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**Plasma protein biomarkers and their association with mutually exclusive cardiovascular phenotypes: the FIBRO-TARGETS case-control analyses**

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*On behalf of the FIBRO-TARGETS investigators*

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## **Abstract**

*Background:* Hypertension, obesity and diabetes are major and potentially modifiable “risk factors” for cardiovascular diseases. Identification of biomarkers specific to these risk factors may help understanding the underlying pathophysiological pathways, and developing individual treatment.

*Methods:* The FIBRO-TARGETS (Targeting Cardiac Fibrosis for Heart Failure Treatment) consortium has merged data from 12 patient cohorts in one common database of >12,000 patients. Three mutually-exclusive main phenotypic groups were identified (“cases”): 1) “hypertensive”; 2) “obese”; and 3) “diabetic”; age-sex matched in a 1:2 proportion with “healthy controls” without any of these phenotypes. Proteomic associations were studied using a biostatistical method based on LASSO and confronted with machine-learning and complex network approaches.

*Results:* The case:control distribution by each cardiovascular phenotype was: hypertension (50:100), obesity (50:98), and diabetes (36:72). Of the 86 studied proteins, 4 were found to be independently associated with hypertension: GDF-15, LEP, SORT-1 and FABP-2; 3 with obesity: CEACAM-8, LEP and PRELP; and 4 with diabetes: GDF-15, REN, CXCL-1 and SCF. GDF-15 (hypertension+diabetes) and LEP (hypertension+obesity) are shared by two different phenotypes. A machine learning approach confirmed GDF-15, LEP and SORT-1 as discriminant biomarkers for the hypertension group, and LEP plus PRELP for the obesity group. Complex network analyses provided insight on the mechanisms underlying these disease phenotypes where fibrosis may play a central role.

*Conclusion:* Patients with “mutually exclusive” phenotypes display distinct bioprofiles that might underpin different biological pathways, potentially leading to fibrosis.

**Key-words:** cardiovascular diseases; phenotypes; proteomics; LASSO; decision tree; complex networks.

## **Introduction**

Cardiovascular diseases (CVDs) are the leading cause of death and health loss worldwide<sup>1</sup>. Hence, cardiovascular prevention (primary and secondary) is essential to reduce CVDs burden<sup>2</sup>. Hypertension, obesity and diabetes are major and potentially modifiable “risk factors” for CVD<sup>3, 4</sup>, that, if left untreated, may lead to major CV events and reduce life expectancy.

In order to achieve diagnostic and therapeutic advances, it is important to identify new pathophysiological mechanisms that may be associated with specific disease phenotypes. Therefore, phenotyping cardiovascular (CV) “risk factors” and diseases may help in the identification of specific biomarkers relevant for understanding the underlying mechanisms, and may allow the study of pathway-specific therapies including personalized approaches to treatment<sup>5</sup>.

To this aim, the FIBRO-TARGETS (Targeting Cardiac Fibrosis for Heart Failure Treatment: <http://www.FIBRO-TARGETS.eu/>) consortium has merged data from 12 patient cohorts in a common database consisting of >12,000 patients with a large spectrum of CV clinical phenotypes<sup>6</sup>. Patients from the merged database were organized in mutually exclusive “cases” of patients with clinically-phenotyped conditions: hypertension, obesity, and diabetes with matched “controls” to identify protein bioprofiles specific to each of these phenotypes.

## **Methods**

### **Study population**

FIBRO-TARGETS is a multinational academic–industrial consortium funded by the European Commission Seventh Framework Programme (FP7). The consortium was built to create synergies and collaborative efforts between clinical investigators, basic scientists, and small- and medium-sized enterprises, combining complementary approaches, disciplines, technologies, resources, and expertise. It aims at bridging pre-clinical findings related to myocardial interstitial fibrosis to the clinical setting with the objective of changing and improving outcomes through the advent of tailored anti-fibrotic therapies. For the present study 406 subjects were selected from 2 cohorts (HVC and STANISLAS<sup>7</sup>), that included both “healthy” and “cardiovascular risk” participants to serve as either cases or controls. Patients from the merged database were organized into three mutually-exclusive clinical phenotypes (“cases”): 1) hypertensive (defined by a systolic blood pressure  $\geq 140$  mmHg or diastolic blood pressure  $\geq 90$  mmHg or ongoing treatment for hypertension)<sup>8</sup>; 2) obese (body mass index [BMI]  $\geq 30$  kg/m<sup>2</sup>)<sup>9</sup>; and 3) diabetic (defined by a random blood glucose  $\geq 200$  mg/dL or a fasting blood glucose  $\geq 126$  mg/dL or a glycated hemoglobin  $\geq 6.5\%$  or treatment for diabetes)<sup>10</sup>. Cases for a given clinical phenotype could not have other condition but the one of interest (*i.e.*, “mutually-exclusive”). Cases were age-sex matched in a 1:2 proportion with “healthy controls” free of any of these phenotypes. For a given phenotype, a maximum of 50 triplets (1:2) were selected. With regards to diabetes we were able to identify only 36 patients without any other condition.

### **Studied biomarkers**

Plasma samples were analysed for protein biomarkers using the Olink Proseek® Multiplex CVD-II panel. The use of the proximity extension assay (PEA) technology<sup>11</sup> (allowing the measurement of 92 proteins within a very limited volume of plasma - 5µL), where 92 oligonucleotide-labelled antibody probe pairs per panel are allowed to bind to their respective targets in the sample in 96-well plate format. When binding to their correct targets, they give rise to new DNA amplicons with each ID-barcoding their respective antigens. The amplicons are subsequently quantified using a Fluidigm BioMark™ HD real-time PCR platform. The platform provides log<sub>2</sub>-normalized protein expression (NPX) data. A detailed description of the Olink® technology is depicted in the **Supplemental Addenda 1**. The abbreviations, full names and respective Olink® multiplex panels of the studied proteins are described in the **Supplemental Table 1**.

Six additional biomarkers (for a total of 98) were also measured by “standard methods” and independently of the Olink® technology. These were: galectin-3 (Gal-3), growth differentiation factor-15 (GDF-15), matrix metalloproteinase-1 (MMP-1), stromal cell derived factor-1 alfa (SDF-1α) expression level were assessed using cyplex protein simple ELLA technology; N-terminal pro-peptide of type III collagen (PIIINP) was measured in plasma with the PIIINP-EL-US kit and neutrophil gelatinase-associated lipocalin (NGAL) in serum was measured by sandwich ELISA assay. The assays were performed in a “blind” fashion to case/control status with cases and controls randomly distributed across plates. The proteomic results were then merged with the baseline data, which included the case-control status, matching variables and clinical risk factors.

### **Statistical and machine-learning methods**

Continuous variables are described as mean ± standard deviation or median (inter-quartile range), as appropriate. Categorical variables were described as frequencies and percentages. In order to compare the phenotypic groups and due to matched nature of the data, p-values were computed using paired t-test or Wilcoxon signed rank test for continuous variables and with Cochran-Mantel-Haenszel test for categorical ones. Olink® biomarkers with more than 25% non-detectable values (n=12; see also the Supplemental Table 1 for information on these specific biomarkers) were excluded, hence the total number of studied biomarkers was =86. The association of the candidate biomarkers and each outcome was examined as one continuous or three binary variables (>1st tertile [T1], >median, >2nd tertile [T2]) in univariable models. Among these four representations, the one presenting the strongest association with the phenotype of interest was kept. In the next step, a LASSO (Least Absolute Shrinkage and Selection Operator) approach was performed with each clinical phenotype as outcome and the 86 biomarkers of interest as explanatory variables (the LASSO method penalizes the sum of the absolute values of the regression coefficients leading to some coefficients shrinking to zero and thus simultaneously performs variable selection). In order to find the best subset of biomarkers associated with each phenotype, we used the following principle consisting of two stages of selection: 1) for each outcome, the LASSO was computed 1000x and we kept a subset of biomarkers that were retained in >80% of the models; 2) then we performed a forward selection procedure on this subset of

biomarkers to only keep those that were significantly associated with the studied phenotype (forward elimination was selected instead of backward to avoid excessive “overfitting” during the stepwise procedure). The odds ratios (ORs) were then presented with their respective 95% confidence intervals (95%CI). Since proteins were measured using NPX (Normalized Protein eXpression) values (the details about NPX values can be found here: <https://www.olink.com/question/what-is-npx/>), the OR for each protein estimates the increase in the odds of HF associated with a doubling in the protein concentration. The statistical analyses were performed using the R<sup>®</sup> software, clogitL1 R package (<http://www.R-project.org>). A p-value <0.05 was considered as statistically significant after correcting for false discoveries with the 1000x repetition procedure.

In the machine-learning (ML) analyses, the case:control proportion (1:2) in each phenotypic group led us to reweight the instances in the datasets using the “distribution class balancer” method, so that each class in each dataset had the same total weight. Then we used a simple and robust supervised classification method known as decision tree (J48 algorithm) because it can provide models explaining the classification process in the form of “explicit decision trees” that identify “cases” by the levels of the expressed biomarkers in an unsupervised fashion i.e. the program chooses the best level of proteins expression to define the cutoff. To improve and quantify classification performance of the models, the J48 algorithm was combined with AdaBoostM1, an iterative metaclassifier. Classification models were evaluated by “leave one out cross validation” (*i.e.*, learn with the whole dataset minus one example, test with this example and repeat to test each example) repeated 1000x. For each phenotypic group, the biomarkers selected by the biostatistical approach were considered as input features. Results are given as the Adaboost decision tree with the highest weight and average values of the area under the ROC curve (AUC) over the 1000 cross-validations. The Weka<sup>®</sup> data mining software was used for the ML analyses. The statistical procedures are resumed in the **Figure 1**.

### **Complex network analyses**

The FHF-GKB (Fight Heart Failure - Graph Knowledge Box) resource, representing most available public knowledge about human protein-disease, protein-protein and protein-pathway relationships is a customized upgraded version of the EdgeBox provided by the EdgeLeap company (available from: <https://www.edgeleap.com/edgebox/>). FHF-GKB data is extracted from public data sources resulting in 20,386 protein nodes imported from Uniprot<sup>12</sup>, including all biomarkers involved in this study, 28,176 disease nodes from Disease Ontology<sup>13</sup> and DisGenet<sup>14</sup> and 2,222 pathway nodes from Reactome (v65)<sup>15</sup>. Protein - protein relationships were retrieved from STRING (v10.5)<sup>16</sup> or Reactome or WikiPathways<sup>17</sup> or Mentha<sup>18</sup> or BioGrid<sup>19</sup>, protein – disease associations from DisGenet (2018-08-24) and protein – pathway relationships from Reactome. The FHF-GKB complex network was queried in order to explore pathways and proteins that connect BM to fibrosis. Three distinct nodes from FHF-GKB were merged to represent fibrosis: fibrosis per se (Unified Medical Language System - UMLS code C0016059), myocardial fibrosis (C0151654) and endomyocardial fibrosis (C0553980). Relations between proteins and disease nodes are retrieved from FHF-GKB under stringent score conditions that

imply that at least one animal model or a manually curated report supports the relation in DisGenet. Queries were expressed according to query patterns defining a path structure between two nodes namely BM-fibrosis, BM-protein-fibrosis and BM-pathway-protein-fibrosis, where the BM nodes were taken from the list of BMs identified for each phenotypic group by the statistical approach. The resulting graphs were merged in a figure illustrating all possible paths not longer than three edges, connecting BMs and fibrosis through pathways and proteins.

## **Results**

### **Study population**

The case:control distribution by each cardiovascular phenotype was as follows: hypertension (50:100), obesity (50:98), and diabetes (36:72). **Table 1**. These phenotypes were “mutually-exclusive” *i.e.* cases could only have one only condition and controls could not have any of these conditions. The baseline characteristics of the studied population for each studied cardiovascular phenotype is depicted in **Table 1**.

### **Biomarkers associated with the studied phenotypes: LASSO approach**

In the present section we describe only the biomarkers associated with each phenotype that were retained in >80% of the LASSO runs followed by the forward stepwise multivariable logistic model. The results for all the biomarkers and the respective selection process are presented in the **Supplemental Material Tables 2 to 4**.

#### **Biomarkers associated with hypertension**

Of the 86 proteins studied, 4 were found to be independently associated with hypertension in the statistical approach: GDF-15 and leptin (LEP) were positively associated (*i.e.*, with an odds ratio >1), whereas sortilin-1 (SORT-1) and fatty acid-binding protein 2 (FABP-2) were negatively associated (*i.e.*, with an odds ratio <1). **Table 2**.

#### **Biomarkers associated with obesity**

Three biomarkers were found to be independently associated with obesity in the statistical approach: carcinoembryonic antigen-related cell adhesion molecule 8 (CEACAM-8) and LEP were positively associated, whereas proline/arginine-rich end leucine-rich repeat protein (PRELP) was negatively associated. **Table 2**.

#### **Biomarkers associated with diabetes**

Four biomarkers were found to be independently associated with diabetes in the statistical approach: GDF-15 and renin (REN) were positively associated, whereas chemokine (C-X-C motif) ligand 1 (CXCL-1) and stem-cell factor (SCF) were negatively associated. **Table 2**.

### **Interpretation of the biostatistical model: machine-learning approach**

The biomarkers retained in the best statistical models were used as selected features in a ML approach aimed at producing descriptive and predictive models of each phenotypic group. The three decision

trees obtained of the hypertension, obesity and diabetes groups are represented in **Figure 2A to C**. The decision trees are organized according to sequential testing of discriminant biomarkers.

For the hypertension group, three such biomarkers are retained: LEP, GDF-15 and SORT-1 but not FABP-2, however, the AUC of the predictive model is rather low (0.61). For obesity only one discriminant biomarker is retained (LEP) for an AUC of 0.77. For diabetes, the four biomarkers of the statistical model were used: CXCL-1, REN, GDF-15 and SCF for an AUC of 0.70.

### **Synthesis of the findings**

The above-described findings are summarized in the **Table 3** and in the **Supplemental Tables 5 & 6**. Interestingly, LEP is shared between the hypertension and obesity, whereas GDF-15 is shared between hypertension and diabetes phenotypes.

Decision trees obtained using machine learning provide insight into the way biomarkers can be used to discriminate between healthy and at-risk “pro-fibrotic” patients.

### **Induced network analyses**

The three networks underpinning the identified proteins for each phenotypic group are presented in **Figures 3A to C**. Relations between each biomarker and fibrosis (including myocardial and endomyocardial fibrosis) may point to potential common disease mechanisms. In the hypertension group and under the conditions used for network retrieval, relevant paths are found only for the LEP biomarker. The same paths are therefore present in the obesity group but they co-exist with specific ones involving the PRELP and CEACAM-8 biomarkers such as diseases of glycosylation and neutrophil degranulation respectively. The neutrophil degranulation pathway is also present in the diabetes group but linked to the CXCL-1 biomarker. In this group other specific pathways appear involving the REN and SCF biomarkers such as metabolism of angiotensinogen and signalling by interleukins, respectively. A resume of the findings is displayed in **Figure 4**.

### **Discussion**

In the present study we identified 9 biomarkers (from which 7 are exclusive, and 2 overlap) associated with the “mutually-exclusive” phenotypes: hypertension (GDF-15, LEP, SORT-1); obesity (LEP, PRELP); and diabetes (GDF-15, REN, CXCL-1, SCF). Two biomarkers overlapped in different phenotypes: GDF-15 [hypertension+diabetes] and LEP [hypertension+obesity]. To the best of our knowledge, this is the first study to assess proteomic bioprofiles in “mutually exclusive” cardiovascular phenotypes, highlighting the distinct bioprofiles that may underpin different biological pathways specific of each condition. However, the overlap observed between cardiovascular “at-risk” phenotypes (hypertension, obesity and diabetes), suggests that the transition to cardiovascular disease may share mechanistic pathways. The identification of these proteomic bioprofiles may help in the understanding of the different mechanisms that are associated with these cardiovascular diseases and potentially enable the development of CVD targeted diagnostic tools and treatments<sup>20, 21</sup>. The utilization of two independent statistical methods for the reproducibility of the results reinforced the



internal validity of our findings. While still requiring further comparison in larger samplings, those analytical approaches resulted in sound biological results externally comforted in animal models and humans (see discussion below).

### **Hypertension**

Patients with hypertension (compared to the matched controls) had higher levels of GDF-15 and LEP and lower levels of SORT-1. GDF-15 is a stress responsive cytokine, highly expressed in cardiomyocytes, adipocytes, macrophages, endothelial cells, and vascular smooth muscle cells in normal and pathological conditions<sup>22</sup>. GDF-15 increases during tissue injury and inflammatory states such as left ventricular hypertrophy and endothelial dysfunction caused by hypertension and/or diabetes and has been shown to predict incident CVD and adverse CV outcomes<sup>5, 23, 24</sup>. Leptin excess has been associated with hypertension, obesity, insulin-resistance/diabetes, subclinical inflammation, atherosclerosis, and incident CVDs<sup>25, 26</sup>. On the other hand, patients with hypertension expressed lower levels of SORT-1, that plays a role in the secretion of proinflammatory cytokines (such as IL-6 and TNF $\alpha$ ) and is implicated in LDL-cholesterol metabolism. Moreover, SORT-1 likely plays an important role in the development of cardiovascular disease and atherosclerosis beyond LDL-cholesterol regulation<sup>27, 28</sup>. As SORT-1 attenuates inflammatory response, it is thus expected that patients with hypertension and/or myocardial infarction express lower levels of this protein. Lower levels of FABP-2 (a cytoplasmic lipid chaperon expressed in adipocytes and macrophages) were identified only using the biostatistical model in hypertensive patients. While additional bibliographic data regarding its association with hypertension are yet lacking, lower levels of FABP-2 have been associated with an increased risk of diabetes<sup>29</sup>.

### **Obesity**

Obese patients expressed higher levels of LEP and lower levels of PRELP using both statistical approaches. The positive association with LEP is sound (as described in the “hypertension” subsection) and the PRELP directly inhibits complement pathways downregulating the inflammatory cascade. PRELP is a component of member of the small leucine-rich proteoglycans<sup>30</sup> and is present in the extracellular matrix playing an essential role in tissue repair and scar formation<sup>31</sup>. The lower expression of PRELP in obese patients (vs. controls) is thus plausible from a pathophysiological viewpoint. CEACAM-8 levels were also increased in obese patients (with the “biostatistical” approach only). This marker has been implicated in cell adhesion, cellular invasiveness, angiogenesis, and inflammation<sup>32</sup>, which are key processes in the pathophysiology of obesity. However, data directly relating CEACAM-8 and obesity are lacking and further study is required.

### **Diabetes**

Patients with diabetes expressed higher concentrations of GDF-15 and REN, and lower concentrations of CXCL-1 and SCF, compared to the matched controls. GDF-15 has been associated with diabetes and CVD, as above described (hypertension subsection). Excessive expression of REN has been associated with diabetes and coronary artery disease<sup>33, 34</sup>. Patients with diabetes expressed lower levels

of SCF. Supporting our findings, lower levels of SCF have also been associated with increased rate of coronary events and all-cause death<sup>35, 36</sup>. Contrary to our findings, CXCL-1 levels have been found to be elevated in patients with diabetes mellitus<sup>37, 38</sup>.

### **The link of the studied proteins to fibrosis**

The machine learning techniques used in the current manuscript aim to help in revealing the underlying mechanistic pathways associated with the study biomarkers, by enriching our observations with varied data sources available via the FHF-GKB. The query patterns with “direct” link biomarker-fibrosis were inexistent i.e. no “intrinsic” profibrotic activity of the measured biomarkers has yet been reported in the literature. Enlarging the query to “biomarker-protein-fibrosis” and to “biomarker-pathway-protein-fibrosis” showed intermediary proteins that are involved in pathways that link the study biomarkers to fibrosis. Our findings are resumed in the figure 4, with the detected biomarkers shown in circles, the enriched pathways deduced by the “machine learning” procedure in orange diamond, structured into biological processes that may lead to the development of fibrosis.

### **Limitations**

Several limitations should be highlighted in the present study. First, this is an observational case-control study, hence causality cannot be ascertained. The ML approach is performed on a small number of patients in each group and this can lead to somewhat overfitted descriptive and predictive models despite of extensive cross-validation. The bioinformatical approach, also does not allow causality assessment. Second, this study lacks external validation, hence these data cannot be extrapolated to other populations. Third, the proteomics assay does not provide standard concentration units, making comparisons with clinically applied cut-offs difficult. Finally, we did not use large unbiased screens but rather selected protein biomarkers based on mechanistic hypotheses. Therefore, we cannot exclude the role of other mechanisms not targeted with our proteomics screen.

### **Conclusion**

Patients with “mutually exclusive” cardiovascular phenotypes have distinctive bioprofiles that might underpin different biological pathways, which may be associated with fibrosis via intermediate links, such as inflammation. Our results support the FIBROTARGETS consortium objectives aiming the development of treatments personalized for specific phenotypic groups of patients.

### **Conflict of interest**

All authors are actively involved in the FIBROTARGETS consortium.

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Figure 1. Summary of the analyses performed for each phenotypic group

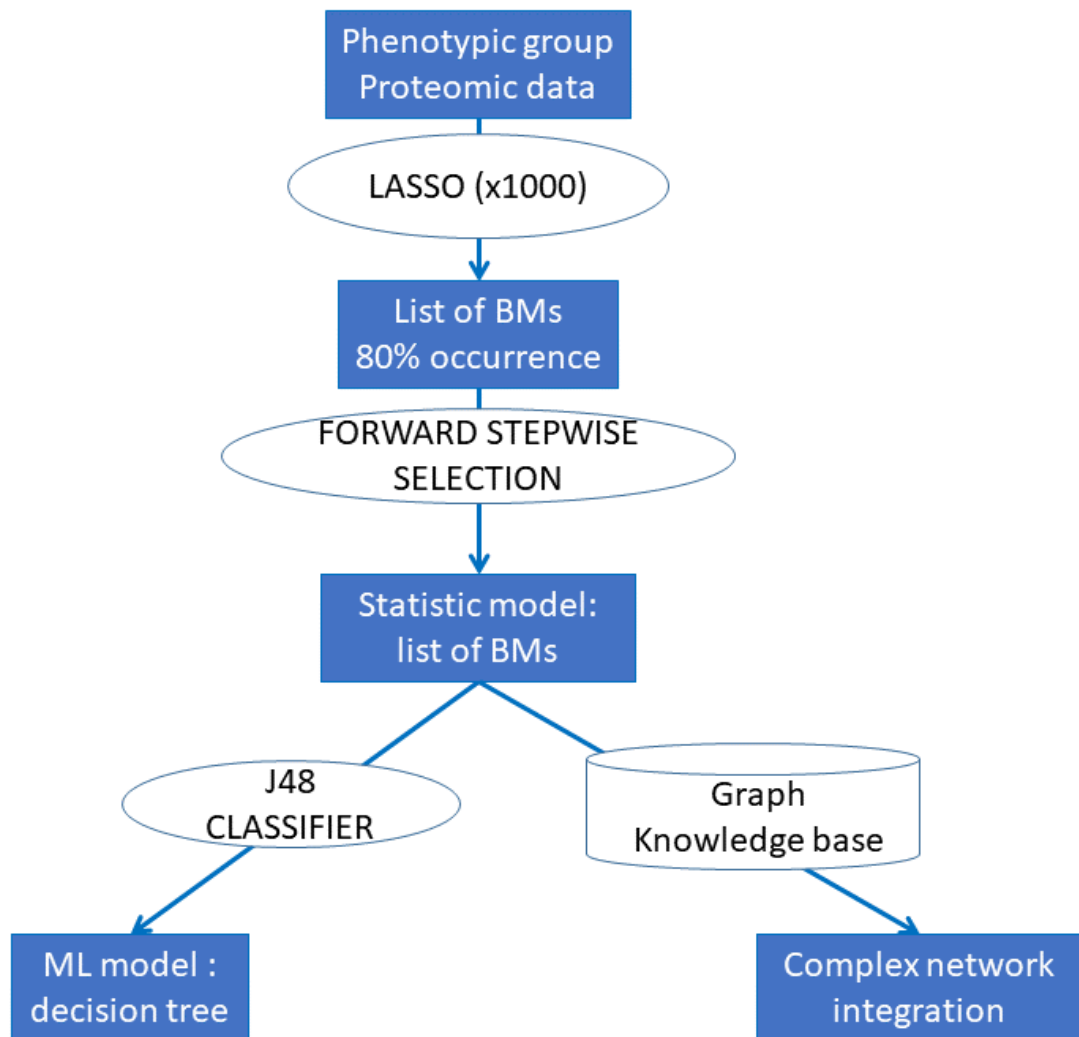
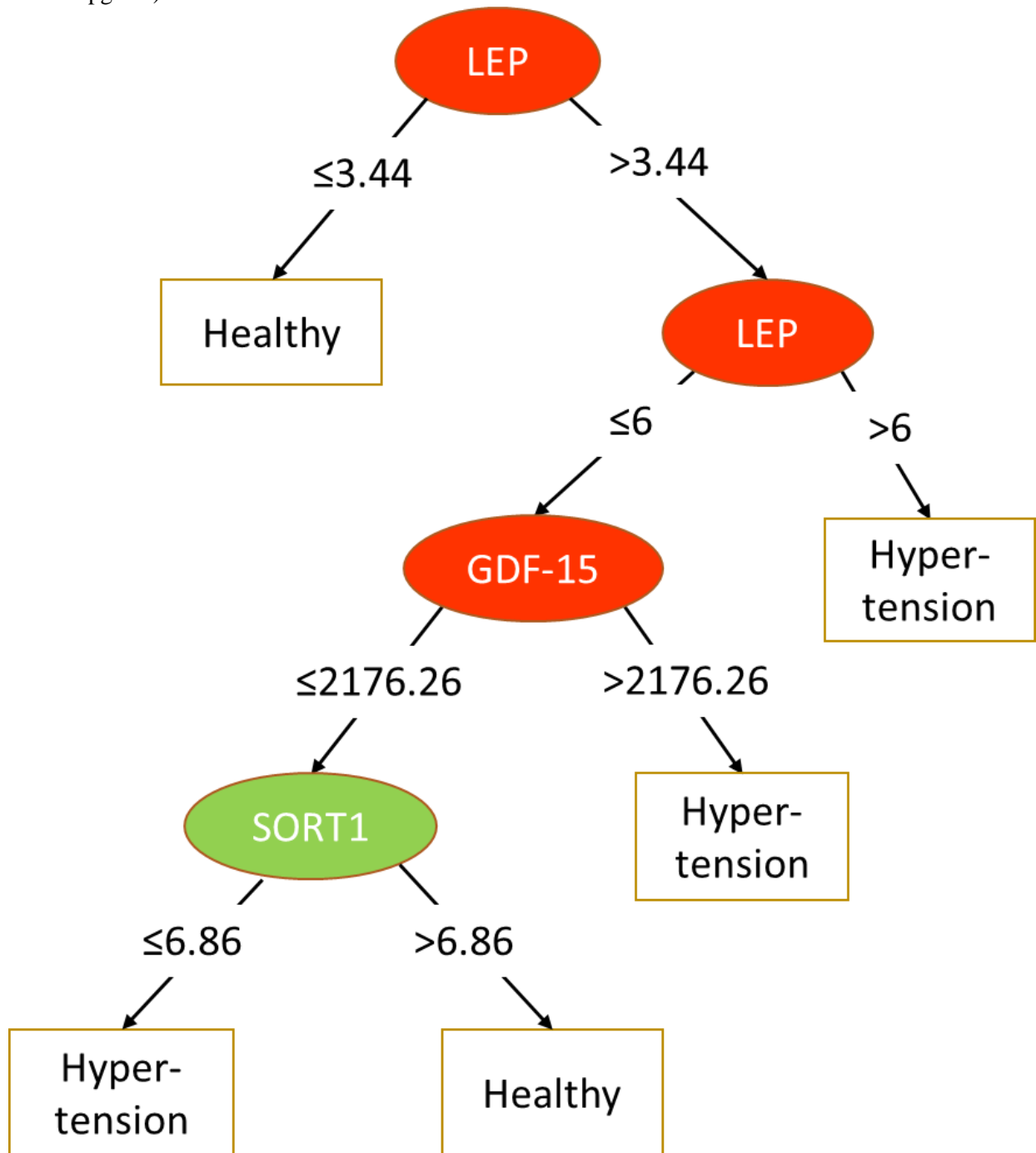


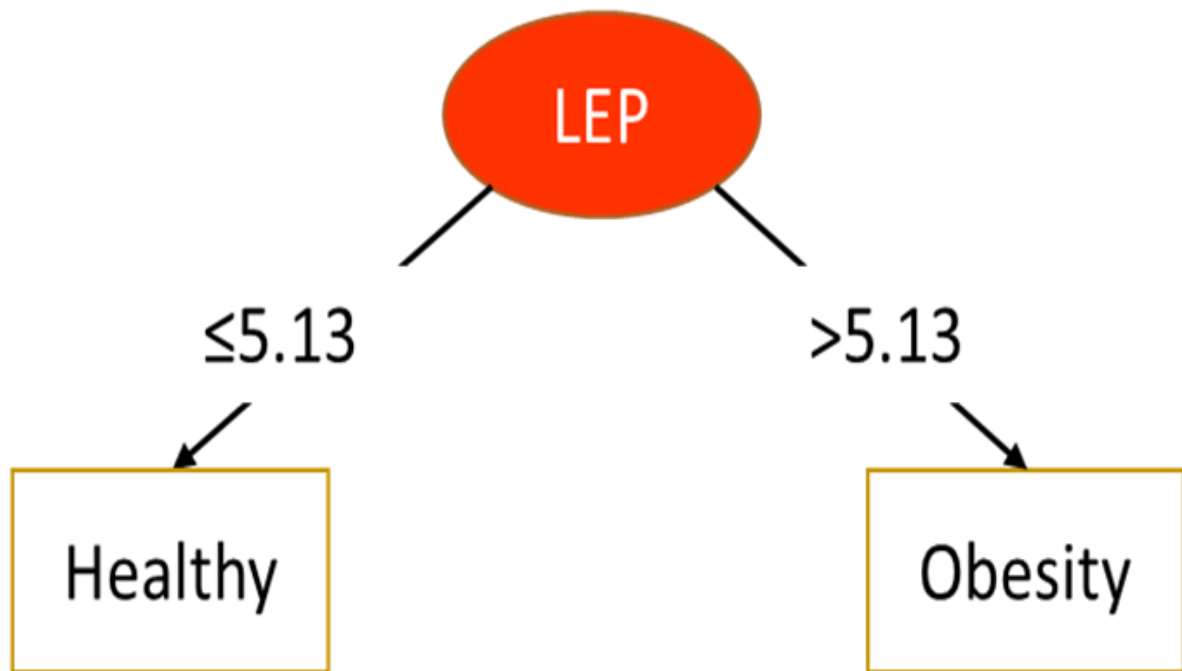


Figure 2. Decision trees for each disease phenotype

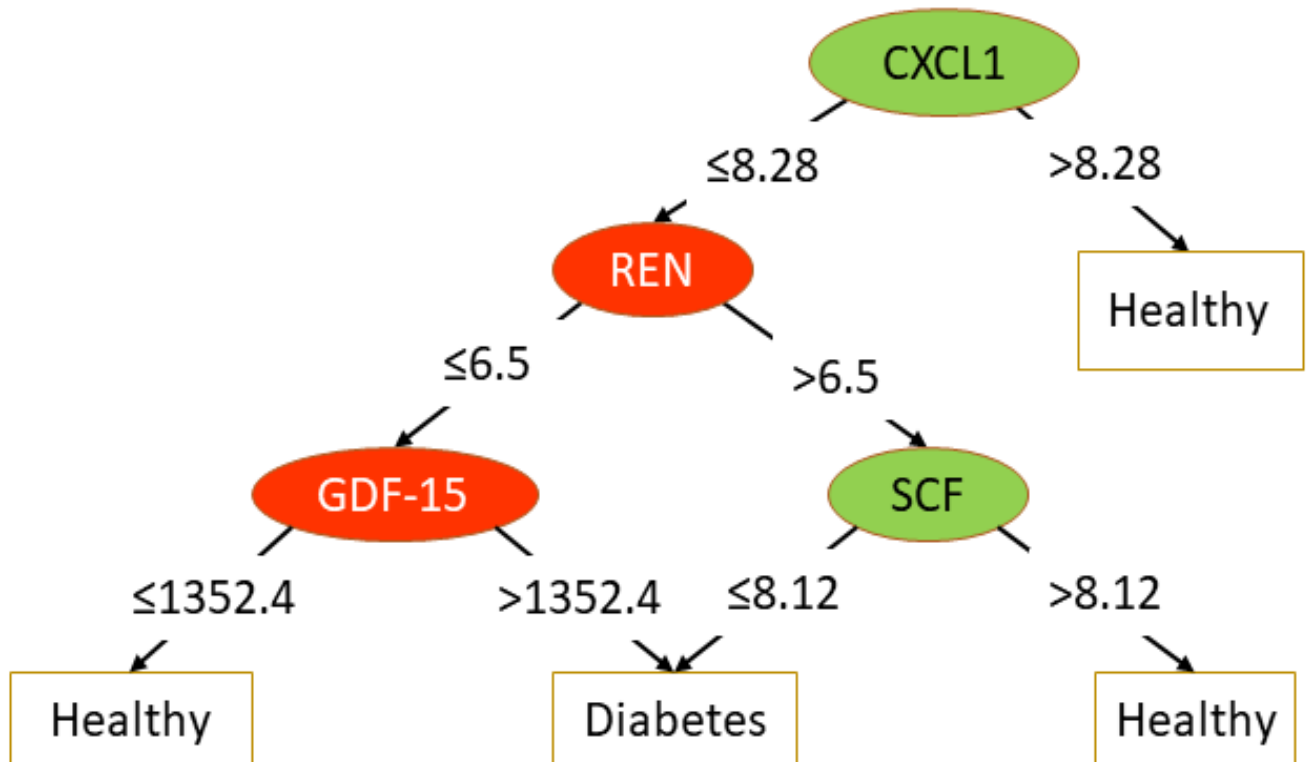
A. Hypertension (AUC =0.61) (LEP and SORT-1 are expressed in NPX values, and GDF-15 in pg/mL)



B. Obesity (AUC =0.77) (LEP is expressed in NPX values)



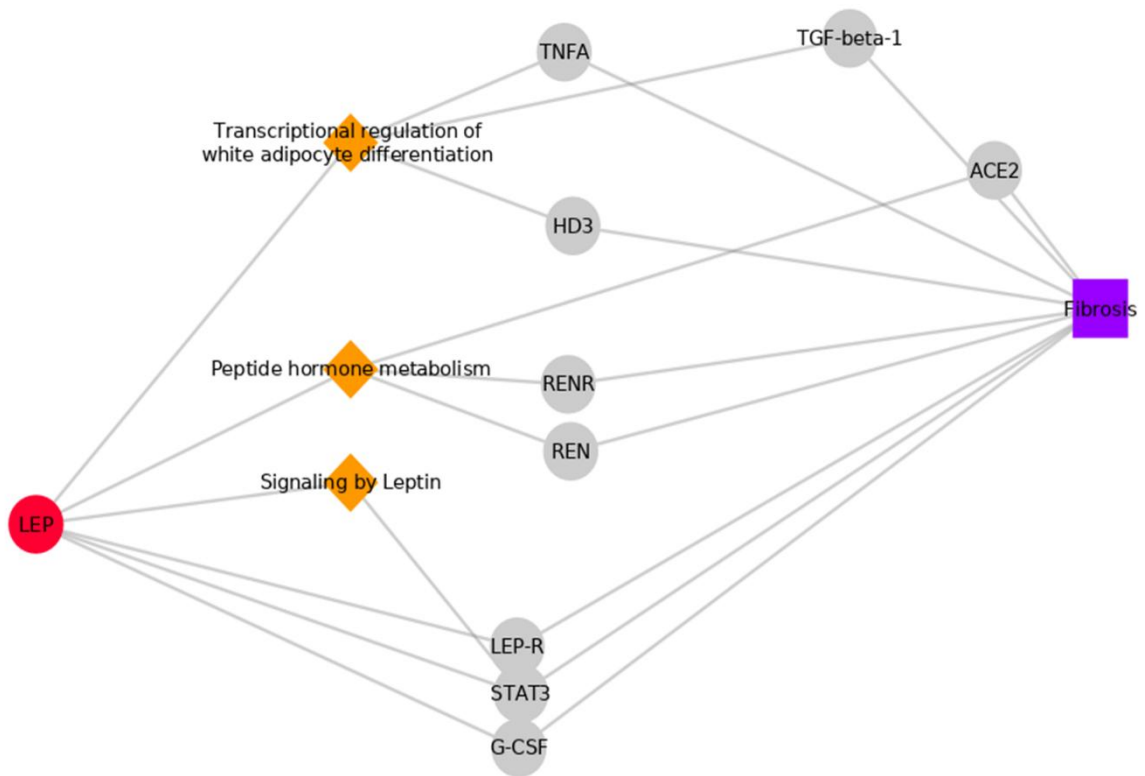
C. Diabetes (AUC =0.70) (CXCL1, REN, and SCF are expressed in NPX values, and GDF-15 in pg/mL)



Legend: red, biomarkers positively (odds ratio >1) associated with the outcome; green, biomarkers negatively (odds ratio <1) associated with the outcome.

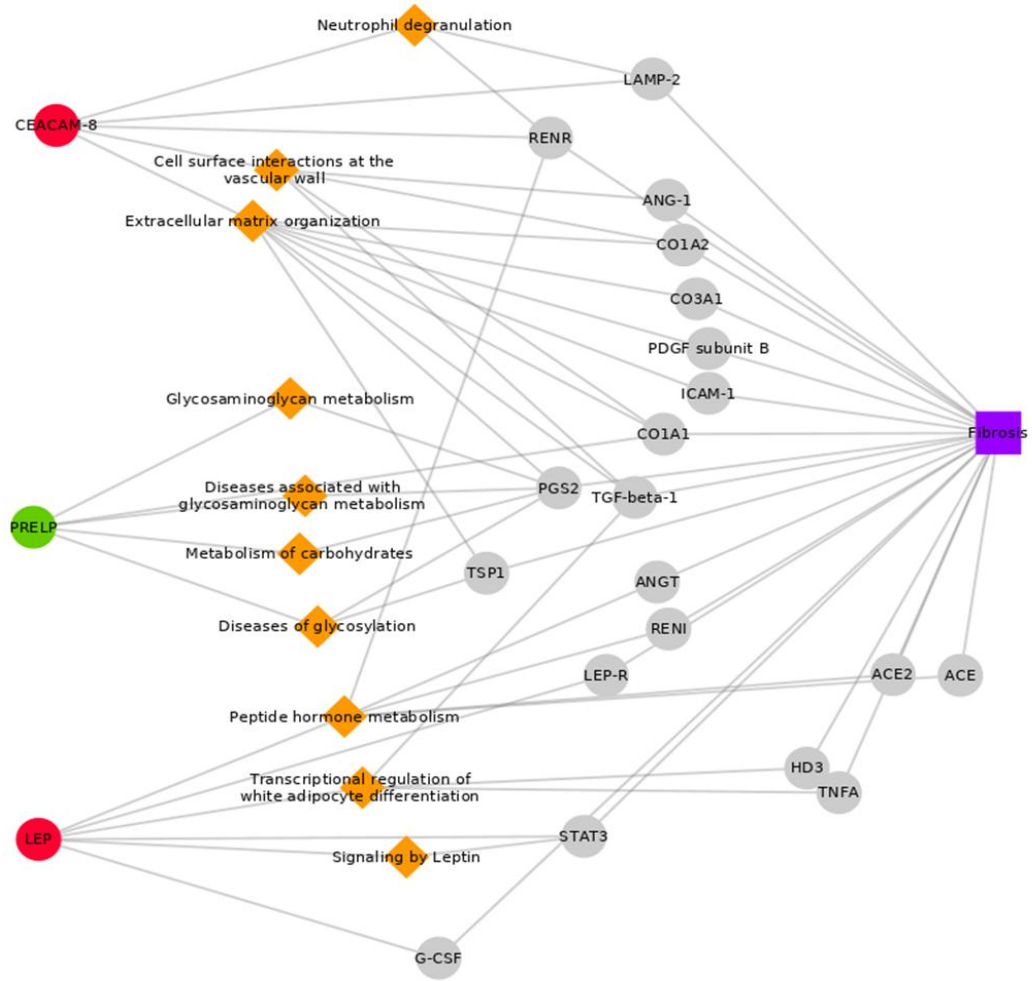
Figure 3. Graphs relating biomarkers to the fibrosis disease node.

A. Hypertension



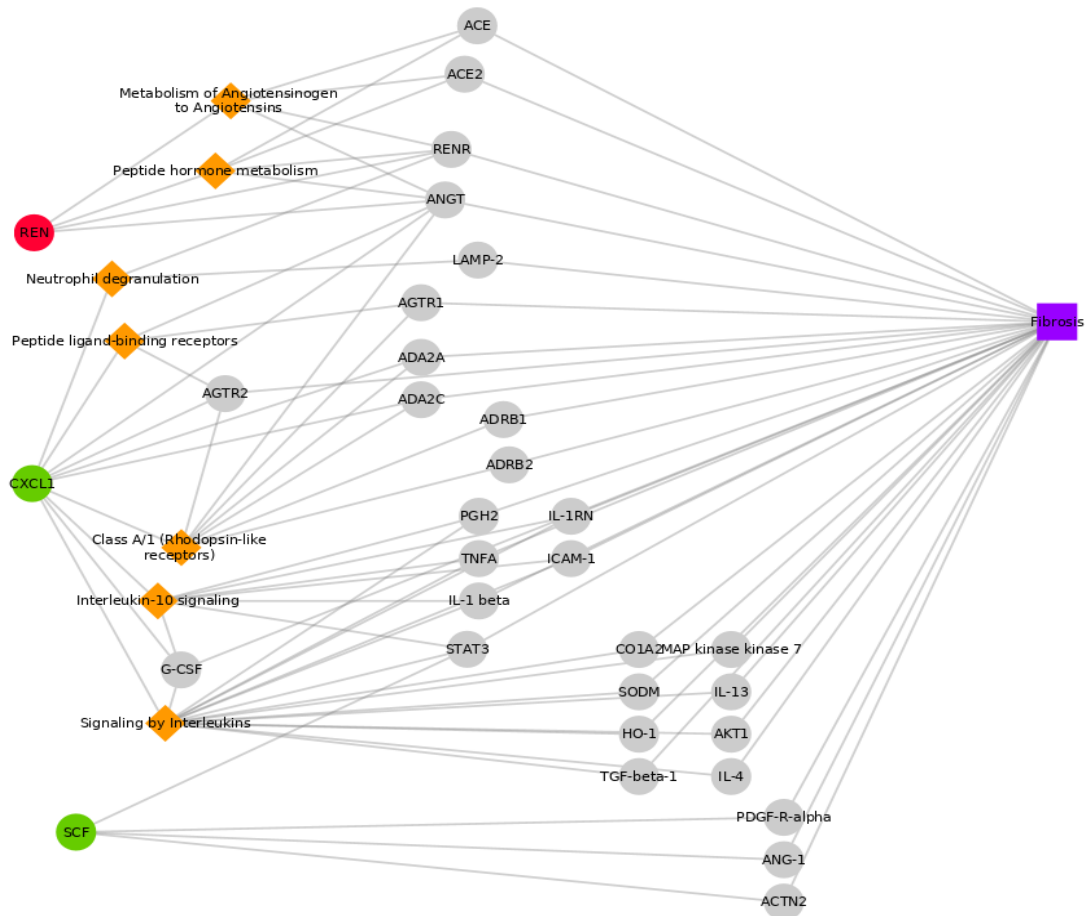
Query patterns: BM- Fibrosis (no result); BM-Protein-Fibrosis; BM-Pathway-Protein-Fibrosis.  
No pathway found for GDF-15, SORT-1, and FABP2

## B. Obesity



Query patterns: BM- Fibrosis (no result); BM-Protein-Fibrosis; BM-Pathway-Protein-Fibrosis.

### C. Diabetes

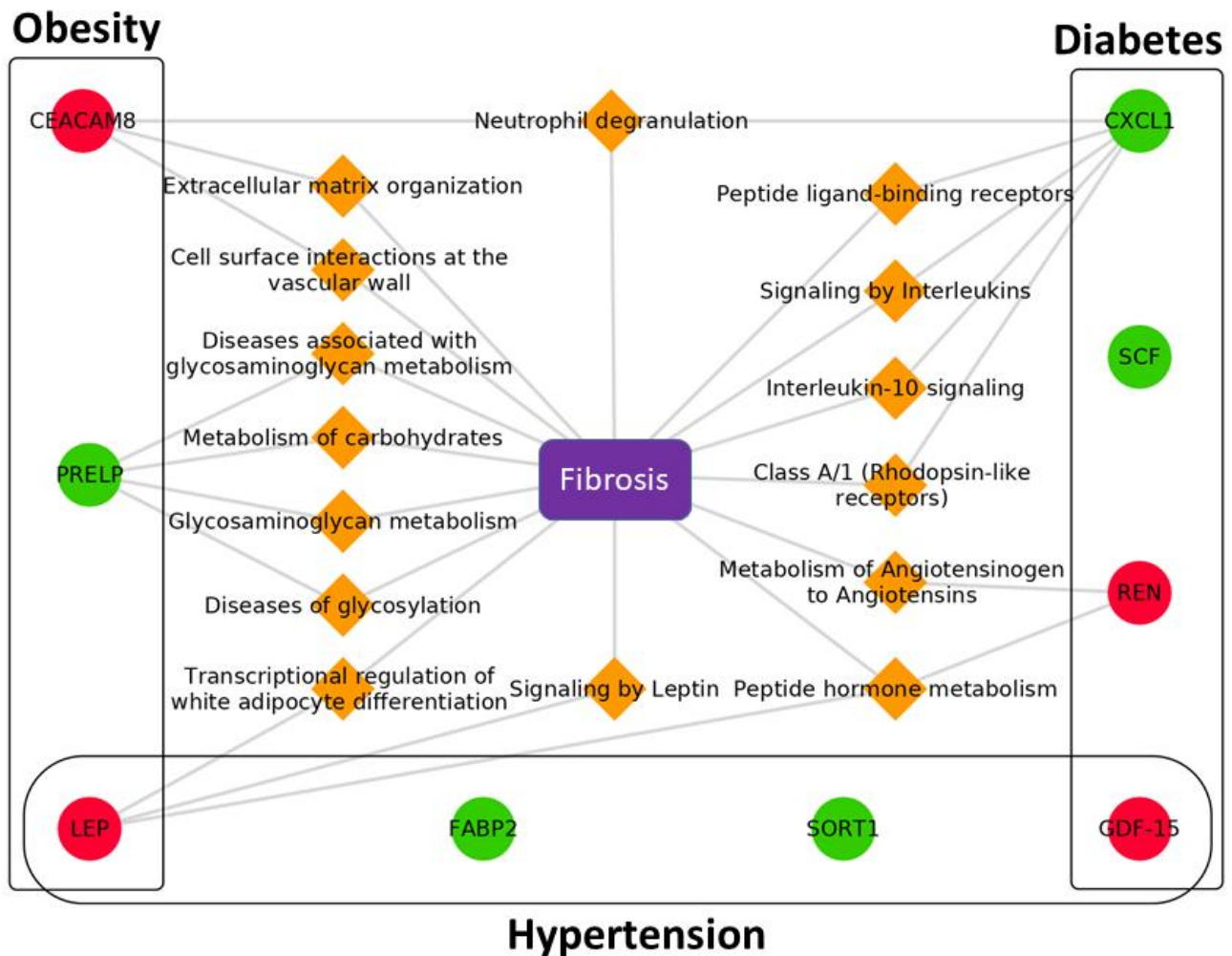


Query patterns: BM- Fibrosis (no result); BM-Protein-Fibrosis; BM-Pathway-Protein-Fibrosis.

No pathway found for GDF-15.

Legend: “circles” are proteins (red/green for biomarkers and grey for intermediate proteins), “orange diamond” are pathways, and “violet squares” are diseases.

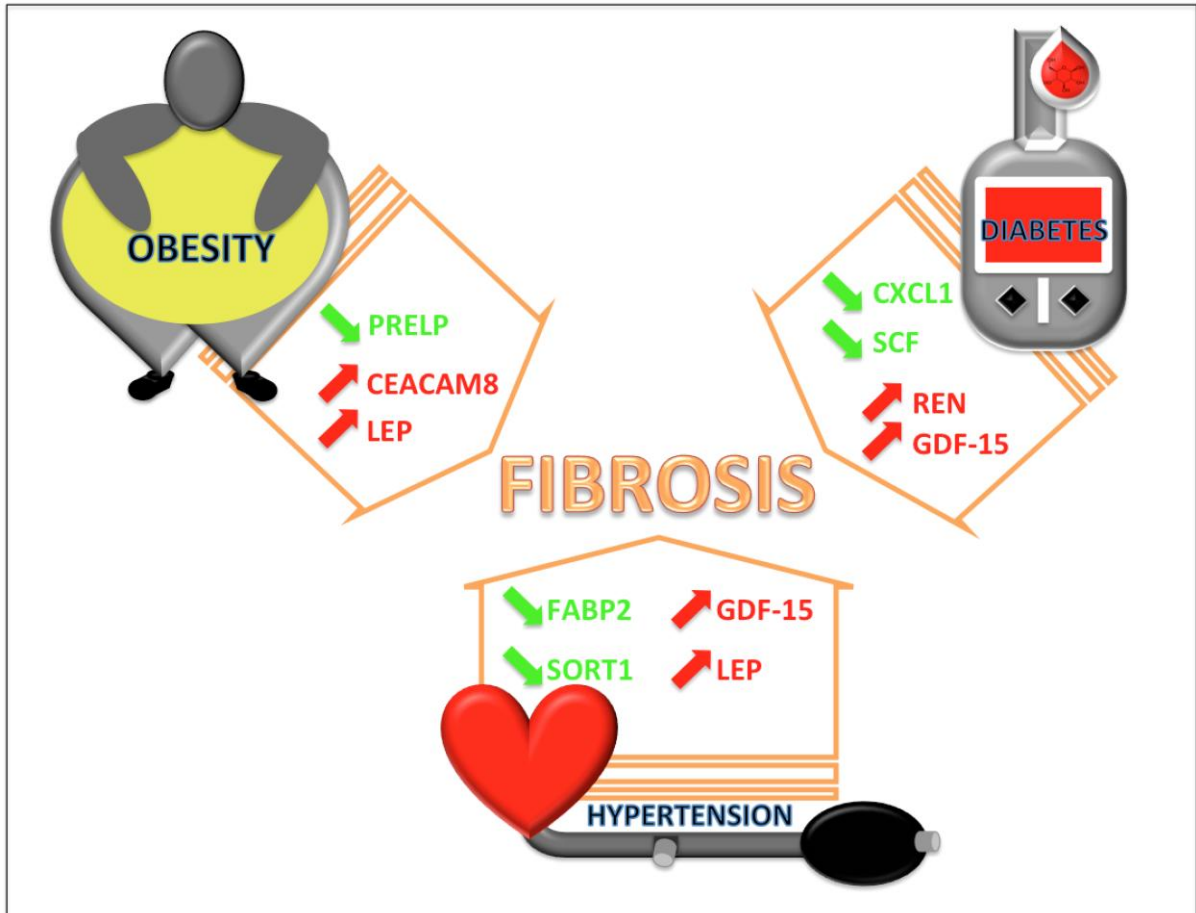
Figure 4. Biomarkers and their associated pathways leading to Fibrosis.



Legend: “circles” are proteins (red, increased expression /green, decreased expression for biomarkers), “orange diamond” are pathways, and “violet squares” are diseases.

## Graphic Abstract

### Plasma protein biomarkers and their association with mutually exclusive cardiovascular phenotypes: the FIBRO-TARGETS case-control analyses



Patients with “mutually exclusive” phenotypes (blue: obesity, hypertension and diabetes) display distinct protein bioprofiles (green decreased expression; red increased expression) that might underpin different biological pathways (orange arrow), potentially leading to fibrosis.



Table 1. Description of clinical factors according to phenotypes

Variables	Hypertension			Obesity			Diabetes		
	Controls (N=100)	Cases (N=50)	p	Controls (N=98)	Cases (N=50)	p	Controls (N=72)	Cases (N=36)	p
Age, y-old	55.4 ± 9.1	55.9 ± 9.3		48.9 ± 12.1	48.5 ± 12.5		58.8 ± 12.5	59.0 ± 12.5	
Women	54 (54.0%)	27 (54.0%)		65 (66.3%)	33 (66.0%)		32 (44.4%)	16 (44.4%)	
Systolic BP, mmHg	128 ± 18	146 ± 20	< 0.0001	125 ± 14	128 ± 17	0.27	127 ± 16	130 ± 15	0.23
Diastolic BP, mmHg	75 ± 11	86 ± 14	< 0.0001	74 ± 9	76 ± 12	0.26	75 ± 11	76 ± 9	0.48
Heart rate, bpm	68 ± 13	66 ± 13	0.42	68 ± 11	69 ± 10	0.47	66 ± 13	71 ± 12	0.015
Total cholesterol, mmol/L	5.8 ± 1.0	6.0 ± 1.3	0.32	5.8 ± 1.1	5.4 ± 1.0	0.052	5.7 ± 0.9	5.0 ± 0.9	0.0005
LDL cholesterol, mmol/L	3.7 ± 0.9	3.8 ± 1.0	0.65	3.7 ± 0.9	3.4 ± 0.9	0.074	3.6 ± 0.8	3.0 ± 0.7	0.001
HDL cholesterol, mmol/L	1.6 ± 0.5	1.4 ± 0.4	0.027	1.6 ± 0.4	1.4 ± 0.4	0.0009	1.5 ± 0.4	1.4 ± 0.7	0.24
Triglycerides, mmol/L	1.0 (0.8 - 1.4)	1.3 (1.1 - 2.3)	0.001	1.1 (0.8 - 1.4)	1.2 (0.9 - 2.0)	0.009	1.2 (0.8 - 1.5)	1.3 (0.8 - 1.9)	0.22
eGFR, mL/min/1.73m <sup>2</sup>	89 ± 12	86 ± 16	0.20	94 ± 15	94 ± 16	0.94	85 ± 16	87 ± 17	0.46
LVEF, %	64 ± 6	64 ± 7	0.75	64 ± 5	65 ± 7	0.58	63 ± 9	62 ± 9	0.38
E/A ratio	1.00 (0.88 - 1.26)	1.03 (0.80 - 1.32)	0.85	1.10 (0.90 - 1.42)	1.07 (0.83 - 1.37)	0.26	1.00 (0.83 - 1.21)	0.90 (0.76 - 1.11)	0.097
Antihypertensive treatment	12 (12.0%)	44 (88.0%)	< 0.0001	9 (9.2%)	9 (18.0%)	0.13	13 (18.1%)	9 (25.0%)	0.33
ACE inhibitor	5 (5.0%)	13 (26.0%)	0.0005	2 (2.0%)	5 (10.0%)	0.033	3 (4.2%)	3 (8.3%)	0.39
Beta blocker	6 (6.0%)	25 (50.0%)	< 0.0001	6 (6.1%)	6 (12.0%)	0.24	11 (15.3%)	7 (19.4%)	0.54
ARB	2 (2.0%)	12 (24.0%)	-	0 (0.0%)	3 (6.0%)	-	1 (1.4%)	3 (8.3%)	0.077

Legend : BP, blood pressure ; eGFR, estimated glomerular filtration rate; LVEF, left ventricular ejection fraction; ACE, angiotensin-converting enzyme; ARB, angiotensin receptor blocker.

Table 2. Biomarkers retained in >80% LASSO plus stepwise forward

<b>Hypertension</b>	OR (95%CI)	p-value
GDF-15 > T2 (836.98)	5.82 (1.64 - 20.6)	0.006
LEP	4.60 (1.97 - 10.7)	0.0004
SORT-1	0.41 (0.23 - 0.73)	0.002
FABP-2 > T1 (7.34)	0.35 (0.13 - 0.92)	0.033
<b>Obesity</b>	OR (95%CI)	p-value
LEP	4508 (22.7 - 893772)	0.002
CEACAM-8 > median (2.42)	63.5 (2.94 - 1368)	0.008
PRELP	0.05 (0.01 - 0.39)	0.004
<b>Diabetes</b>	OR (95%CI)	p-value
GDF-15	3.53 (1.15 - 10.9)	0.028
REN > median (6.50)	15.4 (1.92 - 123)	0.010
CXCL-1	0.27 (0.09 - 0.77)	0.014
SCF > T2 (8.20)	0.23 (0.06 - 0.92)	0.038

Legend: GDF-15, growth differentiation factor 15; LEP, leptin; SORT-1, sortilin; FABP-2, fatty acid binding protein-2; CEACAM-8, cell adhesion molecule 8; PRELP, proline/arginine-rich end leucine-rich repeat protein; REN, renin; CXCL-1, chemokine (C-X-C motif) ligand 1; SCF, stem-cell factor; OR, odds ratio; CI, confidence interval.

The OR corresponds to a doubling in the protein concentration (NPX units).

Table 3. Summary of the biomarkers retained both in the LASSO and in the bioinformatical approach for each studied phenotype

Biomarker	<b>Hypertension</b>	<b>Obesity</b>	<b>Diabetes*</b>
CXCL-1			+
SCF	•		+
REN			+
LEP	•	•	
GDF-15	•		+
SORT-1	•		
FABP-2			
PRELP		•	
CEACAM-8			
CD4			
PSGL			
AGRP			

+ biomarkers retained in the biostatistical model.

• Green, biomarkers retained both in the biostatistical and bioinformatical models.

\*Machine-learning was not applicable to the diabetes phenotype due to the low number of cases.