarkers in patients with atrial fibrillation versus sinus rhythm in the index (n=1,620) and validation cohort (n=1,219) of BIOSTAT-CHF.

Figure 2. Volcano plots of differential biomarker expression in patients with atrial fibrillation versus sinus rhythm in the index (A, n=1,620) and validation cohort (B, n=1,219) of BIOSTAT-CHF. Y-axis = significance, x-axis = effect size (positive = up-regulated, negative = down-regulated), labelled biomarkers are significantly differentially expressed proteins.

Figure 3. Results of pathway overrepresentation analyses of patients with atrial fibrillation versus sinus rhythm in the index cohort (**A** [n=1,620]) and the validation cohort (**B** [n=1,219]) of BIOSTAT-CHF. The nodes in blue represent the 24 biomarkers that were significantly upregulated in patients with atrial fibrillation as compared with those in sinus rhythm in the index cohort (A), and 8 biomarkers that were significantly upregulated in these patients in the validation cohort (B). The nodes in red reveal the overrepresented pathways of these biomarkers. Based on current knowledge, the blue nodes below the figures are biomarkers that were not found to be overrepresented in a specific biological pathway.

1 **Table 1.** Baseline characteristics of patients included in the index cohort.

	Atrial fibrillation n=648 (40%)	Sinus rhythm n=972 (60%)	P-value
Clinical			
Age (years)	72 ± 10	65 ± 13	<0.001
Women (%)	164 (25)	298 (31)	0.02
BMI (kg/m ²)	28 ± 6	27 ± 6	<0.001
NYHA Class I/II/III/IV (%)	9/50/37/4	12/53/32/4	0.07
Systolic blood pressure (mmHg)	125 ± 21	126 ± 23	0.43
Diastolic blood pressure (mmHg)	76 ± 13	75 ± 14	0.23
Heart rate (b.p.m.)	91 ± 24	80 ± 18	<0.001
Smoking			<0.001
Never	270 (42)	322 (33)	
Past	318 (49)	450 (46)	
Current	58 (9)	200 (21)	
History of (%)			
Coronary artery disease*	244 (38)	448 (46)	0.001
Valvular surgery	71 (11)	31 (3)	<0.001
Stroke	72 (11)	71 (7)	0.011
Peripheral artery disease	69 (11)	88 (9)	0.33
Hypertension	419 (65)	597 (61)	0.20
Diabetes	210 (32)	308 (32)	0.80
COPD	116 (18)	140 (14)	0.07
Renal disease	213 (33)	213 (22)	<0.001
Physical examination (%)			
Rales	356 (56)	486 (52)	0.10
Oedema	393 (70)	399 (50)	<0.001
Hepatomegaly	113 (18)	106 (11)	<0.001
KCCQ – Quality of Life			
Functional status score	43 [25, 64]	54 [34, 75]	<0.001
Clinical summary score	41 [24, 61]	50 [32, 71]	<0.001
Overall score	41 [26, 61]	51 [34, 70]	<0.001
Laboratory data			

NT-proBNP (ng/L)	3430 [1872, 6387]	2293 [925, 5347]	<0.001
Haemoglobin (g/L)	13.3 [11.9, 14.6]	13.4 [12.1, 16.6]	0.28
Creatinine (μmol/L)	104 [88, 131]	97 [80, 121]	<0.001
Echocardiographic data			
LVEF, %	33 ± 12	29 ± 10	<0.001
Left atrial diameter, mm	50 ± 8	46 ± 8	<0.001
Interventricular wall thickness, mm	11 ± 2	10 ± 2	<0.001
Posterior wall thickness, mm	11 ± 2	10 ± 2	<0.001
Medication at baseline (%)			
ACE i or ARB	440 (68)	726 (75)	0.003
β blocker	523 (81)	810 (83)	0.20
MRA	318 (49)	516 (53)	0.13
Diuretics	648 (100)	971 (100)	1.00
Amiodarone	81 (13)	110 (11)	0.52
Digoxin	246 (38)	60 (6)	<0.001
Verapamil/diltiazem	22 (3)	7 (1)	<0.001
Class 1c antiarrhythmic drugs	2 (1)	2 (1)	1.00
Ivabradine	0 (0)	23 (2)	<0.001
Vitamin K antagonist	461 (71)	130 (13)	<0.001
Direct oral anticoagulants	7 (1)	0 (0)	0.004

*Coronary artery disease: previous myocardial infarction, percutaneous coronary intervention (PCI) and/or coronary artery bypass graft (CABG). BMI=body mass index, NYHA=New York Heart Association, LVEF=left ventricular ejection fraction, BP = blood pressure, COPD=chronic obstructive pulmonary disease, KCCQ=Kansas City Cardiomyopathy Questionnaire, NT-proBNP=N-terminal pro-B-type natriuretic peptide, ACE i=angiotensin converting enzyme inhibitor, ARB=angiotensin receptor blocker, MRA=mineralocorticoid receptor antagonist. P-values for group comparisons were tested using Student's t tests, Mann-Whitney U tests, or Chi-square tests where appropriate.

1 **Table 2.** Baseline characteristics of patients included in the validation cohort.

	Atrial fibrillation n=468 (38%)	Sinus rhythm n=751 (62%)	P-value
Clinical			
Age (years)	75 ± 10	72 ± 11	<0.001
Women (%)	148 (32)	291 (39)	0.01
BMI (kg/m ²)	29 ± 6	29 ± 6	0.13
NYHA Class I/II/III/IV (%)	0/39/46/14	2/42/44/13	0.22
Systolic blood pressure (mmHg)	126 ± 21	127 ± 23	0.38
Diastolic blood pressure (mmHg)	72 ± 15	68 ± 12	<0.001
Heart rate (b.p.m.)	87 ± 27	72 ± 18	<0.001
Smoking			<0.001
Never	275 (59)	358 (48)	
Past	148 (32)	279 (37)	
Current	43 (9)	110 (15)	
History of (%)			
Coronary artery disease*	194 (42)	462 (62)	<0.001
Valvular surgery	39 (8)	39 (5)	0.04
Stroke	105 (23)	105 (14)	<0.001
Peripheral artery disease	96 (21)	160 (22)	0.79
Hypertension	274 (59)	434 (58)	0.80
Diabetes	149 (32)	225 (30)	0.53
COPD	83 (18)	135 (18)	0.99
Renal disease	220 (47)	312 (42)	0.11
Physical examination (%)			
Rales	219 (49)	301 (42)	0.02
Oedema	290 (68)	385 (57)	0.001
Hepatomegaly	20 (5)	23 (3)	0.36
KCCQ – Quality of Life			
Functional status score	45 [25, 65]	46 [27, 71]	0.05
Clinical summary score	41 [25, 65]	45 [26, 70]	0.07
Overall score	42 [30, 60]	45 [30, 68]	0.03
Laboratory data			
NT-proBNP (ng/L)	2105 [1045, 4204]	872 [311, 2807]	<0.001
Haemoglobin (g/L)	13.5 [12.1, 14.7]	13.1 [11.7, 14.3]	0.004
Creatinine (μmol/L)	98 [82, 123]	95 [77, 121]	0.04
Echocardiographic data			
LVEF, %	43 ± 13	41 ± 13	0.03
Left atrial diameter, mm	48 ± 7	43 ± 7	<0.001
Interventricular wall thickness, mm	13 ± 3	12 ± 4	0.29
Posterior wall thickness, mm	12 ± 4	11 ± 5	0.54
Medication at baseline (%)			
ACE i or ARB	322 (69)	540 (72)	0.28

β blocker	339 (72)	540 (72)	0.89
MRA	144 (31)	229 (31)	0.97
Diuretics	457 (98)	746 (99)	0.02
Amiodarone	12 (3)	27 (4)	0.41
Digoxin	193 (41)	12 (2)	<0.001
Verapamil/diltiazem	18 (4)	13 (2)	0.036
Class 1c antiarrhythmic drugs	0 (0)	0 (0)	1.00
Ivabradine	1 (1)	33 (4)	<0.001
Vitamin K antagonist	327 (70)	87 (12)	<0.001
Direct oral anticoagulants	22 (5)	6 (1)	<0.001

*Coronary artery disease: previous myocardial infarction, percutaneous coronary intervention (PCI) and/or coronary artery bypass graft (CABG). BMI=body mass index, NYHA=New York Heart Association, LVEF=left ventricular ejection fraction, BP = blood pressure, COPD=chronic obstructive pulmonary disease, KCCQ=Kansas City Cardiomyopathy Questionnaire, NT-proBNP=N-terminal pro-B-type natriuretic peptide, ACE i=angiotensin converting enzyme inhibitor, ARB=angiotensin receptor blocker, MRA=mineralocorticoid receptor antagonist. P-values for group comparisons were tested using Student's t tests, Mann-Whitney U tests, or Chi-square tests where appropriate.

Figure 1. Venn diagram displaying the number of significantly upregulated and downregulated biomarkers in patients with atrial fibrillation versus sinus rhythm in the index (n=1,620) and validation cohort (n=1,219) of BIOSTAT-CHF.

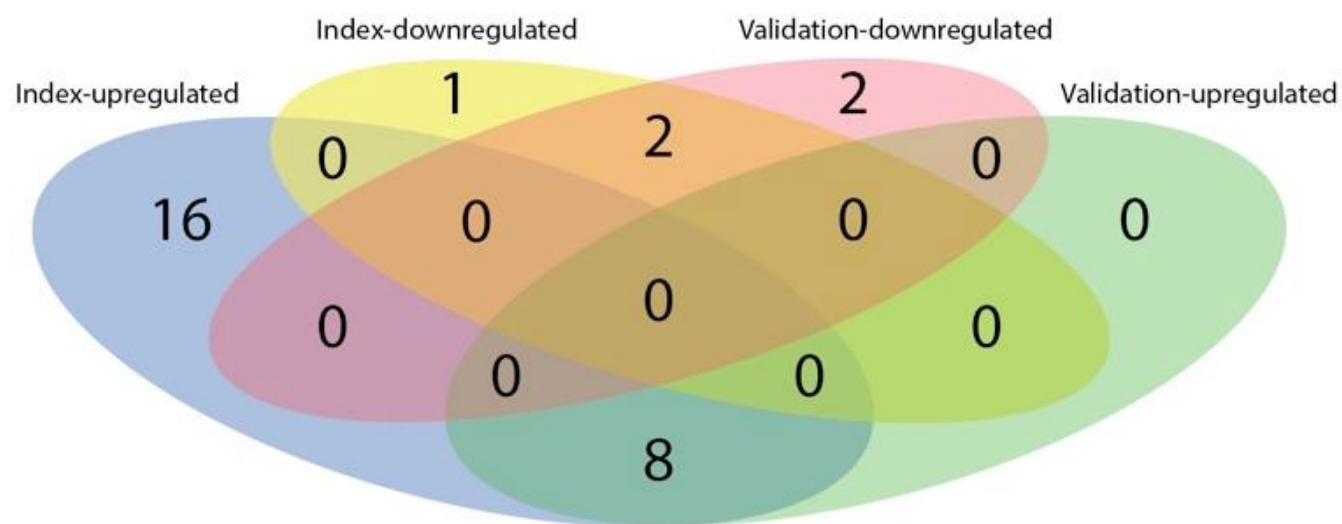


Figure 2. Volcano plots of differential biomarker expression in patients with atrial fibrillation versus sinus rhythm in the index (A, n=1,620) and validation cohort (B, n=1,219) of BIOSTAT-CHF. Y-axis = significance, x-axis = effect size (positive = up-regulated, negative = down-regulated), labelled biomarkers are significantly differentially expressed proteins.

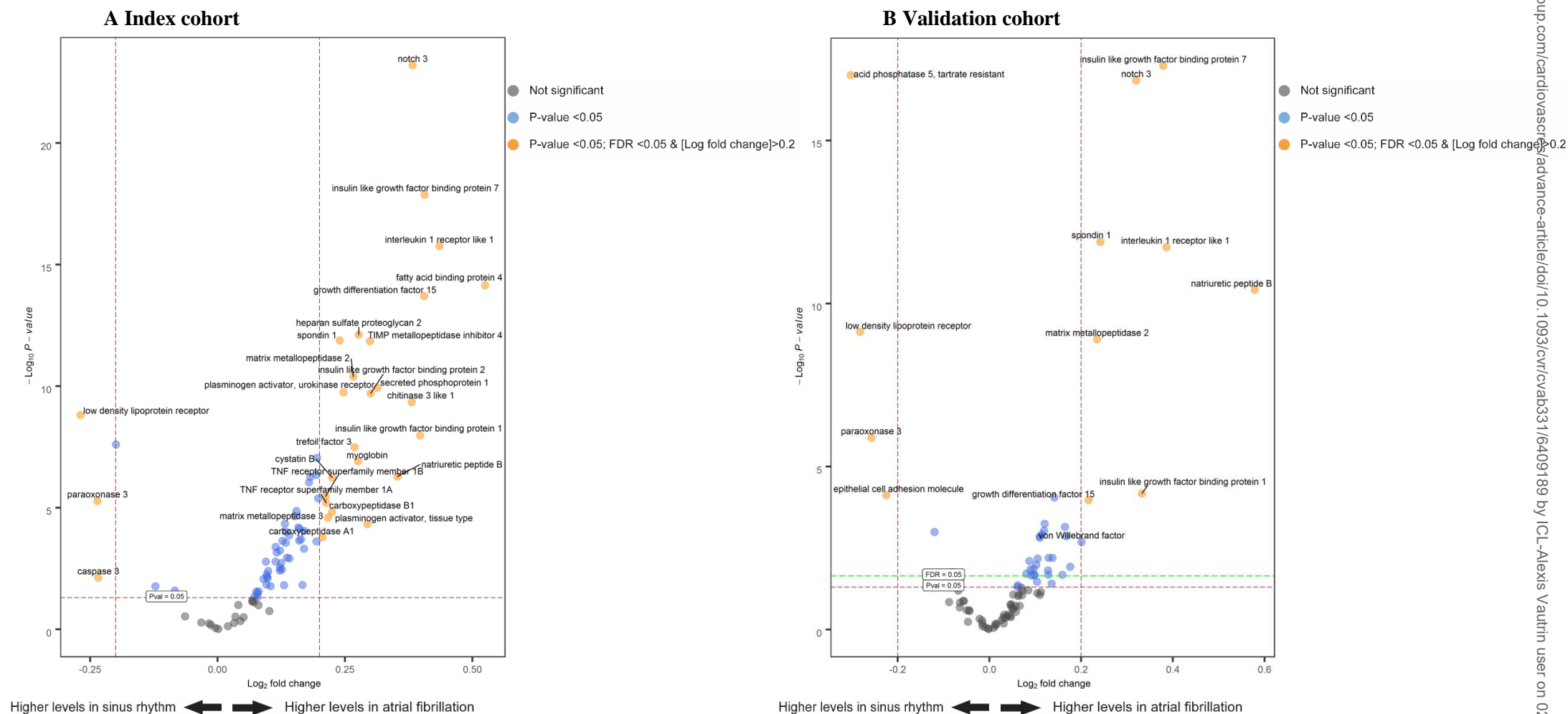
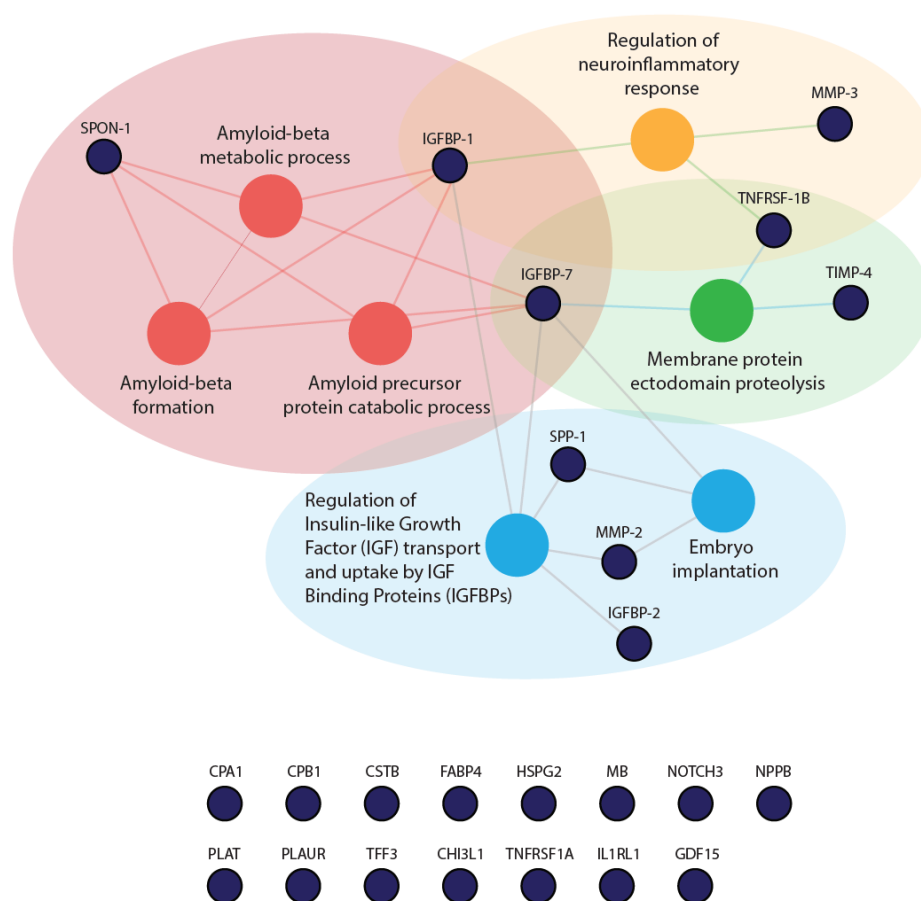
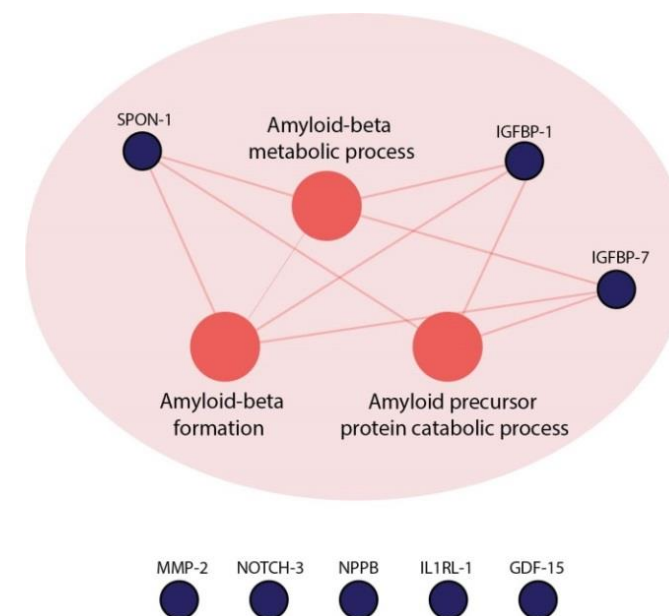


Figure 3. Results of pathway overrepresentation analyses of patients with atrial fibrillation versus sinus rhythm in the index cohort (**A** [n=1,620]) and the validation cohort (**B** [n=1,219]) of BIOSTAT-CHF. The nodes in blue represent the 24 biomarkers that were significantly upregulated in patients with atrial fibrillation as compared with those in sinus rhythm in the index cohort (**A**), and 8 biomarkers that were significantly upregulated in these patients in the validation cohort (**B**). The nodes in red reveal the overrepresented pathways of these biomarkers. Based on current knowledge, the blue nodes below the figures are biomarkers that were not found to be overrepresented in a specific biological pathway.

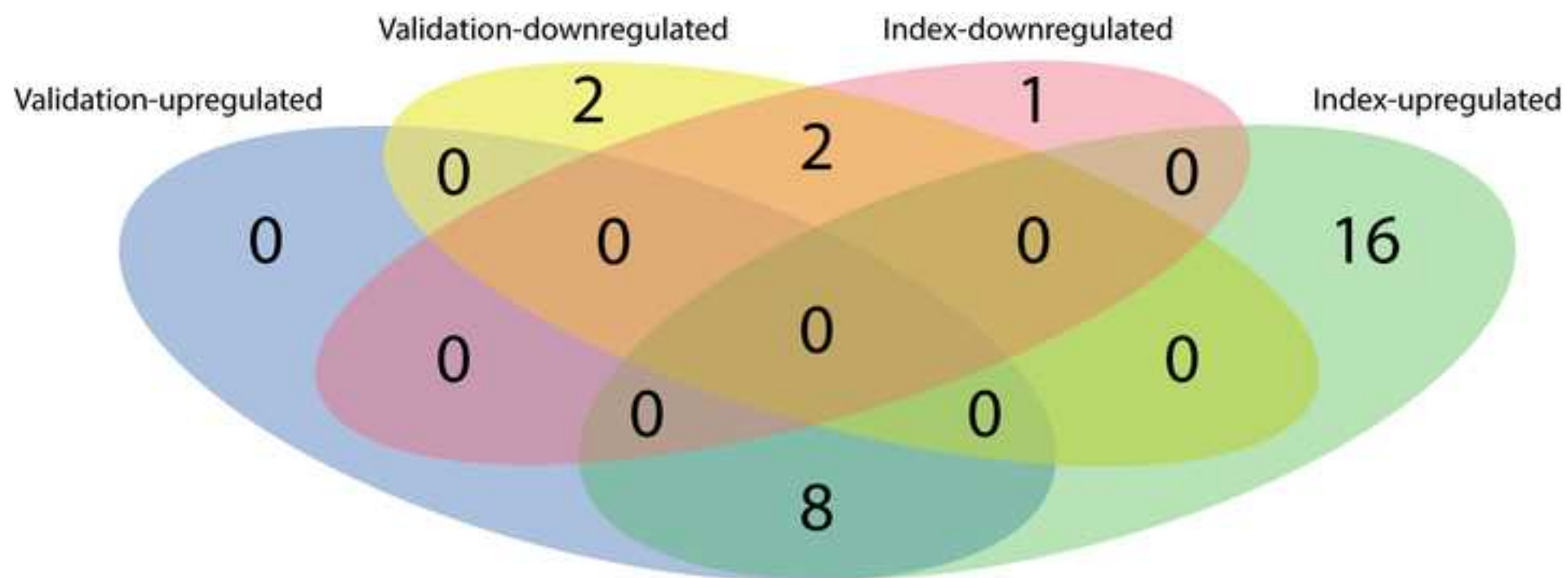
A.



B.



1 **Abbreviations:** SPON-1=spondin 1, IGFBP-1=insulin like growth factor binding protein 1, IGFBP-7=insulin like growth factor binding protein 7, MMP-3=matrix metalloproteinase 3,
2 TNFRSF1B= TNF receptor superfamily member 1B, TIMP-4= Metalloproteinase inhibitor 4, SPP-1= secreted phosphoprotein 1, MMP-2=matrix metalloproteinase 2, IGFBP-2= insulin like
3 growth factor binding protein 2, CPA1= carboxypeptidase A1, CPB1= carboxypeptidase B1, CSTB= cystatin B, FABP4= Fatty acid-binding protein, adipocyte, HSPG2= heparan sulfate
4 proteoglycan 2, MB= Myoglobin, NOTCH3= Neurogenic locus notch homolog protein 3, NPPB=natriuretic peptide B, PLAT= plasminogen activator, tissue type, PLAUR= plasminogen
5 activator, urokinase receptor, TFF3= trefoil factor 3, CHI3L1= chitinase 3 like 1, TNFRSF1A= TNF receptor superfamily member 1A, IL1RL1=interleukin 1 receptor like 1, GDF-15=growth
6 differentiation factor 15.



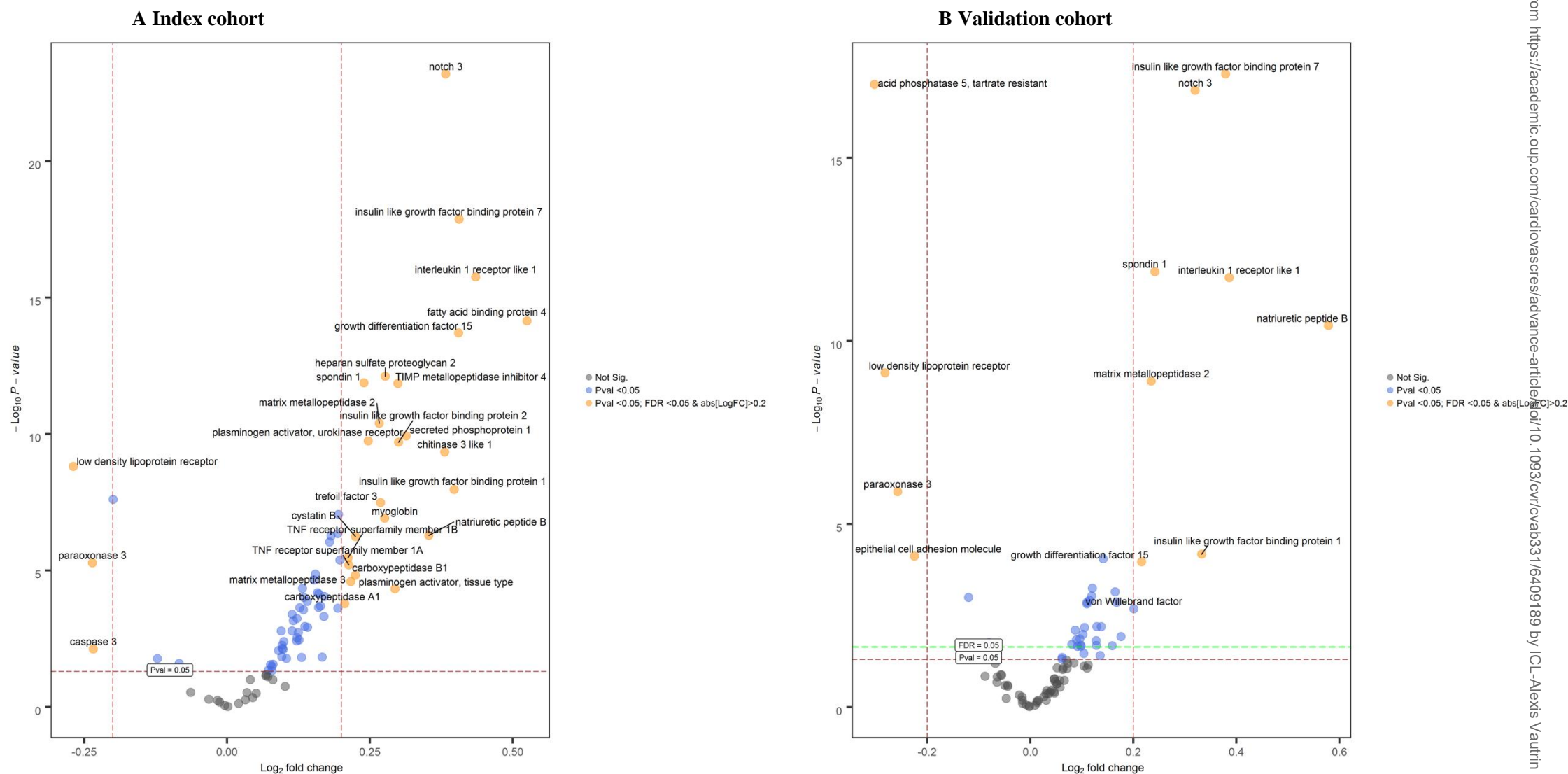
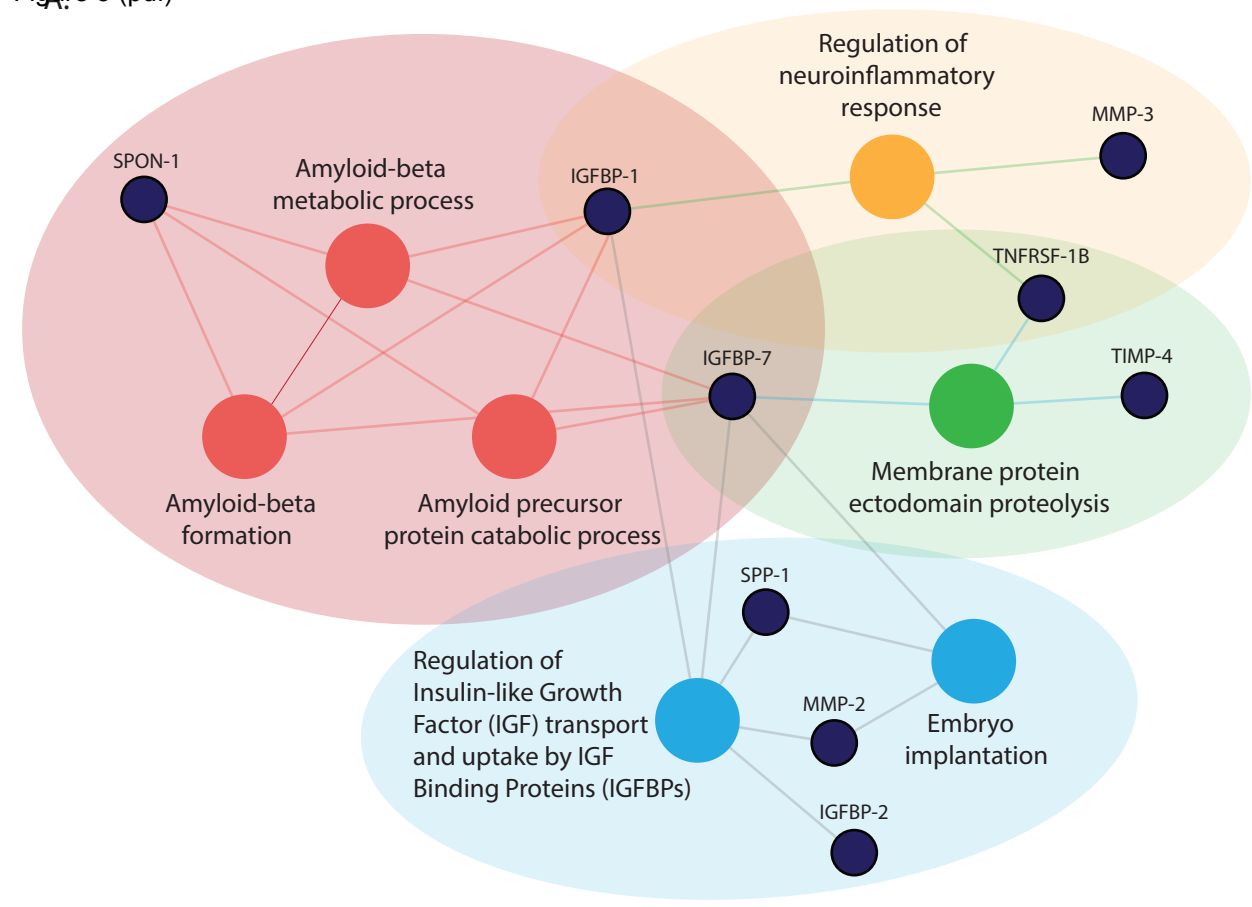


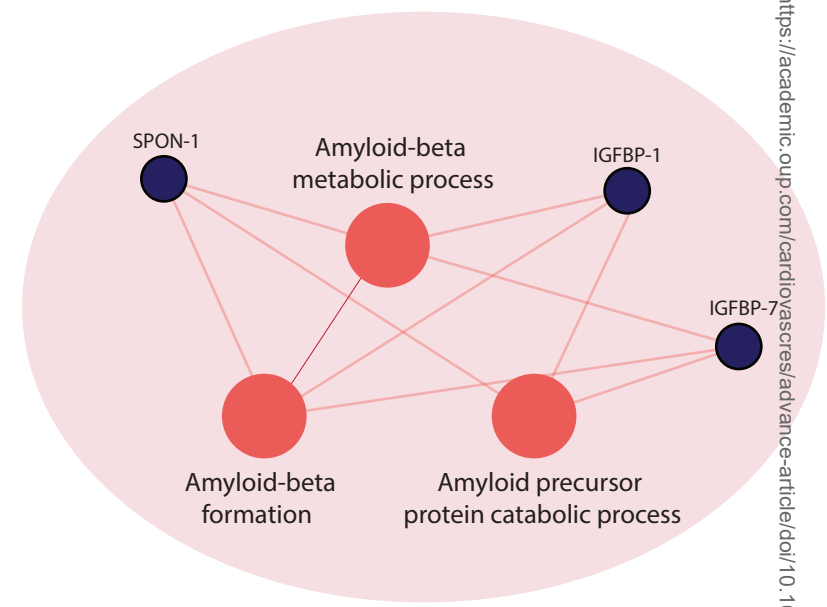
Figure 3 (pdf)



- | | | | | | | | |
|------|-------|------|--------|----------|--------|--------|------|
| CPA1 | CPB1 | CSTB | FABP4 | HSPG2 | MB | NOTCH3 | NPPB |
| PLAT | PLAUR | TFF3 | CHI3L1 | TNFRSF1A | IL1RL1 | GDF15 | |

B.

[Click here to access/download;Figure\(s\);Figure 3.pdf](#)



- | | | | | |
|-------|---------|------|---------|--------|
| MMP-2 | NOTCH-3 | NPPB | IL1RL-1 | GDF-15 |
| | | | | |

Pathophysiological Pathways in Patients with Heart Failure and Atrial Fibrillation

