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# Pathophysiological pathways in patients with heart failure and atrial fibrillation

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1 **Abstract.**

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

6 **Methods and Results.** From a panel of 92 biomarkers from different pathophysiological  
7 domains available in 1,620 patients with HF, we first tested which biomarkers were  
8 dysregulated in patients with HF and AF (n=648) compared with patients in sinus rhythm  
9 (n=972). Secondly, pathway overrepresentation analyses were performed to identify  
10 biological pathways linked to higher plasma concentrations of biomarkers in patients who had  
11 HF and AF. Findings were validated in an independent HF cohort (n=1,219, 38% with AF).  
12 Patient with AF and HF were older, less often women, and less often had a history of  
13 coronary artery disease compared with those in sinus rhythm. In the index cohort, 24  
14 biomarkers were upregulated in patients with AF and HF. In the validation cohort, 8  
15 biomarkers were upregulated, which all overlapped with the 24 biomarkers found in the index  
16 cohort. The strongest up-regulated biomarkers in patients with AF were spondin-1 (fold  
17 change 1.18,  $p=1.33 \times 10^{-12}$ ), insulin-like growth factor-binding protein-1 (fold change 1.32,  
18  $p=1.08 \times 10^{-8}$ ), and insulin-like growth factor-binding protein-7 (fold change 1.33,  $p=1.35 \times 10^{-$   
19  $18$ ). Pathway overrepresentation analyses revealed that the presence of AF was associated with  
20 activation amyloid-beta metabolic processes, amyloid-beta formation, and amyloid precursor  
21 protein catabolic processes with a remarkable consistency observed in the validation cohort.

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

25 **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.<sup>1-3</sup> 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.<sup>2,3</sup> 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.<sup>4,5</sup> Moreover, patients with AF and HF have a  
9 poorer quality of life, and worse outcome as compared with those without AF.<sup>4,6</sup> 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,  $\beta$ -blockers did not  
12 improve clinical outcomes in patients with AF and HFrEF.<sup>7</sup> The potential lack of  $\beta$ -blocker  
13 efficacy suggests differences in pathophysiology between HF patients with and without AF,  
14 but the exact mechanisms remain poorly understood and understudied.<sup>7</sup>  
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.<sup>8,9</sup> 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.<sup>10,11</sup> 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.

## 1 **Methods**

### 2 *Patient population*

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

### 15 *Definitions*

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

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

#### 5 *Biomarkers*

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

#### 15 *Statistical analyses*

16 Normally distributed continuous variables were displayed as mean ± standard deviation, non-  
17 normally distributed variables as median with the first and third quartile (Q1-Q3). Categorical  
18 variables were presented as numbers with percentages. Group comparisons were tested using  
19 Student’s t tests, Mann-Whitney U tests, or Chi-square tests where appropriate. Differences in  
20 expression of the 92 biomarkers between patients with AF versus sinus rhythm were tested  
21 using Linear Models for Microarray data analysis (Limma) software (version 3.34.9), using a  
22 log<sub>2</sub> fold change cutoff of 0.2, and a false discovery rate <0.05 according to the Benjamini-  
23 Hochberg method. The biomarkers that were upregulated in patients with AF compared to  
24 those in sinus rhythm were further studied by using pathway overrepresentation analysis.  
25 Pathway overrepresentation analysis was performed with knowledge from established

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

20

## 21 **Results**

### 22 **Index cohort**

#### 23 *Clinical characteristics*

24 Of 1,620 patients with HF enrolled, 648 (40%) had AF and 972 (60%) were in sinus rhythm.

25 Patients with AF were older, less often women, had a higher BMI and a higher heart rate



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

#### 5 *Biomarker concentrations*

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

#### 14 *Pathway overrepresentation analyses of upregulated biomarkers*

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

### 21 **Validation cohort**

#### 22 *Clinical characteristics*

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

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

#### 5 *Biomarker concentrations*

6 In the validation cohort, eight biomarkers were significantly upregulated in patients with AF,  
7 all of which overlapped with the 24 biomarkers that were found to be significantly  
8 upregulated in the index cohort (*Figure 1*). The eight biomarkers that were upregulated in AF  
9 patients in both HF cohorts included IGFBP7 (fold change 1.30,  $p=5.13 \times 10^{-18}$ ), NOTCH3  
10 (fold change 1.25,  $p=1.44 \times 10^{-17}$ ), spondin 1 (SPON1, fold change 1.18,  $p=1.29 \times 10^{-12}$ ),  
11 IL1RL1 (fold change 1.31,  $p=3.44 \times 10^{-11}$ ), natriuretic peptide B (fold change 1.49,  $p=3.78 \times 10^{-11}$ ),  
12 matrix metalloproteinase 2 (MMP2, fold change 1.18,  $p=1.25 \times 10^{-9}$ ), IGFBP1 (fold change  
13 1.26,  $p=6.68 \times 10^{-5}$ ), and growth differentiation factor 15 (GDF15, fold change 1.16,  
14  $p=1.07 \times 10^{-4}$ ) (*Supplementary Table 2*). Low-density lipoprotein receptor (LDLR) and  
15 paraoxonase 3 (PON3) were significantly downregulated in patients with AF in both the index  
16 and the validation cohort.

#### 17 *Pathway overrepresentation analyses of upregulated biomarkers*

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

#### 21 *Overlap index and validation cohort*

22 The three amyloid-beta-related pathways were found in both the index and validation cohort,  
23 and were related to three upregulated biomarkers in patients with AF: SPON1 (fold change  
24 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,

1  $p=1.35 \times 10^{-18}$ ). After adjusting for the clinical covariates age, sex, body mass index, heart rate,  
2 a history of coronary artery disease, and renal disease, the concentrations of SPON-1, IGFBP-  
3 1, and IGFBP-7 remained associated with the presence of AF (all  $p < 0.001$ ), in both the index  
4 and the validation cohort. These associations were similar among HF phenotypes  
5 (HF<sub>r</sub>EF/HF<sub>m</sub>rEF/HF<sub>p</sub>EF;  $p$  for interaction = 0.42) and across LVEF as a continuous variable  
6 ( $p$  for interaction = 0.96).

7

## 8 **Discussion**

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

### 20 *Individual biomarkers*

21 In the present study, the concentrations of SPON-1, IGFBP-1 and IGFBP-7 were closely  
22 linked to the three amyloid-beta-related pathways. Although IGFBP-1 and IGFBP-7 are  
23 linked to a wide range of biological processes associated with inhibition and stimulation of  
24 cell growth, the current knowledge indicates a more specific role for SPON-1.<sup>20</sup> SPON-1 is an  
25 extracellular matrix cell adhesion glycoprotein that is expressed in multiple organs including

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

### 16 *Amyloid-beta in heart failure and atrial fibrillation*

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

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

### 13 *Clinical relevance*

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

## 1 *Strengths and Limitations*

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

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

1 analyses. There was, however, no interaction between the amyloid-beta-related biomarkers  
2 and the HF subgroups, suggesting a pathophysiological role of amyloid-beta in HF patients  
3 across the full LVEF spectrum. Not all (combinations of) biomarkers were annotated by the  
4 publicly available databases even though these biomarkers were deemed to be significantly  
5 up- or downregulated in our analyses (e.g. NOTCH3 and IL1RL1), since the content of these  
6 databases is based on what is currently known about these biomarkers. Therefore, the results  
7 of the current pathway overrepresentation analyses might change over time, when the  
8 knowledge on the (combination of) biomarkers has increased. The current findings might  
9 reflect underlying pathophysiological processes specific to elderly patients with AF and HF,  
10 since the mean age of these patients was 72 and 75 years in the two cohorts respectively. The  
11 number of women included in the cohorts was limited (n=462 [index] and n=439 [validation],  
12 32%) and mainly comprised postmenopausal women. Even though we have attempted to  
13 define the AF and sinus rhythm group mutually exclusive, it is possible that patients with  
14 asymptomatic paroxysmal AF were misclassified. Based on the current definition, we may  
15 have predominantly included patients with persistent/permanent AF and less patients with  
16 paroxysmal AF. Moreover, we do not have data on the duration of AF since no continuous  
17 rhythm monitoring was incorporated in the study protocol, which might have influenced the  
18 biomarker concentrations. Unfortunately, we also do not have information on cognitive  
19 function, other neurologic diseases, nor information on systemic or cardiac amyloidosis of  
20 patients enrolled in BIOSAT-CHF, which could have strengthened our hypothesis linking  
21 AF – HF to Alzheimer’s disease and/or amyloidosis. As with all cross-sectional studies, we  
22 cannot prove causality.

## 23 **Conclusion**

24 In two independent cohorts of patients with HF, the presence of AF was associated with  
25 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



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## 3 **Author Contribution Statement**

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

## 10 **Conflict of Interest**

11 AAV and/or his institution received grants and/or consultancy reimbursements from Amgen,  
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### 13 **Data availability**

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

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37

1 **Figure Legends.**

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

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

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

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

	<b>Atrial fibrillation</b> n=648 (40%)	<b>Sinus rhythm</b> n=972 (60%)	<b>P-value</b>
<b>Clinical</b>			
Age (years)	72 ± 10	65 ± 13	<0.001
Women (%)	164 (25)	298 (31)	0.02
BMI (kg/m <sup>2</sup> )	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)	
<b>History of (%)</b>			
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
<b>Physical examination (%)</b>			
Rales	356 (56)	486 (52)	0.10
Oedema	393 (70)	399 (50)	<0.001
Hepatomegaly	113 (18)	106 (11)	<0.001
<b>KCCQ – Quality of Life</b>			
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
<b>Laboratory data</b>			



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
<b>Echocardiographic data</b>			
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
<b>Medication at baseline (%)</b>			
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

1

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

8

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

	<b>Atrial fibrillation</b> n=468 (38%)	<b>Sinus rhythm</b> n=751 (62%)	<b>P-value</b>
<b>Clinical</b>			
Age (years)	75 ± 10	72 ± 11	<0.001
Women (%)	148 (32)	291 (39)	0.01
BMI (kg/m <sup>2</sup> )	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)	
<b>History of (%)</b>			
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
<b>Physical examination (%)</b>			
Rales	219 (49)	301 (42)	0.02
Oedema	290 (68)	385 (57)	0.001
Hepatomegaly	20 (5)	23 (3)	0.36
<b>KCCQ – Quality of Life</b>			
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
<b>Laboratory data</b>			
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
<b>Echocardiographic data</b>			
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
<b>Medication at baseline (%)</b>			
ACE i or ARB	322 (69)	540 (72)	0.28

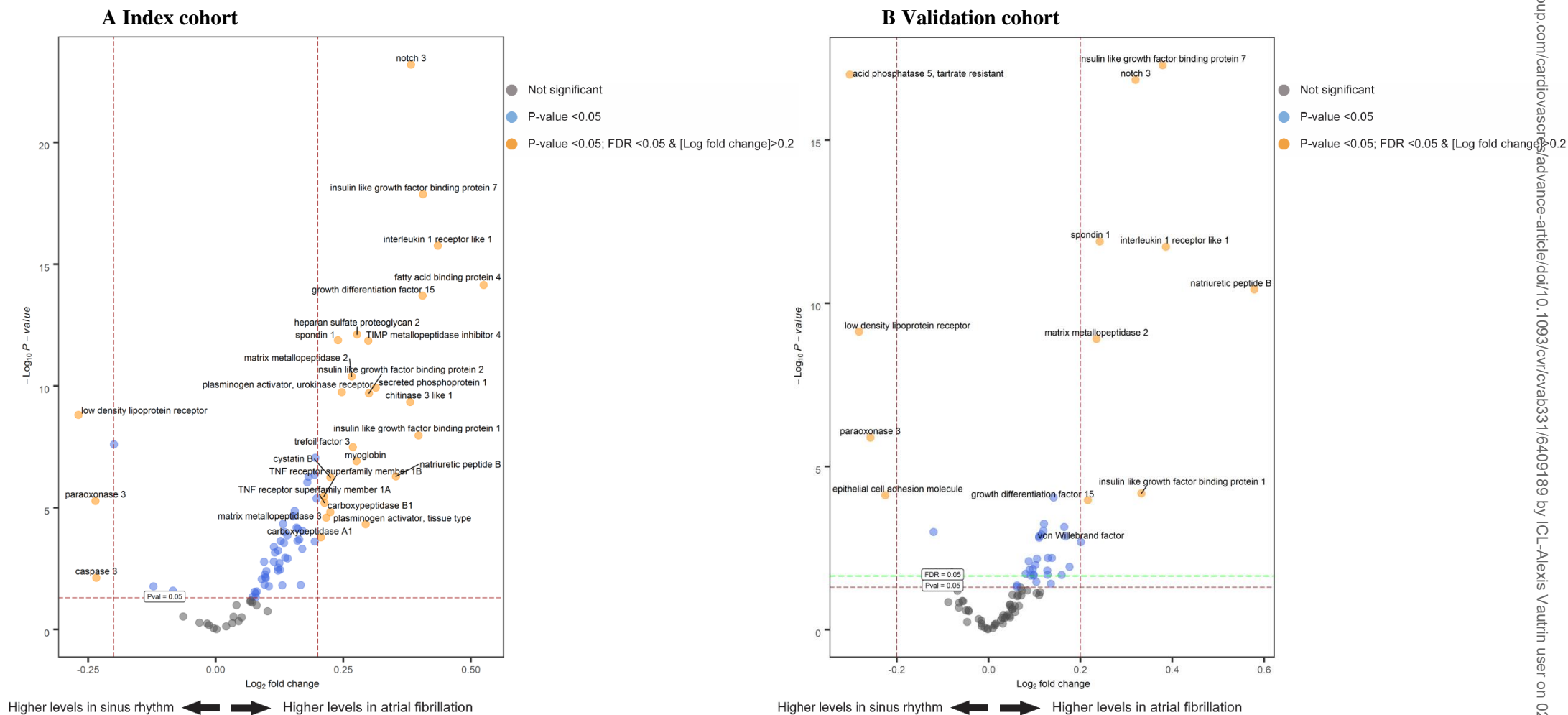
β blocker	339 (72)	540 (72)	0.89
MRA	144 (31)	229 (31)	0.97
Diuretics	457 (98)	746 (99)	0.02
Amiodarone	12 (3)	27 (4)	0.41
Digoxin	193 (41)	12 (2)	<0.001
Verapamil/diltiazem	18 (4)	13 (2)	0.036
Class 1c antiarrhythmic drugs	0 (0)	0 (0)	1.00
Ivabradine	1 (1)	33 (4)	<0.001
Vitamin K antagonist	327 (70)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

1

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

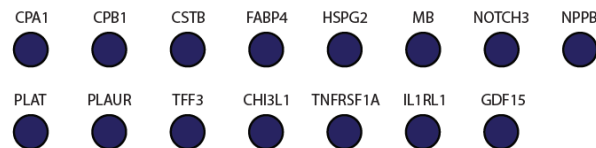
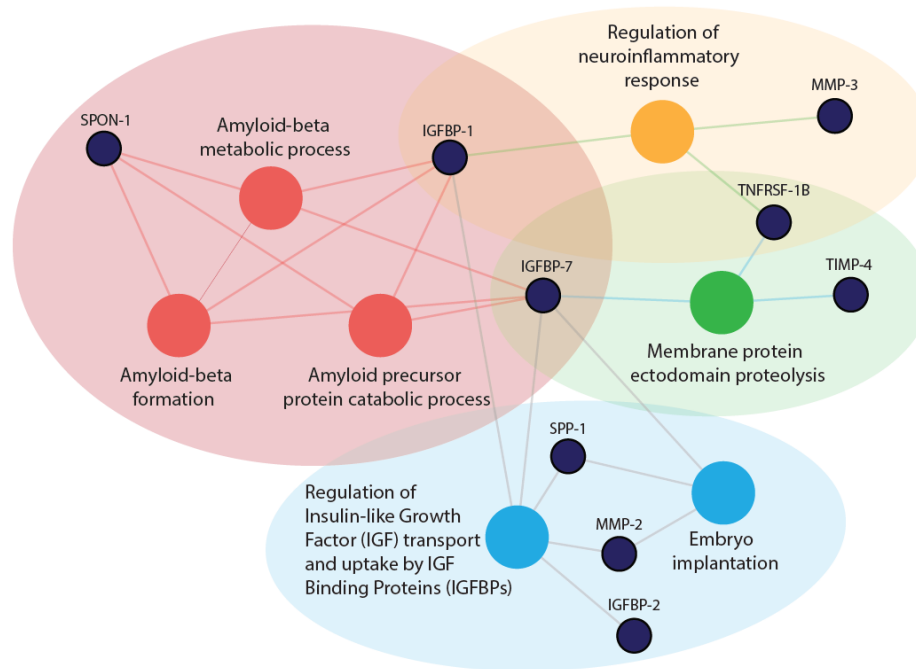


**Figure 2.** Volcano plots of differential biomarker expression in patients with atrial fibrillation versus sinus rhythm in the index (A, n=1,620) and validation cohort (B, n=1,219) of BIostat-CHE. Y-axis = significance, x-axis = effect size (positive = up-regulated, negative = down-regulated), labelled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

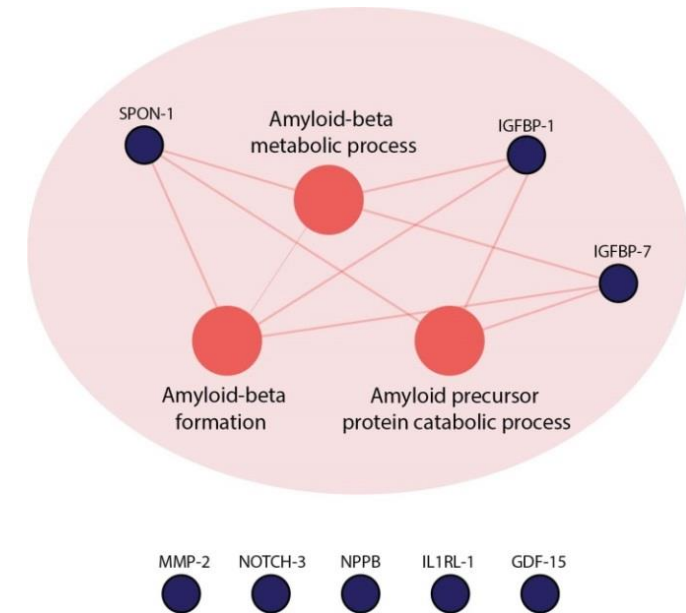


**Figure 3.** Results of pathway overrepresentation analyses of patients with atrial fibrillation versus sinus rhythm in the index cohort (**A** [n=1,620]) and the validation cohort (**B** [n=1,219]) of BIOSTAT-CHF. The nodes in blue represent the 24 biomarkers that were significantly upregulated in 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.

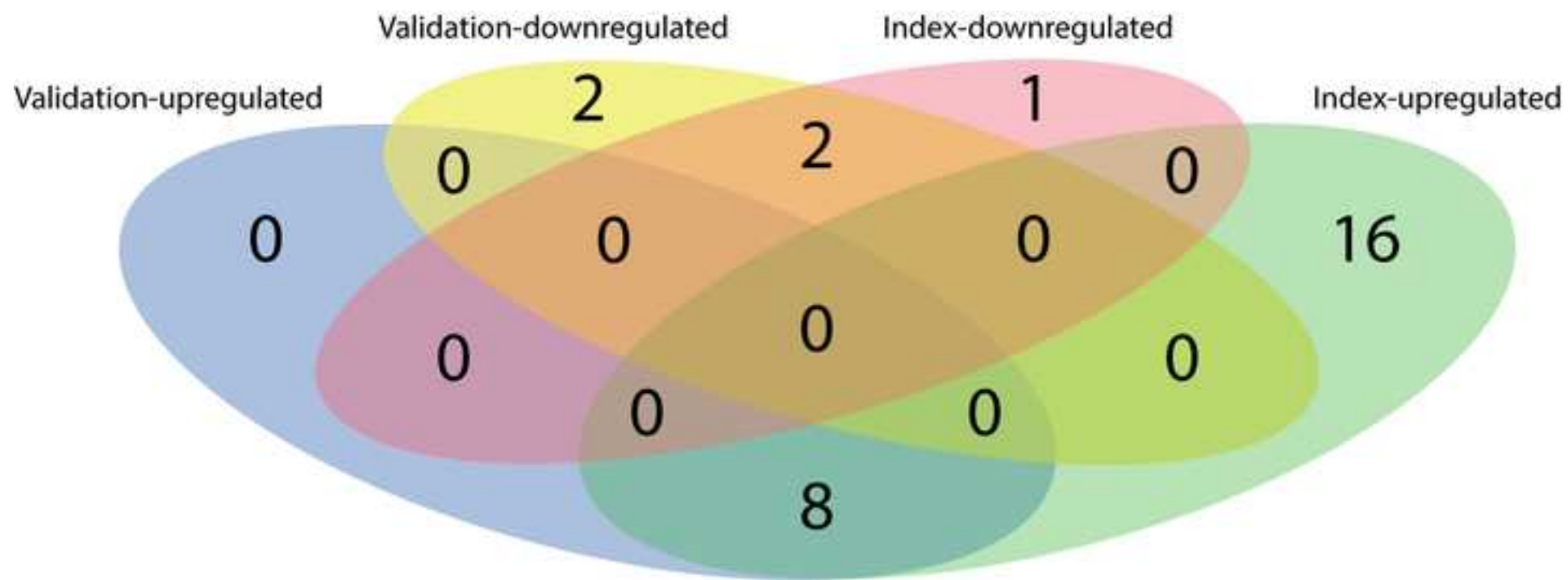
**A.**



**B.**

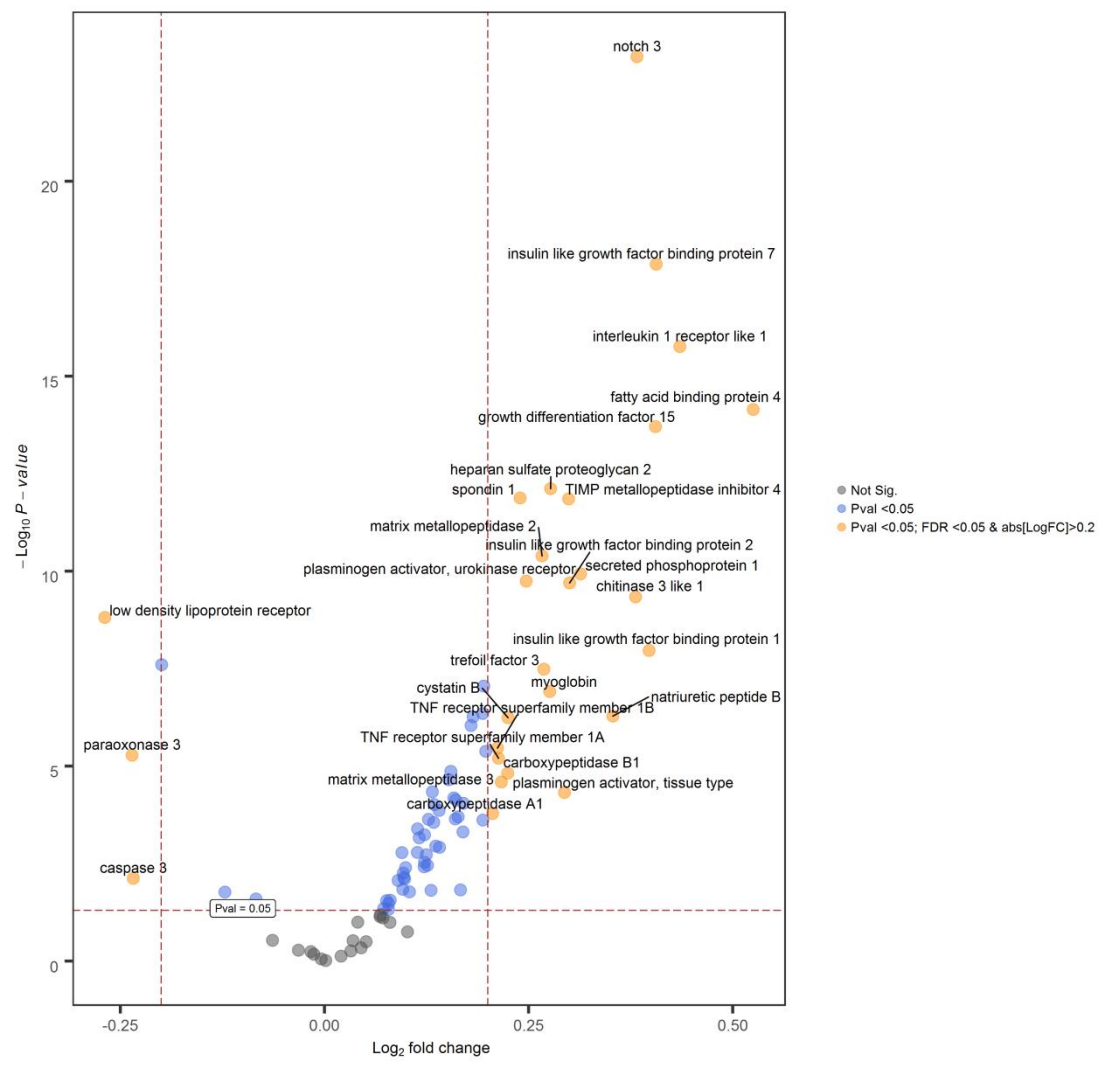


1 **Abbreviations:** SPON-1=spondin 1, IGFBP-1=insulin like growth factor binding protein 1, IGFBP-7=insulin like growth factor binding protein 7, MMP-3=matrix metalloproteinase 3,  
2 TNFRSF1B= TNF receptor superfamily member 1B, TIMP-4= Metalloproteinase inhibitor 4, SPP-1= secreted phosphoprotein 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.





### A Index cohort



### B Validation cohort

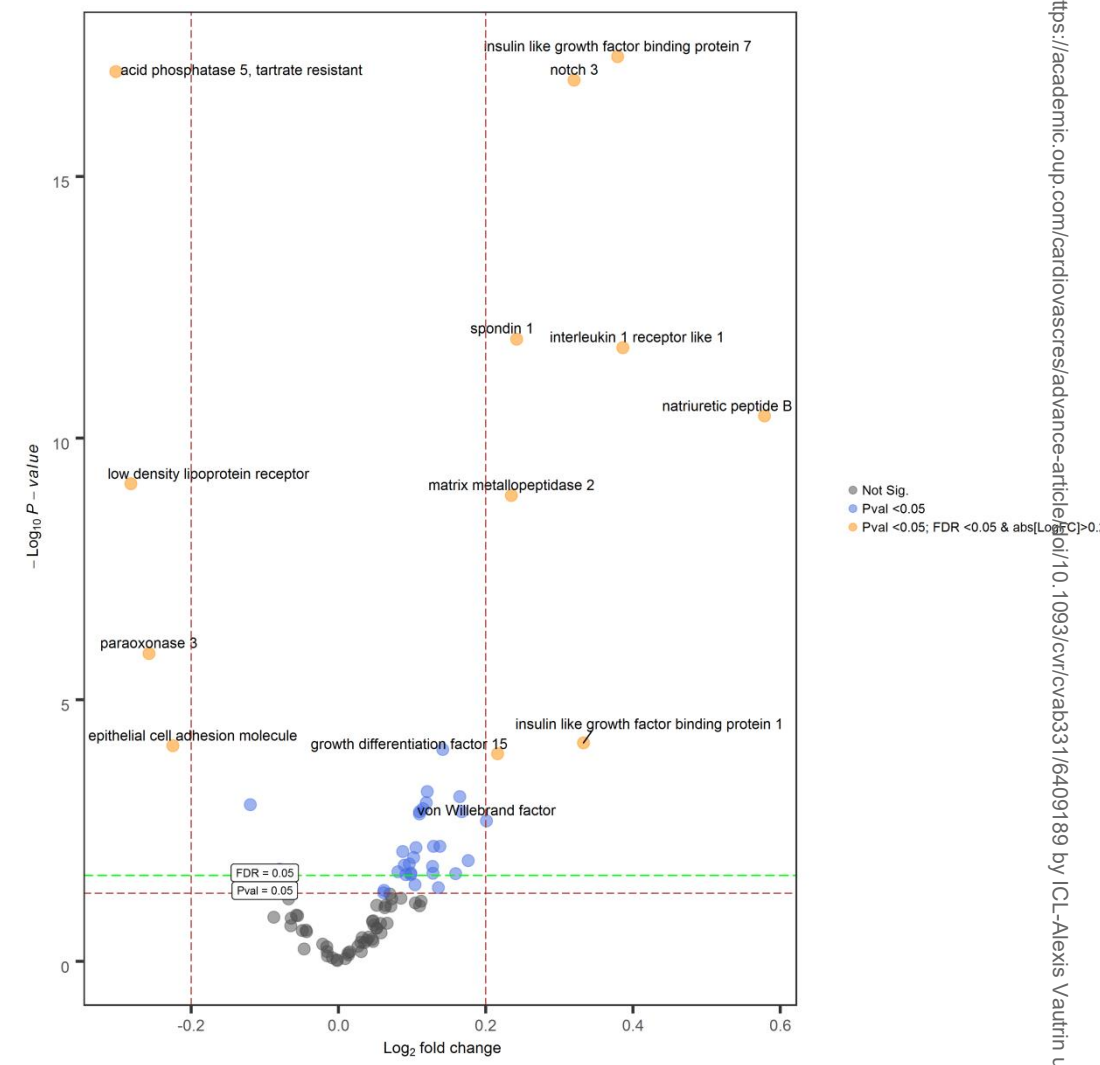
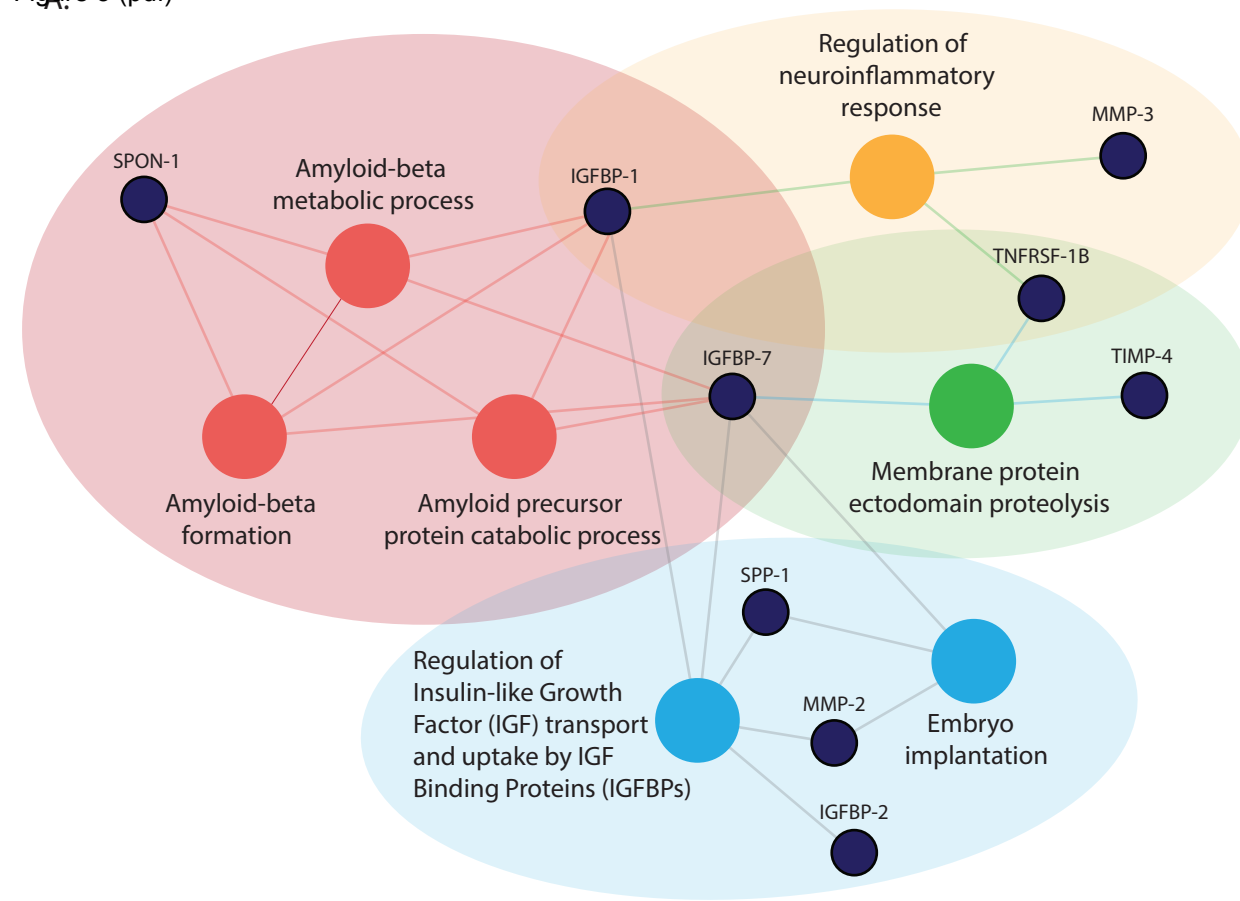


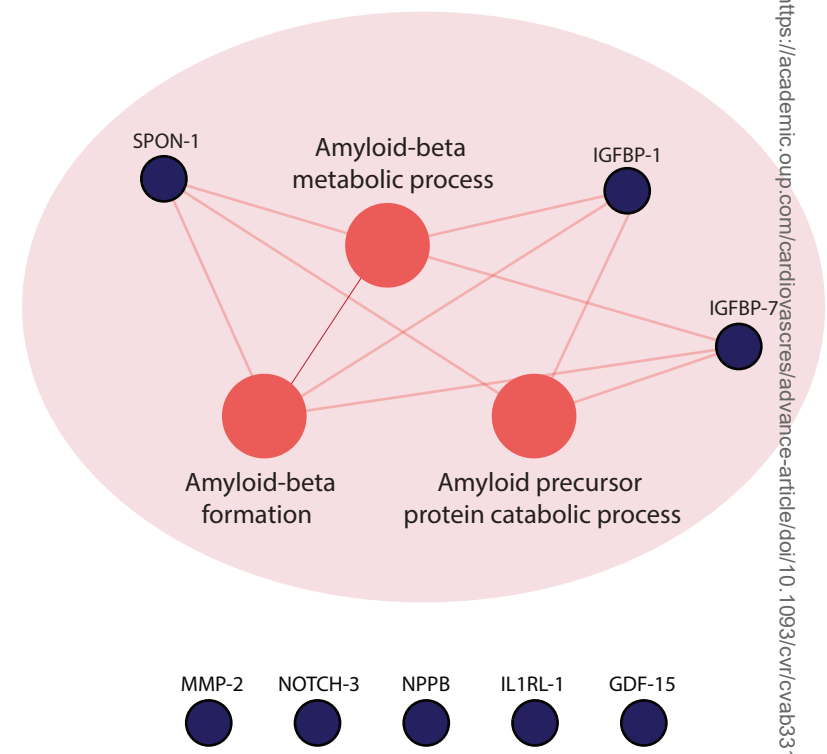
Figure 3 (pdf)



- |      |       |      |        |          |        |        |      |
|------|-------|------|--------|----------|--------|--------|------|
| 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

