Universidade de Aveiro Departamento de Química
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Estudo do dimorfismo sexual na insuficiência cardíaca pela análise proteómica da urina

Study of sexual dimorphism in heart failure by proteomic analysis of urine

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Dissertação apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Mestre em Bioquímica, ramo Bioquímica Clínica, realizada sob a orientação científica da Doutora Rita Marisa Nogueira Ferreira, Investigadora da Unidade de Investigação Cardiovascular da Faculdade de Medicina da Universidade do Porto, do Professor Doutor Francisco Manuel Lemos Amado, Professor Associado com Agregação do Departamento de Química da Universidade de Aveiro e do Professor Doutor Mário André da Silva Santos, Cardiologista do Centro Hospitalar Universitário do Porto.

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Dedico este trabalho à minha família.

o júri

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palavras-chave Insuficiência cardíaca; dimorfismo sexual; proteómica; urina

resumo

A insuficiência cardíaca (IC) é considerada uma epidemia global, com uma prevalência crescente associada ao envelhecimento da população. A insuficiência cardíaca com fração de ejeção reduzida (ICFER) e a insuficiência cardíaca com fração de ejeção preservada (ICFEP) são dois tipos de IC com prevalências diferentes em homens e mulheres. O sexo masculino e a doença isquémica do coração estão relacionados com a ICFER, enquanto o elevado número de comorbilidades, o sexo feminino e o envelhecimento são fatores de risco da ICFEP.

Embora tenham sido descritas diferenças sexuais na epidemiologia da IC, ainda é necessário entender o papel do sexo no desenvolvimento da doença. Assim, o objetivo desta dissertação foi avaliar os mecanismos moleculares associados ao dimorfismo sexual na IC, através da análise proteómica da urina de pacientes com IC e de indivíduos controlo (indivíduos sem IC) (50% de mulheres em todos os grupos experimentais), usando uma abordagem metodológica GeLC-MS/MS.

Três proteínas (RBP4, A1BG e ACP2) apresentaram níveis significativamente diferentes no proteoma da urina entre homens e mulheres com ICFER. A1BG e ACP2 mostraram níveis aumentados nos homens, enquanto que os níveis da proteína RBP4 estavam aumentados nas mulheres. Os níveis aumentados de RBP4 na urina das mulheres podem estar associados ao facto de uma maior percentagem de mulheres com ICFER apresentar diabetes *mellitus*.

Relativamente à comparação das mulheres do grupo controlo com as mulheres com ICFER, verificou-se um aumento significativamente da proteína S100A9 nas mulheres com IC, sugerindo a presença de uma resposta inflamatória característica desta condição, provavelmente associada às comorbilidades. A proteína A1BG, que foi encontrada significativamente diferente entre homens e mulheres com ICFER, foi encontrada diminuída nas mulheres com ICFER em comparação com o grupo controlo feminino. Adicionalmente, os homens com ICFER apresentaram níveis significativamente mais baixos da proteína NAGLU em comparação com o grupo controlo masculino.

Em geral, níveis aumentados das proteínas RBP4 e S100A9 nas mulheres com ICFER parecem estar relacionados com uma condição inflamatória. No entanto, no futuro será importante validar os resultados obtidos, bem como aumentar o número de doentes, tendo em consideração as comorbilidades apresentadas.

keywords Heart failure; sexual dimorphism; proteomics; urine

abstract

Heart failure (HF) is a global epidemic, with increasing prevalence due to the world's aging population. Heart failure with reduced ejection fraction (HFrEF) and heart failure with preserved ejection fraction (HFpEF) are two HF types with different prevalence among men and women. Male sex and ischemic heart disease are related with HFrEF, while higher burden of comorbidities, female sex and aging are risk factors of HFpEF.

Although sex differences have been described in HF epidemiology, it is still necessary to understand the role that sex plays in the management of HF. Therefore, the purpose of this study is to assess the molecular mechanisms associated with the sexual dimorphism in HF, through GeLC-MS/MS proteomic analysis of urine of patients with HF and control individuals (individuals without HF) (50% of women in all experimental groups).

Urine proteomics allowed the identification of three proteins (RBP4, A1BG and ACP2) whose levels was significantly different between men and women with HFrEF. Of these proteins, A1BG and ACP2 appeared upregulated in men, and RBP4 was upregulated in women. The urinary levels of RBP4 might be associated with the higher percentage of women with HFrEF presenting diabetes mellitus. Regarding the comparison of control women with HFrEF women, a significantly higher levels of the S100A9 protein in women with HF emphasized the inflammatory response characteristic of this condition, probably linked with comorbidities. A1BG protein, whose levels was found to be significantly different amongst men and women with HFrEF, also appeared downregulated in HFrEF women when compared with the control group. On the other hand, HFrEF men showed lower levels of NAGLU protein when compared to control men.

The positive association of RBP4 and S100A9 proteins in women with HFrEF suggests the presence of an inflammatory condition. However, future studies will be important to validate the results obtained, as well as to increase the sample size, taking into account the comorbidities presented by the participants.

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Abbreviations

A1BG	Alpha-1B-glycoprotein
ACEIs	Angiotensin-converting enzyme inhibitors
ACN	Acetonitrile
ACP2	Lysosomal acid phosphatase
AGC	Auto gain control
AMI	Acute myocardial infarction
AMPK	Adenosine monophosphate-activated protein kinase
AR	Androgen receptor
ARBs	Angiotensin receptor blockers
ARNI	Angiotensin receptor neprilysin inhibitor
BNP	B-type natriuretic peptide
BSA	Bovine serum albumin
CAD	Coronary artery disease
CaMKII	Calcium/calmodulin-dependent protein kinase type II
cAMP	Cyclic adenosine monophosphate
cGMP	Cyclic guanosine monophosphate
CHD	Coronary heart disease
CKD	Chronic kidney disease
CRISP-3	Cysteine-rich secretory protein 3
CRP	C-reactive protein
DBP	Vitamin D-binding protein
DM	Diabetes mellitus
E	Transmitral early flow velocity
E'	Annular velocity
E2	17β-estradiol
ECM	Extracellular matrix
EDHF	Endothelial-derived hyperpolarizing factor
EF	Ejection fraction
eNOS	Endothelial nitric oxide synthase
ER	Estrogen receptor
ERK	Extracellular signal-regulated kinase
ET	Exercise training

ET-1	Endothelin-1
FA	Formic acid
GeLC-MS/MS	Gel electrophoresis combined with liquid chromatography-tandem mass spectrometry
GLUT	Glucose transporter
GPER	G protein-coupled estrogen receptor
GPR30	G protein-coupled estrogen receptor 30
HF	Heart failure
HFmrEF	Heart failure with mid-range ejection fraction
HFpEF	Heart failure with preserved ejection fraction
HFrEF	Heart failure with reduced ejection fraction
HPLC	High-pressure liquid chromatography
HR	Heart rate
HS	Heparan sulfate
hs-CRP	High-sensitivity C-reactive protein
IGF-1	Insulin-like growth factor-1
IL-6	Interleukin-6
iNOS	Inducible nitric oxide synthase
JNK	c-Jun N-terminal protein kinase
LC-MS/MS	Liquid chromatography-tandem mass spectrometry
LV	Left ventricular
LVEF	Left ventricular ejection fraction
MI	Myocardial infarction
MMP	Matrix metalloproteinase
MRAs	Mineralocorticoid receptor antagonists
mRNA	Messenger ribonucleic acid
MyD88	Myeloid-dependent primary response gene 88
MYLIP	Myosin regulatory light chain interacting protein
NAGLU	Alpha-N-acetylglucosaminidase
NF-κB	Nuclear factor kappa B
NO	Nitric oxide
NT-proBNP	N-terminal pro-B-type natriuretic peptide
nsSNPs	Nonsynonymous single-nucleotide polymorphisms

NYHA	New York Heart Association
$ONOO^-$	Peroxynitrite
PCA	Principal component analysis
PGI2	Prostacyclin
PI3K	Phosphoinositide 3-kinase
PKA	Protein kinase A
PKC	Protein kinase C
PKG	Protein kinase G
PPARα	Peroxisome proliferator-activated receptor-α
QoL	Quality of life
RAAS	Renin-angiotensin-aldosterone system
RAGE	Receptor for advanced glycation end-products
RBP4	Retinol-binding protein 4
ROS	Reactive oxygen species
RyR	Ryanodine receptor
S100A9	Protein S100-A9
SBP	Systolic blood pressure
SDS	Sodium dodecyl sulphate
SDS-PAGE	Sodium dodecyl sulfate-polyacrylamide gel electrophoresis
SERCA-2a	Sarco/endoplasmic reticulum Ca^{2+} ATPase 2a
sGC	Soluble guanylate cyclase
SR	Sarcoplasmic reticulum
STRA6	Stimulated by retinoic acid 6
T2DM	Type 2 diabetes mellitus
TGF	Transforming growth factor
TIMP	Tissue-specific inhibitors of metalloproteinase
TLR4	Toll-like receptor 4
TNF-α	Tumor necrosis factor-α
UPS	Ubiquitin proteasome system
VCAM	Vascular cell adhesion molecule
VCO_2	Carbonic dioxide production
VE	Ventilation
VO_2	Oxygen consumption

1 Introduction

Heart failure (HF) represents a major healthcare problem in today's society, being the most frequent cause of hospitalization in people over 65 years [1]. Usually, HF patients present signs and symptoms like fatigue, dyspnea and, in more severe cases, pulmonary and peripheral edema [2]. HF can be divided into HF with reduced ejection fraction (EF) (HFrEF, EF <40%), HF with preserved ejection fraction (HFpEF, EF ≥50%), and HF with mid-range ejection fraction (HFmrEF, EF 40%-49%) [3,4]. Currently, the pathophysiology of HFpEF is less well understood than HFrEF, particularly at the myocardial tissue level, and it is strongly associated with aging and female sex [5], affecting almost half of the population with HF [6]. Also, HFpEF is associated with a higher burden of comorbidities, such as obesity, hypertension, diabetes mellitus (DM), metabolic syndrome, coronary artery disease (CAD), valvular heart disease, and atrial fibrillation [7]. In contrast, HFrEF is more prevalent among men and is a heterogeneous syndrome with different causes underlying systolic dysfunction [4]. Along this line, the heart adapts differently between men and women with HF and clinical phenotype is highly heterogeneous, making its diagnosis and treatment challenging. Despite this higher symptom burden, women have been under-enrolled in clinical trials and preclinical studies of HF include mainly male animals [8]. So, it is crucial to shed light on sex differences in HF, in order to guarantee effective therapies, improving treatment efficiency and optimizing cost-effectiveness [9]. Thus, the aim of the present study was to assess the molecular mechanisms associated with the sexual dimorphism in HF by proteomic analysis of urine, using gel electrophoresis combined with liquid chromatography-tandem mass spectrometry (GeLC-MS/MS), a multidimensional and highly sensitive method [10]. GeLC-MS/MS is considered a key technology for identifying and quantifying proteins and post-translational modifications and for detecting protein-protein interactions. Therefore, the proteomic technologies can be seen as a sophisticated method, which produces very valuable data and allows the identification and quantification of proteins, as well as information regarding their localization, modifications, interactions, activities, and, ultimately, their function [11].

2 Heart failure

Left ventricular (LV) HF is a complex syndrome characterized by adverse LV structural or functional impairment of ventricular filling or ejection of the blood resulting in typical symptoms, such as breathlessness, ankle swelling, and fatigue, that can be accompanied by signs, such as elevated jugular venous pressure, pulmonary crackles, and peripheral edema [2]. Among patients with HF, LV ejection fraction (LVEF) has emerged as a clinically useful phenotypic marker and allow the classification of HF into HFrEF (EF <40%), HFpEF (EF ≥50%), and HFmrEF (EF 40%-49%). The three HF types have different trends in incidence and prevalence [4].

2.1 Epidemiology

The prevalence of HF varies from 1% to 14% based on available data from Europe and USA [2,7]. HF affects mainly older individuals, and the incidence and prevalence tend to increase with the aging of the population [12]. According to community-based studies the proportion of HF cases due to HFpEF is between 40% and 71% (mean 56%) [13]. Furthermore, HF seems to affect differently men and women according to LVEF. Women are approximately 65% less likely to develop HFrEF than men, particularly in their younger years [14].

HFrEF is more common in men because men have a higher incidence of ischemic heart disease, peripheral artery disease, impaired renal function, and chronic obstructive lung disease, which are some risk factors that influence the development of HFrEF [15]. Regarding HFpEF, its phenotype is heterogeneous, encompassing various comorbidities. Specific risk factors of HFpEF include hypertension [16], older age [4], and female sex [5]. However, there are other comorbidities frequently associated with HFpEF patients and likely contributing to disease risk, such as CAD, atrial fibrillation [17], obesity [18], and DM [19]. Importantly, about 50% of patients with HFpEF have five or more comorbidities [20].

HFpEF is more affected by age than HFrEF, and the prevalence of HFpEF at any given age is twice higher in women than in men [21], especially in post-menopausal women [22]. Likewise, the occurrence of hospitalizations is significantly higher among women [22]. These features were already identified by a survey in Portugal, wherein it was shown that the prevalence of HFpEF in community-based settings increases rapidly with advancing age, especially in women, approaching 10% in women aged 80 years or more [23]. Furthermore,

the longest life expectancy in women, together with the higher incidence of HFpEF in the elderly, also contributes to the higher prevalence of HFpEF in women [24].

A recent study analyzed sex-based differences in HF across the EF spectrum [25]. They found that men reported a lower risk of mortality/morbidity in HFpEF and HFmrEF but a higher risk in HFrEF. By contrast, women had a better prognosis in HFrEF [25]. Women, in this study, were older and more symptomatic and more likely to have hypertension and kidney disease but less likely to have ischemic heart disease [25].

Relatively to HFmrEF, patients are younger and more predominantly male compared with those with HFpEF [26]. A study that analyzed sex-based differences in epidemiology and clinical characteristics in patients with HFmrEF and HFpEF, demonstrated that clinical manifestations of HFmrEF and HFpEF differ widely between women and men [27]. They recognized that HFpEF is significantly more prevalent in women than in men, and that the prevalence of HFmrEF is higher in men than in women. Furthermore, women with HFpEF are more symptomatic and have higher body mass index and N-terminal pro-B-type natriuretic peptide (NT-proBNP) levels, impaired diastolic function, and higher prevalence of hypertension in comparison with men [27]. Male patients with HFmrEF are younger, have a higher prevalence of CAD, a more dilated left ventricle, and better diastolic function in comparison with HFpEF patients [27].

2.2 Pathophysiology

Despite the fact that the pathophysiology of HF is complex and not yet entirely understood, it is known that HFrEF is associated with a reduced cardiac output due to a progressive loss of cardiomyocytes, which leads to LV remodeling. Mechanistically, multiple aspects of the cardiac contractile apparatus are dysregulated in HFrEF and lead to adverse LV eccentric hypertrophy. Commonly, it displays alteration of the extracellular matrix (ECM) balance between collagen deposition and degradation [15], and irregular areas of fibrosis are created by collagen which replaces the dead cardiomyocytes [28]. The loss of cardiomyocytes results from various types of cell death such as exaggerated autophagy, apoptosis, or necrosis within the cardiomyocytes [29,30]. Usually, HFrEF results from ischemia, infection, or toxicity [31]. Chronically abnormal loading due to hypertension, valvular disease or tachyarrhythmias, also predisposes to HFrEF [32].

Regarding HFpEF, it was in the past referred to as ‘diastolic heart failure’, which can be defined as the inability to fill the ventricle to an adequate preload volume at acceptably low

pressures [33]. However, diastolic dysfunction is not the only contributor to HFpEF development. Other mechanisms, such as impaired ventricular-vascular coupling [25], chronotropic incompetence [34], and pulmonary hypertension [35] can also be important factors. Exercise intolerance is another common feature among HFpEF patients, and it is defined as the reduced ability to perform activities involving dynamic movement because of symptoms of dyspnea or fatigue, common clinical symptoms among patients with HF [36].

In healthy hearts, 80% of LV filling occurs during early diastole and the remaining 20% occurs with atrial contraction [37]. Patients with early-stage HFpEF might be more reliant on left atrial contraction to achieve LV filling than are healthy controls [38]. In more advanced stages of HFpEF, progressive atrial dilatation and loss of atrial contractile reserve occur, particularly with stress [39]. Atrial dilatation precedes atrial fibrillation and is associated with chronic LV diastolic dysfunction as well as to comorbidities commonly associated with HFpEF [40].

The pathogenesis of diastolic LV dysfunction on HFpEF was recently target of several studies, and the new HFpEF paradigm shifts emphasis from LV afterload excess to coronary microvascular inflammation [31]. The new HFpEF paradigm proposes that myocardial remodeling and dysfunction results from a sequence of events [31]. Firstly, comorbidities and especially obesity induces a systemic pro-inflammatory state, with increased circulating levels of interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α). This pro-inflammatory state leads to coronary microvascular endothelial dysfunction. Both vascular cell adhesion molecule (VCAM) and E-selectin were shown to be highly expressed [41,42] and their expression leads to activation and subendothelial migration of circulating leukocytes [41]. In addition, coronary microvascular endothelial cells produce reactive oxygen species (ROS). The induction of ROS production leads to the formation of peroxynitrite ($ONOO^-$) and the reduction of nitric oxide (NO) bioavailability [31]. So, ROS limits NO bioavailability for adjacent cardiomyocytes. Endothelial nitric oxide synthase (eNOS) produces NO as a dimer, but “uncouples” into monomers in the presence of inflammation/oxidative stress, producing superoxide anion. Indeed, patients with HFpEF present significantly higher expression of the eNOS monomer [43].

The limited NO bioavailability affects soluble guanylate cyclase (sGC) activity and results in decreased levels of cyclic guanosine monophosphate (cGMP) and low protein kinase G (PKG) activity in cardiomyocytes [44]. Low PKG activity removes the brake on

cardiomyocyte hypertrophy, thereby inducing concentric LV remodeling, and stiffens the cardiomyocyte because of hypophosphorylation of the giant cytoskeletal protein titin [45]. Other protein kinases, such as protein kinase A (PKA), protein kinase C (PKC), extracellular signal-regulated kinase (ERK) and Ca^{2+}/Calmodulin-dependent protein kinase type II (CaMKII), can also modulate the phosphorylation state of titin [44,46,47]. The compliance of titin itself is dependent on post-transcriptional and post-translational modifications, including isoform expression and phosphorylation [48]. Titin can be expressed in two main isoforms, N2B and N2BA [49]. Previous data have shown that the ratio between N2B and N2BA determines the titin contribution to passive stiffness, with increased N2B/N2BA ratios resulting in increased stiffness [50]. A significant shift toward the expression of the shorter, stiffer N2B isoform in myocardium from patients with HFpEF was hypothesized as being responsible for the observed higher cardiomyocyte passive stiffness [51]. The ratio of phosphorylated N2BA to N2B was shown to be significantly higher among patients with HFpEF [44]. Specifically, increased phosphorylation was observed at a PKC site on the N2BA isoform and reduced phosphorylation at the PKA/PKG site on the N2B isoform. Low myocardial PKA and PKG activity and cGMP concentration have also been demonstrated in HFpEF [51–53]. In addition to titin, also collagen has an important role in the myocardial stiffness [44]. Both stiff cardiomyocytes and increased collagen deposition by myofibroblasts cause diastolic LV dysfunction, the major cardiac functional deficit in HFpEF [44].

2.3 Role of comorbidities on the predisposition of men and women to develop heart failure

Comorbidities play an important role in the development of HF. In fact, the increase in the prevalence of HFpEF reflects an increase in the prevalence of known risk factors, including DM, obesity and hypertension, which are somewhat more common in women than in men [54]. While a diabetic woman has three times the risk of non-diabetic women to develop ischemic heart disease and subsequent HF, the risk is only doubled in diabetic men compared with non-diabetic men [55,56]. DM can be associated with obesity and both may influence the development of HF because both promote fibrosis, endothelial dysfunction and a worse vascular compliance [57]. Obesity has important implications for cardiovascular morbidity and mortality, and it has been identified as a major risk factor for hypertension and coronary heart disease (CHD) [4]. An impairment of systolic function was associated with obesity in

women but not in men [58]. Moreover, obese women also have a worse diastolic function, compared to men [58], which makes obese women more susceptible to develop HFpEF.

Regarding hypertension, women with HF are more likely than men to present hypertension, and the risk of developing HF, in this case, is 3-fold in women, compared with 2-fold in men [59]. This could be explained by a higher augmentation index between peripheral and central blood pressure in women compared with men, which leads to a pattern of pressure overload concentric cardiac hypertrophy and ultimately HFpEF [56]. Also, women have a larger left atrial volume index and a lower EF than men in the setting of atrial fibrillation [60]. Atrial fibrillation appears to increase the risk of HF in women, but possibly not in men [61].

Aging is another factor that also plays an important role in HF because it is associated with a reduction in cardiomyocyte renewal [62], and an increase in systemic inflammation that impairs NO bioavailability and promotes fibrotic remodeling of the ECM [31]. Moreover, LV volume increases modestly with age in men but not in women and it is smaller in women than in men after adjustment for age [12]. Also, aging affects the compliance of the heart and vessels [63], increases diastolic LV elastance and vascular stiffness, which is more pronounced in women [12].

Several cardiovascular risk factors were shared among HFmrEF, HFrEF, and HFpEF, but patients with HFmrEF were more likely to have hypertension [26]. Furthermore, ischemic heart disease has also a significant prevalence in patients with HFmrEF similar to those with HFrEF. Besides, functional differences were apparent, with decreased LV contractility and increased LV diastolic stiffness in HFmrEF compared with HFpEF [26].

In addition to comorbidities, cardiac structure and function of men and women may also be related to the predisposition of both sexes to develop HF.

2.4 Cardiac structure and function in men and women and their relation with heart failure development

Healthy women and men have different LV dimensions. In comparison with men, women have smaller and stiffer heart vessels [64], smaller ventricular chambers, and poorer diastolic function which leads to lower stroke volumes [12]. Moreover, women have greater arterial elastance and wave reflection with aging [12] and a higher prevalence of coronary microvascular dysfunction, which is closely linked to HFpEF, than age-matched men [65]. Previous studies have shown that women under aerobic exercise rely more on the chronotropic response, while men preferentially rely on preload and Frank-Starling

mechanism to improve cardiac output [66,67]. However, women can maintain a cardiac output as men through higher resting heart rate (HR) and higher peripheral oxygen extraction, secondary to a greater vasodilatory response [66]. For that, the chronotropic incompetence may play a crucial role in reducing exercise tolerance, decreasing peak oxygen consumption in women with HFpEF [34].

Women have higher systolic and diastolic LV elastance (stiffness) than men at a given age, and these differences are accentuated with aging, the mechanism involved can be explained by coronary microvascular inflammation [45], as previously mentioned.

A recent study showed that women had a greater degree of impairment of left ventricular diastolic reserve, which has been ascribed as a central element in HFpEF pathophysiology [68]. Women also displayed relatively more advanced degrees of systemic and pulmonary vascular dysfunction. These findings agreed with established evidence regarding increased arterial stiffening with aging in women compared to men [12]. Furthermore, women are more sensitive to afterload-induced diastolic dysfunction than men [12,69], strengthening the relationship between higher arterial elastance and impaired diastolic reserve [68].

At a cellular level, the number of cardiomyocytes, which was the same at birth in both sexes, suffers a relatively attenuated decline in aging women, lower tendency toward cardiomyocyte hypertrophy and eccentric LV remodeling present in women compared with men [70].

Noncardiac factors like female hormones, such as estrogens, whose decrease can play an important role in impaired calcium (Ca^{2+}) handling [71,72], reduction of vasodilatation [73] and myocardial fibrosis [74], can also influence the development of HF. The combination of these factors in cardiac structure and function, together with the underlying sex differences, may explain the predominance of HFpEF and HFrEF in women and men respectively, as demonstrated in Figure 1.

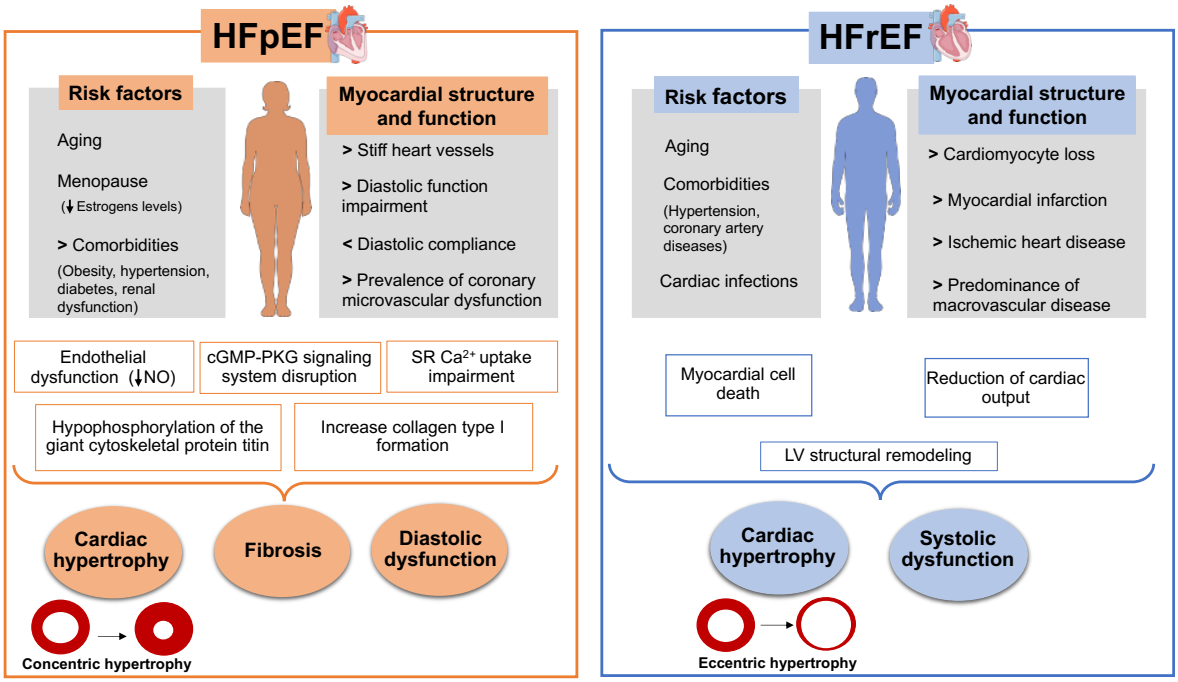


Figure 1. Sex-related differences that may predispose women and men to the development of HFpEF and HFrEF, respectively. HFpEF is a syndrome more prevalent in post-menopausal women, while HFrEF is more prevalent in men. In the case of HFpEF, risk factors such as aging, obesity, diabetes and hypertension can lead to endothelial dysfunction, as well as to hypophosphorylation of titin and collagen accumulation, resulting in concentric hypertrophy, fibrosis and diastolic dysfunction. Regarding HFrEF, elevated blood pressure and coronary artery diseases have an impact on LV remodeling, culminating in eccentric hypertrophy and systolic dysfunction. Sex differences in myocardial structure and function further support the prevalence of HFpEF and HFrEF in women and men, respectively. Abbreviations: cGMP-PKG, cyclic guanosine monophosphate-protein kinase G; HFpEF, heart failure with preserved ejection fraction; HFrEF, heart failure with reduced ejection fraction; LV, left ventricular; NO, nitric oxide; SR, sarcoplasmic reticulum. Figure made using Servier Medical Art (https://smart.servier.com/).

2.5 Impact of sex differences in molecular mechanisms associated with heart failure – Role of estrogens

As previously mentioned, HFpEF patients are mainly post-menopausal women. So, estrogen decline after menopause may have influence in the development of HFpEF. Indeed, the loss of estrogens can have a crucial role in HF development and progression because several processes like endothelial dysfunction, impaired angiogenesis, perivascular fibrosis, and blood vessel rarefaction are estrogen-mediated and are associated with microvascular dysfunction, which contributes to HFpEF [75]. Furthermore, HFpEF phenotype is associated with a pro-inflammatory state [31], and 17β-estradiol (E2) has been hypothesized to prevent it in pre-menopausal women [74]. Apart, a pro-inflammatory state can also be influenced by

the immune system, and sex differences in immune system biology have been described. Women show a stronger immunity capacity than men. However, the disadvantage is that females have higher expression of pro-inflammatory genes, higher levels of inflammatory cytokines, greater activation of CD4 and CD8 T-cells, and overall heightened systemic inflammation [76]. In the same way, women have higher circulating levels of C-reactive protein (CRP) than men [77].

Men and women differ in the systemic concentrations of estrogens, their relative ratios, as well as in the distribution of isoforms, and splice variants of estrogen receptors (ERs). These differences in hormonal concentrations lead to differential downstream signaling and responses [78]. Mechanistically, the physiological effects of estrogen are mediated by estrogen binding to ERs. There are three ERs (ERα, ERβ, and an orphan G-protein-coupled estrogen receptor, now known as GPER or GPR30), and the interaction with ERs signaling occurs *via* the traditional regulation of transcription as well as by activating membrane signaling cascades [75]. ERα and ERβ are localized in both the nucleus and the cytosol, and after binding estrogen they can translocate to the nucleus, bind to DNA and modulate gene expression [75]. Although ERs are mostly present in the nucleus, where they act as transcription factors, a small pool of ERs has been reported to lay in the vicinity of the plasma membrane, where they stimulate eNOS activity involving protein kinases such as phosphoinositide 3-kinase (PI3K) and Akt (also known as protein kinase B) [79].

ERs are located on the myocardium and vascular endothelium, smooth muscle cells, and adventitial cells in both sexes [80]. Cardiac fibroblasts and cardiomyocytes were found to express functional ERα and ERβ, and their expression differs between sexes [81]. In general, men's hearts have higher ERβ messenger ribonucleic acid (mRNA) expression compared to women's hearts, but there are no differences in mRNA expression of ERα [82]. However, in the presence of aortic valve stenosis, women's hearts showed a 2.5-fold greater increase in ERβ expression compared to men's hearts [82]. High ERβ expression may contribute to the reduced degree of cardiac hypertrophy seen in women taking hormone-replacement therapy compared to non-treated women [83]. Also, sex-based differences linked to estrogens receptors were found on the mitochondrial membrane in cardiac tissue [84]. ERs and GPR30 *via* E2 can trigger transcriptional changes in nuclear and mitochondrial genes influencing mitochondrial function, cell survival, and ultimately cardioprotection [85]. It was observed that the heart of female rats seems to have less mitochondrial content when compared to that

of male [86]. Furthermore, female mitochondria were more efficient in ATP generation when compared with the male ones, taking into account the observed increased in ATP synthase activity/ ATP synthase protein ratio [86]. Also, female rat cardiac mitochondria seem to produce less ROS, and this may be associated with the protective role of estrogens [87]. ERs can also modulate the Ca^{2+} influx in the L-channels and the activity of the sarcoplasmic reticulum (SR) [71,72]. Heart function depends on the coordinated action of individual cardiomyocytes, which are composed mainly by contractile proteins and mitochondria [9]. Indeed, HF is linked to several bioenergetic abnormalities including decreased energy metabolism, increased apoptosis, production of ROS, and dysfunctional Ca^{2+} signaling, and these abnormalities can largely be attributed to changes in mitochondrial homeostasis [88]. When these mechanisms are dysregulated, the contraction and relaxation of cardiomyocytes are affected, and HF may develop [89]. Contraction begins with an action potential that causes Ca^{2+} release from voltage-gated L-type Ca^{2+} channels in the sarcolemma and within the T-tubules. The resulting increase in Ca^{2+} cytosolic concentration triggers Ca^{2+}-induced Ca^{2+} release from ryanodine receptors (RyR), located on the SR. The propagating Ca^{2+} binds to cardiac troponin C and induces conformational changes between tropomyosin and actin on the thin filament. This exposes the myosin-binding sites on actin, enabling myosin to bind to it, thus activating the cross-bridge cycle (systole). Cardiac relaxation (diastole) requires the active uptake of Ca^{2+} into the SR through sarco/endoplasmic-reticulum Ca^{2+}ATPase-2a (SERCA-2a) and also active Ca^{2+} efflux through the Na^{+}/Ca^{2+} exchanger [90]. Some studies observed smaller contractions and a reduced Ca^{2+} transient in ventricular myocytes from female rats [91–93], possibly explained by altered SR Ca^{2+} release due to sex differences in cyclic adenosine monophosphate (cAMP) and PKA [94]. Intracellular cAMP levels were measured in ventricular myocytes from adult C57BL/6 male and female mice. Under basal conditions, female cells had significantly lower levels of cAMP in comparison to males. Thus, the lower intracellular cAMP would cause less PKA activation and result in lower levels of phosphorylation of excitation-contraction coupling targets in females than in males [94]. In addition, estrogen has also been shown to modulate the renin-angiotensin-aldosterone system (RAAS), nitric oxide synthase, and natriuretic peptide systems, thereby limiting myocardial remodeling and fibrosis, promoting fluid and electrolyte homeostasis, and attenuating microvascular dysfunction [83].

2.5.1 Role of estrogens in endothelial dysfunction

Vascular tone is strictly regulated by vasoconstricting and vasodilating factors that can be systemically and locally derived and modulated by Ca^{2+} influx into the cells [95,96]. Vascular tone can be regulated by the nervous system, which stimulates vasoconstriction through α-adrenergic receptors and vasodilation at the systemic level through β-adrenergic receptors [74]. HFpEF patients often present with dysregulation of the vascular tone involving a disturbed balance of secretion of vasoconstricting and vasodilating factors by the endothelium of the microvasculature [74]. NO, produced from L-arginine by eNOS, is an important regulator of the vascular tone [95]. NO increases cGMP concentration in vascular smooth muscle cells by stimulation of sGC activity. Elevated cGMP levels activate PKG, which diminishes Ca^{2+} levels in the cell through multiple mechanisms, and consequently, vasorelaxation is induced [97].

Vasodilatation pathways can be modulated by estrogens [74]. E2 can positively regulate eNOS activity and thereby NO production by binding to the ERα present in endothelial cells [98]. The increased availability of NO increases diastolic function in animal models and in the human heart [99]. Higher levels of cardiac endothelium NO in pre-menopausal women compared with men contributes to better endothelial-dependent vasodilation in women, and polymorphisms in the NO system have greater effects in women than in men [100]. Endogenous B-type natriuretic peptide (BNP) is known to act similarly to NO to preserve diastolic function in congestive HF, in dogs, and BNP modulates cGMP in the same direction as NO [101]. Interestingly, the plasma levels of BNP are higher in women and increases more modestly in congestive HF [102,103].

Estrogens can also stimulate other vasodilating factors like prostacyclin (PGI2), endothelial-derived hyperpolarizing factor (EDHF), and adenosine. E2 stimulates PGI2 production through ERα and cyclooxygenase-1 signaling [104]. The EDHF might protect pre-menopausal women from impaired vasodilation and the development of HFpEF because EDHF can compensate for NO loss under pathological conditions and a relevant study indicated that EDHF-mediated vasodilation is more prevalent in pre-menopausal women than in post-menopausal women or men [100].

Relatively to vasoconstricting factors, such as endothelin-1 (ET-1), are also influenced by E2 [95]. ET-1 has effects on arterial subendothelial smooth muscle cells, and decreased circulating levels of the vasoconstrictor ET-1 were seen in pre-menopausal women, which

have a lower blood pressure than men at a similar age [105]. Also, E2 can decrease oxidative stress by upregulating the levels and activity of mitochondrial enzymes, like manganese superoxide dismutase [106]. Baseline levels of ROS are lower in women than in men due to differences in phosphorylation patterns of mitochondrial proteins, for example, aldehyde dehydrogenase 2 [85]. Moreover, ROS formation can be suppressed by GPR30 signaling [107], and GPR30 seems to be more prevalent among women, protecting them from oxidative stress [108].

2.5.2 Role of estrogens on fibrosis

Replacement fibrosis is more related with HFrEF and perivascular fibrosis (the formation of fibrosis around blood vessels) in the microvasculature independent of epicardial stiffening is more associated with HFpEF [109,110]. A decrease in estrogens can lead to an increase in collagen synthesis, which is crucial to ventricular stiffness [111]. Fibrillar collagen is the primary determinant of LV diastolic stiffness in human hearts, and estrogen reduces cardiac fibroblast proliferation and collagen synthesis [112]. An inhibition of collagen cross-linking leads to a decrease in total collagen, disrupts collagen integrity, and leads to a reduction in LV stiffness [113]. A recent study showed that rat cardiac fibroblast ERα activation by E2 leads to inhibition of collagen I and III production in females, while E2 binding to ERβ promotes collagen production in males [114]. However, it was shown that increased levels of ERβ after myocardial infarction (MI) protects female mice from inflammation and fibrosis [115]. Sex and age-dependent regulation of collagen have been reported in humans [116]. The myocardial ECM is mainly composed of fibrillar collagens, types I and III [117]. Collagen degradation is mediated by matrix metalloproteinases (MMP) [118]. MMP activity is, in turn, inhibited by tissue-specific inhibitors of metalloproteinases (TIMP) [118]. An important factor for the maintenance of ECM integrity and stability is the transforming growth factor (TGF)-β. In fact, activation of the TGF-β signaling pathway may induce factors with potent fibrogenic actions [119]. In younger individuals, women were found to present less collagen types I and III than men, while in older individuals, women had more collagen types I and III than men. Also, aging alone is associated with an increase in the levels of collagen type III in women [116]. In a study with separate analyses in each sex after MI, male mice with cardiomyocyte-specific ERβ overexpression showed a decrease in deposition of collagen I and III in myocardium [120]. Chronic β-adrenergic stimulation in spontaneously hypertensive rats was reported to lead to an increase in collagen deposition

in males but not in females, making that males more susceptible to develop LV dilation and eccentric LV remodeling than females [121]. Apart, it was also observed that the side effects of β-adrenergic stimulation are related to increased HR and, in special, male animal cardiomyocytes respond with a larger increase in diastolic Ca^{2+} and SR Ca^{2+} content in comparison to female [91]. So, the sex differences in collagen regulation likely contribute to the distinct cardiac remodeling in males and females following hypertrophy. In addition to sex differences in collagen, sex differences in contractile proteins, such as myosin regulatory light chain interacting protein (MYLIP), have also been reported [122]. E2 influences cardiac gene regulation, and since E2 levels rise in older and/or obese men, pharmacological targeting of MYLIP in men with elevated E2 levels has shown a possible decrease in their risk for the development or progression of cardiovascular disease [122].

Relatively to perivascular fibrosis and cardiac hypertrophy, present in HFpEF patients, it can be reduced by E2-induced GPR30 activation, which results in the suppression of inducible NOS (iNOS) activity [123]. iNOS is usually activated during infections and chronic inflammation, where it continuously produces NO [95]. Also, iNOS impairs vasoconstriction by activating sGC, but simultaneously reduces vasodilatation by limiting tetrahydrobiopterin (BH_4) availability for eNOS, thus inducing vascular dysfunction [124].

So, it is clear that estrogens play an important role in molecular mechanisms associated with HF, such as impaired Ca^{2+} handling [71,72], reduction of vasodilatation [73] and myocardial fibrosis [74]. However, estrogens are not the unique sexual hormones that influence these processes, androgens are also important players [125]. Although the physiological actions of androgens in the heart have remained controversial compared with those of estrogen, testosterone administration in Sprague-Dawley male rats with heart failure reduced LV mass index, improving cardiac function, *via* direct androgen receptor (AR) mediated-signaling pathway [126].Testosterone was also found to regulate the L-type Ca^{2+} channel, as well as other Ca^{2+} regulatory proteins [127]. Indeed, testosterone treatment of ventricular cardiomyocytes isolated from two-day-old rats induced an increase in the L-type Ca^{2+} channel mRNA levels, as well as Na^{+}/Ca^{2+} exchanger mRNA levels [127]. Moreover, androgens seems to influence cardiac fibrosis by upregulation of TGF-β, which is known to induce ECM deposition predisposing men to cardiac fibrosis [128]. Also, a reduction in testosterone levels leads to decreased contractility of individual cardiomyocytes and suppresses Ca^{2+} transients [129]. Therefore, loss of testosterone, in older men, may suppress

contractile function and contribute to systolic dysfunction [129]. So, it has been recognized that sex hormones have a crucial role in HF development and progression, and both estrogens and androgens modulated several processes in cardiac function by acting on ERs and ARs expressed in distinct cells of the heart, including cardiomyocytes, endothelial cells, vascular smooth cells and fibroblasts [130].

2.6 Impact of sex in molecular mechanisms associated with heart failure - Role of energetic metabolism

A rise in glycolysis is observed in hypertrophied hearts which can be associated with a decline in cardiac function [131]. Insulin-dependent glucose is more prominent in women, but the capacity for the storage of glucose in the form of glycogen is similar in women and men [132]. In general, men's hearts have a greater preference for glucose than women's hearts and conversely, women's hearts generally use more fats [133].

Data on cardiac glucose utilization in the presence of systolic dysfunction, show an increase in glycolysis relative to mitochondrial oxidative metabolism including glucose oxidation [134–136]. Myocardial glucose utilization may be decreased by estrogen because it is known to upregulate NO synthases, which cause a reduction in adenosine monophosphate-activated protein kinase (AMPK) stimulated glucose transporter (GLUT) type 4 translocation to the cell surface, thereby inhibiting myocardial glucose uptake. Glucose is transported into the cytosol by glucose transporters including GLUT1 and GLUT4. While GLUT1 is the major glucose transporter in the fetal heart and contributes to constitutive glucose uptake; in the adult heart, GLUT4 is the predominant isoform and mediates the bulk of basal myocardial glucose uptake [131].

Peroxisome proliferator-activated receptor (PPARα) has been suggested to be a key regulator of metabolism. PPARα is known to regulate fatty acid oxidation and has been well-documented to play a role in cardiac hypertrophy and dysfunction [137]. Some studies report a decrease in PPARα with hypertrophy [138,139], and a PPARα agonist has been shown to reduce hypertrophy [140,141]. However, treatment with a different PPARα agonist, did not reduce cardiac hypertrophy and increased contractile dysfunction [139]. So, it was concluded that a decrease in PPARα during hypertrophy is essential for the maintenance of contractile function in hypertrophy.

In a study with female and male mice with cardiac specific overexpression of PPARα, it was observed that mice exhibit cardiac hypertrophy and elevated fatty acid oxidation and

decreased glucose oxidation. However, no sex differences were reported [142]. Although there are baseline differences in PPARα expression in the heart, female mice show lower expression compared to male mice [143]. In PPARα-KO mice, inhibition of carnitine palmitoyltransferase 1 results in the death of 100% of the male mice, but only 25% of the female mice, suggesting sex differences in the response to changes in PPARα [144]. Thus, several pathological processes influence in a different way the risk of men and women to develop HF.

3 Sex-associated differences in patients' response to heart failure treatment

In HF, treatment approaches intend to improve patients' clinical status, functional capacity and quality of life (QoL), as well as prevent hospital admission and reduce mortality [2,7]. At present, evidence-based therapy of HF is largely restricted to HFrEF [145], compared with HFpEF, and many of the clinical trials have been underpowered to examine the sex-specific differences in therapeutic effect [146].

3.1 Pharmacological therapy

Although several studies have been highlighting differences between men and women in the prevalence of HF, women are less well represented in pharmacological trials, and medical therapy is not given to patients based on sex differences [146].

Preliminary data suggest that men and women respond differently to many drugs [147]. In fact, it is known that women generally have lower body weight, a higher proportion of body fat, and lower plasma volume than men, and these factors can contribute to a longer duration of action of lipophilic drugs and higher peak plasma concentrations of hydrophilic drugs in women [148]. Furthermore, lower cardiac output in women results in lower hepatic flow and lower glomerular filtration rate, and women have lower expression of some drug-specific cytochrome P450 isoenzymes, which could also contribute to higher plasma levels of both hydrophilic and lipophilic drugs in women [149]. Despite that, HF guidelines recommend the up-titration of angiotensin-converting enzyme inhibitors (ACEIs) or angiotensin receptor blockers (ARBs) and beta-blockers (β-blockers) to the same target doses in men and women [2,7].

Specific pharmacological HFpEF therapies with an impact on its mortality have not been developed yet, and in this case treatment recommendations focus on the management of patients' symptoms, risk factors and comorbidities [7]. HFrEF patients are also treated

without sex distinction. Indeed, a recent randomized controlled trial of pharmacological therapy in patients with HFrEF [150] demonstrated that despite significant differences in baseline characteristic between women and men in two trials PARADIGM-HF (Prospective comparison of Angiotensin Receptor Neprilysin Inhibitor (ARNI) with ACEI to Determine Impact on Global Mortality and morbidity in Heart Failure) [151] and ATMOSPHERE (Aliskiren tiral to Minimize OutcomeS in Patients with Heart Failure) [152], men and women were generally treated similarly. The rates of use of a diuretic, β-blocker, and mineralocorticoid receptor antagonist (MRA) were very similar in women and men. Although, women were slightly more likely to receive digitalis (32.4% *vs.* 30.6%) and ARBs (16.4% *vs.* 11.9%) compared with men, and less likely to receive an ACEIs (84.7% *vs.* 88.7%). Of the 15 415 patients enrolled in these trials, 21.8% were women. They were older, had higher blood pressure, higher HR, and were more often obese, compared with men. However, despite women with HFrEF have less comorbidities compared with men, and better outcomes than men, presenting better survival, and lower rates of hospitalization; women have more symptoms and worse health-related QoL than men. They also report much more anxiety or depression [150].

To the best of our knowledge, only fourteen studies analyzed the influence of sex differences on the response to HF treatment (Table 1) [153–166]. Most of these studies involve patients with HFrEF (~ 71%). In addition, women represent less than 30% of the total patients in most of the studies. Data presented in Table 1 indicate that the average age of the patients included is 64.5 years old, and most of these patients belong to New York Heart Association (NYHA) functional class II, III and IV. Moreover, in the case of women, hypertension and DM are the more frequent comorbidities, and by contrast, most of the men have CHD and MI [153–166].

In general, the survival benefit with ACE inhibitors in patients with HF seems to be lower in women than in men [147]. ACEIs block angiotensin-converting enzyme that converts angiotensin I to angiotensin II. Decreased production of angiotensin II enhances natriuresis, lowers blood pressure, and prevents remodeling of smooth muscle and cardiomyocytes. Lowered arterial and venous pressure reduces cardiac preload and afterload [167]. Also, it was hypothesized that ACEIs interfere with the degradation of bradykinin which is a peptide that causes vasodilation [167].

The significant decrease in morbidity and mortality achieved with β-blockers along with their prominent effect in reverse remodeling have established three β-blockers, (bisoprolol, metoprolol succinate, and carvedilol) as therapy in the pharmacological management of systolic HF [168]. In the case of metoprolol, men show a significant benefit [154]. By contrast, women show somewhat better benefits in terms of reduction in all-cause mortality and hospitalization with carvedilol [157]. Metoprolol and bisoprolol interact primarily with β-receptors. By contrast, carvedilol blocks α_1-, β_1-, and β_2-receptors, resulting in increased anti-hypertensive effects [169]. Further studies are needed to provide definitive conclusions on the effect of β-blockers on both sexes.

ARBs are often used as an alternative therapy in patients with HF who cannot tolerate ACEIs or, increasingly rare, as an addition to ACE-inhibitor therapy [2]. The effects of ARBs seem to be similar, with a reduction in mortality and morbidity in both sexes. However, the largest trial to data, PARAGON-HF trial (Prospective Comparison of Angiotensin Receptor Neprilysin Inhibitor with Angiotensin Receptor Blocker Global Outcomes in Heart Failure and Preserved Left Ventricular Ejection Fraction), reported a strong sex specific response to sacubitril-valsartan compared to valsartan, where a great benefit was seen in women with HFpEF than in men [162]. The primary outcome valued was a composite of first and recurrent hospitalizations for HF and death from cardiovascular causes. PARAGON-HF represents one of the largest populations of women with HFpEF ever studied, wherein 2479 women (51.7%) and 2317 men (48.3%) were involved. Women were older than men and were more obese. The median LVEF in women was 60% and in men it was 55%. Women had a worse NYHA class distribution, and more symptoms of HF. Sacubitril/valsartan, compared with valsartan, reduced the probability of cardiovascular death and total hospitalizations for HF by 27% in women with HFpEF, but with no effect in men [162]. The reasons why women might have a more favorable response to neprilysin inhibition than men may be related to the lower natriuretic peptide levels in women. Furthermore, in this study, more women than men had mild left ventricular systolic dysfunction, and sacubitril-valsartan appears to be beneficial in patients with left ventricular systolic dysfunction; in particular, this medication is more effective for high LVEF values, as is the case for women in the study [162].

Table 1. Sex-specific responses to pharmacological therapies for heart failure.

Treatment (drug class)	HF phenotype (LVEF)	n patients (% women)	Mean age		Comorbidities			NYHA functional class	Primary end point	Sex-related differences in efficacy	Study
			M (y)	W (y)		M (%)	W (%)				
Enalapril (ACEI)	HFrEF (≤ 35%)	485 (11%)	59.1	59.1	Ischemic heart disease	83.20		I-II	All-cause mortality	Significant benefit in men; trend towards benefit in women	SOLVED-Investigators [153]
					Myocardial infarction	79.95					
					Hypertension	37.05					
					Diabetes mellitus	15.25					
					Idiopathic dilated cardiomyopathy	9.35					
Metoprolol (β-blocker)	HFrEF (< 40%)	3991 (23%)	63.0	65.0	Hypertension	42.0	51.0	II-IV	All-cause mortality and hospitalization	Significant benefit in men; no significant benefit in women	MERIT-HF [154]
					Previous myocardial infraction	51.0	40.0				
					Diabetes mellitus	23.0	30.0				
					Atrial fibrillation	18.0	10.0				
Bisoprolol (β-blocker)	HFrEF (≤ 35%)	2647 (19%)	60.0	65.0	Primary cardiomyopathy	48.0	40.0	III-IV	All-cause mortality	Women showed somewhat better benefit compared to men	CIBIS-II [155]
					Hypertension	24.0	35.0				
					Atrial fibrillation	20.0	17.0				
					Diabetes mellitus	11.0	14.0				
Carvedilol (β-blocker)	HFrEF (< 25%)	2300 (20%)	63.4	63.4	Not described			II-III	All-cause mortality	Similar benefit in both sexes	COPERNICUS [156]
Carvedilol (β-blocker)	HFrEF (≤ 35%)	1094 (23%)	58.1	58.1	Not described			II-IV	All-cause mortality and hospitalization	Women showed somewhat better benefit compared to men	US Carvedilol HF study [157]

Valsartan (ARB)	HFrEF (< 40%)	5010 (20%)	62.5	63.3	Coronary heart disease	60.9	42.3	II-IV	Mortality and mortality and morbidity combined	Similar benefit in both sexes	Val-HEFT [158]
					Idiopathic cardiomyopathy	28.5	41.7				
					Diabetes mellitus	24.9	27.8				
					Hypertension	6.0	9.6				
					Other	4.6	6.5				
Candesartan (ARB)	HFrEF (≤ 40%)	4576 (26%)	64.4	67.8	Hypertension	9.0	21.0	II-IV	CV mortality and HF hospitalization	Similar benefit in both sexes	CHARM [159]
					Valvular disease	2.0	4.0				
					Atrial fibrillation	2.0	3.0				
Irbesartan (ARB)	HFpEF (≥ 45%)	4133 (60%)	72.0	72.0	Hypertension	88.5		II-IV	All-cause mortality and hospitalization	Similar no significant treatment effects in both sexes	I-PRESERVE [160]
					Angina	40.0					
					Atrial fibrillation	29.0					
					Diabetes mellitus	27.5					
					Myocardial infarction	23.5					
Candesartan (ARB)	HFpEF (> 40%)	3023 (40%)	67.2	67.2	Diabetes mellitus	64.3		II-IV	All-cause mortality	Similar benefit in both sexes	CHARM-Preserved [161]
					Angina	44.4					
					Myocardial infarction	44.4					
					Atrial fibrillation	29.2					
Sacubitril/Valsartan (ARNI) *vs.* Valsartan (ARB)	HFpEF (≥ 40%)	4796 (51.7%)	71.8	73.6	Hypertension	94.6	96.5	I-IV	First and recurrent hospitalizations for HF and death from cardiovascular causes	Sacubitril/valsartan, compared with valsartan, reduced the probability of cardiovascular death and total hospitalizations for HF by 27% in women, but with no effect in men	PARAGON-HF [148]
					Diabetes mellitus	45.8	40.4				
					Atrial fibrillation	35.8	29.4				
					Angina pectoris	31.2	26.8				
					Renal disease	27.5	23.9				
					Myocardial infarction	30.0	15.7				
					COPD	16.6	11.5				
					Anemia	14.7	14.8				
					Cancer	8.9	9.2				

Spironolactone (MRA)	HFrEF (≤ 35%)	1663 (27%)	65.0	65.0	Not described			II-IV	All-cause mortality	Similar benefit in both sexes	RALES [163]
Spironolactone (MRA)	HFpEF (≥ 45%)	1767 (49.9%)	71.0	72.1	Hypertension	88.0	91.0	I-IV	CV death, cardiac arrest, or HF hospitalization	There were no sex differences in response to primary outcomes; Spironolactone therapy was associated with reduced all-cause mortality in women but no in men	TOPCAT [164]
					Dyslipidemia	74.0	68.0				
					Coronary artery disease	54.0	38.0				
					Diabetes mellitus	49.0	40.0				
					Atrial fibrillation	45.0	39.0				
					Angina	32.0	23.0				
					Myocardial infarction	26.0	14.0				
					COPD	19.0	14.0				
Eplerenone (MRA)	HFrEF (≤ 40%)	6632 (29%)	64.0	64.0	Hypertension	60.0		After acute MI	All-cause mortality	Similar benefit in both sexes	EPHESUS [165]
					Diabetes mellitus	32.0					
					Acute myocardial infarction	27.0					
					Heart failure	14.0					
Digoxin	HFrEF (≤ 45%)	6800 (22%)	64.0	66.0	Prior myocardial infarction	68.0	54.4	I-IV	All-cause mortality	Increased mortality in women; no significant effect in men	DIG [166]
					Hypertension	42.9	54.1				
					Diabetes mellitus	26.9	33.6				
					Current angina	26.3	28.3				

Abbreviations: ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; CHF, chronic heart failure; COPD, chronic obstructive pulmonary disease; CV, cardiovascular; HF, heart failure; HFpEF, heart failure with preserved ejection fraction; HFrEF, heart failure with reduced ejection fraction; LVEF, left ventricular ejection fraction; M, men; MI, myocardial infarction; MRA, mineralocorticoid receptor antagonist; n, number of patients; NYHA, New York Heart Association; W, women.

Relatively to MRAs, like spironolactone and eplerenone, they block receptors that bind to aldosterone and, with different degrees of affinity, other steroid hormones (e.g. corticosteroids, androgens) receptors. The response to MRAs seems to be similar in both sexes; however, spironolactone therapy was associated with reduced all-cause mortality in women but not in men [164]. Digoxin shows a significantly increased risk of death among women and no significant effect in men [166].

Until now, clinical trials have not identified an efficient treatment to HFpEF with optimal drug doses based on sex-specific response to therapy. Therefore, it is essential to study novel approaches to the prevention and treatment of this condition [170], and to increase women inclusion in HF clinical trials because treatment guidelines are predominantly based on male-derived data.

3.2 Non-pharmacological therapy – Exercise training

Exercise training (ET) is being considered an effective adjunct treatment in HF. In HFrEF, ET has found to improve exercise capacity and reduce morbidity [171], and in HFpEF, ET has demonstrated improvements in cardiorespiratory fitness, diastolic function, QoL and general health [172].

Some studies have been done with HFrEF and HFpEF patients that performed an exercise program of endurance and resistance training during 12 weeks [173–176]. The session time, in general, was between 20 to 40 minutes, 2 or 3 times *per* week. These patients have a high proportion of comorbidities such as CAD, hypertension, DM, and overweight. The goal of these studies was mostly to evaluate ET impact on QoL, peak of oxygen consumption (VO_2), cardiac structure and function [173–176]. Regarding HFrEF patients, it was demonstrated that aerobic interval training enhances cardiac systolic function, as indicated by increased LVEF and cardiac power index peak. Also, it enhanced cardiac hemodynamic response to exercise and heightened peak VO_2 and O_2 uptake efficiency slope [173]. In HFpEF patients, ET improved cardiac diastolic function as reflected by the decrease in E/E' ratio (ratio of transmitral early flow velocity to annular velocity), increased blood distribution to *vastus lateralis* muscle and increased O_2 extraction by *vastus lateralis* muscle during exercise. Also, ET regimen heightened peak VO_2, O_2 uptake efficiency slope, and lowered the VE/VCO_2 (ratio between minute ventilation and carbonic dioxide production) slope [173]. Another study also showed that ET improved emotional status, physical and social dimensions of QoL, and symptoms of depression in HFpEF patients. General health

perception also improved significantly [174]. Furthermore, ET in HFpEF patients improved exercise capacity and physical dimensions of QoL. This benefit was associated with atrial reverse remodeling and improved left ventricular diastolic function [175].

The largest randomized controlled trial testing the efficacy and safety of ET on clinical outcomes is the multicenter HF-ACTION (Heart Failure: A Controlled Trial Investigating Outcomes of Exercise Training), in which over 2300 stable patients with HFrEF were randomized to 36 supervised sessions of aerobic ET and home-based ET in addition to usual care or usual care alone. Results showed that ET group experienced a modest 4% improvement in peak VO_2, reduced the risk of cardiovascular disease and HF hospitalizations [177]. However, this trial shows a reduced improvement in peak VO_2 because, normally, ET demonstrated improvements in peak VO_2 between 10% and 15% [178]. So, one of the reasons for the small improvement in cardiorespiratory fitness in the HF-ACTION trial was the low adherence to the prescribed ET because only 30% of patients completed the training protocol [177].

One of the hallmarks of HFpEF is endothelial dysfunction, and exercise training has been seen to improve it in patients with CAD and DM [179,180], wherein several mechanisms besides the modulation of eNOS have been discussed [181]. It is known that women have a higher eNOS expression in skeletal muscle and endothelium than men, which leads to a more pronounced vasodilator response to exercise [182]. Although the exact mechanisms responsible for the above described ET-induced cardiovascular benefits are not clear, several hypotheses have been proposed. At the myocardium level, exercise training was seen to improve LVEF by inducing a positive LV remodeling [183], also ET seems to attenuate cardiac fibrosis [184] and enhance myocardial function [181]. Mechanistically, ET can reduce the activation of the ubiquitin-proteasome system (UPS) and myostatin expression in the myocardium in HF models [185], as well as induce the activation of insulin-like growth factor-1 (IGF-1) [186]. Further mechanisms are the modulation of Ca^{2+} handling [187] or even the modulation of stem cell proliferation [188]. Also, ET induces beneficial anti-inflammatory effects including, for example, a decrease in serum CRP levels in healthy individuals and a decrease in IL-6 plasma levels [189], which may protect against inflammation-mediated myocardial fibrosis and dysfunction. Regarding the peripheral vasculature, regular physical activity can reduce mitochondrial reactive oxygen species production, enhance cellular antioxidant defense proteins, and reduce mitochondrial fission,

a sign of mitochondrial dysfunction [190]. So, taking into consideration all these beneficial effects of ET, it may contribute to improve compliance, reduce stiffness, and afterload, finally reducing the risk of cardiac dysfunction.

Similarly to the studies performed with pharmacological therapy, also in exercise-based cardiac rehabilitation trials, women have been underrepresented [182]. Importantly, one trial was done only in women (n=32) with HFpEF [176], in which the results showed a greater increase in distance walked on the 6-min walk test and improved QoL scores in the participants in the intervention group who underwent a combined program of exercise and education (n=16), compared with the participants in the control group who were assigned to an education program only (n=16) [176].

A very limited number of studies was performed on the sex-associated differences in HFpEF patients' response to exercise training. A recent study focused on the importance of sex differences in cardiac and skeletal muscle responses to exercise [191]. A total of 295 patients with an LVEF of 50% or more and NYHA class II to IV were included. Results showed that women (59%) exhibited worse peripheral O_2 extraction with exercise, worse right ventricular and LV systolic reserve, and worse diastolic reserve response to exercise compared with men [191]. These findings are consistent with another study about differences in exercise hemodynamics between 47 men and 114 women with HFpEF, which reported that women had greater impairments in diastolic reserve and peripheral oxygen kinetics [68]. These sex differences persisted even after adjustment for hemoglobin values, which has been proposed to be a major driver of differences in oxygen transport and utilization in men and women. So, these differences in exercise response may suggest that women and men respond differently to exercise training [191].

4 Aims

HF is a major healthcare problem that seems to affect women and men in different ways. HFrEF is more prevalent in men; whereas HFpEF is twice more prevalent in women. HFpEF is mainly a disease of the elderly and post-menopausal women. However, women remain underrepresented in clinical trials and most of the preclinical studies only include male animals. A recent study reported a sex specific response to sacubitril-valsartan compared to valsartan, where a superior benefit was reported in women with HFpEF. Thus, it is critical to comprehend the sex-associated mechanisms in HF and understand the role that sex plays in the management of this disorder. So, the aim of this work was to study the molecular mechanisms associated with the sexual dimorphism in HF. To achieve this, urine samples were collected from male and female patients with HF and from control individuals, for proteome characterization using a mass spectrometry-based approach and bioinformatics analysis to the identification of the biological processes associated with sex in HF.

5 Materials and Methods

5.1 Experimental scheme

The experimental scheme followed in this work is summarized in Figure 2 and will be detailed in section 5.2 to 5.7.

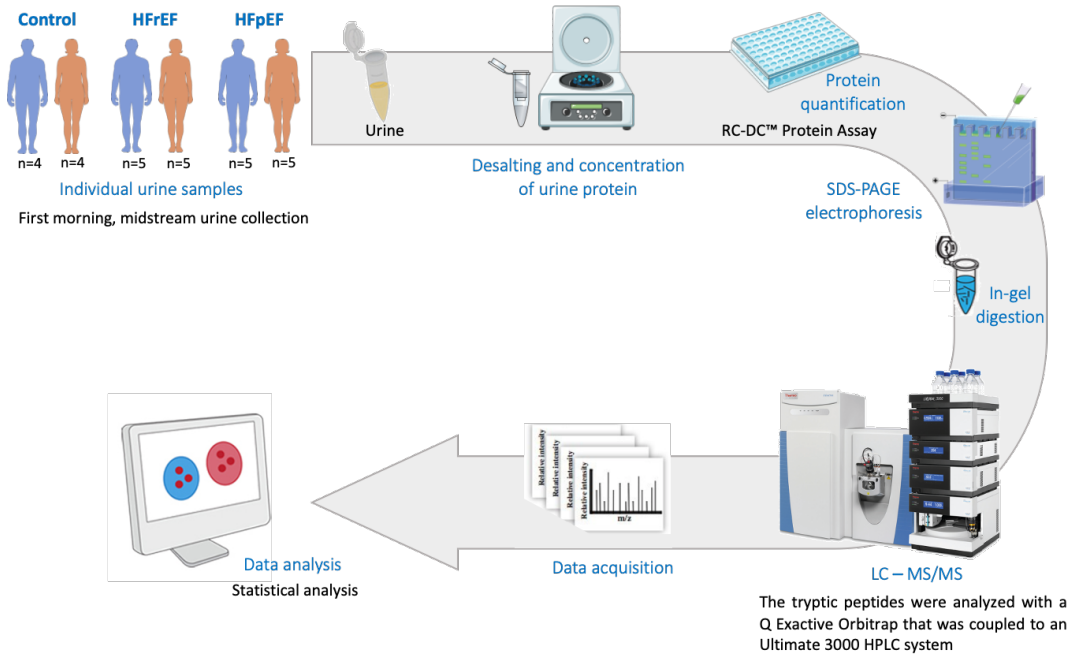


Figure 2. Schematic representation of the experimental procedure. In order to study the molecular mechanisms associated with the sexual dimorphism in HF, urine samples were collected from all participants. Urine proteome was characterized by GeLC-MS/MS. Abbreviations: HFpEF, heart failure with preserved ejection fraction; HFrEF, heart failure with reduced ejection fraction; HPLC, high-pressure liquid chromatography; LC, liquid chromatography; MS, mass spectrometry; RC-DC, reducing agent compatible and detergent compatible; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Figure made using Servier Medical Art (https://smart.servier.com/) and Planet Orbitrap (https://planetorbitrap.com/).

5.2 Study population

Subjects enrolled in the present study included 10 HFrEF patients (5 men and 5 women) and 10 HFpEF patients (5 men and 5 women). Eight subjects (4 men and 4 women) without HF were included as controls. The demographic and clinical characteristics of enrolled subjects are summarized in Table 2.

The present study was approved by the Hospital Ethics Committee and followed the Declaration of Helsinki. HFrEF subjects included in the study are enrolled in the Centro Hospitalar Universitário do Porto and HFpEF and control participants are followed in the Hospital de São João do Porto. All of them gave their written informed consent after being

explained the nature and purpose of the present study. The eligibility criteria included HFrEF and HFpEF patients: i) with ≥ 18 years old; ii) diagnosed with HFrEF or HFpEF according to criteria of the European Society of Cardiology (2016) [2]; iii) with clinical stability for ≥ 6 weeks and iv) receiving optimal medical treatment for ≥ 6 weeks. Control group included individuals: i) with ≥ 18 years old; ii) with cardiovascular risk factors and iii) that do not had criteria to be diagnosed with HF.

5.3 Sample collection, processing and protein quantification

A first morning, midstream urine collection was performed. Urine samples were centrifuged at 3000 g for 10 min (4ºC), and the supernatant was stored at -80ºC until further analysis. Then, urine samples were passed through a 10-kDa filter (Vivaspin 500–10 kDa, Sartorius Biotech) to concentrate samples. The final retentate was resuspended in 100 mL of 0.3 M Tris and 4% sodium dodecyl sulphate (SDS). Total protein content was estimated in the fraction corresponding to the retentate using the RC-DC protein assay kit (Bio-Rad®, Hercules, CA, USA) according to the manufacture's recommendations and using bovine serum albumin (BSA) as standard. A calibration curve with standard solutions of BSA at concentrations between 0.15625 and 10 mg/mL was made. The absorbance was measured at 750 nm in a microplate reader (Multiskan GO, Thermo Fischer Scientific®, Northumberland, UK).

5.4 Protein separation by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE)

The proteome sample (40 µg) was mixed (1:2) with loading buffer (0.5 M Tris-HCl pH 6.8, 4% (w/v) SDS, 15% (v/v) glycerol, 1 mg/mL bromophenol blue and 20% (v/v) β-mercaptoethanol) and incubated at 100°C for 5 min. Subsequently, the samples were separated using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) in a 12.5% gel prepared as previously described [192]. The gels were run for 45 min at 180 V in running buffer (250 mM glycine, 25 mM Tris, pH 8.6 and 0.1% (w/v) SDS). Following electrophoretic separation, gels were incubated in fixation solution (40% (v/v) methanol and 10% (v/v) acetic acid) for 30 min, stained with Colloidal Coomassie Blue G-250 and destained with 25% (v/v) methanol until an optimal contrast was achieved. The gels were posteriorly scanned in the ChemiDoc Imaging System v.2.3.0.07 (Bio-Rad®, Hercules, CA, USA).

5.5 In-gel digestion

Each lane of the gels was cut in eight sections and washed with 25 mM ammonium bicarbonate (NH_4HCO_3) for 30 min. After this time the supernatant was removed, and the gel was washed three times in a solution of 50% acetonitrile (ACN)/25 mM NH_4HCO_3 for more 30 min. Following, the gel was totally dehydrated in 100% ACN, 30 min. Proteins in each section were reduced with 10 mM dithiothreitol in 25 mM NH_4HCO_3, 45 min at 56ºC, alkylated with a solution of 55 mM iodoacetamide in 25 mM NH_4HCO_3 (30 min in the dark) and washed again three times with 25 mM NH_4HCO_3, 50% ACN/25 mM NH_4HCO_3 (15 min) and 100% ACN (10 min), respectively; before being digested with trypsin (Pierce, Life Technologies, USA) overnight (37ºC). The resulting peptides mixture was extracted from the gel fractions with 5% formic acid (FA) (20 min) and 5% FA/50% ACN (20 min, two times), pulled into one section for each line, and dried using vacuum centrifugation [193].

5.6 Protein identification by liquid chromatography-tandem MS (LC-MS/MS)

The tryptic peptides were resuspended in 1% (v/v) FA and analyzed with a Q Exactive Orbitrap (Thermo Fisher Scientific, Bremen) through the EASY-Spray nano-ESI source (Thermo Fisher Scientific, Bremen) that was coupled to an Ultimate 3000 (Dionex, Sunnyvale, CA) high-pressure liquid chromatography (HPLC) system. The trap (5 mm × 300 µm internal diameter (ID)) and the EASY-Spray analytical (150 mm × 75 µm) columns used were C18 Pepmap100 (Dionex, LC Packings) having a particle size of 3 µm. Peptides were trapped at 30 µL/min in 96% solvent A (0.1% FA). Elution was achieved with the solvent B (0.1% FA/80 % acetonitrile v/v) at 300 nL/min. The 92 min gradient used was as follows: 0–3 min, 96% solvent A; 3–70 min, 4–25% solvent B; 70–90 min, 25–40% solvent B; 90–92 min, 90% solvent B; 90–100 min, 90% solvent B; 101-120 min, 96% solvent A. The mass spectrometer was operated at 1.6 kV in the data dependent acquisition mode. A MS^2 method was used with a FT survey scan from 400 to 1600 m/z (resolution 70 000; auto gain control (AGC) target 1E6). The 10 most intense peaks were subjected to higher-energy collisional dissociation fragmentation (resolution 17 500; AGC target 5E4, normalized collision energy 28%, max. injection time 100 ms, dynamic exclusion 35 s).

Processing of the raw data generated from LC-MS/MS analysis was carried out using Proteome Discoverer 2.2 (Thermo Fischer Scientific, Bremen, Germany), with the MS Amanda 2.0 (University of Applied Sciences Upper Austria, Research Institute of Molecular

Pathology) and Sequest HT (SEQUEST HR algorithm, license Thermo Scientific, University of Washington, USA). Database search parameters were as follows: carbamidomethylation of cysteine, oxidation of methionine, acetylation, phosphorylation and the allowance for up to two missed tryptic cleavages. The peptide mass tolerance set to 10 ppm and fragment ion mass tolerance set to 0.02 Da. To achieve a 1% false discovery rate (FDR), the Percolator 2.0 (Thermo) node was implemented for a decoy database search strategy and peptides were filtered for high confidence and a minimum length of six amino acids, and proteins were filtered for a minimum number of peptide sequences of one. For each protein, a minimal number of identified unique peptides was set to two peptides. In the case of the proteins that have been identified in 80% of samples of each experimental group, the missing values were replaced by 1/5 of the minimum positive value of each variable. Forty percent or more of missing values in the samples of each experimental group lead to remove features (Table S1). Input data were searched against the UniProtKB-SwissProt database under taxonomy *Homo sapiens* (Release in Jun 2020). Due to technical problems, protein identification in HFpEF urine samples by LC-MS/MS was not possible.

5.7 Bioinformatics tools

From the data obtained by LC-MS/MS, the analysis of the differences between groups were explored using the free available MetaboAnalyst 4.0 software [194], which allowed the visualization of the data in principal component analysis (PCA), heat map, volcano plot and boxplots. The Jvenn [195] and FunRich (version 3.1.3) [196,197] were also used. Jvenn is a Venn diagram creation tool that highlights the distribution of the proteins identified in the data sets, showing the unique and overlapping proteins. Processes up to six peptide/protein data sets and retrieve the list of unique peptides for each set [195]. FunRich is a functional enrichment analysis tool, which can handle a variety of protein data sets, identifies the most prevalent biological processes in the groups, the cellular origin of the identified proteins and molecular function [196,197].

5.8 Statistical analysis

Statistical analysis was performed with Graph Pad Prism software, version 8.2.1. Continuous variables are presented as mean ± standard deviation. Categorical variables are presented as counts and proportions. Data were tested for normal distribution using the Shapiro-Wilk test.

Comparison of continuous variables between the groups was made by a two-way analysis of variance (ANOVA) or Kruskal-Wallis test for non-normally distributed data. Frequency distribution was compared using the Fisher's exact test. Results were considered significantly different when $p<0.05$.

6 Results

6.1 Characteristics of participants

The clinical and demographic characteristics of the patients with HFrEF, HFpEF and control individuals (50% of women in all groups of participants) are described in Table 2. The age of the patients was significantly different between men and women with HFrEF ($p<0.05$), with women being younger. However, this feature does not seem to be a particularity of this condition, because women with HFrEF tend to be older than men [150]. Also, HFrEF participants were significantly younger than their respective HFpEF sex match ($p<0.05$ in men and $p<0.0001$ in women), which is in concordance with other cohort studies. Commonly, patients with incident HFpEF are older than those with incident HFrEF [198–200]. Additionally, in this study, the HFrEF patients' age is closer to the age of the control group; in contrast to the ages of HFpEF patients, which was significantly higher than those the control group ($p<0.001$ in men and $p<0.01$ in women). Regarding the etiology of HF, 40% of HFrEF (either in men and women) had ischemic etiology, as well as 40% of HFpEF men. Most of HF patients belong to NYHA functional class II and III (60% of HFrEF men, 100% of HFrEF women, 80% of HFpEF men and 80% of HFpEF women). Men with HFrEF had a significant lower level of LVEF in comparison with HFpEF men ($p<0.001$) and control men ($p<0.01$). The same was observed to HFrEF women, which show a LVEF significantly lower than HFpEF women ($p<0.01$) and control women ($p<0.01$). However, this feature is expected due to the nature of the HFrEF disease. Significant sex differences were verified in systolic blood pressure (SBP) between men and women with HFpEF ($p<0.001$), and between women with HFrEF and HFpEF ($p<0.001$), with HFpEF women, in both cases, showing a higher SBP. No significant differences were observed in diastolic blood pressure between patients' groups.

Table 2. Demographic and clinical characteristics of the study participants.

Characteristic	Control			HFrEF			HFpEF		
	Men (n=4)	Women (n=4)	p-value[a]	Men (n=5)	Women (n=5)	p-value[b]	Men (n=5)	Women (n=5)	p-value[c]
Age, yrs	60.50±13.33	63.50±5.75	0.9884	68.00±4.85	54.00±6.16	0.0390	83.40±4.51$^{###,\ \S}$	84.20±4.27$^{**,\ \&\&\&\&}$	>0.999
Ischemic etiology, no (%)	0 (0)	0 (0)	>0.9999	2 (40)	2(40)	>0.999	2 (40)	0 (0)	0.4444
NYHA II-III, no (%)	1 (25)	0 (0)	>0.9999	3 (60)	5 (100)**	0.4444	4 (80)	4 (80)*	>0.999
LVEF, %	60.50±3.54	63.33±3.51	0.9975	35.40±6.19$^{\#\#}$	41.40±12.10**	0.7557	61.20±3.70§§§	60.80±5.26$^{\&\&}$	>0.999
SBP, mmHg	130.80±15.59	136.30±1.50	0.9910	123.60±15.57	112.20±11.52	0.7524	110.0±4.24	161.20±18.91$^{\&\&\&}$	0.0001
DBP, mmHg	73.50±8.35	76.00±7.57	0.9994	66.40 ±18.09	63.50±2.52	0.9984	66.00±8.97	78.00±9.62	0.5019
BMI, kg/m^2	26.36±4.99	32.30±5.17	0.5560	30.72±5.34	27.61±7.09	0.9180	27.94±3.03	27.66±3.24	>0.999
Comorbidities, no (%)									
HTA	2 (50)	4 (100)	0.4286	3 (60)	0 (0)**	0.1667	5 (100)	5 (100)$^{\&\&}$	>0.999
DM	0 (0)	2 (50)	0.4286	0 (0)	4 (80)	0.0476	0 (0)	1 (20)	>0.999
Dyslipidemia	3 (75)	4 (100)	>0.999	3 (60)	3 (60)	>0.999	3 (60)	2 (40)	>0.999
AF	0 (0)	0 (0)	>0.999	0 (0)	0 (0)	>0.999	0 (0)	1 (20)	>0.999
Obesity	1 (25)	3 (75)	0.4857	2 (40)	2 (40)	>0.999	2 (40)	1 (20)	>0.999
CAD	2 (50)	0 (0)	0.4286	3 (60)	3 (60)	>0.999	0 (0)	0 (0)	>0.999
PAD	2 (50)	0 (0)	0.4286	0 (0)	2 (40)	0.4444	0 (0)	0 (0)	>0.999
OSA	1 (25)	3 (75)	0.4857	2 (40)	0 (0)*	0.4444	1 (20)	3 (60)	0.5238
Stroke	0 (0)	1 (25)	>0.999	0 (0)	0 (0)	>0.999	1 (20)	0 (0)	>0.999
AMI	0 (0)	1 (25)	>0.999	1 (20)	2 (40)	>0.999	1 (20)	0 (0)	>0.999
COPD	1(25)	0 (0)	>0.999	0 (0)	0 (0)	>0.999	1 (20)	0 (0)	>0.999
Chronic kidney disease	0 (0)	1 (25)	>0.999	1 (20)	0 (0)	>0.999	2 (40)	3 (60)	>0.999
Lifestyle habits, no (%)									
Smoking status									
Never smoked	0 (0)	3 (75)	0.1429	0 (0)	2 (40)	0.4444	3 (60)	5 (100)	0.4444
Ex-smoker	1 (25)	1 (25)	>0.999	5 (100)$^{\#}$	1 (20)	0.0476	2 (40)	0 (0)	0.4444
Current smoker	3 (75)	0 (0)	0.1429	0 (0)$^{\#}$	2 (40)	0.4444	0 (0)$^{\#}$	0 (0)	>0.999

Table 2. Continued.

Characteristic	Control			HFrEF			HFpEF		
	Men (n=4)	Women (n=4)	p-value[a]	Men (n=5)	Women (n=5)	p-value[b]	Men (n=5)	Women (n=5)	p-value[c]
Baseline treatments and prior interventions, no (%)									
Aspirin	1 (25)	1 (25)	>0.999	4 (80)	3 (60)	>0.999	3 (60)	0 (0)	0.1667
Clopidogrel	0 (0)	0 (0)	>0.999	0 (0)	2 (40)	0.4444	0 (0)	0 (0)	>0.999
Ticagrelor	0 (0)	0 (0)	>0.999	0 (0)	0 (0)	>0.999	0 (0)	0 (0)	>0.999
Statins	2 (50)	2 (50)	>0.999	4 (80)	3 (60)	>0.999	5 (100)	2 (40)	0.1667
Ezetimibe	1 (25)	0 (0)	>0.999	0 (0)	0 (0)	>0.999	0 (0)	1 (20)	>0.999
Beta blockers	0 (0)	2 (50)	0.4286	5 (100)[##]	5 (100)	>0.999	4 (80)[#]	2 (40)	0.5238
ACEI/ARB	3 (60)	1 (20)	0.4857	3 (60)	3 (60)	>0.999	4 (80)	2 (40)	0.5238
Sacubitril/valsartan	0 (0)	0 (0)	>0.999	1 (20)	2 (40)	>0.999	0 (0)	0 (0)	>0.999
Dapaglifozine	0 (0)	0 (0)	>0.999	1 (20)	3 (60)	0.5238	0 (0)	0 (0)	>0.999
Spironolactone/Eplerenone	0 (0)	0 (0)	>0.999	4 (80)[#]	5 (100)[**]	>0.999	0 (0)[§]	0 (0)[&&]	>0.999
Furosemide	1 (25)	1 (25)	>0.999	3 (60)	3 (60)	>0.999	3 (60)	4 (80)	>0.999
Warfarin/acenocoumarol	0 (0)	0 (0)	>0.999	0 (0)	0 (0)	>0.999	1 (20)	0 (0)	>0.999
NOAC	0 (0)	0 (0)	>0.999	0 (0)	0 (0)	>0.999	0 (0)	1 (0)	>0.999
Amiodarone	0 (0)	0 (0)	>0.999	1 (20)	0 (0)	>0.999	0 (0)	0 (0)	>0.999
Digoxin	0 (0)	0 (0)	>0.999	0 (0)	0 (0)	>0.999	0 (0)	0 (0)	>0.999
Alopurinol	1 (25)	0 (0)	>0.999	1 (20)	0 (0)	>0.999	1 (20)	0 (0)	>0.999
OAD	0 (0)	1 (25)	>0.999	2 (40)	5 (100)[*]	0.1667	1 (20)	1 (20)[&]	>0.999
Insulin	0 (0)	0 (0)	>0.999	0 (0)	1 (20)	>0.999	0 (0)	0 (0)	>0.999
Anxiolytics	2 (50)	0 (0)	0.4286	1 (20)	1 (20)	>0.999	0 (0)	2 (40)	0.4444
Antidepressants	0 (0)	2 (50)	0.4286	0 (0)	1 (20)	>0.999	0 (0)	2 (40)	0.4444
ICD	0 (0)	0 (0)	>0.999	1 (20)	1 (20)	>0.999	0 (0)	0 (0)	>0.999
CRT	0 (0)	0 (0)	>0.999	2 (40)	2 (40)	>0.999	0 (0)	0 (0)	>0.999

Table 2. Continued.

Characteristic	Control			HFrEF			HFpEF		
	Men (n=4)	Women (n=4)	p-value[a]	Men (n=5)	Women (n=5)	p-value[b]	Men (n=5)	Women (n=5)	p-value[c]
Laboratory results									
Creatinine (mg/dL)	0.79±0.18	0.70±0.10	0.9990	1.08±0.14	0.72±0.14	0.6237	1.26±0.70	0.91±0.37	0.6399
NT-proBNP (pg/mL)	39.40±8.71	86.65±18.87	>0.999	866.40±846.7	246.40±108.8	0.2460	486.4±340.7	648.5±318.9	0.9924
Hemoglobin (g/dL)	12.98±1.54	13.08±1.79	>0.999	13.88±1.30	12.98±2.25	0.9401	12.52±1.39	11.28±0.21	0.8391
HbA1c (DCCT) (%)	5.55±0.31	6.08±0.97	0.8565	5.90±0.72	7.08±0.70	0.0809	5.90±0.32	5.40±0.64[&&]	0.8222
Total cholesterol (mg/dL)	173.00±27.47	147.30±40.37	0.9289	172.20±47.03	163.20±41.29	0.9989	129.60±41.58	184.80±25.71	0.2457
Triglycerides (mg/dL)	154.00±78.97	152.30±6.81	>0.999	147.60±57.20	143.00±57.65	>0.999	73.80±26.55	87.75±9.81	0.9980
HDL (mg/dL)	61.00±19.90	46.00±0.00	0.4525	42.20±5.17	39.25±3.10	0.9981	38.75±2.36	58.60±13.89	0.0995
LDL (mg/dL)	81.25±38.17	90.00±22.61	0.9993	100.40±42.12	88.20±37.57	0.9919	63.00±5.29	105.20±29.89	0.5436
Iron (µg/dL)	77.50±34.51	62.50±13.03	0.9899	133.60±51.75	55.40±39.62	0.0216	90.00±24.32	57.40±31.48	0.6914
Transferrin saturation (%)	22.25±7.68	16.25±6.13	0.9480	37.00±9.98	13.80±10.80	0.0112	27.00±10.68	18.60±10.57	0.7418
Ferritin (ng/mL)	134.60±75.80	109.80±94.15	0.9941	200.8±63.94	29.25±30.32	0.0100	59.08±25.02[§]	99.12±55.77	0.9199
Sodium (mmol/L)	140.50±3.11	141.50±1.29	0.9862	139.20±3.11	140.00±0.00	0.9936	141.00±1.87	142.80±1.92	0.7850
Potassium (mmol/L)	4.20±0.12	4.38±0.13	0.9978	4.94±0.28	4.38±0.59	0.6349	4.36±0.68	4.60±0.94	0.9839
Albumin (g/dL)	4.25± 0.32	4.26±0.15	>0.999	4.60±0.19	4.40±0.45	0.9085	3.96±0.28[§]	3.97±0.35	>0.999
CRP (ultra) (mg/L)	2.88±1.53	4.43±2.24	0.9531	2.98±1.57	3.88±3.31	0.9929	3.44±2.93	4.73±3.06	0.9734
Leukocytes $x10^3/\mu L$	7.53±0.52	6.27±1.00	0.9024	7.68±1.35	9.68±2.31	0.4721	7.32±2.59	5.75±1.07[&]	0.7495
Neutrophils $x10^3/\mu L$	4.75±0.58	3.67±0.76	0.8407	4.87±1.14	5.85±1.83	0.8332	4.94±1.71	3.52±0.93	0.5913
Lymphocytes $x10^3/\mu L$	1.79±0.20	1.89±0.44	>0.999	2.01±0.69	3.02±0.52	0.2108	1.71±1.21	1.57±0.19[&]	0.9995

Values are mean ± standard deviation or number (%). p-value[a]: Control men *vs.* Control women; p-value[b]: HFrEF men *vs.* HFrEF women; p-value[c]: HFpEF men *vs.* HFpEF women. To obtain the NT-proBNP values of the HFpEF and the Control group, a formula for converting B-type natriuretic peptide into NT-proBNP values was used [201]. [#]p<0.05 *vs.* Control men, [##]p<0.01 *vs.* Control men, [###]p<0.001 *vs.* Control men; [§]p<0.05 *vs.* HFrEF men, [§§]p<0.01 *vs.* HFrEF men, [§§§]p<0.001 *vs.* HFrEF men; [*]p<0.05 *vs.* Control women, [**]p<0.01 *vs.* Control women; [&]p<0.05 *vs.* HFrEF women, [&&]p<0.01 *vs.* HFrEF women, [&&&]p<0.001 *vs.* HFrEF women, [&&&&]p<0.0001 *vs.* HFrEF women. Abbreviations: ACEI/ARB, angiotensin-converting enzyme inhibitor/angiotensin receptor blocker; AF, atrial fibrillation; AMI, acute myocardial infarction; BMI, body mass index; CAD, coronary artery disease; COPD, chronic obstructive pulmonary disease; CRP, C-reactive protein; CRT, cardiac resynchronization therapy; DBP, diastolic blood pressure; DM, diabetes mellitus; HbA1c (DCCT), glycated hemoglobin (Diabetes Control and Complications Trial); HDL, high density lipoprotein; HTA, arterial hypertension; ICD, implantable cardioverter defibrillator; LDL, low density lipoprotein; LVEF, left ventricular ejection fraction; NOAC, novel oral anticoagulant; NYHA, New York Heart Association; NT-proBNP, N-terminal proB-type natriuretic peptide; OAD, oral anti-diabetic; OSA, obstructive sleep apnea; PAD, peripheral artery disease; SBP, systolic blood pressure; TSH, thyroid-stimulating hormone.

Regarding the comorbidities presented by the participants, most of them had hypertension (60% of HFrEF men, 100% of HFpEF men, 100% of HFpEF women, 50% of control men and 100% of control women). A significant higher percentage of HFrEF women presented DM (80% *vs*. 0% HFrEF men, $p<0.05$), and though not reaching significance, peripheral artery disease (40% *vs.* 0% HFrEF men) and acute myocardial infarction (AMI) (40% *vs.* 20% HFrEF men) were higher in HFrEF women than men. When comparing with women, a higher percentage of HFrEF men had obstructive sleep apnea (40% *vs.* 0% HFrEF women) and chronic kidney disease (CKD) (20% *vs.* 0% HFrEF women). Also, a significant percentage of HFrEF men (100% *vs.* 20% HFrEF woman, $p<0.05$) were ex-smokers. Regarding HFpEF, although without statistical significance, a higher percentage of women presented obstructive sleep apnea (60% *vs.* 20% HFpEF men), CKD (60% *vs.* 40% HFpEF men) and atrial fibrillation (20% *vs.* 0% HFpEF men) and a higher percentage of HFpEF men had dyslipidemia (60% *vs.* 40% HFpEF women) and obesity (40% *vs.* 20% HFpEF women).

In general, the participants were treated with β-blockers (100% HFrEF men and women; 80% HFpEF men and 40% HFpEF women), ACEI/ARB (60% HFrEF men and women; 80% HFpEF men and 40% HFpEF women), aspirin (80% HFrEF men and 60% HFrEF women; 60% HFpEF men), statins (80% HFrEF men and 60% HFrEF women; 100% HFpEF men and 40% HFpEF women) and furosemide (60% HFrEF men and women; 60% HFpEF men and 80% HFpEF women). Moreover, a significantly higher proportion of HFrEF patients were treated with spironolactone/eplerenone in comparison with HFpEF patients ($p<0.05$ in men; $p<0.01$ in women). Also, oral anti-diabetics were more often used by HFrEF women in comparison with HFpEF women ($p<0.05$). No significant differences were observed in the use of device therapies (implantable cardioverter defibrillator and cardiac resynchronization therapy).

Regarding the laboratory results, HFrEF men presented significantly higher levels of iron and ferritin in comparison with HFrEF women ($p<0.05$), as well as higher percentage of transferrin saturation in comparison with HFrEF women ($p<0.05$). Also, higher levels of ferritin and albumin were verified in HFrEF men in comparison with HFpEF men ($p<0.05$). In the case of HFrEF women, they showed higher levels of glycated hemoglobin (HbA1c (DCCT)) in comparison with HFpEF women ($p<0.01$), and the levels of leukocytes and lymphocytes were also higher than those in HFpEF women ($p<0.05$). A possible explanation

for the higher levels of leukocytes and lymphocytes in HFrEF women compared to HFpEF women may be related to the number of women with HFrEF having DM. In fact, a higher recruit of leukocytes and their subsets (neutrophils, monocytes, and lymphocytes) has been linked with inflammation associated with DM that leads to the progression of cardiovascular diseases [202]. During inflammation, leukocytes function to resolve injury and defend the host through several mechanisms, and evidence clearly shows that DM induces oxidative stress, intracellular Ca^{2+} abnormalities, metabolic alterations, mitochondrial dysfunction, and inflammation that directly contribute to the development of structural abnormalities and increased incidence of HF [203,204]. Thus, higher levels of leukocytes and lymphocytes in women with HFrEF, when compared to women with HFpEF, may be associated with the fact that most women with EF <40% (80%) have DM and, added to this, higher levels of glycated hemoglobin.

6.2 Characterization of urine proteome profile in heart failure patients

LC-MS/MS analysis of urine samples from HFrEF patients and control individuals allowed the identification of 833 distinct proteins. Of these, a total of 81 proteins were exclusively identified in the HFrEF women group, 55 proteins in the HFrEF men group, 20 proteins in the control women group and 79 proteins were exclusive in the control men group (Figure 3). Manual inspection of the proteins exclusively identified in each group showed that none was identified in 100% of the group's samples and most of them were only present in 20% of the group's individuals and so, its discriminatory power is questionable. According to FunRich software [196,197] (Release data: July 30, 2020), most of the quantified proteins belong to "metabolism", "energy pathways" and "cell growth and/or maintenance" biological processes. Regarding the molecular function, "extracellular matrix structural constituent", "receptor activity", "transporter activity" and "structural molecule activity" were highlighted. Moreover, "exosomes", "cytoplasm", and "extracellular origin" are the cellular components more relevant.

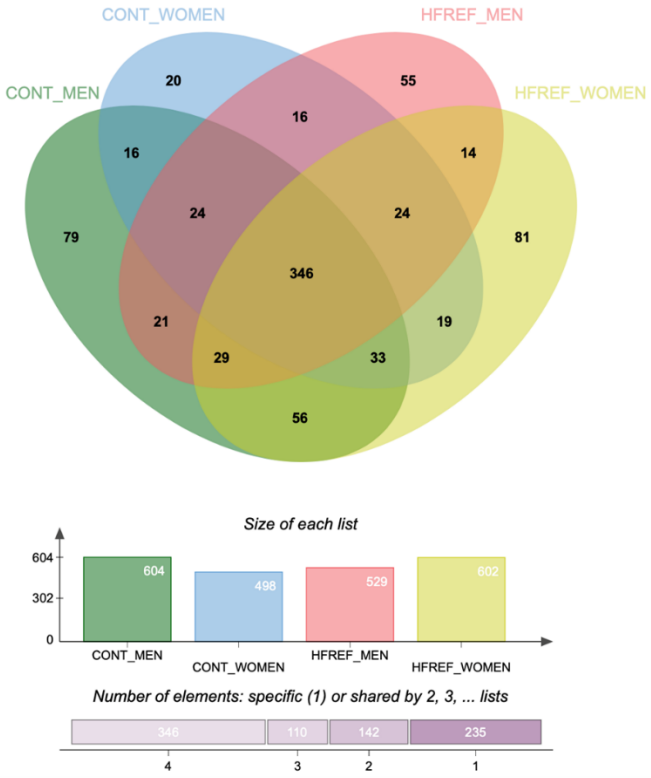


Figure 3. Comparative analysis of the urine proteome between the experimental groups performed with Jvenn [195]. Abbreviations: CONT, control; HFREF, heart failure with reduced ejection fraction.

Proteomic data visualization (clustering and heat map) performed with MetaboAnalyst software (https://www.metaboanalyst.ca) allowed the comparative analysis between groups. Therefore, to reduce the dimensionality of the data and visualize sample grouping, we performed PCA, heat map and volcano plot analysis on the proteomic data set. PCA revealed a slight proteome dispersion between the control groups analyzed by sex (Fig. 4A), along the second dimension, which is related to the variability of the distributions; whereas the first dimension is influenced by the values of the mean within cohorts; although one man clearly stood out from the group. Regarding HFrEF patients, there was no clear proteome separation into two clusters, though one woman and one man with HFrEF appear to behave differently and distance themselves from the intersection (Fig. 4B). Comparing control men group with HFrEF men (Fig. 4C), we verify that there was some proteome split, and clearly two individuals, one from each condition, distanced themselves. On the PCA of the control women group and women with HFrEF, there was a close relationship among these two groups (Fig. 4D), although two women with HFrEF were outside the main cluster, suggesting a different proteome profile.

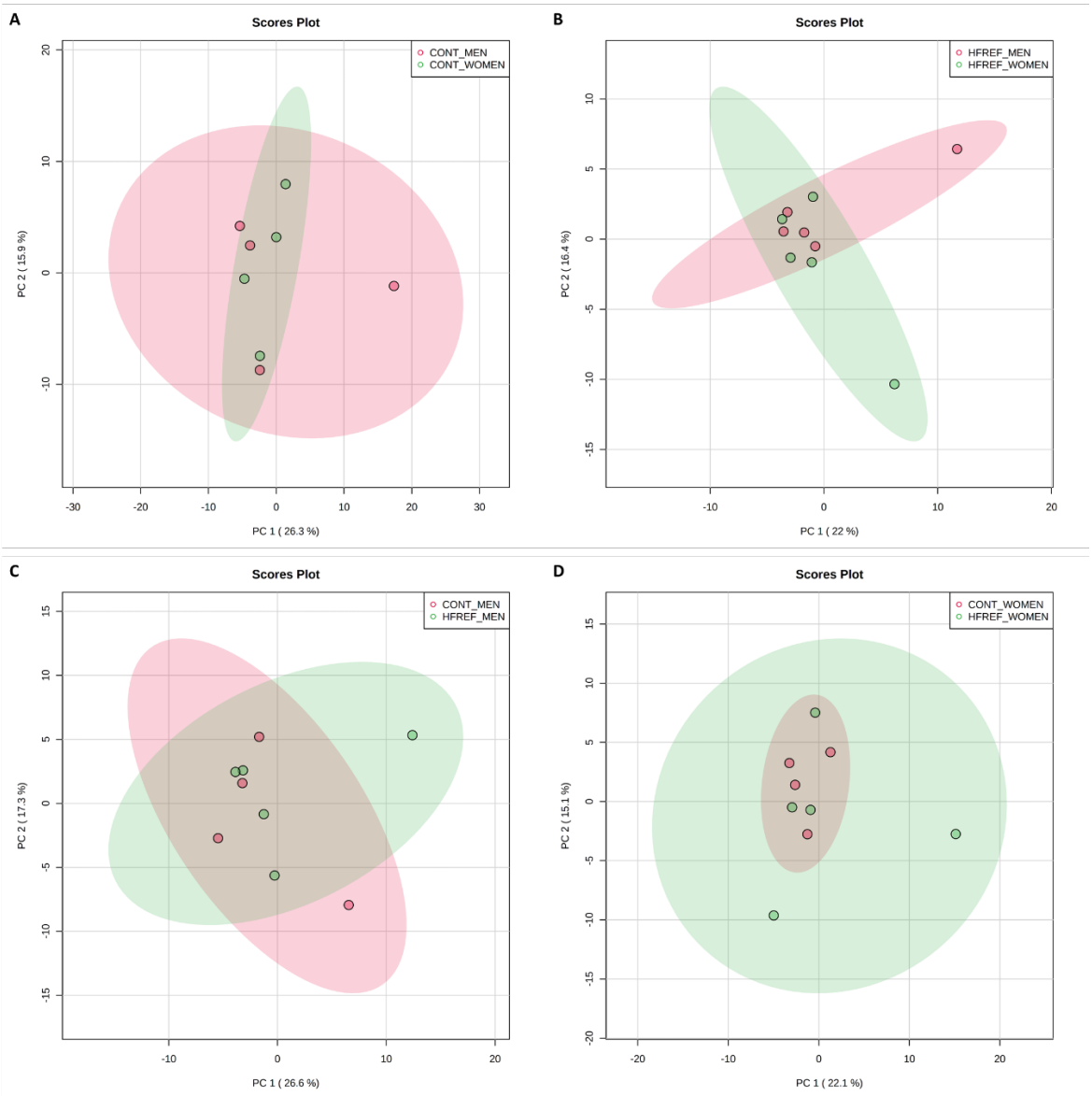
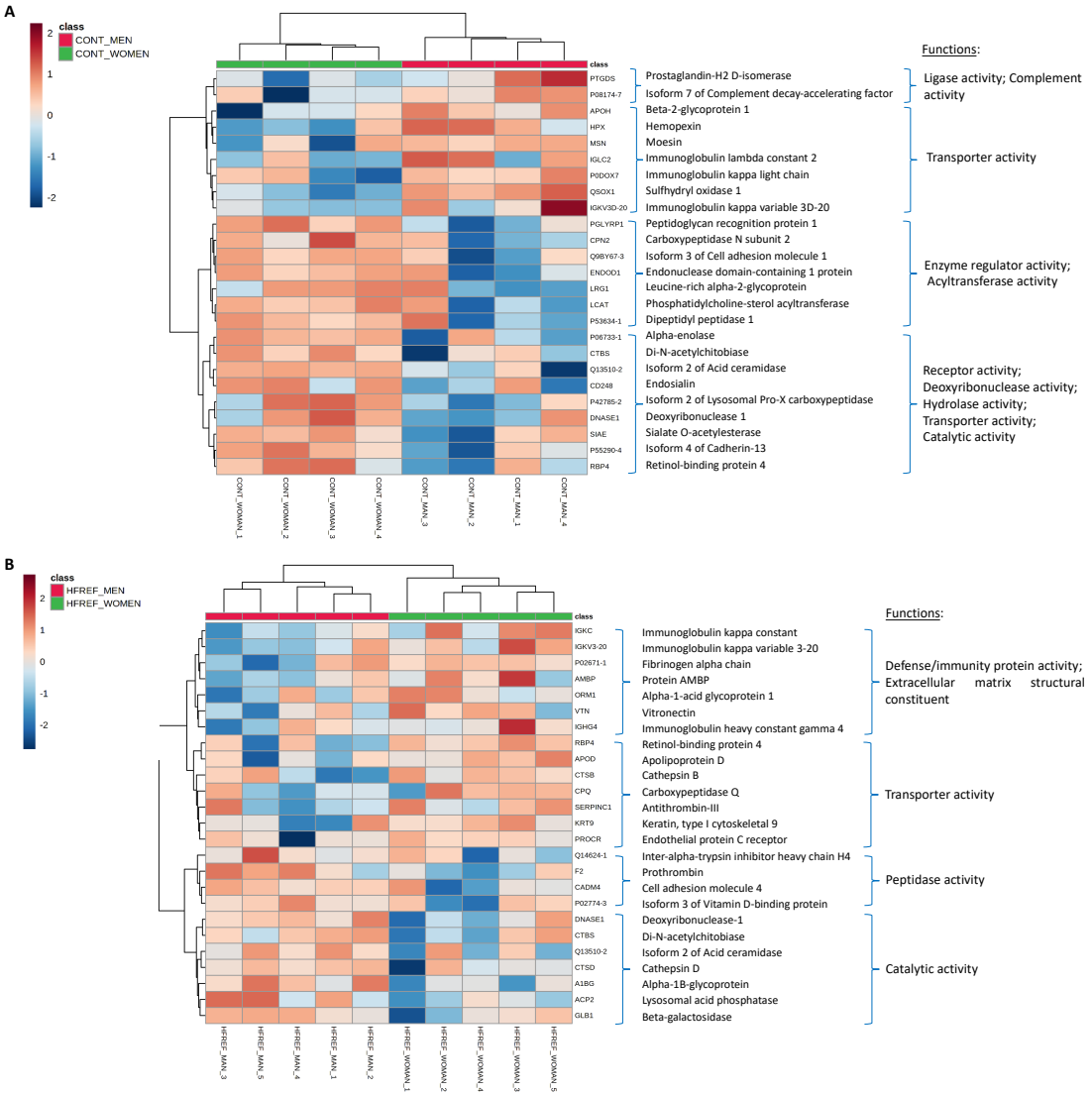


Figure 4. Principal component analysis of urine proteins of A) Control men and women, B) HFrEF men and women, C) Control and HFrEF men and D) Control and HFrEF women. Abbreviations: CONT, control; HFREF, heart failure with reduced ejection fraction.

Regarding the heat maps results (Fig. 5), we verified for the top 25 proteins (top 25 of features ranked by t-tests to retain the most contrasting patterns) from the control group, as well as HFrEF patients, clearly clustered men and women individuals (Fig. 5A and Fig. 5B, respectively). When HFrEF men were compared with HFrEF women, the top 25 proteins profile showed four main groups of functions, which are “Defense/immunity protein activity and extracellular matrix structural constituent”, “Transporter activity”, “Peptidase activity” and “Catalytic activity”.


A
class
CONT_MEN
CONT_WOMEN
2
1
0
-1
-2
class
PTGDS
P08174-7
APOH
HPX
MSN
IGLC2
P0DOX7
QSOX1
IGKV3D-20
PGLYRP1
CPN2
Q9BY67-3
ENDOD1
LRG1
LCAT
P53634-1
P06733-1
CTBS
Q13510-2
CD248
P42785-2
DNASE1
SIAE
P55290-4
RBP4
Prostaglandin-H2 D-isomerase
Isoform 7 of Complement decay-accelerating factor
Beta-2-glycoprotein 1
Hemopexin
Moesin
Immunoglobulin lambda constant 2
Immunoglobulin kappa light chain
Sulfhydryl oxidase 1
Immunoglobulin kappa variable 3D-20
Peptidoglycan recognition protein 1
Carboxypeptidase N subunit 2
Isoform 3 of Cell adhesion molecule 1
Endonuclease domain-containing 1 protein
Leucine-rich alpha-2-glycoprotein
Phosphatidylcholine-sterol acyltransferase
Dipeptidyl peptidase 1
Alpha-enolase
Di-N-acetylchitobiase
Isoform 2 of Acid ceramidase
Endosialin
Isoform 2 of Lysosomal Pro-X carboxypeptidase
Deoxyribonuclease 1
Sialate O-acetylesterase
Isoform 4 of Cadherin-13
Retinol-binding protein 4
Functions:
Ligase activity; Complement activity
Transporter activity
Enzyme regulator activity; Acyltransferase activity
Receptor activity; Deoxyribonuclease activity; Hydrolase activity; Transporter activity; Catalytic activity
CONT_WOMAN_1
CONT_WOMAN_2
CONT_WOMAN_3
CONT_WOMAN_4
CONT_MAN_3
CONT_MAN_2
CONT_MAN_1
CONT_MAN_4
B
class
HFREF_MEN
HFREF_WOMEN
2
1
0
-1
-2
class
IGKC
IGKV3-20
P02671-1
AMBP
ORM1
VTN
IGHG4
RBP4
APOD
CTSB
CPQ
SERPINC1
KRT9
PROCR
Q14624-1
F2
CADM4
P02774-3
DNASE1
CTBS
Q13510-2
CTSD
A1BG
ACP2
GLB1
Immunoglobulin kappa constant
Immunoglobulin kappa variable 3-20
Fibrinogen alpha chain
Protein AMBP
Alpha-1-acid glycoprotein 1
Vitronectin
Immunoglobulin heavy constant gamma 4
Retinol-binding protein 4
Apolipoprotein D
Cathepsin B
Carboxypeptidase Q
Antithrombin-III
Keratin, type I cytoskeletal 9
Endothelial protein C receptor
Inter-alpha-trypsin inhibitor heavy chain H4
Prothrombin
Cell adhesion molecule 4
Isoform 3 of Vitamin D-binding protein
Deoxyribonuclease-1
Di-N-acetylchitobiase
Isoform 2 of Acid ceramidase
Cathepsin D
Alpha-1B-glycoprotein
Lysosomal acid phosphatase
Beta-galactosidase
Functions:
Defense/immunity protein activity; Extracellular matrix structural constituent
Transporter activity
Peptidase activity
Catalytic activity
HFREF_MAN_3
HFREF_MAN_5
HFREF_MAN_4
HFREF_MAN_1
HFREF_MAN_2
HFREF_WOMAN_1
HFREF_WOMAN_2
HFREF_WOMAN_4
HFREF_WOMAN_3
HFREF_WOMAN_5

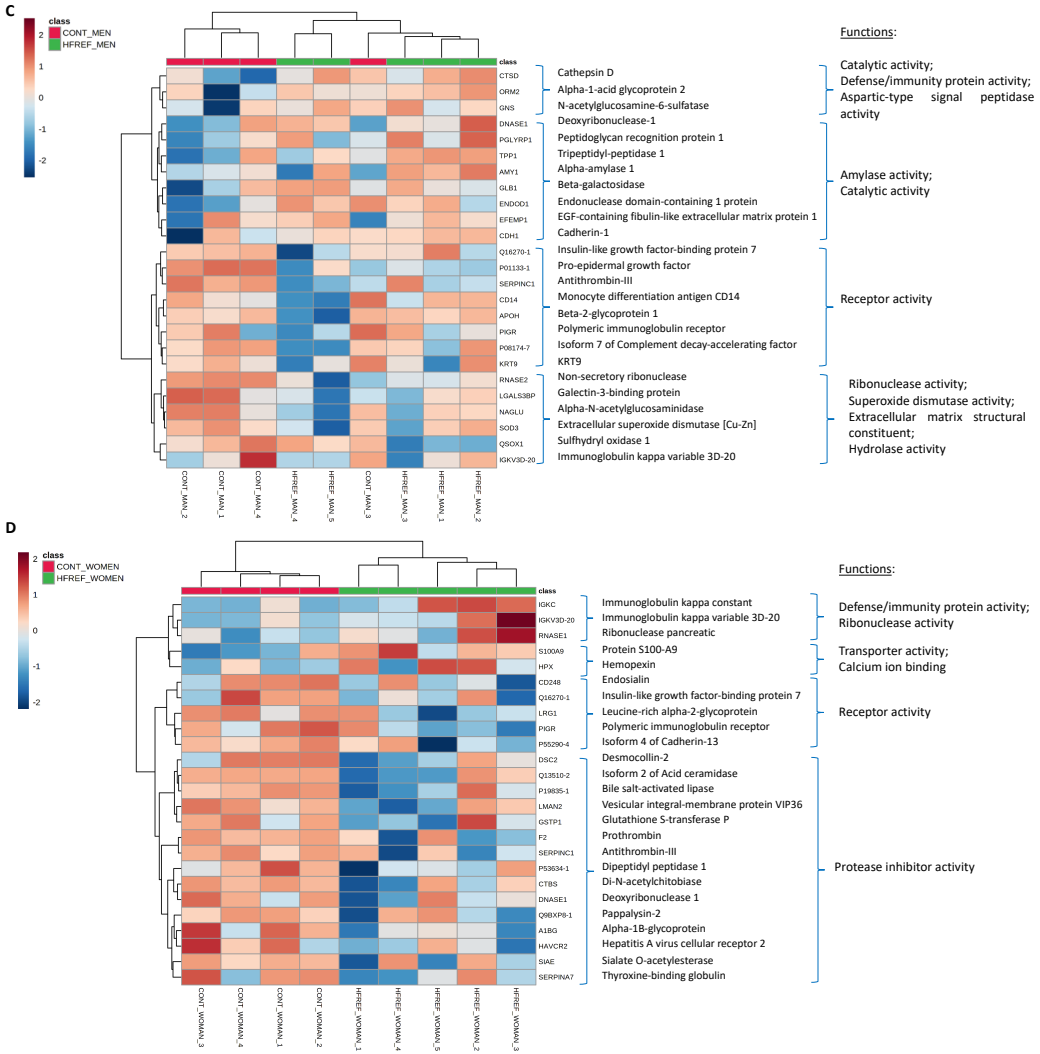


Figure 5. Heat map and clustering for the top 25 proteins from A) Control men and women, B) HFrEF men and women, C) Control and HFrEF men and D) Control and HFrEF women. The molecular function was obtained using the FunRich software [196,197] (Release data: July 30, 2020). Abbreviations: CONT, control; HFREF, heart failure with reduced ejection fraction.

Volcano plots and their respective boxplots were used to summarize the number of proteins whose levels was significantly different in the groups, once compared by sex (Fig. S1 and Fig. S2, respectively). Significant proteins were selected by fold change (>2 or <-2-fold) and adjusted Mann-Whitney $p<0.05$. Table 3 summarizes the list of proteins with significant differences between the various comparisons.

Table 3. List of proteins with statistically significant changes in levels in the different experimental groups.

	Control men *versus* Control women					**HFrEF men *versus* HFrEF women**			
	Gene Name	**Protein Name**	**Fold change**	**p-value**		**Gene Name**	**Protein Name**	**Fold change**	**p-value**
Decreased levels	PGLYRP1	Peptidoglycan recognition protein 1	0.11	0.014	**Decreased levels**	RBP4	Retinol-binding protein 4	0.28	0.043
	Q13510-2	Isoform 2 of Acid ceramidase	0.24	0.044					
	P55290-4	Isoform 4 of Cadherin-13	0.17	0.048	**Increased levels**	A1BG	Alpha-1B-glycoprotein	3.69	0.022
	CPN2	Carboxypeptidase N subunit 2	0.14	0.049		ACP2	Lysosomal acid phosphatase	22.96	0.038
Increased levels	QSOX1	Sulfhydryl oxidase 1	96.35	0.001					
	HPX	Hemopexin	3.52	0.036					

	Control men *versus* HFrEF men					**Control women *versus* HFrEF women**			
	Gene Name	**Protein Name**	**Fold change**	**p-value**		**Gene Name**	**Protein Name**	**Fold change**	**p-value**
Increased levels	NAGLU	Alpha-N-acetylglucosaminidase	3.33	0.043	**Decreased levels**	S100A9	Protein S100A9	0.20	0.046
					Increased levels	A1BG	Alpha-1B-glycoprotein	4.60	0.038

When control men group was compared to the control women group, a total of four proteins (peptidoglycan recognition protein 1, isoform 2 of acid ceramidase, isoform 4 of cadherin-13 and carboxypeptidase N subunit 2) were downregulated and two (sulfhydryl oxidase 1 and hemopexin) were upregulated. Comparing sex in the case of individuals with HFrEF, three proteins stood out, which were retinol-binding protein 4 (RBP4), a protein that mediates retinol transport in blood [205], alpha-1B-glycoprotein (A1BG), a member of the immunoglobulin superfamily [206], which is related with diverse functions based on molecular recognition especially in the immune system and in cell adhesion [207], and lysosomal acid phosphatase (ACP2), a lysosomal enzyme with catalytic activity [208]. Of these proteins, A1BG and ACP2 appeared upregulated in men and downregulated in women, and RBP4 was found to be upregulated in women when compared with men. Interestingly, A1BG also appeared downregulated in HFrEF women when compared to control women group. Furthermore, the levels of protein S100-A9 (S100A9), a Ca^{2+}-binding protein [209], is higher in HFrEF women than in control women group, and in the case of comparing the control men group with HFrEF men, only one protein appeared differently expressed, which was alpha-N-acetylglucosaminidase protein (NAGLU). The main function of NAGLU is to facilitate the breakdown of heparan sulfate (HS) glycosaminoglycan in lysosomes [210].

7 Discussion

HF is a global epidemic, with increasing prevalence due to the world's aging population [12]. HFrEF and HFpEF are two distinct conditions with respect to epidemiology and clinical manifestations [4]. Although previous data indicate that the prevalence of HFrEF and HFpEF is distinct in men and women [4], it is still necessary to understand the role that sex plays in the management of HF. To overcome this gap, and in order to better understand the pathophysiology of HF, a high throughput MS-based approach was used to characterize the urine proteome from men and women. Indeed, proteomic technologies have been used in HF study [211]. For instance, a tandem MS approach firstly reported an association of S100A8 with HFpEF, by showing that S100A8 was increased in the plasma of the HFpEF patients when compared with controls [212]. Moreover, in HFpEF women patients, 17 serum proteins were found to be significantly different from non-HFpEF patients, using antibody microarrays. Of these proteins, angiogenin was considered as a potential prognostic and diagnostic biomarker in HFpEF [213]. Regarding HFrEF, oxidative stress-induced modification of proteins was observed to be increased in the plasma of HFrEF patients [214]. A mass spectrometry analysis performed by Voyager-DE STR MALDI-TOF spectrometer allowed the identification of two proteins that undergo oxidation in the plasma, α-1-antitrypsin and fibrinogen [214]. Also, LC-MS/MS analysis of myocardial tissue of HFrEF patients showed an increase in oxidative stress-induced proteins, mainly α-cardiac actin and creatine kinase M-type [215]; suggesting that increased oxidative stress in a failing heart may contribute to the pathogenesis of HF [215]. The proteome profile of peripheral blood mononuclear cells obtained by MALDI-TOF MS, showed 18 proteins differentially expressed in patients with chronic HF when compared to the control group, including cytoskeleton, cell cycle progression, stress response and repair of DNA, and energy metabolism proteins [216].

Urine was the chosen biological sample for this analysis once it can be collected in a non-invasive way and a large amount of sample can be easily obtained. Moreover, urine proteome is not dominated by highly abundant proteins, like albumin in the case of serum and plasma [217]. Also, the protein composition and fragmentation of urine are relatively stable in comparison with other biofluids such as plasma or serum, which are more predisposed to proteolytic degradation during and after sampling [218]. Urine has been used to explore biomarkers in HFrEF. Recently, peptides from constituents of the ECM, like fragments of

type I and III of fibrillar collagens were identified in HFrEF patients, using CE-MS-based analysis. In fact, collagen deposition contributes to LV structural remodeling [219]. A similar study identified 107 specific peptide biomarkers in HFrEF patients with CKD through analysis of urine proteome by CE-MS [220]. Most of the peptides belongs to types I and III collagen [220]. Specifically, in the heart, collagen type I provides rigidity and determines stiffness [221], and collagen type III contributes to elasticity [222]. Besides, this study also developed a machine-based classifier that was able to classify HFrEF patients with CKD and CKD patients without HFrEF with 84% sensitivity and 91% specificity [220]. However, there are few proteomic studies carried out in patients with HFpEF with LVEF ≥50%, and, to the best of our knowledge, no study has yet evaluated sexual differences at the urine proteomic level in HFrEF and HFpEF.

Due to technical issues, this work did not allow the study of urine proteome of HFpEF patients. However, the clinical and demographic characteristics of patients with HFpEF (Table 2) showed that these patients are the oldest ones, with women being slightly older than men. In fact, patients with incident HFpEF tend to be older than those with incident HFrEF [198–200]. As previously claimed, age is an important factor in development of HF because it is associated with a reduction in cardiomyocyte renewal [62], and an increase in systemic inflammation that impairs NO bioavailability and promotes fibrotic remodeling of the ECM [31]. In addition to age, also comorbidities play a crucial role in HFpEF development [31]. All participants with HFpEF had hypertension and a higher percentage of women presented obstructive sleep apnea, CKD and atrial fibrillation compared to HFpEF men. By contrast, a higher percentage of HFpEF men had dyslipidemia and obesity.

Multivariate PCA together with hierarchical cluster analysis were used for data visualization and analysis of proteomics results. In the PCA (Fig. 4), the proteomic profiles were not clear to show proteome separation between the groups. However, in all sets there were patients spaced from the main cluster. This evidence highlights that the clinical condition of each individual interferes with their proteome profile. Looking to data obtained in the volcano plot analysis (Table 3), we verified three proteins (RBP4, A1BG and ACP2) whose levels was significantly different comparing men and women with HFrEF. RBP4 presented a higher levels in women when compared to men. This protein belongs to the lipocalin superfamily, being mainly secreted by the liver and adipose tissue. Its main function is to mediate the transport of retinol (vitamin A) in the circulation [205]. However, it has been

suggested that RBP4, when secreted by adipose tissue, is an important adipokine that contributes to insulin resistance, related with DM [223]. Indeed, several studies in human subjects support a positive association between RBP4, insulin resistance, and type 2 diabetes mellitus (T2DM) [224–227]. High circulatory levels of RBP4 has been associated to a decreased expression of the insulin-response glucose transporter, GLUT-4, in adipose tissue and skeletal muscle, leading to insulin resistance and impaired glucose tolerance [228]. Mechanistically, RBP4 can act independently of retinol to impair insulin signaling in adipocytes indirectly, by inducing pro-inflammatory cytokine production from macrophages. This process is mediated in part, by toll-like receptor 4 (TLR4) cell surface receptor and not by the specific RBP4 receptor, STRA6 (stimulated by retinoic acid 6), and involves the c-Jun N-terminal protein kinase (JNK) signaling pathway [229]. The association between RBP4 and markers of systemic inflammation have also been described in obese individuals [230], and inflammation represents a potential mediator of RBP4-associated metabolic alterations [231]. Obesity is related with insulin resistance with increased secretion of cytokines and other bioactive substances from adipose tissue, as well as the number of adipose macrophages [232]. Adipose production of insulin-sensitizing adipokines with pro-inflammatory properties, such as RBP4, is increased in the obese state, going in line with the fact that RBP4 has been linked to insulin resistance [233].

In this study, the significantly higher urinary levels of RBP4 observed in women with HFrEF when compared with men can be related with the fact that 80% of HFrEF women had DM in contrast with none HFrEF male patient (Table 2). Accordingly, recently it was found that urinary RBP4 concentrations were higher in patients with pre-diabetes or T2DM than in subjects with normal glucose tolerance [234]. Moreover, urinary RBP4 concentrations were positively associated with several cardiometabolic parameters including insulin resistance, inflammation, and arterial stiffness, as well as with serum levels of high-sensitivity C-reactive protein (hs-CRP) [234]. hs-CRP is recognized as a marker of inflammation and in the data, HFrEF women showed higher circulation levels of CRP when compared with HFrEF men (Table 2). Importantly, RBP4 has been shown to increase expression of TLR4 and myeloid-dependent primary response gene 88 (TLR4/MyD88) which are associated with inflammatory response and cardiomyocyte hypertrophy contributing to cardiac hypertrophy and ischemic heart disease; in addition, RBP4 levels are positively regulated by angiotensin II levels [235]. Thus, in HFrEF, the significantly higher urinary levels of RBP4 among

women compared with men can be associated with the higher percentage of women in this study population of HFrEF patients presenting DM. Further studies, considering the comparison between women and men with HFrEF with the same percentage of comorbidities as DM, can possibly help to elucidate this issue.

The studies that reported sexual differences in plasma RBP4 protein concentration are controversial. A recent study showed that male compared with female C57BL/6J mice display significant gender differences in circulating RBP4 levels from 6 weeks of age, extending more than 1 year, with male mice displaying higher circulating RBP4 levels [236]. By contrast, other study showed that serum RBP4 levels were decreased in male patients with CAD, especially those with AMI, than in controls and no significant change occurred in the levels of RBP4 for women between both groups. Lower RBP4 levels were positively correlated with decreased testosterone levels in male patients with CAD [237]. Another study reported that elevated serum RBP4 levels were associated with incident CHD among women. However, this evidence belongs to a study carried out only in women, which showed that RBP4 levels were related with a 3-fold increased risk of incident CHD in women [238]. Importantly, a recent study found that serum RBP4 concentration was positively correlated with several risk factors of CVD, such as higher age, higher systolic blood pressure and triglycerides, lower HDL levels and smoking status [239]. Furthermore, when adjusted for gender, they showed a significant correlation with RBP4 only in women [239]. Then, they proposed that RBP4 can serve as an independent predictor of CVD in women [239]. These CVD subjects had also other conditions including hypertension, dyslipidemia and DM, and the treatment medications included both cardiovascular and anti-diabetic drugs [239]. So, RBP4 protein has been studied in the context of CVDs and its levels seems to be distinct in men and women.

Another protein, whose levels was found to be significantly different amongst men and women with HFrEF, as well as between the control women group and women with HFrEF, was A1BG. This protein is a member of the immunoglobulin superfamily and previous information showed that A1BG forms a complex with cysteine-rich secretory protein 3 (CRISP-3), a protein present in exocrine secretions and in secretory granules of neutrophilic granulocytes that is believed to play a role in innate immunity [207]. Although the molecular function of A1BG is yet poorly understood, it is related with diverse functions based on molecular recognition especially in the immune system and in cell adhesion, and is primarily

expressed in the liver [207]. There association between the A1BG protein and HF condition is rather poor [240]; however, it is ubiquitously expressed in many tissues under normal condition and was found to be overexpressed in several types of cancer [241,242]. In this study, A1BG appeared with significantly lower levels in HFrEF women when compared with men, suggesting that this protein might be involved in immune-mediated inflammatory reaction in a higher level in men than in women. Of the few studies that reported sex differences in A1BG levels, higher serum levels of A1BG were shown in pre-menopausal women, in their forties and fifties, when compared to men of the same age; however, there was no reference to A1BG levels for post-menopausal women [243]. Other evidence showed increased levels of circulating A1BG in female C57BL/6 mice; nonetheless, this study failed to detect any levels of A1BG in male C57BL/6 mice [244]. Interestingly, intending to identify genetic markers that are related with cardiovascular drug response, outcomes and adverse events in patients with hypertension, non-synonymous single-nucleotide polymorphisms (nsSNPs) in A1BG was found to be associated with anti-hypertensive treatment related adverse cardiovascular outcomes [245].

ACP2 is a lysosomal enzyme that is localized on the lysosomal membrane [208]. The *ACP2* gene encodes the major beta subunit of lysosomal acid phosphatase in humans and belongs to the histidine acid phosphatase family, which hydrolyze orthophosphoric monoesters to alcohol and phosphate [208]. Although the exact physiological and biochemical functions of ACP2 remain unclear, a high levels level of ACP2 was found in human malignant cell tumors, more specifically in colorectal cancer [246]. Recently, ACP2 was detected in abundance in the urine of patients with diabetic kidney disease with a good prognosis, and was poorly detected in diabetic kidney patients with a poor prognosis [247]. In this study, ACP2 appeared with significantly higher levels in the urine of men than of women with HFrEF. However, to the best of our knowledge, to date there was no association between ACP2 and DM or HF condition. Moreover, it is the first time that sex differences were associated with ACP2.

Regarding the significant differences in the urine proteome of the control women group *versus* women with HFrEF, S100A9 protein levels was found to be significantly higher in women with HFrEF than in control group. This is a Ca^{2+}-binding protein that commonly appears with its partner, S100A8, forming a heterodimer (S100A8/S100A9) [209]. S100A8/S100A9 are predominantly produced by myeloid cells (monocytes and neutrophils)

and are known to exhibit increased levels in many inflammatory and autoimmune diseases, being associated with the activation of innate immune pathways and stimulation of TLR4 and the receptor for advanced glycation end-products (RAGE)-mediated cascades [248,249]. Binding to TLR4 and RAGE activates the NF-κB (nuclear factor kappa B) *via* the RAGE-MAPK signaling pathway, resulting in the amplification of the pro-inflammatory cascade [250]. The levels of S100A8 and S100A9 could be upregulated by a number of conditions such as oxidative stress, specific cytokines, and growth factors in many types of cells, such as fibroblasts, mature macrophages, vascular endothelial cells, and keratinocytes [251].

Increased levels of S100A8 and S100A9 has been demonstrated both in animal models of myocardial ischemia and in human MI patients [252,253]. During the acute phase of the MI, S100A8/S100A9 is rapidly released from the site of the ischemic injury and increases in the coronary and systemic circulation before the markers of myocardial damage myoglobin and troponin [254]. S100A8/S100A9 is secreted mainly by neutrophils that infiltrate the ischemic myocardium and resolve the thrombus reaching high levels in the post-MI circulation [254]. Increased systemic S100A8/S100A9 levels have been associated with a negative long-term prognosis [255]. Treatment with recombinant S100A8/S100A9 amplified myocardial injury and aggravated HF in a mouse model of ischemia/reperfusion [255]. Common findings showed that in patients with acute coronary syndromes high plasma levels of S100A8/S100A9 during the acute event were associated with late development of systolic dysfunction and with a higher incidence of HF hospitalizations, 1 year after the coronary event [256]. The same study found that in mouse models of reperfusion and non-reperfusion MI, early short-term treatment with a small molecule inhibitor that blocks interactions between S100A9 and its receptors TLR4 and RAGE had lasting protective effects, attenuating systolic dysfunction, and increasing cardiac output for at least three weeks after infarction [256]. The beneficial actions of the S100A9 inhibitor were attributed to an attenuated recruitment of neutrophils and inflammatory monocytes, and to the activation of a reparative phenotype in infiltrating macrophages [256]. These findings provide important information on the pathophysiological role of S100 proteins in mediating inflammatory injury and adverse remodeling following MI [256].

Previous data found that serum and urinary levels of S100A8/S100A9 were associated with chronic low-grade inflammation in patients with T2DM [257]. In fact, urinary concentrations of S100A8/S100A9 have been reported as early markers of disease activity for conditions

associated with inflammation and increased neutrophil activity [258]. Thus, the higher levels of S100A9 in the data in women with HFrEF compared with women of the control group may probably be associated with an inflammatory response characteristic of HFrEF and its associated comorbidities.

Recently, it was found that plasma S100A8/S100A9 concentration, in individuals aged 63 to 68 years, with no previous history of CV disease, is positively influenced by circulating neutrophil numbers, smoking, body mass index, glycosylated hemoglobin, and low-density lipoprotein; whereas, HDL was negatively associated with S100A8/A9 [259]. In this study, women with HFrEF showed higher levels of neutrophils and glycosylated hemoglobin, and 40% of women with HFrEF are current smokers in contrast to no current smoker in the female control group. Therefore, these factors may have influenced the higher levels of S100A9 in urine of HFrEF women.

Proteomic analysis of the urine of control men and men with HFrEF revealed significantly reduced NAGLU levels in men with HFrEF. NAGLU is an acid hydrolase that facilitates the breakdown of HS glycosaminoglycan in lysosomes [210]. A recent research showed that the reduced levels of NAGLU in plasma may be associated with tissue protection/repair mechanisms [260]. A proteomic analysis of plasma samples from patients with stable angina, AMI and healthy control subjects, showed a significantly reduced levels of four proteins, including NAGLU, in AMI compared to patients with stable angina [260]. This reduction in the plasma levels of NAGLU has been speculated to represent a mechanism of atheroprotection, since it was previously reported that the HS content of the blood vessel walls is decreased in atherosclerosis [261]. Furthermore, increasing cholesterol content during the development of atherosclerosis in the human aorta is accompanied by decreasing amounts of HS [262]. HS has been proposed to be anti-atherogenic through inhibition of lipoprotein retention, inflammation, and smooth muscle cell proliferation [263].

A significantly higher level of iron and ferritin, as well as a higher percentage of transferrin saturation in HFrEF men than in women was verified in the data (Table 2). These differences might be particularly important in the case of sex differences in HFrEF. Ferritin is an iron storage protein, which can be found in various organs, such as the liver, heart, and kidney. Serum ferritin concentrations are directly proportional to intracellular ferritin concentrations and thus, it is considered to be the best indicator of body iron stores [264]. Generally, there is an increase in iron stores with age, being more pronounced in females than in males [265].

Ferritin is decreased in patients with iron deficiency anemia, and it is increased in patients with insulin resistance or inflammation [264]. In the data, women with HFrEF showed lower levels of iron and ferritin than the male counterparts, and there are evidences that iron deficiency seems to be much more common than anemia across the overall HF population, with nearly two-fold higher prevalence according to some reports [266,267]. In fact, one of the common causes of anemia is iron deficiency. In the laboratory results of HFrEF patients (Table 2), there is no evidence of anemia in these groups. However, HFrEF women showed iron deficiency, once the serum ferritin levels are lower than 100 μg/L and transferrin saturation is lower than 20% [268].

Serum ferritin levels can be inversely associated with 25 hydroxyvitamin D (25(OH)D) in men, and positively associated with 25(OH)D level in pre-menopausal women [269]. Heat map results showed that the isoform 3 of the vitamin D-binding protein (DBP) is highly expressed in the urine of men than in women with HFrEF. DBP can be lost in the urine along with 25(OH)D and other vitamin D metabolites when megalin and cubilin are absent to complex with DBP or DBP-25(OH)D [270]. This may suggest that the higher levels of DBP in male urine may be related to lower plasma levels of vitamin D and higher levels of ferritin. Serum 25 (OH)D level has been positively associated with increased levels of testosterone and estrogen, in men and women, respectively [271,272]. Estrogen directly reduces hepatic hepcidin expression through a functional estrogen response element in the promoter region of the hepcidin gene [273]. Decreased hepcidin expression leads to body iron overload; in contrast, increased hepcidin expression causes iron deficiency anemia [274]. Evidences showed that serum hepcidin is higher in post-menopausal women than pre-menopausal women [275]. In the case of men, it has been more controversial. A study suggested that testosterone increases ferritin levels by inhibiting hepcidin [276]. Contrast evidences reported that testosterone was inversely associated with ferritin in normal weight subjects. However, it was not associated with ferritin in overweight and obese subjects [277].

This study presents some limitations. The small number of patients may not be representative of the broader HF population. Also, the clinical heterogeneity of the patients may underpower the conclusions, since the results must be intertwined with the view that there are different characteristics among participants. Therefore, in the future, it would be important to have a more homogeneous group of participants, particularly in terms of comorbidities. Finally, the protein concentrations in the urine is influenced by several factors

such as physical exercise, diet and lifestyle [278], which becomes a limitation in terms of comparison among individuals. In spite of these limitations, this study underscores the power of the used proteomics approach.

8 Conclusion and future perspectives

Significant sex-based differences exist among the HF population divided by EF spectrum; however, there is still a gap in relation to the role that sex plays in the management of HF. To add new insights on this issue, urine samples were collected from men and women with HF, and the proteome was characterized by mass spectrometry, in order to identify the biological processes associated with sex in HF.

According to the results obtained it was possible to conclude that:

i) The proteomic profile of control women when compared with HFrEF women, showed one positively regulated protein (S100A9) and another poorly regulated protein (A1BG) in women with HFrEF. The higher levels of S100A9 in urine may emphasize the inflammatory response characteristic of HFrEF;

ii) HFrEF men showed lower levels of NAGLU protein compared to control men;

iii) Three proteins (RBP4, A1BG and ACP2) were expressed differently among men and women with HFrEF. Of these proteins, A1BG and ACP2 appeared upregulated in men, and RBP4 was found to be upregulated in women. The urinary levels of RBP4 may be related with the higher percentage of women with HFrEF presenting DM.

Altogether, these results suggest that an inflammatory condition is present in HFrEF patients, in particular in HFrEF women, given the increased urinary levels of RBP4 and S100A9, probably associated with comorbidities. Thus, in future studies, it will be important to validate the obtained results, as well as to increase sample size, taking into consideration the comorbidities presented by the participants.

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Supplementary data

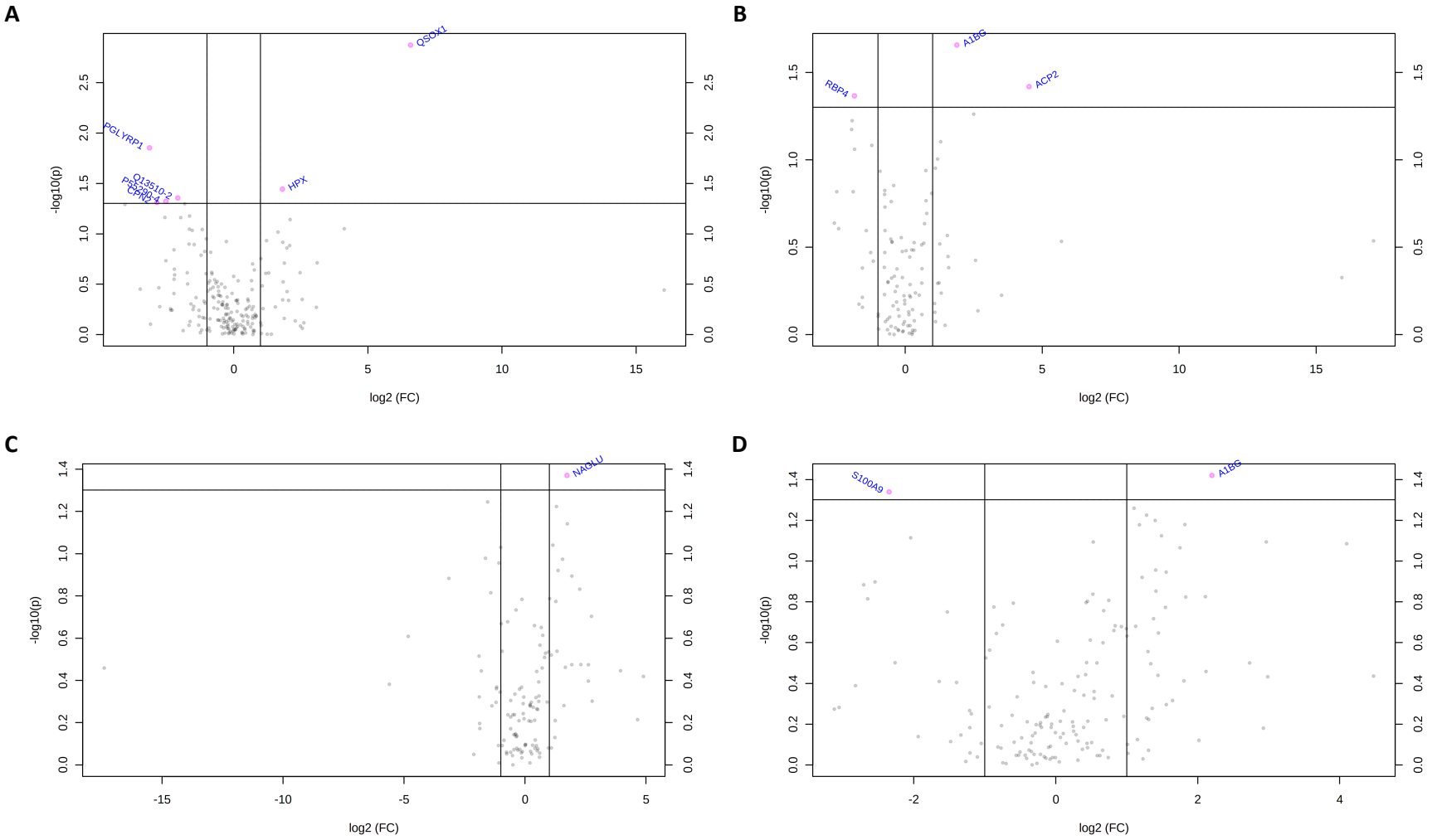

Figure S 1. Volcano plots of A) Control men *versus* Control women, B) HFrEF men *versus* HFrEF women, C) Control men *versus* HFrEF men and D) Control women *versus* HFrEF women. Significant features (in pink) had p<0.05.

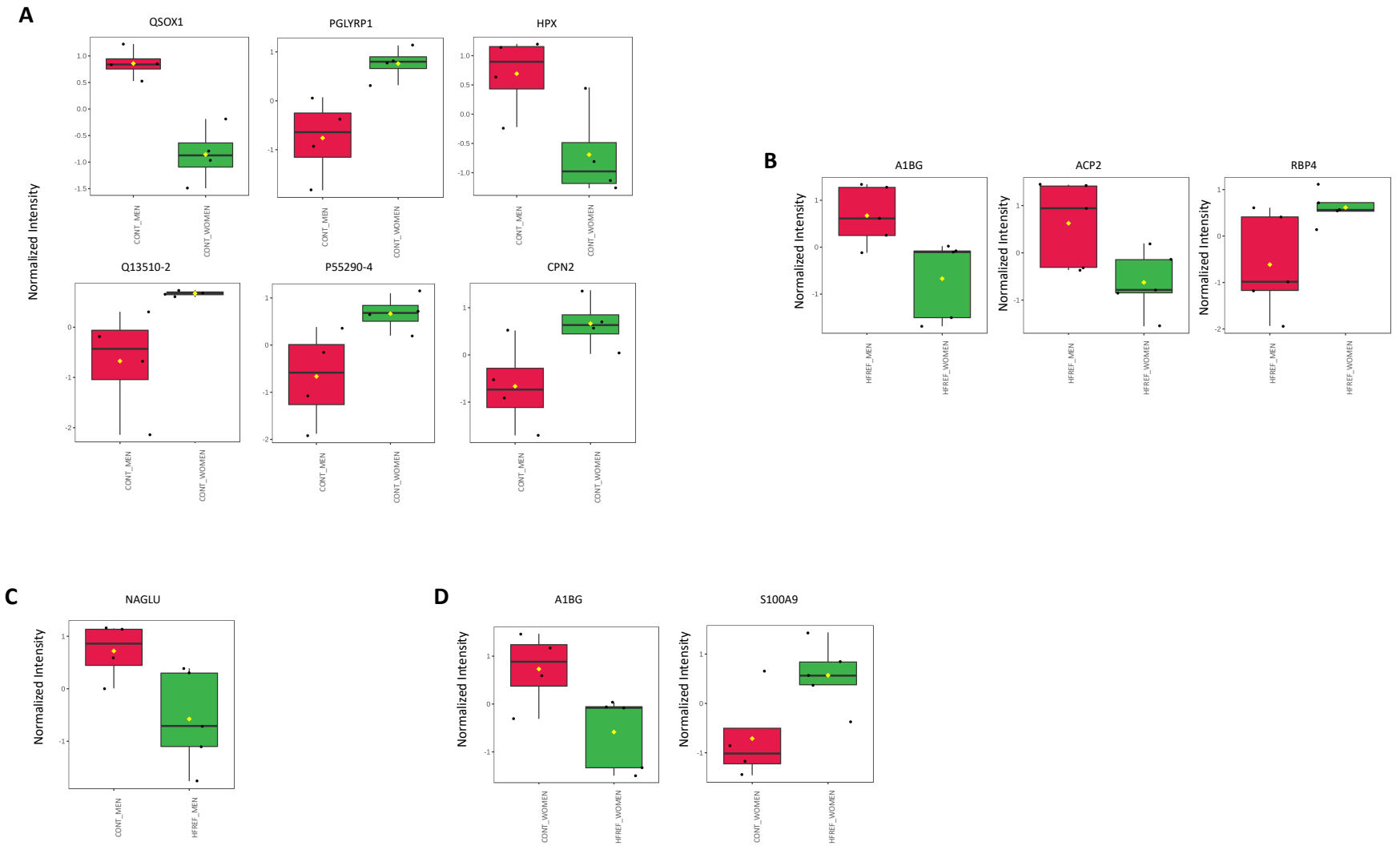

Figure S 2. Boxplots of the proteins that contributed to the significant changes between A) Control men *versus* Control women, B) HFrEF men *versus* HFrEF women, C) Control men *versus* HFrEF men and D) Control women *versus* HFrEF women. Abbreviations: CONT, control; HFREF, heart failure with reduced ejection fraction.

Table S 1. List of proteins identified in the GeLC-MS/MS analysis and their respective abundances, used to explore the differences among groups with the MetaboAnalyst 4.0 software [194].

		Abundances							
Accession	**Description**	**Control man**	**Control man**	**Control man**	**Control man**	**Control woman**	**Control woman**	**Control woman**	**Control woman**
A0A0C4DH25	Immunoglobulin kappa variable 3D-20 [OS=Homo sapiens]	6.17E+07	1.7E+07	2.24E+08	1.2E+08	4.3E+07	1.6E+07	1.34E+07	1.39E+07
O43451	Maltase-glucoamylase, intestinal [OS=Homo sapiens]	5.91E+07	9.08E+06	1.63E+08	2.99E+08	8.05E+07	7.91E+07	2.02E+08	4.31E+07
O60494	Cubilin [OS=Homo sapiens]	6.08E+08	9.05E+06	1.34E+08	3.42E+08	2.72E+08	4.02E+08	1.85E+08	9.84E+07
O75594	Peptidoglycan recognition protein 1 [OS=Homo sapiens]	1.86E+08	3.89E+07	5.01E+07	1.06E+09	3.57E+08	6.65E+07	1.62E+07	3.88E+07
O75882-1	Attractin [OS=Homo sapiens]	6.49E+07	5.73E+06	5.67E+07	9.85E+07	9.47E+07	3.26E+07	5.25E+07	1.77E+07
P00450	Ceruloplasmin [OS=Homo sapiens]	1.69E+08	1.3E+08	1.64E+09	8.97E+08	5.29E+08	3.2E+08	7.11E+07	6.28E+08
P00734	Prothrombin [OS=Homo sapiens]	1.49E+08	4.64E+07	1.75E+08	5.71E+08	1.63E+08	1.95E+08	2.17E+08	1.5E+08
P01008	Antithrombin-III [OS=Homo sapiens]	1.72E+07	8.93E+06	3.14E+07	3.5E+07	2.24E+07	6.64E+07	4.89E+06	9.48E+06
P01009-1	Alpha-1-antitrypsin [OS=Homo sapiens]	3.15E+08	5.6E+08	1.07E+09	7.28E+08	9.26E+08	9.41E+08	9.2E+07	4.73E+08
P01011-1	Alpha-1-antichymotrypsin [OS=Homo sapiens]	2.39E+08	2.17E+08	7.09E+08	2.04E+09	8.99E+08	3.08E+08	2.9E+08	3.17E+08
P01042-2	Isoform LMW of Kininogen-1 [OS=Homo sapiens]	2.79E+09	7.15E+08	2.19E+09	5.74E+09	3.38E+09	3.67E+09	2.1E+09	1.34E+09
P01133-1	Pro-epidermal growth factor [OS=Homo sapiens]	8.24E+08	6.7E+07	1.98E+08	7.86E+08	1.11E+09	9.45E+08	3.66E+08	1.43E+08

P01619	Immunoglobulin kappa variable 3-20 [OS=Homo sapiens]	4.61E+07	1.22E+08	1.62E+08	1.09E+09	3.2E+08	1.17E+08	1.52E+08	1.1E+08
P01833	Polymeric immunoglobulin receptor [OS=Homo sapiens]	7.91E+08	5.22E+08	8.55E+08	2.13E+09	8.18E+08	9.63E+08	6.32E+08	3.39E+08
P01834	Immunoglobulin kappa constant [OS=Homo sapiens]	2.64E+09	2.89E+09	8.48E+09	8.95E+09	7.53E+09	2.66E+09	2.87E+09	2.73E+09
P01859	Immunoglobulin heavy constant gamma 2 [OS=Homo sapiens]	7.2E+08	1.06E+09	6.63E+09	1.8E+09	2.51E+09	1.13E+09	1.26E+09	5.16E+08
P01876	Immunoglobulin heavy constant alpha 1 [OS=Homo sapiens]	1.09E+09	2.26E+09	2.01E+09	2.26E+09	1.1E+09	1.77E+09	5.19E+08	6.82E+08
P02533	Keratin, type I cytoskeletal 14 [OS=Homo sapiens]	4.48E+07	1.39E+07	7.96E+06	1.54E+07	2.83E+07	2.86E+07	1.21E+07	5.69E+06
P02671-1	Fibrinogen alpha chain [OS=Homo sapiens]	6.92E+07	2.19E+07	3.36E+07	3.26E+08	1.21E+08	2.52E+07	2.64E+07	1.97E+07
P02749	Beta-2-glycoprotein 1 [OS=Homo sapiens]	1.48E+07	2.46E+07	5.08E+07	4.46E+08	8.6E+07	1.11E+08	1.1E+08	2E+07
P02750	Leucine-rich alpha-2-glycoprotein [OS=Homo sapiens]	9.95E+06	1.36E+07	7.38E+08	1.13E+09	2.24E+08	6.27E+07	6.25E+07	7.26E+08
P02753	Retinol-binding protein 4 [OS=Homo sapiens]	2.54E+07	2.01E+07	4.7E+07	2.15E+08	1.61E+08	6.95E+07	7.71E+07	4.3E+06
P02760	Protein AMBP [OS=Homo sapiens]	2.77E+09	8.37E+08	1.4E+10	4.27E+10	8.75E+09	4.69E+09	2.1E+10	9.26E+09
P02763	Alpha-1-acid glycoprotein 1 [OS=Homo sapiens]	1.58E+08	2.44E+08	3.97E+09	2.86E+09	2.87E+08	3.33E+08	2.87E+08	2.11E+09
P02765	Alpha-2-HS-glycoprotein [OS=Homo sapiens]	2.31E+07	5.53E+07	1.52E+08	2.11E+08	2.47E+08	1.04E+08	1.14E+09	3.09E+07
P02768-1	Serum albumin [OS=Homo sapiens]	3.44E+10	4.57E+10	1.2E+11	3.93E+10	2.68E+10	6.63E+10	5.58E+10	2.79E+10

P02774-3	Isoform 3 of Vitamin D-binding protein [OS=Homo sapiens]	7.12E+07	1.72E+08	1.12E+08	1.81E+08	1.34E+08	2.08E+08	1.27E+08	2.88E+07
P02787	Serotransferrin [OS=Homo sapiens]	7.69E+08	1.61E+09	6.92E+09	8.47E+08	6.46E+08	2.67E+09	1.52E+09	2.43E+09
P02790	Hemopexin [OS=Homo sapiens]	1.66E+08	2.73E+08	2.89E+08	6.91E+08	2.69E+08	3.85E+08	2.41E+08	1.37E+08
P04217	Alpha-1B-glycoprotein [OS=Homo sapiens]	1.82E+08	7.2E+07	1.61E+09	1.61E+09	6.52E+08	3.39E+08	9.28E+07	1.18E+09
P04264	Keratin, type II cytoskeletal 1 [OS=Homo sapiens]	1.28E+09	5.14E+08	4.38E+08	5.76E+08	1.19E+09	2.51E+09	7.19E+08	4.32E+08
P04406	Glyceraldehyde-3-phosphate dehydrogenase [OS=Homo sapiens]	2.36E+07	6.62E+07	6.91E+06	3.79E+07	7.61E+07	4.4E+07	1.81E+07	5.36E+07
P04746	Pancreatic alpha-amylase [OS=Homo sapiens]	1.43E+09	3.78E+08	6.66E+08	3.88E+09	1.08E+09	7.98E+08	5.73E+08	4.21E+07
P05090	Apolipoprotein D [OS=Homo sapiens]	5.06E+08	1.76E+08	1.06E+09	5.27E+09	2.13E+09	3.54E+08	3.25E+08	9.4E+08
P05154	Plasma serine protease inhibitor [OS=Homo sapiens]	7.67E+08	1.59E+08	4.95E+08	1.55E+09	1.22E+09	8.3E+08	7.25E+07	4.58E+08
P05155-3	Isoform 3 of Plasma protease C1 inhibitor [OS=Homo sapiens]	2.55E+08	1.82E+07	1.48E+08	3.59E+08	4.81E+08	3.69E+08	3.65E+07	9.53E+07
P06396	Gelsolin [OS=Homo sapiens]	5.86E+07	1.75E+08	7.07E+07	2E+09	1.6E+08	6.78E+07	2.06E+08	1.14E+08
P06733-1	Alpha-enolase [OS=Homo sapiens]	1.66E+07	2.66E+08	5.77E+06	3.14E+07	4.05E+07	2.01E+07	1.58E+07	2.52E+07
P06870-1	Kallikrein-1 [OS=Homo sapiens]	4.13E+07	3.77E+07	1.71E+08	2.67E+08	2.89E+08	1.12E+08	5.29E+08	4.44E+07
P07195	L-lactate dehydrogenase B chain [OS=Homo sapiens]	1.54E+07	2.08E+07	1.39E+07	5.05E+07	5.53E+07	2.09E+07	6.47E+06	1.97E+07
P07339	Cathepsin D [OS=Homo sapiens]	9.92E+07	2.95E+07	3.98E+08	5.67E+08	1.58E+08	1.16E+08	1.16E+09	1.58E+08

P07911-5	Isoform 5 of Uromodulin [OS=Homo sapiens]	1.33E+09	3.75E+08	5.95E+09	4.1E+10	5.06E+10	3.04E+09	2.24E+09	1.59E+10
P07998	Ribonuclease pancreatic [OS=Homo sapiens]	1.87E+08	1.58E+07	1.19E+08	5.89E+08	3.52E+08	2.11E+08	5.25E+08	1.01E+08
P08174-7	Isoform 7 of Complement decay-accelerating factor [OS=Homo sapiens]	7.92E+07	1.75E+07	2.74E+08	6.66E+08	2.97E+08	3.92E+07	1.17E+08	1.19E+08
P08571	Monocyte differentiation antigen CD14 [OS=Homo sapiens]	1.32E+08	3.75E+07	7.15E+08	1.02E+09	8.4E+08	1.49E+08	4.63E+08	2.17E+08
P09211	Glutathione S-transferase P [OS=Homo sapiens]	6.88E+06	2.04E+08	3.64E+06	3.14E+07	3.01E+07	1.87E+07	1.7E+07	3.38E+07
P0DOX2	Immunoglobulin alpha-2 heavy chain [OS=Homo sapiens]	6.75E+07	1.5E+08	1.42E+08	1.56E+08	6.72E+07	8.12E+07	3.57E+07	2E+07
P0DOX5	Immunoglobulin gamma-1 heavy chain [OS=Homo sapiens]	1.15E+09	1.19E+09	7.59E+08	2.99E+09	1.53E+09	1.99E+09	3.95E+08	8.39E+08
P0DOY2	Immunoglobulin lambda constant 2 [OS=Homo sapiens]	4.56E+08	6.52E+08	7.84E+08	4.07E+09	5.86E+08	2.93E+08	4.4E+08	4.9E+08
P10153	Non-secretory ribonuclease [OS=Homo sapiens]	9.21E+08	7.41E+07	4.94E+08	9.31E+08	7.72E+08	4.75E+08	3.66E+08	1.26E+08
P10253	Lysosomal alpha-glucosidase [OS=Homo sapiens]	5.53E+08	2.7E+07	1.08E+09	5.31E+08	6.69E+08	4.65E+08	5.72E+08	3.02E+08
P10451-5	Isoform 5 of Osteopontin [OS=Homo sapiens]	7.47E+08	4.47E+06	3.48E+08	1.11E+09	6.15E+08	7.38E+08	6.92E+08	3.63E+08
P10909-2	Isoform 2 of Clusterin [OS=Homo sapiens]	7.35E+07	1.72E+07	1.52E+08	4.38E+08	1.29E+08	1.27E+08	5.73E+07	7.87E+07
P11117	Lysosomal acid phosphatase [OS=Homo sapiens]	9.97E+07	8.68E+06	1.26E+08	4.82E+07	5.52E+07	9.99E+07	1.02E+08	2.91E+07

P11142-1	Heat shock cognate 71 kDa protein [OS=Homo sapiens]	1.19E+07	4.18E+07	4.57E+06	8.16E+06	1.05E+07	1.17E+07	5.83E+06	2.9E+06
P12109	Collagen alpha-1(VI) chain [OS=Homo sapiens]	2.48E+08	1.92E+07	1.84E+08	3.42E+08	4.04E+08	2.19E+08	5.68E+07	9.38E+07
P12830	Cadherin-1 [OS=Homo sapiens]	8.52E+07	1.05E+07	3.66E+08	1.1E+09	3.04E+08	8.1E+07	4.44E+08	1.3E+08
P13645	Keratin, type I cytoskeletal 10 [OS=Homo sapiens]	7.03E+08	2.02E+08	2.61E+08	2.78E+08	3.6E+08	7.35E+08	2.93E+08	1.92E+08
P13647	keratin, type II cytoskeletal 5 [OS=Homo sapiens]	8.63E+06	4.79E+06	4.79E+06	4.7E+06	1.52E+07	1.76E+07	1.84E+07	8.34E+06
P13987	CD59 glycoprotein [OS=Homo sapiens]	3.23E+08	1.31E+08	3.04E+08	1.21E+09	5.81E+08	2.21E+08	2.62E+08	2.37E+08
P15144	Aminopeptidase N [OS=Homo sapiens]	1.75E+08	2.42E+06	3.7E+08	6.15E+08	2.7E+08	8.2E+07	2.54E+08	7.88E+07
P15311	Ezrin [OS=Homo sapiens]	3.2E+07	6.16E+06	6.42E+06	2.18E+07	3.78E+07	6.4E+06	3.48E+07	2.74E+07
P15586	N-acetylglucosamine-6-sulfatase [OS=Homo sapiens]	7.16E+07	1.26E+07	2.75E+08	2.62E+08	1.32E+08	4.85E+07	1.37E+08	8.53E+07
P16070	CD44 antigen [OS=Homo sapiens]	3.19E+08	5.89E+07	5.1E+08	7.92E+08	5.85E+08	3.71E+08	3.74E+08	2.53E+08
P16444	Dipeptidase 1 [OS=Homo sapiens]	3.74E+07	2.74E+06	7.5E+07	1.02E+08	7.54E+07	7.59E+06	2.83E+07	1.44E+07
P17900	Ganglioside GM2 activator [OS=Homo sapiens]	1.44E+07	4.98E+06	2.13E+08	1.92E+08	1.1E+08	1.87E+07	9.35E+07	8.64E+07
P19652	Alpha-1-acid glycoprotein 2 [OS=Homo sapiens]	1.7E+07	2.88E+07	1.83E+09	7.63E+08	1.54E+08	1.35E+08	3.04E+08	5.26E+08
P19835-1	Bile salt-activated lipase [OS=Homo sapiens]	3.64E+07	2E+06	2.89E+07	1.06E+08	4.58E+07	3.78E+07	1.01E+08	1.71E+07
P19961	Alpha-amylase 2B [OS=Homo sapiens]	4.42E+07	7.15E+06	7.61E+07	6.15E+07	7.36E+07	1.57E+07	2.79E+07	3.74E+06

P24855	Deoxyribonuclease-1 [OS=Homo sapiens]	1.31E+08	6.57E+06	8.56E+07	1.77E+08	1.89E+08	1.68E+08	3.41E+07	1.25E+07
P25311	Zinc-alpha-2-glycoprotein [OS=Homo sapiens]	3.98E+08	2.78E+08	7.71E+09	3.9E+09	7.37E+08	6.98E+08	1.49E+09	4.83E+09
P35527	Keratin, type I cytoskeletal 9 [OS=Homo sapiens]	4.13E+08	1.76E+08	8.12E+07	1.28E+08	6.41E+08	1.2E+09	2.92E+08	1.48E+08
P35555	Fibrillin-1 [OS=Homo sapiens]	7.29E+06	2.68E+06	1.31E+07	4.11E+07	1.37E+07	7.46E+06	1.13E+07	1.28E+07
P35908	Keratin, type II cytoskeletal 2 epidermal [OS=Homo sapiens]	2.04E+08	6.75E+07	6.6E+07	8.32E+07	7.14E+07	3.36E+08	8.94E+07	9.86E+07
P41222	Prostaglandin-H2 D-isomerase [OS=Homo sapiens]	8.09E+08	2.43E+08	1.85E+09	1.32E+10	2.09E+09	4.25E+08	2.1E+09	1.33E+09
P54802	Alpha-N-acetylglucosaminidase [OS=Homo sapiens]	7.64E+07	7.43E+06	4.05E+08	2.1E+08	1.9E+08	6.99E+07	2.14E+08	1.42E+08
P60174	Triosephosphate isomerase [OS=Homo sapiens]	4.36E+06	5.45E+07	1.85E+06	2.54E+07	5.27E+07	9.4E+06	8.23E+06	2.32E+07
P80188	Neutrophil gelatinase-associated lipocalin [OS=Homo sapiens]	1.54E+07	8.29E+08	1.7E+07	4.17E+07	7.36E+07	1.55E+07	7.7E+07	1.3E+07
P98160	Basement membrane-specific heparan sulfate proteoglycan core protein [OS=Homo sapiens]	2.3E+08	7.79E+07	1.23E+08	4.19E+09	1.01E+09	2.82E+08	7.62E+08	1.3E+08
P98164	Low-density lipoprotein receptor-related protein 2 [OS=Homo sapiens]	4.76E+08	8.47E+06	4.84E+07	2.56E+08	1.42E+08	4.55E+08	2.1E+08	6.6E+07
Q01459	Di-N-acetylchitobiase [OS=Homo sapiens]	4.25E+07	2.96E+06	1.18E+08	8.79E+07	8.59E+07	3.98E+07	9.47E+07	3.72E+07
Q08380	Galectin-3-binding protein [OS=Homo sapiens]	6.24E+08	7E+07	7.78E+08	1.11E+09	7.58E+08	8.56E+08	2.07E+08	2.91E+08

Q12805	EGF-containing fibulin-like extracellular matrix protein 1 [OS=Homo sapiens]	7.88E+07	1.83E+07	2.78E+07	1.29E+09	1.92E+08	1.36E+08	4.66E+07	2.91E+07
Q12907	Vesicular integral-membrane protein VIP36 [OS=Homo sapiens]	3.24E+08	1.44E+07	3.2E+08	2.49E+09	1.04E+09	2.12E+08	4.34E+07	3.14E+08
Q13510-2	Isoform 2 of Acid ceramidase [OS=Homo sapiens]	2.35E+07	4.96E+06	1.09E+08	4.86E+07	4.14E+07	4.67E+07	3.75E+08	4.31E+07
Q14624-1	Inter-alpha-trypsin inhibitor heavy chain H4 [OS=Homo sapiens]	6.03E+08	2.23E+08	4.62E+08	4.7E+09	1.18E+09	8.84E+08	4.56E+08	6.1E+08
Q16270-1	Insulin-like growth factor-binding protein 7 [OS=Homo sapiens]	3.56E+08	2.92E+07	2.03E+08	4.45E+08	3.4E+08	3.42E+08	3.35E+07	9.55E+07
Q16769	Glutaminyl-peptide cyclotransferase [OS=Homo sapiens]	2.4E+08	3.73E+07	1.45E+08	4.31E+08	6.03E+08	1.05E+08	1.84E+08	1.04E+08
Q6EMK4	Vasorin [OS=Homo sapiens]	5.68E+08	5.69E+07	3.49E+08	1.12E+09	8.63E+08	7.16E+08	5.22E+08	3.6E+08
Q8TDQ0	Hepatitis A virus cellular receptor 2 [OS=Homo sapiens]	1.17E+07	2.43E+06	3.45E+07	7.31E+07	1.37E+07	1.82E+07	4.14E+07	1.77E+07
Q8WVN6	Secreted and transmembrane protein 1 [OS=Homo sapiens]	9.21E+07	2.91E+07	7.99E+07	1.49E+09	3.6E+08	5.49E+07	7.84E+07	3.11E+07
Q96PD5-2	Isoform 2 of N-acetylmuramoyl-L-alanine amidase [OS=Homo sapiens]	3.38E+07	1.46E+07	2.24E+08	4.71E+07	4.82E+07	8.62E+07	1.23E+08	5.58E+07
Q9HCU0	Endosialin [OS=Homo sapiens]	6.58E+07	1.01E+07	4.59E+07	2.48E+08	7.24E+07	8.61E+07	1.35E+08	7.04E+07
Q9UNN8	Endothelial protein C receptor [OS=Homo sapiens]	1.78E+08	1.68E+07	2.33E+08	2.7E+08	7.47E+08	2.11E+08	5.61E+07	1.47E+08

Q9Y5Y7	Lymphatic vessel endothelial hyaluronic acid receptor 1 [OS=Homo sapiens]	2.29E+07	1.12E+07	3.54E+08	1.18E+09	1.35E+08	3.92E+07	1.07E+08	1.2E+08
O00187-1	Mannan-binding lectin serine protease 2 [OS=Homo sapiens]	2E+07		3.43E+07	1.22E+09	4.14E+08	5.03E+07	4.74E+07	7.35E+07
O00391	Sulfhydryl oxidase 1 [OS=Homo sapiens]	2.36E+07	8.82E+06	2.2E+07	7.22E+07	1.03E+08	1.68E+07		1E+07
O14498	Immunoglobulin superfamily containing leucine-rich repeat protein [OS=Homo sapiens]	1.31E+07		4.6E+06	3.92E+07	3.41E+07	1.17E+07	1.04E+07	9.23E+06
O14773	Tripeptidyl-peptidase 1 [OS=Homo sapiens]	6.28E+07		6.01E+07	4.73E+07	8.56E+07	6.16E+07	8.4E+07	8.07E+07
O94919	Endonuclease domain-containing 1 protein [OS=Homo sapiens]	7.24E+06		6.02E+07	2.74E+08	5.55E+07	1.2E+07	2.29E+07	5.19E+07
P00558	Phosphoglycerate kinase 1 [OS=Homo sapiens]	8.67E+06	1.39E+08		3.53E+07	3.56E+07	1.76E+07	6.76E+06	2.75E+07
P00747	Plasminogen [OS=Homo sapiens]	4.85E+06	1.34E+07	2.93E+07	2E+07	3.91E+07	1.54E+07		3.21E+06
P01019	Angiotensinogen [OS=Homo sapiens]	7.93E+06	5.75E+06	3.4E+07	4.39E+07	2.69E+07	4.2E+07	6.51E+06	
P01024	Complement C3 [OS=Homo sapiens]	2.72E+07	2.04E+08	2.16E+07	3.29E+07	1.79E+07	1.37E+08		1.02E+07
P01034	Cystatin-C [OS=Homo sapiens]	3.75E+07		2.07E+07	5.31E+07	4.33E+07	2.89E+07	2.69E+07	1.88E+07
P01591	Immunoglobulin J chain [OS=Homo sapiens]	1.46E+07	4.22E+07	2.53E+07	2.99E+07	1.8E+07	3.23E+07		1.19E+07
P01614	Immunoglobulin kappa variable 2D-40 [OS=Homo sapiens]		6.76E+07	2.14E+08	5.65E+08	1.42E+08	4.84E+07	6.66E+07	7.24E+07
P01860	Immunoglobulin heavy constant gamma 3 [OS=Homo sapiens]		2.29E+09	3.34E+07	8.09E+06	2.65E+07	1.73E+06	1.19E+08	3.54E+06

P01861	Immunoglobulin heavy constant gamma 4 [OS=Homo sapiens]	4.04E+07	6.29E+07	3.43E+07	9.03E+06	2.95E+07	2.85E+07		3.98E+06
P02766	Transthyretin [OS=Homo sapiens]	1.61E+07	1.53E+07	1.58E+07	1.79E+07	1.94E+07	3.35E+07	5.61E+06	
P02788	Lactotransferrin [OS=Homo sapiens]	2.29E+08	3.44E+09	5.32E+07	7.61E+08	2.5E+07	2.07E+07		6.86E+07
P04004	Vitronectin [OS=Homo sapiens]	4.04E+07		3.03E+07	8.03E+07	6.93E+07	5E+07	7.81E+06	1.46E+07
P04066	Tissue alpha-L-fucosidase [OS=Homo sapiens]	3.14E+06		8.65E+06	9.7E+06	1.11E+07	4.39E+06	2.35E+07	6.81E+06
P04083	Annexin A1 [OS=Homo sapiens]	2.72E+07	1.19E+08		4.98E+07	2.26E+08	2.24E+08	6.01E+07	3.82E+08
P04180	Phosphatidylcholine-sterol acyltransferase [OS=Homo sapiens]	1.54E+07		4.03E+07	2E+07	3.01E+07	1.31E+07	1.68E+07	7.18E+05
P04745	Alpha-amylase 1 [OS=Homo sapiens]	1.06E+08	3.51E+06	4.38E+06	2.96E+08	3.43E+09	4.9E+06		3E+08
P05062	Fructose-bisphosphate aldolase B [OS=Homo sapiens]	2.04E+07		1.58E+07	7.36E+07	1.32E+08	2E+07	6.55E+06	2.54E+07
P05451	Lithostathine-1-alpha [OS=Homo sapiens]	2.23E+07		8.85E+07	2.13E+09	1.61E+08	2.36E+07	2.95E+08	1.38E+07
P05543	Thyroxine-binding globulin [OS=Homo sapiens]	3.38E+07		2.14E+08	2.56E+08	2.34E+08	2.9E+07	6.7E+07	7.04E+07
P06280	Alpha-galactosidase A [OS=Homo sapiens]	1.19E+07		1.76E+07	1.13E+07	6.43E+06	6.91E+06	3.56E+07	1.62E+07
P06310	Immunoglobulin kappa variable 2-30 [OS=Homo sapiens]	3.16E+07		1.48E+07	4.72E+08	1.5E+07	2.77E+07	2.8E+06	1.95E+06
P06702	Protein S100-A9 [OS=Homo sapiens]	6.97E+06	8.2E+08		2.71E+07	9.12E+07	1.33E+07	3.31E+07	5.72E+07
P06865	Beta-hexosaminidase subunit alpha [OS=Homo sapiens]	1.31E+07		1.78E+07	8.09E+06	1.91E+07	4.3E+06	7.96E+07	9E+06

P07858	Cathepsin B [OS=Homo sapiens]	2.47E+07		3.11E+07	3.62E+07	1.35E+07	1.96E+07	3.53E+07	1.97E+07
P08294	Extracellular superoxide dismutase [Cu-Zn] [OS=Homo sapiens]	5.7E+07	2.69E+06	1.87E+07	1.26E+08	7.82E+07	1.07E+07		5.49E+06
P0DJD8	Pepsin A-3 [OS=Homo sapiens]	1.28E+08		1.33E+09	6.15E+09	1.24E+09	1.74E+08	7.97E+08	4.56E+08
P0DMV8	Heat shock 70 kDa protein 1A [OS=Homo sapiens]	6.51E+06	1.09E+08	1.49E+06		1.31E+07	2.75E+06	2.86E+06	7.44E+06
P0DOX7	Immunoglobulin kappa light chain [OS=Homo sapiens]	1.59E+07	1.34E+07	3.6E+07	1.87E+10	2.14E+07	4.24E+06	3.43E+06	
P10451-1	Osteopontin [OS=Homo sapiens]	7.1E+07		3.4E+07	8.82E+07	7.1E+07	5.35E+07	4.74E+07	3.27E+07
P14384	Carboxypeptidase M [OS=Homo sapiens]	1.91E+07		8.55E+06	1.26E+07	4.36E+07	1.46E+07	2.45E+07	6.12E+06
P14543-1	Nidogen-1 [OS=Homo sapiens]	3.94E+07		2.54E+07	2.65E+08	5.86E+07	5.31E+06	1.11E+07	1.76E+07
P14618	Pyruvate kinase PKM [OS=Homo sapiens]	9.4E+06	3.07E+08		5.95E+06	1.66E+07	5.21E+06	2.94E+07	1.38E+07
P15151-1	Poliovirus receptor [OS=Homo sapiens]	1.53E+07		1.12E+08	2.89E+07	2.9E+07	2.25E+07	1.83E+07	9.27E+06
P15289-1	Arylsulfatase A [OS=Homo sapiens]	4.74E+07		2.53E+08	1.04E+08	1.01E+08	2.79E+07	1.11E+08	5.4E+07
P15941-2	Isoform 2 of Mucin-1 [OS=Homo sapiens]	4.67E+07		3.06E+07	2.8E+07	7.09E+07	6.23E+07	2.29E+07	2.21E+07
P16278	Beta-galactosidase [OS=Homo sapiens]	9.76E+07		1.66E+08	3.36E+07	3.77E+07	8.89E+07	4.13E+08	4.62E+07
P16870	Carboxypeptidase E [OS=Homo sapiens]	1.72E+07		1.21E+07	2.42E+07	1.09E+07	1.53E+07	1.14E+07	3.21E+06
P22352	Glutathione peroxidase 3 [OS=Homo sapiens]	8.47E+06		2.14E+07	3.21E+07	2.49E+07	1.26E+07	5.46E+06	7.15E+06
P22792	Carboxypeptidase n subunit 2 [OS=Homo sapiens]	5.45E+07		1.01E+08	1.15E+08	1.4E+08	3.68E+07	5.75E+06	1.16E+07

P26038	Moesin [OS=Homo sapiens]	7.29E+05	3.21E+08	5.83E+07	7.75E+07	9.67E+05	2.47E+07		7.72E+05
P26992	Ciliary neurotrophic factor receptor subunit alpha [OS=Homo sapiens]	8.43E+06		2.01E+07	4.49E+07	3.29E+07	7.97E+06	8.09E+06	5.64E+05
P29508	Serpin B3 [OS=Homo sapiens]	1.87E+07	3.53E+07		7.79E+06	6.7E+08	2.71E+08	9.86E+07	1.52E+08
P30530	Tyrosine-protein kinase receptor UFO [OS=Homo sapiens]	1.06E+08		1.7E+08	1.25E+08	1.76E+08	1.17E+08	9.47E+07	6.91E+07
P30740	Leukocyte elastase inhibitor [OS=Homo sapiens]	9.61E+06	5.77E+08		1.48E+07	6.24E+07	3.68E+07	1.93E+07	4.45E+07
P42785-2	Isoform 2 of Lysosomal Pro-X carboxypeptidase [OS=Homo sapiens]	1.37E+07		2.92E+07	2.07E+07	3.26E+07	1.53E+07	1.89E+07	7.53E+06
P43121	Cell surface glycoprotein MUC18 [OS=Homo sapiens]	6.52E+06		1.9E+07	3.07E+07	2.71E+07	5.74E+06	2.61E+06	1.25E+07
P43652	Afamin [OS=Homo sapiens]		7.92E+06	9.77E+07	2.77E+06	7.5E+06	2.71E+07	1.95E+07	1.71E+07
P53634-1	Dipeptidyl peptidase 1 [OS=Homo sapiens]	1.51E+07		8.05E+07	4.16E+07	4.64E+07	2.19E+07	1.07E+08	2.07E+07
P55290-4	Isoform 4 of Cadherin-13 [OS=Homo sapiens]	1.64E+07		9.72E+06	6.17E+07	3.38E+07	6.63E+06	2.52E+07	1.15E+07
P62979	Ubiquitin-40S ribosomal protein S27a [OS=Homo sapiens]	3.9E+07	3.77E+07	1.67E+07	4.82E+07	2.59E+07	1.21E+07	2.1E+07	
Q02487	Desmocollin-2 [OS=Homo sapiens]	1.09E+07		1.06E+07	4.82E+07	1.49E+07	1.63E+06	1.88E+07	1.61E+07
Q07075	Glutamyl aminopeptidase [OS=Homo sapiens]	4.87E+06		1.33E+08	4.39E+07	2.67E+07	2.56E+07	6.26E+07	4.44E+07
Q12794-1	Hyaluronidase-1 [OS=Homo sapiens]	2.27E+07		7.98E+06	6.15E+06	1.64E+07	7.88E+06	1.47E+07	3.91E+06

Q15113	Procollagen C-endopeptidase enhancer 1 [OS=Homo sapiens]	6.39E+06		1.45E+07	1.13E+08	2.62E+07	6.06E+06	1.15E+07	1.79E+07
Q16651	Prostasin [OS=Homo sapiens]	1.12E+07		7.21E+07	2.75E+07	2.48E+07	8.75E+06	2.79E+07	6.67E+06
Q6UX06	Olfactomedin-4 [OS=Homo sapiens]	1.92E+07	1.18E+07	1.29E+07	2.05E+07	1.87E+07		4.17E+06	1.63E+06
Q6UXB8-1	Peptidase inhibitor 16 [OS=Homo sapiens]	8.51E+06		1.43E+08	3.04E+08	3E+07	2.44E+07	6.02E+07	1.64E+08
Q7Z5L0-1	Vitelline membrane outer layer protein 1 homolog [OS=Homo sapiens]	4.33E+07		7.15E+06	4.97E+07	1.74E+08	5.22E+07	6.19E+06	1.73E+07
Q8IYS5-7	Isoform 7 of Osteoclast-associated immunoglobulin-like receptor [OS=Homo sapiens]	6.11E+07		2.67E+07	9.83E+07	2.33E+07	2.53E+07	4.93E+07	2.12E+07
Q8NFZ8	Cell adhesion molecule 4 [OS=Homo sapiens]	7.75E+07		6.08E+07	1.69E+08	1.02E+08	2.89E+07	5.26E+07	2.99E+07
Q8WZ75-1	Roundabout homolog 4 [OS=Homo sapiens]	5.24E+07		7.78E+07	2.7E+08	3.78E+08	1.32E+08	5.34E+06	7.22E+07
Q92820	Gamma-glutamyl hydrolase [OS=Homo sapiens]	1.61E+07		1.48E+07	1.71E+07	3.73E+07	2.09E+07	1.27E+08	6.48E+07
Q96RW7-1	Hemicentin-1 [OS=Homo sapiens]	2.4E+07		1.12E+07	1.55E+08	6.76E+07	4.62E+07	4.93E+07	1.78E+07
Q99519	Sialidase-1 [OS=Homo sapiens]	9.51E+06		1.71E+07	7.57E+06	1.65E+07	5.68E+06	1.28E+07	3.86E+06
Q9BY67-3	Isoform 3 of Cell adhesion molecule 1 [OS=Homo sapiens]	1.35E+07		2.44E+07	1.92E+07	5.6E+07	2.19E+07	4.86E+06	5.14E+06
Q9HAT2	Sialate O-acetylesterase [OS=Homo sapiens]	1.91E+07		3.06E+07	4.26E+07	4.68E+07	3.38E+07	7.92E+06	1.24E+07
Q9NZP8	Complement C1r subcomponent-like protein [OS=Homo sapiens]	4.12E+07		3.45E+07	7.77E+07	8.21E+07	5.68E+07	2.44E+07	1.49E+07

Q9UHL4	Dipeptidyl peptidase 2 [OS=Homo sapiens]	2.13E+06		2.12E+07	4.56E+06	8.52E+06	1.22E+07	4.47E+07	2.29E+07
Q9UKU9	Angiopoietin-related protein 2 [OS=Homo sapiens]	1.58E+07		1.74E+06	1.53E+08	8.11E+07	2.03E+06	1.45E+06	6.03E+06
Q9Y646	Carboxypeptidase Q [OS=Homo sapiens]	3.63E+07		7.38E+07	3.5E+07	5.05E+07	8.18E+06	2.62E+08	7.82E+07
A0A0B4J1X5	Immunoglobulin heavy variable 3-74 [OS=Homo sapiens]	3.93E+06		1.49E+07	1.58E+07	1.03E+07		4.84E+06	2.7E+06
O00241	Signal-regulatory protein beta-1 [OS=Homo sapiens]	4.11E+06		1.19E+07	2.44E+07	4.2E+06	1.83E+06		4.45E+06
O43895	Xaa-Pro aminopeptidase 2 [OS=Homo sapiens]	4.83E+06		2.72E+07	1.12E+07	4.73E+06	5.79E+06	1.8E+07	
O75144-2	Isoform 2 of ICOS ligand [OS=Homo sapiens]	2.14E+07		3.74E+07	4.33E+07	3.26E+07	2.7E+07	2.21E+07	
O95336	6-phosphogluconolactonase [OS=Homo sapiens]	1.75E+07		2.56E+07	1.15E+08	6.54E+07	9.72E+06		4.27E+07
O96009	Napsin-A [OS=Homo sapiens]	1.94E+07		8.6E+07	1.03E+07	3.72E+07		1.04E+08	1.39E+07
P00738	Haptoglobin [OS=Homo sapiens]		1.13E+08	6.68E+09	6.7E+08	7.06E+07		3.74E+07	3.65E+08
P00749	Urokinase-type plasminogen activator [OS=Homo sapiens]	3.18E+07		5.59E+06	2.33E+07	8.65E+07	3.87E+07		3.51E+06
P02649	Apolipoprotein E [OS=Homo sapiens]	7.32E+06		2.61E+06	2.65E+07	3.42E+07	8.82E+06		4.64E+06
P05156	Complement factor I [OS=Homo sapiens]	1.78E+07		3.67E+07	8.36E+07	3.18E+07	1.53E+07		3.23E+06
P07686	Beta-hexosaminidase subunit beta [OS=Homo sapiens]	5.98E+06		2.98E+07	3.07E+07	8.87E+06		1.83E+08	1.98E+07
P08195-4	Isoform 4 of 4F2 cell-surface antigen heavy	8.71E+06		1.17E+07	3.26E+07	2.56E+07	4.76E+06		6.09E+06

	chain [OS=Homo sapiens]							
P08473	Neprilysin [OS=Homo sapiens]	1.57E+07	4.78E+07	1.65E+07	2.86E+07	1.43E+07	1.19E+07	
P09467	Fructose-1,6-bisphosphatase 1 [OS=Homo sapiens]	5.7E+06	3.98E+06	1.64E+07	1.82E+07	4.1E+06	3.76E+06	
P09603	Macrophage colony-stimulating factor 1 [OS=Homo sapiens]	1.13E+07	2.09E+07	8.05E+07	1.81E+07		2.99E+07	4.25E+06
P09668	Pro-cathepsin H [OS=Homo sapiens]	7.1E+06	1.28E+07	2.56E+07	8.45E+06	6.15E+06	1.24E+07	
P13473-3	Isoform LAMP-2C of Lysosome-associated membrane glycoprotein 2 [OS=Homo sapiens]	3.6E+07	8.44E+07	6.35E+07	4.47E+07	3.36E+07	7.06E+07	
P15328	Folate receptor alpha [OS=Homo sapiens]	1.4E+07	6.54E+06	6.44E+07		2.46E+07	8.33E+06	6.04E+06
P19022	Cadherin-2 [OS=Homo sapiens]	5.93E+07	4.61E+07	2.65E+07	3.51E+07	1.51E+07	8.41E+07	
P22891-2	Isoform 2 of Vitamin K-dependent protein Z [OS=Homo sapiens]	9.84E+07	6.4E+07	1.2E+08	4.88E+07	1.05E+08		1.36E+07
P33908	Mannosyl-oligosaccharide 1,2-alpha-mannosidase IA [OS=Homo sapiens]	2.59E+07	3.4E+06	8.41E+06	3.79E+07	3.51E+07		5.15E+06
P39059	Collagen alpha-1(XV) chain [OS=Homo sapiens]	4.99E+07	9.58E+07	2.37E+08	1.67E+08	6.25E+07	1.45E+07	
P39060-3	Collagen alpha-1(XVIII) chain [OS=Homo sapiens]	2.87E+07	1.32E+06	7.41E+07	6.64E+07	3E+06		2.2E+06
P43251-2	Isoform 2 of Biotinidase [OS=Homo sapiens]	1.78E+07	7.4E+07	8.45E+07	2.71E+07	1.37E+07	1.75E+07	
Q03154	Aminoacylase-1 [OS=Homo sapiens]	8.32E+06	5.62E+06	1.05E+07	9.29E+07	1.15E+07		9.93E+06

Q03403	Trefoil factor 2 [OS=Homo sapiens]	3.94E+07	5.24E+07	1.48E+08	2.53E+07	2.91E+07	2.62E+07	
Q5JS37	NHL repeat-containing protein 3 [OS=Homo sapiens]	8.58E+06	2.71E+07	4.51E+06		1.03E+07	3.81E+07	9.23E+06
Q6UVK1	Chondroitin sulfate proteoglycan 4 [OS=Homo sapiens]	1.99E+07	7.41E+06	5.3E+07	2.88E+07	2E+07		1.31E+07
Q6UXG3	CMRF35-like molecule 9 [OS=Homo sapiens]	1.73E+07	1.15E+07	2.16E+07	1.37E+07	8.06E+06	7.89E+06	
Q86UN3	Reticulon-4 receptor-like 2 [OS=Homo sapiens]	8.93E+06	6.7E+06	2.45E+07	1.37E+07	7.56E+06	7.69E+06	
Q92859	Neogenin [OS=Homo sapiens]	1.74E+06	2.87E+06	1.68E+06	8.15E+06	1.4E+06	1.76E+06	
Q93088	Betaine--homocysteine S-methyltransferase 1 [OS=Homo sapiens]	3.17E+07	1.91E+07	2.87E+07	6.22E+07	3.15E+07		2.42E+07
Q96DA0	Zymogen granule protein 16 homolog B [OS=Homo sapiens]	5.87E+07	2.94E+07	3.29E+07	1.47E+07	1.74E+07	1.29E+07	
Q96IU4	Protein ABHD14B [OS=Homo sapiens]	5.58E+06	1.67E+07	6.29E+07	1.77E+07	5.44E+06		1.55E+07
Q9H3G5	Probable serine carboxypeptidase CPVL [OS=Homo sapiens]	2.36E+07	2.79E+07	5.3E+07	4.07E+07	4.36E+07		1E+07
Q9H8L6	Multimerin-2 [OS=Homo sapiens]	2.13E+07	3.45E+07	5.3E+07	6.51E+07	2.16E+07	1.66E+07	
Q9HCN6-3	Isoform 3 of Platelet glycoprotein VI [OS=Homo sapiens]	1.05E+07	1.16E+07	8.02E+07	2.12E+07	9.22E+06	1.31E+07	

Table S1. Continued.

		Abundances									
Accession	**Description**	**HFrEF man**	**HFrEF man**	**HFrEF man**	**HFrEF man**	**HFrEF man**	**HFrEF woman**	**HFrEF woman**	**HFrEF woman**	**HFrEF woman**	**HFrEF woman**
O14773	Tripeptidyl-peptidase 1 [OS=Homo sapiens]	6.57E+07	5.46E+07	4.42E+07	1.48E+07	1.45E+07	1.45E+07	6.86E+07	1.21E+08	4E+07	4.49E+07
O60494	Cubilin [OS=Homo sapiens]	3.14E+08	6.89E+08	1.01E+08	2.71E+07	7.85E+06	5.02E+06	2.26E+08	5.2E+07	3.99E+07	9E+07
O75594	Peptidoglycan recognition protein 1 [OS=Homo sapiens]	5.25E+08	9.38E+08	5.87E+07	3.9E+07	1.23E+07	7.12E+07	1.02E+09	9.16E+08	8.21E+07	1.11E+08
O94919	Endonuclease domain-containing 1 protein [OS=Homo sapiens]	2.74E+07	6.31E+07	8.11E+06	5.35E+06	1.24E+07	1.12E+06	1.07E+08	2.5E+08	8.79E+06	1.28E+07
P00450	Ceruloplasmin [OS=Homo sapiens]	1.49E+08	1.02E+09	2.97E+07	4.2E+07	7.02E+08	3.28E+08	9.1E+08	2.04E+08	2.28E+08	5.29E+08
P00734	Prothrombin [OS=Homo sapiens]	1.52E+08	3.69E+08	8.55E+07	7.54E+07	4.68E+07	1.09E+08	2.18E+08	3.39E+08	1E+08	2.25E+08
P01009-1	Alpha-1-antitrypsin [OS=Homo sapiens]	9.44E+08	5.96E+08	1.18E+08	5.58E+07	1.06E+09	8.16E+08	1.12E+09	3.79E+08	8.87E+07	1.05E+09
P01011-1	Alpha-1-antichymotrypsin [OS=Homo sapiens]	3.7E+08	1.5E+09	1.78E+08	6.73E+07	4.81E+08	2.29E+08	1.45E+09	1.72E+09	3.67E+08	5.18E+08
P01133-1	Pro-epidermal growth factor [OS=Homo sapiens]	2.27E+09	2.03E+09	3.08E+08	1.22E+08	4E+07	5.04E+07	1.01E+09	1.43E+08	1.96E+08	5.64E+08
P01619	Immunoglobulin kappa variable 3-20 [OS=Homo sapiens]	3.27E+08	1.67E+09	6.78E+07	1.3E+08	1.49E+08	5.14E+08	1.13E+09	4.93E+09	2.39E+08	1.63E+08
P01833	Polymeric immunoglobulin	2.75E+09	4.05E+09	6.42E+08	1.67E+08	3E+08	7.51E+07	2.26E+09	1.41E+09	2.97E+08	1.86E+09

	receptor [OS=Homo sapiens]										
P01834	Immunoglobulin kappa constant [OS=Homo sapiens]	5.14E+09	8.24E+09	1.13E+09	2.46E+09	3.99E+09	3.01E+09	2.71E+10	2.15E+10	4.73E+09	2.5E+10
P01859	Immunoglobulin heavy constant gamma 2 [OS=Homo sapiens]	3.09E+09	1.57E+09	2.82E+08	1.04E+09	1.32E+09	6.92E+08	2.67E+09	3.16E+09	5.84E+08	2.2E+10
P01871-2	Isoform 2 of Immunoglobulin heavy constant mu [OS=Homo sapiens]	2.9E+07	8.85E+07	9.85E+06	6.27E+06	6.89E+07	1.06E+08	3.53E+08	5.82E+08	1.42E+07	7.19E+07
P01876	Immunoglobulin heavy constant alpha 1 [OS=Homo sapiens]	1.57E+09	8.22E+09	1.53E+09	1.63E+08	1.66E+09	8.05E+08	4.2E+09	7.17E+08	1.14E+09	1.11E+07
P02751-15	Isoform 15 of Fibronectin [OS=Homo sapiens]	1.43E+08	2.51E+08	5.23E+08	1.46E+07	1.19E+07	1.43E+07	1.48E+08	8.78E+07	3.63E+07	4.51E+07
P02760	Protein AMBP [OS=Homo sapiens]	9.23E+09	1.57E+10	1.97E+09	2.94E+09	4.1E+09	7.32E+09	3.44E+10	7.04E+10	1.12E+10	3.32E+09
P02763	Alpha-1-acid glycoprotein 1 [OS=Homo sapiens]	1.63E+08	4.43E+08	7.16E+07	5.6E+07	1.67E+09	7.57E+07	7.38E+08	2.25E+09	3.24E+08	3.02E+08
P02765	Alpha-2-HS-glycoprotein [OS=Homo sapiens]	3.14E+08	2.11E+08	1.49E+07	6.56E+06	3.42E+06	2.52E+08	3.68E+08	1.07E+09	7.94E+07	4.71E+07
P02768-1	Serum albumin [OS=Homo sapiens]	4.32E+10	7.62E+10	1.66E+10	1.13E+10	1.74E+11	4.19E+10	5.32E+10	6.15E+10	3.67E+10	8.26E+10
P02774-3	Isoform 3 of Vitamin D-binding protein [OS=Homo sapiens]	1.47E+08	1.42E+08	1.64E+07	6.34E+06	2.27E+07	2.6E+08	1.27E+08	2.75E+08	7.54E+07	2.08E+08
P02787	Serotransferrin [OS=Homo sapiens]	1.1E+09	1.34E+09	3.21E+08	2.58E+08	7.83E+09	2.74E+09	1.64E+09	3.92E+09	1.35E+09	3.75E+09
P02788	Lactotransferrin [OS=Homo sapiens]	1.84E+08	1.22E+08	1.73E+09	1.93E+07	2.22E+08	2.34E+08	1.33E+09	2.05E+06	1.1E+08	2.91E+09

P02790	Hemopexin [OS=Homo sapiens]	3.14E+08	5.24E+08	5.18E+07	2.92E+07	7.49E+07	4.16E+08	6.01E+08	6.96E+08	1.63E+08	6.71E+08
P04217	Alpha-1B-glycoprotein [OS=Homo sapiens]	1.52E+08	6.5E+08	2.23E+07	3.26E+07	6.96E+08	2.97E+08	1.54E+09	3.59E+08	1.58E+08	1.76E+08
P04264	Keratin, type II cytoskeletal 1 [OS=Homo sapiens]	2.48E+08	3.93E+08	5.34E+08	2.8E+08	4.05E+08	5.29E+08	6.36E+08	4.37E+08	2.22E+08	5.34E+08
P04746	Pancreatic alpha-amylase [OS=Homo sapiens]	3.47E+09	2.04E+09	3.07E+08	3.41E+08	6.41E+07	7.38E+07	5.48E+09	1.85E+09	7.75E+08	8.23E+08
P05090	Apolipoprotein D [OS=Homo sapiens]	7.15E+08	3.14E+09	3.18E+08	2.23E+08	2.26E+08	2.36E+08	2.38E+09	4.05E+09	5.45E+08	7.9E+08
P05154	Plasma serine protease inhibitor [OS=Homo sapiens]	2.86E+09	2.84E+09	5.51E+08	1.44E+08	6.66E+07	7.88E+07	1.88E+09	1.42E+08	3.21E+08	1.45E+09
P06396	Gelsolin [OS=Homo sapiens]	3.24E+08	5.75E+08	2.33E+07	6.67E+07	3.36E+07	9.84E+07	1.34E+09	1.95E+09	1.68E+08	7.37E+07
P06870-1	Kallikrein-1 [OS=Homo sapiens]	7.98E+08	2.45E+08	1.05E+08	3.56E+07	9.12E+07	2.97E+07	1.77E+08	1.19E+08	7.64E+06	1.1E+08
P07339	Cathepsin D [OS=Homo sapiens]	4.81E+08	6.05E+08	2.28E+08	2.56E+07	5.61E+07	2.39E+07	6.42E+08	1.75E+09	1.22E+08	1.5E+08
P07858	Cathepsin B [OS=Homo sapiens]	2.6E+07	5.55E+07	2.19E+07	3.85E+06	6.22E+06	6.91E+06	8.66E+07	3.19E+08	4.24E+06	1.59E+07
P07911-5	Isoform 5 of Uromodulin [OS=Homo sapiens]	1.05E+11	3.04E+10	2.51E+09	2.55E+09	6E+09	8.43E+08	6.69E+10	4.14E+09	1.02E+09	1.97E+09
P07998	Ribonuclease pancreatic [OS=Homo sapiens]	2.08E+08	5.83E+08	6.93E+07	8.98E+07	7.46E+07	6.52E+07	2.93E+08	5.46E+08	4.79E+07	1.62E+08
P08174-7	Isoform 7 of Complement decay-accelerating factor [OS=Homo sapiens]	1.34E+08	8.4E+08	2.13E+07	3.74E+07	3.42E+07	1.7E+07	5.24E+08	3.84E+08	6.95E+07	5.01E+07
P08571	Monocyte differentiation	2.98E+08	3.01E+08	6.49E+07	1.2E+07	8.42E+07	7.86E+07	1.08E+09	2.95E+09	1.17E+08	4.4E+07

	antigen CD14 [OS=Homo sapiens]										
P0DOX2	Immunoglobulin alpha-2 heavy chain [OS=Homo sapiens]	6E+07	3.28E+08	4.42E+07	8.59E+06	5.91E+07	4.72E+07	2.53E+08	1.16E+08	6.99E+07	4.23E+07
P0DOX5	Immunoglobulin gamma-1 heavy chain [OS=Homo sapiens]	6.84E+08	2.33E+09	3.63E+08	2.03E+08	1.22E+09	6.97E+08	4.75E+09	3.36E+09	8.6E+08	4.42E+09
P0DOY2	Immunoglobulin lambda constant 2 [OS=Homo sapiens]	5.38E+08	2.68E+09	1.87E+08	2.84E+08	9.58E+08	8.6E+08	3.9E+09	4.58E+09	6.17E+08	5.15E+08
P10153	Non-secretory ribonuclease [OS=Homo sapiens]	1.27E+09	2.21E+09	1.41E+08	1.92E+08	7.38E+07	8.01E+07	7.37E+08	1E+09	1.59E+08	3.86E+08
P10253	lysosomal alpha-glucosidase [OS=Homo sapiens]	8.21E+08	1.33E+09	1.73E+08	5.64E+07	3.13E+07	1.61E+07	4.85E+08	6.49E+08	1.77E+08	9.27E+07
P12109	Collagen alpha-1(VI) chain [OS=Homo sapiens]	8.99E+08	7.46E+08	6.2E+07	3.12E+07	8.12E+06	1.32E+07	4.43E+08	1.31E+08	3.25E+07	1.68E+08
P12830	Cadherin-1 [OS=Homo sapiens]	7.96E+07	8.52E+08	4.13E+07	2.33E+07	4.44E+07	4.52E+07	8.26E+08	1.19E+09	9.89E+07	2.46E+07
P13645	Keratin, type I cytoskeletal 10 [OS=Homo sapiens]	1.31E+08	1.63E+08	2.77E+08	1.64E+08	2.63E+08	2.81E+08	2.66E+08	3.37E+08	7.67E+07	2.39E+08
P13987	CD59 glycoprotein [OS=Homo sapiens]	7.72E+08	1.5E+09	2.36E+08	1.57E+08	1.03E+08	1.12E+08	4.99E+08	1.1E+09	1.46E+08	6.08E+08
P15586	N-acetylglucosamine-6-sulfatase [OS=Homo sapiens]	1.25E+08	2.51E+08	6.23E+07	1.77E+07	4.83E+07	3.09E+06	2.45E+08	5.95E+08	3.43E+07	4.17E+07
P16070	CD44 antigen [OS=Homo sapiens]	8.24E+08	1.4E+09	1.54E+08	8.43E+07	6.17E+07	3.21E+07	6.52E+08	5.2E+08	1.61E+08	3.71E+08
P16444	Dipeptidase 1 [OS=Homo sapiens]	2.22E+08	1.96E+08	4.01E+07	1.4E+07	1.93E+06	2.45E+06	1.01E+08	2.04E+07	7.67E+06	7.65E+07
P19652	Alpha-1-acid glycoprotein 2 [OS=Homo sapiens]	4.31E+07	8.64E+07	2.87E+07	3.72E+06	1.79E+08	6.61E+07	2.53E+08	1.35E+09	6.92E+07	7.68E+07

P19961	Alpha-amylase 2B [OS=Homo sapiens]	3.33E+08	3.59E+08	4.94E+07	1.94E+07	5.11E+06	4.59E+06	7.26E+07	5.94E+07	3.93E+07	7.5E+07
P25311	Zinc-alpha-2-glycoprotein [OS=Homo sapiens]	5.71E+08	7.35E+08	3.15E+08	1.15E+08	3.23E+09	4.65E+08	2.75E+09	9.15E+09	1.23E+09	3.93E+08
P35527	Keratin, type I cytoskeletal 9 [OS=Homo sapiens]	7.42E+07	6.94E+07	1.24E+08	6.15E+07	1.14E+08	1.7E+08	1.7E+08	7.48E+07	3.79E+07	1.2E+08
P35908	Keratin, type II cytoskeletal 2 epidermal [OS=Homo sapiens]	5.36E+07	5.06E+07	1.23E+08	2.33E+07	9.92E+07	8.22E+07	9.51E+07	1.32E+08	1.41E+07	6.89E+07
P41222	Prostaglandin-H2 D-isomerase [OS=Homo sapiens]	1.15E+09	5.83E+09	6.99E+08	8.79E+08	1.41E+09	1.17E+09	7.51E+09	1.24E+10	1.6E+09	5.46E+08
P54802	Alpha-N-acetylglucosaminidase [OS=Homo sapiens]	3.24E+08	2.92E+08	6.01E+07	9.34E+06	2.85E+07	5.56E+06	2.99E+08	2.65E+08	3.29E+07	4.58E+07
P80188	Neutrophil gelatinase-associated lipocalin [OS=Homo sapiens]	1.82E+07	4.44E+07	3.36E+07	4.32E+06	1.67E+07	1.47E+08	3.72E+08	5.73E+07	1.86E+07	3.95E+08
P98160	Basement membrane-specific heparan sulfate proteoglycan core protein [OS=Homo sapiens]	1.51E+09	4.68E+09	1.77E+08	5.11E+08	4.59E+07	5.64E+08	2.7E+09	9.4E+09	4.1E+08	4.01E+08
P98164	Low-density lipoprotein receptor-related protein 2 [OS=Homo sapiens]	1.24E+08	5.3E+08	1.29E+08	1.81E+07	1.14E+07	3.41E+06	2.81E+08	5.74E+07	3.83E+07	2.5E+07
Q08380	Galectin-3-binding protein [OS=Homo sapiens]	1.56E+09	1.95E+09	3.5E+08	9.53E+07	1.34E+08	3.63E+07	1.1E+09	2.6E+08	2.34E+08	5.68E+08
Q12805	EGF-containing fibulin-like	3.44E+08	1.26E+09	1.05E+08	2.09E+08	4.75E+06	5.35E+07	8.8E+08	4.14E+08	1.49E+08	1.13E+08

	extracellular matrix protein 1 [OS=Homo sapiens]										
Q12907	Vesicular integral-membrane protein VIP36 [OS=Homo sapiens]	6.17E+08	1.09E+09	2.83E+07	9.58E+07	1.16E+08	1.76E+08	2.71E+09	1.6E+09	5.88E+07	1.83E+08
Q14624-1	Inter-alpha-trypsin inhibitor heavy chain H4 [OS=Homo sapiens]	2.6E+09	3.24E+09	2.91E+08	3.3E+08	9.87E+07	4.99E+08	3.68E+09	2.83E+09	5.68E+08	1.39E+09
Q16270-1	Insulin-like growth factor-binding protein 7 [OS=Homo sapiens]	9.05E+08	6.65E+08	1.91E+08	4.18E+07	6.8E+06	2.42E+07	4.09E+08	6.39E+07	1.09E+08	3.78E+08
Q16769	Glutaminyl-peptide cyclotransferase [OS=Homo sapiens]	1E+09	4.99E+08	7.32E+07	4.45E+07	3.38E+07	5.2E+07	5.94E+08	5.39E+08	5.9E+07	6.92E+08
Q6EMK4	Vasorin [OS=Homo sapiens]	1.54E+09	2.14E+09	2.62E+08	1.46E+08	9.12E+07	5.82E+07	1.24E+09	9.73E+08	2.67E+08	5.62E+08
Q8NFZ8	Cell adhesion molecule 4 [OS=Homo sapiens]	2.39E+08	2.33E+08	4.39E+07	1.63E+07	2.96E+06	5.27E+06	8.22E+07	1.38E+08	2.11E+07	1.2E+08
Q8WZ75-1	Roundabout homolog 4 [OS=Homo sapiens]	2.77E+08	5.61E+07	6.33E+06	1.35E+07	1.07E+07	4.3E+06	1.17E+08	1.47E+07	3.89E+06	2.92E+07
Q9UNN8	Endothelial protein C receptor [OS=Homo sapiens]	2E+08	1.88E+08	4.59E+07	1.8E+07	2.09E+07	6.48E+06	2.77E+08	3.4E+08	3.35E+07	1.68E+08
A0A0B4J1X5	Immunoglobulin heavy variable 3-74 [OS=Homo sapiens]	3.07E+07	2.07E+07	1.14E+07	4.15E+06		1.83E+07	2.5E+07	2.69E+07	4.23E+06	1.37E+07
A0A0C4DH25	Immunoglobulin kappa variable 3D-20 [OS=Homo sapiens]	6.26E+07	1.72E+08		2.05E+07	2.1E+07	2.98E+07	1.31E+08	4.2E+08	2.59E+07	2.28E+07
O43451	Maltase-glucoamylase,	1.66E+08	4.96E+08	4.96E+07	2.18E+07	2.68E+06		3.27E+08	7.45E+07	7.43E+07	2.61E+07

	intestinal [OS=Homo sapiens]										
P00738	Haptoglobin [OS=Homo sapiens]	4.65E+08	1.01E+08	1.33E+07		8.53E+08	7.3E+08	3.34E+08	8.76E+08	1.69E+07	3.27E+08
P01008	Antithrombin-III [OS=Homo sapiens]	1.62E+07	3.89E+07	5.99E+06		4.69E+06	4.82E+07	6.68E+07	7.65E+07	1.95E+07	2.97E+07
P01764	Immunoglobulin heavy variable 3-23 [OS=Homo sapiens]	1.03E+07	6.18E+07	6.39E+06	2.73E+06	5.88E+06	3.86E+06		7.41E+07	1.4E+07	7.41E+06
P01860	Immunoglobulin heavy constant gamma 3 [OS=Homo sapiens]	8.32E+06	3.11E+07	7.97E+06	3.72E+06	1.15E+06	1.05E+09	9.51E+06	2.55E+07		6E+07
P01861	Immunoglobulin heavy constant gamma 4 [OS=Homo sapiens]	7.31E+06	3.11E+07		1.72E+06	8.99E+06	3.76E+07	3.07E+07	1.37E+08	4.81E+06	6.37E+07
P02533	Keratin, type I cytoskeletal 14 [OS=Homo sapiens]	1.36E+07		2.92E+06	5.09E+06	1.11E+07	1.36E+07	2.66E+07	3.67E+06	4.35E+07	4.33E+06
P02671-1	Fibrinogen alpha chain [OS=Homo sapiens]	3.04E+08	5.55E+08	2.49E+07	1.49E+07		1.63E+08	3.6E+08	2.76E+08	2.68E+07	1.4E+08
P02749	Beta-2-glycoprotein 1 [OS=Homo sapiens]	2.05E+08	4.53E+08	3.98E+07	2.29E+07		2.72E+07	1.07E+08	2.99E+08	2.93E+07	7E+07
P02750	Leucine-rich alpha-2-glycoprotein [OS=Homo sapiens]	5.91E+07	1.41E+08	1.15E+07		2.26E+08	6.2E+07	1.12E+09	1.69E+09	1.14E+08	2.73E+07
P02753	Retinol-binding protein 4 [OS=Homo sapiens]	8.21E+07	1.21E+08	2.3E+07	3.49E+06		4.39E+08	1.32E+08	9.92E+08	2.98E+07	3.14E+07
P04004	Vitronectin [OS=Homo sapiens]	1.36E+08	9.7E+07	1.17E+07	3.9E+06		6.58E+07	6.76E+07	1.63E+07	2.25E+07	4.22E+07
P05155-3	Isoform 3 of Plasma protease C1 inhibitor [OS=Homo sapiens]	1.01E+09	3.28E+08	3.29E+07	2.25E+07		8.13E+06	5.05E+08	1.74E+08	1.89E+07	1.89E+08

P09603	Macrophage colony-stimulating factor 1 [OS=Homo sapiens]	2.9E+07	2.73E+07	5E+06	2.68E+06		2.66E+06	2.94E+07	4.16E+07	3.16E+06	1.15E+07
P10909-2	Isoform 2 of Clusterin [OS=Homo sapiens]	5.25E+08	3.34E+08	3.22E+08		5.99E+06	3.81E+07	1.75E+08	8.94E+07	4.06E+07	1.61E+08
P11117	Lysosomal acid phosphatase [OS=Homo sapiens]	1.84E+08	9.47E+07	5.35E+07	1.08E+07	5.73E+06		3.66E+07	1.56E+08	3.41E+07	3.17E+07
P15144	Aminopeptidase N [OS=Homo sapiens]	5.74E+08	5.74E+08	1.26E+08	8.01E+06	1.8E+07		4.17E+08	3.51E+08	4.58E+07	8.29E+07
P16278	Beta-galactosidase [OS=Homo sapiens]	1.9E+08	1.57E+08	4.03E+07	4.33E+06	4.54E+06		4.03E+07	1.95E+08	1.63E+07	3.16E+07
P24855	Deoxyribonuclease-1 [OS=Homo sapiens]	5.05E+08	3.94E+08	6.28E+07	1.42E+07	1.03E+07		1.81E+08	3.63E+08	4.13E+07	2.47E+08
P30530	Tyrosine-protein kinase receptor UFO [OS=Homo sapiens]	2.34E+08	3.63E+08	3.8E+07	3.89E+07	1.39E+07		1.7E+08	5.71E+07	9.87E+06	5.35E+07
Q01459	Di-N-acetylchitobiase [OS=Homo sapiens]	4.65E+07	7.28E+07	1.34E+07	2.06E+06	1.48E+07		1.38E+08	2.11E+08	1.24E+07	6.87E+06
Q03403	Trefoil factor 2 [OS=Homo sapiens]	2.58E+07	9.85E+07	1.67E+07	1.09E+07		7.12E+07	5E+07	8.72E+07	4.58E+07	8.94E+07
Q13510-2	Isoform 2 of Acid ceramidase [OS=Homo sapiens]	9.06E+07	7.65E+07	3.08E+07	6.97E+06	7.79E+06		7.27E+07	1.39E+08	1.03E+07	1.09E+07
Q8TDQ0	Hepatitis A virus cellular receptor 2 [OS=Homo sapiens]	6.29E+07	8.94E+07	1.28E+07	9.24E+06	3.95E+06	5.53E+06	5.04E+07		1.18E+07	3.47E+07
Q8WVN6	Secreted and transmembrane protein 1 [OS=Homo sapiens]	1.04E+09	1.27E+09	3.87E+07	6.2E+07		9.83E+07	5.63E+08	1.48E+09	3.6E+07	3.81E+08
Q9HCU0	Endosialin [OS=Homo sapiens]	3.7E+08	2.15E+08	3.73E+07	4.16E+07		1.1E+07	2.1E+08	2.76E+08	6.85E+07	1.09E+08
Q9UKU9	Angiopoietin-related protein 2 [OS=Homo sapiens]	8.25E+07	1.79E+08	1.81E+06	6.96E+06		2.48E+06	2.44E+08	2.06E+07	2.8E+06	1.28E+07

O75882-1	Attractin [OS=Homo sapiens]	1.16E+08	1.45E+08	2.39E+07	4.06E+06			1.04E+08	3.91E+07	5.53E+06	1.31E+07
P01024	Complement C3 [OS=Homo sapiens]	2.5E+07	1.05E+07	2.51E+07		1.96E+07	3.55E+08	6.79E+07		2.66E+07	2.2E+08
P01034	Cystatin-C [OS=Homo sapiens]	3.65E+07	4.83E+07	3.16E+07	6.03E+06		1.95E+07		8.86E+07	2.22E+07	4.68E+07
P01614	Immunoglobulin kappa variable 2D-40 [OS=Homo sapiens]	2.1E+08	6.64E+08		7E+07	1.86E+08	1.25E+08	4.54E+08	1.84E+09	2.88E+07	
P04745	Alpha-amylase 1 [OS=Homo sapiens]	1.47E+08	8.19E+09	3.15E+07		2.84E+08	4.09E+08	1.01E+09	1.69E+08		2.64E+07
P07195	L-lactate dehydrogenase B chain [OS=Homo sapiens]	5.34E+07	4.26E+07	1.81E+07	5.06E+06			1.81E+08	2.02E+08	1.78E+07	1.18E+07
P14543-1	Nidogen-1 [OS=Homo sapiens]	8.14E+07	1.72E+08	1.12E+07	2.32E+06		1.98E+07	1.75E+08	2.03E+08		5.96E+07
P15328	Folate receptor alpha [OS=Homo sapiens]	6.23E+07	3.51E+07	6.45E+06	3.34E+06			2.97E+07	2.83E+07	6.29E+06	2.64E+07
P19835-1	Bile salt-activated lipase [OS=Homo sapiens]	9.78E+07	1.12E+08	1E+07	3.47E+06			9.3E+07	2.2E+07	1.09E+07	7.42E+07
P22891-2	Isoform 2 of Vitamin K-dependent protein Z [OS=Homo sapiens]	5.9E+07	5.39E+07		1.81E+07	5.43E+06		3.8E+07	5.42E+07	5.51E+06	4.64E+07
P35555	Fibrillin-1 [OS=Homo sapiens]	1.98E+07	3.84E+07	6.08E+06	4.92E+06			2.57E+07	3.8E+07	6.92E+06	1.68E+07
Q07507	Dermatopontin [OS=Homo sapiens]	4.9E+07	9.38E+07	6.22E+06	5.88E+06		1.65E+07	1.29E+08	1.27E+08	2.01E+07	
Q96RW7-1	Hemicentin-1 [OS=Homo sapiens]	7.85E+07	1.26E+08	1.1E+07		6.58E+06		1.43E+08	1.79E+08	1.82E+07	2.84E+07
Q9BXP8-1	Pappalysin-2 [OS=Homo sapiens]	1.95E+08	5.54E+08	1.93E+07	9.09E+06			1.95E+08	2.01E+07	3.7E+06	6.74E+06
Q9HAT2	Sialate O-acetylesterase [OS=Homo sapiens]	3.81E+07	9.94E+07	1.15E+07		1.22E+06		5.34E+07	7.19E+07	9.56E+06	3.85E+06

Q9Y646	Carboxypeptidase Q [OS=Homo sapiens]	6.55E+07	7.15E+07	1.87E+07	8.16E+06	8.47E+07	1.5E+08	1.01E+07	1.18E+07

Table S1. Continued

		Abundances								
Accession	**Description**	**Control man**	**Control man**	**Control man**	**Control man**	**HFrEF man**	**HFrEF man**	**HFrEF man**	**HFrEF man**	**HFrEF man**
O00391	Sulfhydryl oxidase 1 [OS=Homo sapiens]	2.36E+07	8.82E+06	2.2E+07	7.22E+07	8.84E+07	7.24E+07	2.28E+07	3.42E+06	1.11E+06
O43451	Maltase-glucoamylase, intestinal [OS=Homo sapiens]	5.91E+07	9.08E+06	1.63E+08	2.99E+08	1.66E+08	4.96E+08	4.96E+07	2.18E+07	2.68E+06
O60494	Cubilin [OS=Homo sapiens]	6.08E+08	9.05E+06	1.34E+08	3.42E+08	3.14E+08	6.89E+08	1.01E+08	2.71E+07	7.85E+06
O75594	Peptidoglycan recognition protein 1 [OS=Homo sapiens]	1.86E+08	3.89E+07	5.01E+07	1.06E+09	5.25E+08	9.38E+08	5.87E+07	3.9E+07	1.23E+07
P00450	Ceruloplasmin [OS=Homo sapiens]	1.69E+08	1.3E+08	1.64E+09	8.97E+08	1.49E+08	1.02E+09	2.97E+07	4.2E+07	7.02E+08
P00734	Prothrombin [OS=Homo sapiens]	1.49E+08	4.64E+07	1.75E+08	5.71E+08	1.52E+08	3.69E+08	8.55E+07	7.54E+07	4.68E+07
P01009-1	Alpha-1-antitrypsin [OS=Homo sapiens]	3.15E+08	5.6E+08	1.07E+09	7.28E+08	9.44E+08	5.96E+08	1.18E+08	5.58E+07	1.06E+09
P01011-1	Alpha-1-antichymotrypsin [OS=Homo sapiens]	2.39E+08	2.17E+08	7.09E+08	2.04E+09	3.7E+08	1.5E+09	1.78E+08	6.73E+07	4.81E+08
P01133-1	Pro-epidermal growth factor [OS=Homo sapiens]	8.24E+08	6.7E+07	1.98E+08	7.86E+08	2.27E+09	2.03E+09	3.08E+08	1.22E+08	4E+07
P01619	Immunoglobulin kappa variable 3-20 [OS=Homo sapiens]	4.61E+07	1.22E+08	1.62E+08	1.09E+09	3.27E+08	1.67E+09	6.78E+07	1.3E+08	1.49E+08

P01833	Polymeric immunoglobulin receptor [OS=Homo sapiens]	7.91E+08	5.22E+08	8.55E+08	2.13E+09	2.75E+09	4.05E+09	6.42E+08	1.67E+08	3E+08
P01834	Immunoglobulin kappa constant [OS=Homo sapiens]	2.64E+09	2.89E+09	8.48E+09	8.95E+09	5.14E+09	8.24E+09	1.13E+09	2.46E+09	3.99E+09
P01859	Immunoglobulin heavy constant gamma 2 [OS=Homo sapiens]	7.2E+08	1.06E+09	6.63E+09	1.8E+09	3.09E+09	1.57E+09	2.82E+08	1.04E+09	1.32E+09
P01871-2	Isoform 2 of Immunoglobulin heavy constant mu [OS=Homo sapiens]	7.84E+06	8.07E+06	1.27E+07	4.44E+08	2.9E+07	8.85E+07	9.85E+06	6.27E+06	6.89E+07
P01876	Immunoglobulin heavy constant alpha 1 [OS=Homo sapiens]	1.09E+09	2.26E+09	2.01E+09	2.26E+09	1.57E+09	8.22E+09	1.53E+09	1.63E+08	1.66E+09
P02760	Protein AMBP [OS=Homo sapiens]	2.77E+09	8.37E+08	1.4E+10	4.27E+10	9.23E+09	1.57E+10	1.97E+09	2.94E+09	4.1E+09
P02763	Alpha-1-acid glycoprotein 1 [OS=Homo sapiens]	1.58E+08	2.44E+08	3.97E+09	2.86E+09	1.63E+08	4.43E+08	7.16E+07	5.6E+07	1.67E+09
P02765	Alpha-2-HS-glycoprotein [OS=Homo sapiens]	2.31E+07	5.53E+07	1.52E+08	2.11E+08	3.14E+08	2.11E+08	1.49E+07	6.56E+06	3.42E+06
P02768-1	Serum albumin [OS=Homo sapiens]	3.44E+10	4.57E+10	1.2E+11	3.93E+10	4.32E+10	7.62E+10	1.66E+10	1.13E+10	1.74E+11
P02774-3	Isoform 3 of Vitamin D-binding protein [OS=Homo sapiens]	7.12E+07	1.72E+08	1.12E+08	1.81E+08	1.47E+08	1.42E+08	1.64E+07	6.34E+06	2.27E+07
P02787	Serotransferrin [OS=Homo sapiens]	7.69E+08	1.61E+09	6.92E+09	8.47E+08	1.1E+09	1.34E+09	3.21E+08	2.58E+08	7.83E+09
P02788	Lactotransferrin [OS=Homo sapiens]	2.29E+08	3.44E+09	5.32E+07	7.61E+08	1.84E+08	1.22E+08	1.73E+09	1.93E+07	2.22E+08

P02790	Hemopexin [OS=Homo sapiens]	1.66E+08	2.73E+08	2.89E+08	6.91E+08	3.14E+08	5.24E+08	5.18E+07	2.92E+07	7.49E+07
P04217	Alpha-1B-glycoprotein [OS=Homo sapiens]	1.82E+08	7.2E+07	1.61E+09	1.61E+09	1.52E+08	6.5E+08	2.23E+07	3.26E+07	6.96E+08
P04264	Keratin, type II cytoskeletal 1 [OS=Homo sapiens]	1.28E+09	5.14E+08	4.38E+08	5.76E+08	2.48E+08	3.93E+08	5.34E+08	2.8E+08	4.05E+08
P04746	Pancreatic alpha-amylase [OS=Homo sapiens]	1.43E+09	3.78E+08	6.66E+08	3.88E+09	3.47E+09	2.04E+09	3.07E+08	3.41E+08	6.41E+07
P05090	Apolipoprotein D [OS=Homo sapiens]	5.06E+08	1.76E+08	1.06E+09	5.27E+09	7.15E+08	3.14E+09	3.18E+08	2.23E+08	2.26E+08
P05154	Plasma serine protease inhibitor [OS=Homo sapiens]	7.67E+08	1.59E+08	4.95E+08	1.55E+09	2.86E+09	2.84E+09	5.51E+08	1.44E+08	6.66E+07
P06396	Gelsolin [OS=Homo sapiens]	5.86E+07	1.75E+08	7.07E+07	2E+09	3.24E+08	5.75E+08	2.33E+07	6.67E+07	3.36E+07
P06870-1	Kallikrein-1 [OS=Homo sapiens]	4.13E+07	3.77E+07	1.71E+08	2.67E+08	7.98E+08	2.45E+08	1.05E+08	3.56E+07	9.12E+07
P07339	Cathepsin D [OS=Homo sapiens]	9.92E+07	2.95E+07	3.98E+08	5.67E+08	4.81E+08	6.05E+08	2.28E+08	2.56E+07	5.61E+07
P07911-5	Isoform 5 of Uromodulin [OS=Homo sapiens]	1.33E+09	3.75E+08	5.95E+09	4.1E+10	1.05E+11	3.04E+10	2.51E+09	2.55E+09	6E+09
P07998	Ribonuclease pancreatic [OS=Homo sapiens]	1.87E+08	1.58E+07	1.19E+08	5.89E+08	2.08E+08	5.83E+08	6.93E+07	8.98E+07	7.46E+07
P08174-7	Isoform 7 of Complement decay-accelerating factor [OS=Homo sapiens]	7.92E+07	1.75E+07	2.74E+08	6.66E+08	1.34E+08	8.4E+08	2.13E+07	3.74E+07	3.42E+07
P08571	Monocyte differentiation antigen CD14 [OS=Homo sapiens]	1.32E+08	3.75E+07	7.15E+08	1.02E+09	2.98E+08	3.01E+08	6.49E+07	1.2E+07	8.42E+07

P0DOX2	Immunoglobulin alpha-2 heavy chain [OS=Homo sapiens]	6.75E+07	1.5E+08	1.42E+08	1.56E+08	6E+07	3.28E+08	4.42E+07	8.59E+06	5.91E+07
P0DOX5	Immunoglobulin gamma-1 heavy chain [OS=Homo sapiens]	1.15E+09	1.19E+09	7.59E+08	2.99E+09	6.84E+08	2.33E+09	3.63E+08	2.03E+08	1.22E+09
P0DOY2	Immunoglobulin lambda constant 2 [OS=Homo sapiens]	4.56E+08	6.52E+08	7.84E+08	4.07E+09	5.38E+08	2.68E+09	1.87E+08	2.84E+08	9.58E+08
P10153	Non-secretory ribonuclease [OS=Homo sapiens]	9.21E+08	7.41E+07	4.94E+08	9.31E+08	1.27E+09	2.21E+09	1.41E+08	1.92E+08	7.38E+07
P10253	Lysosomal alpha-glucosidase [OS=Homo sapiens]	5.53E+08	2.7E+07	1.08E+09	5.31E+08	8.21E+08	1.33E+09	1.73E+08	5.64E+07	3.13E+07
P11117	Lysosomal acid phosphatase [OS=Homo sapiens]	9.97E+07	8.68E+06	1.26E+08	4.82E+07	1.84E+08	9.47E+07	5.35E+07	1.08E+07	5.73E+06
P12109	Collagen alpha-1(VI) chain [OS=Homo sapiens]	2.48E+08	1.92E+07	1.84E+08	3.42E+08	8.99E+08	7.46E+08	6.2E+07	3.12E+07	8.12E+06
P12830	Cadherin-1 [OS=Homo sapiens]	8.52E+07	1.05E+07	3.66E+08	1.1E+09	7.96E+07	8.52E+08	4.13E+07	2.33E+07	4.44E+07
P13645	Keratin, type I cytoskeletal 10 [OS=Homo sapiens]	7.03E+08	2.02E+08	2.61E+08	2.78E+08	1.31E+08	1.63E+08	2.77E+08	1.64E+08	2.63E+08
P13987	CD59 glycoprotein [OS=Homo sapiens]	3.23E+08	1.31E+08	3.04E+08	1.21E+09	7.72E+08	1.5E+09	2.36E+08	1.57E+08	1.03E+08
P15144	Aminopeptidase N [OS=Homo sapiens]	1.75E+08	2.42E+06	3.7E+08	6.15E+08	5.74E+08	5.74E+08	1.26E+08	8.01E+06	1.8E+07
P15586	N-acetylglucosamine-6-sulfatase [OS=Homo sapiens]	7.16E+07	1.26E+07	2.75E+08	2.62E+08	1.25E+08	2.51E+08	6.23E+07	1.77E+07	4.83E+07
P16070	CD44 antigen [OS=Homo sapiens]	3.19E+08	5.89E+07	5.1E+08	7.92E+08	8.24E+08	1.4E+09	1.54E+08	8.43E+07	6.17E+07
P16444	Dipeptidase 1 [OS=Homo sapiens]	3.74E+07	2.74E+06	7.5E+07	1.02E+08	2.22E+08	1.96E+08	4.01E+07	1.4E+07	1.93E+06

P19652	Alpha-1-acid glycoprotein 2 [OS=Homo sapiens]	1.7E+07	2.88E+07	1.83E+09	7.63E+08	4.31E+07	8.64E+07	2.87E+07	3.72E+06	1.79E+08
P19961	Alpha-amylase 2B [OS=Homo sapiens]	4.42E+07	7.15E+06	7.61E+07	6.15E+07	3.33E+08	3.59E+08	4.94E+07	1.94E+07	5.11E+06
P24855	Deoxyribonuclease-1 [OS=Homo sapiens]	1.31E+08	6.57E+06	8.56E+07	1.77E+08	5.05E+08	3.94E+08	6.28E+07	1.42E+07	1.03E+07
P25311	Zinc-alpha-2-glycoprotein [OS=Homo sapiens]	3.98E+08	2.78E+08	7.71E+09	3.9E+09	5.71E+08	7.35E+08	3.15E+08	1.15E+08	3.23E+09
P35527	Keratin, type I cytoskeletal 9 [OS=Homo sapiens]	4.13E+08	1.76E+08	8.12E+07	1.28E+08	7.42E+07	6.94E+07	1.24E+08	6.15E+07	1.14E+08
P35908	Keratin, type II cytoskeletal 2 epidermal [OS=Homo sapiens]	2.04E+08	6.75E+07	6.6E+07	8.32E+07	5.36E+07	5.06E+07	1.23E+08	2.33E+07	9.92E+07
P41222	Prostaglandin-H2 D-isomerase [OS=Homo sapiens]	8.09E+08	2.43E+08	1.85E+09	1.32E+10	1.15E+09	5.83E+09	6.99E+08	8.79E+08	1.41E+09
P54802	Alpha-N-acetylglucosaminidase [OS=Homo sapiens]	7.64E+07	7.43E+06	4.05E+08	2.1E+08	3.24E+08	2.92E+08	6.01E+07	9.34E+06	2.85E+07
P80188	Neutrophil gelatinase-associated lipocalin [OS=Homo sapiens]	1.54E+07	8.29E+08	1.7E+07	4.17E+07	1.82E+07	4.44E+07	3.36E+07	4.32E+06	1.67E+07
P98160	Basement membrane-specific heparan sulfate proteoglycan core protein [OS=Homo sapiens]	2.3E+08	7.79E+07	1.23E+08	4.19E+09	1.51E+09	4.68E+09	1.77E+08	5.11E+08	4.59E+07
P98164	Low-density lipoprotein receptor-related protein 2 [OS=Homo sapiens]	4.76E+08	8.47E+06	4.84E+07	2.56E+08	1.24E+08	5.3E+08	1.29E+08	1.81E+07	1.14E+07

Q01459	Di-N-acetylchitobiase [OS=Homo sapiens]	4.25E+07	2.96E+06	1.18E+08	8.79E+07	4.65E+07	7.28E+07	1.34E+07	2.06E+06	1.48E+07
Q08380	Galectin-3-binding protein [OS=Homo sapiens]	6.24E+08	7E+07	7.78E+08	1.11E+09	1.56E+09	1.95E+09	3.5E+08	9.53E+07	1.34E+08
Q12805	EGF-containing fibulin-like extracellular matrix protein 1 [OS=Homo sapiens]	7.88E+07	1.83E+07	2.78E+07	1.29E+09	3.44E+08	1.26E+09	1.05E+08	2.09E+08	4.75E+06
Q12907	Vesicular integral-membrane protein VIP36 [OS=Homo sapiens]	3.24E+08	1.44E+07	3.2E+08	2.49E+09	6.17E+08	1.09E+09	2.83E+07	9.58E+07	1.16E+08
Q13510-2	Isoform 2 of Acid ceramidase [OS=Homo sapiens]	2.35E+07	4.96E+06	1.09E+08	4.86E+07	9.06E+07	7.65E+07	3.08E+07	6.97E+06	7.79E+06
Q14624-1	Inter-alpha-trypsin inhibitor heavy chain H4 [OS=Homo sapiens]	6.03E+08	2.23E+08	4.62E+08	4.7E+09	2.6E+09	3.24E+09	2.91E+08	3.3E+08	9.87E+07
Q16270-1	Insulin-like growth factor-binding protein 7 [OS=Homo sapiens]	3.56E+08	2.92E+07	2.03E+08	4.45E+08	9.05E+08	6.65E+08	1.91E+08	4.18E+07	6.8E+06
Q16769	Glutaminyl-peptide cyclotransferase [OS=Homo sapiens]	2.4E+08	3.73E+07	1.45E+08	4.31E+08	1E+09	4.99E+08	7.32E+07	4.45E+07	3.38E+07
Q6EMK4	Vasorin [OS=Homo sapiens]	5.68E+08	5.69E+07	3.49E+08	1.12E+09	1.54E+09	2.14E+09	2.62E+08	1.46E+08	9.12E+07
Q8TDQ0	Hepatitis A virus cellular receptor 2 [OS=Homo sapiens]	1.17E+07	2.43E+06	3.45E+07	7.31E+07	6.29E+07	8.94E+07	1.28E+07	9.24E+06	3.95E+06
Q9UNN8	Endothelial protein C receptor [OS=Homo sapiens]	1.78E+08	1.68E+07	2.33E+08	2.7E+08	2E+08	1.88E+08	4.59E+07	1.8E+07	2.09E+07

A0A0C4DH25	Immunoglobulin kappa variable 3D-20 [OS=Homo sapiens]	6.17E+07	1.7E+07	2.24E+08	1.2E+08	6.26E+07	1.72E+08		2.05E+07	2.1E+07
O14773	Tripeptidyl-peptidase 1 [OS=Homo sapiens]	6.28E+07		6.01E+07	4.73E+07	6.57E+07	5.46E+07	4.42E+07	1.48E+07	1.45E+07
O75882-1	Attractin [OS=Homo sapiens]	6.49E+07	5.73E+06	5.67E+07	9.85E+07	1.16E+08	1.45E+08	2.39E+07	4.06E+06	
O94919	Endonuclease domain-containing 1 protein [OS=Homo sapiens]	7.24E+06		6.02E+07	2.74E+08	2.74E+07	6.31E+07	8.11E+06	5.35E+06	1.24E+07
P01008	Antithrombin-III [OS=Homo sapiens]	1.72E+07	8.93E+06	3.14E+07	3.5E+07	1.62E+07	3.89E+07	5.99E+06		4.69E+06
P01024	Complement C3 [OS=Homo sapiens]	2.72E+07	2.04E+08	2.16E+07	3.29E+07	2.5E+07	1.05E+07	2.51E+07		1.96E+07
P01591	Immunoglobulin J chain [OS=Homo sapiens]	1.46E+07	4.22E+07	2.53E+07	2.99E+07	5E+06	4.76E+07	2.58E+07		1.82E+07
P01860	Immunoglobulin heavy constant gamma 3 [OS=Homo sapiens]		2.29E+09	3.34E+07	8.09E+06	8.32E+06	3.11E+07	7.97E+06	3.72E+06	1.15E+06
P01861	Immunoglobulin heavy constant gamma 4 [OS=Homo sapiens]	4.04E+07	6.29E+07	3.43E+07	9.03E+06	7.31E+06	3.11E+07		1.72E+06	8.99E+06
P02533	Keratin, type I cytoskeletal 14 [OS=Homo sapiens]	4.48E+07	1.39E+07	7.96E+06	1.54E+07	1.36E+07		2.92E+06	5.09E+06	1.11E+07
P02671-1	Fibrinogen alpha chain [OS=Homo sapiens]	6.92E+07	2.19E+07	3.36E+07	3.26E+08	3.04E+08	5.55E+08	2.49E+07	1.49E+07	
P02749	Beta-2-glycoprotein 1 [OS=Homo sapiens]	1.48E+07	2.46E+07	5.08E+07	4.46E+08	2.05E+08	4.53E+08	3.98E+07	2.29E+07	

P02750	Leucine-rich alpha-2-glycoprotein [OS=Homo sapiens]	9.95E+06	1.36E+07	7.38E+08	1.13E+09	5.91E+07	1.41E+08	1.15E+07		2.26E+08
P02753	Retinol-binding protein 4 [OS=Homo sapiens]	2.54E+07	2.01E+07	4.7E+07	2.15E+08	8.21E+07	1.21E+08	2.3E+07	3.49E+06	
P04745	Alpha-amylase 1 [OS=Homo sapiens]	1.06E+08	3.51E+06	4.38E+06	2.96E+08	1.47E+08	8.19E+09	3.15E+07		2.84E+08
P05155-3	Isoform 3 of Plasma protease C1 inhibitor [OS=Homo sapiens]	2.55E+08	1.82E+07	1.48E+08	3.59E+08	1.01E+09	3.28E+08	3.29E+07	2.25E+07	
P07195	L-lactate dehydrogenase B chain [OS=Homo sapiens]	1.54E+07	2.08E+07	1.39E+07	5.05E+07	5.34E+07	4.26E+07	1.81E+07	5.06E+06	
P07858	Cathepsin B [OS=Homo sapiens]	2.47E+07		3.11E+07	3.62E+07	2.6E+07	5.55E+07	2.19E+07	3.85E+06	6.22E+06
P08294	Extracellular superoxide dismutase [Cu-Zn] [OS=Homo sapiens]	5.7E+07	2.69E+06	1.87E+07	1.26E+08	1.7E+08	3.37E+08	6.66E+06	4.48E+06	
P10909-2	Isoform 2 of Clusterin [OS=Homo sapiens]	7.35E+07	1.72E+07	1.52E+08	4.38E+08	5.25E+08	3.34E+08	3.22E+08		5.99E+06
P16278	Beta-galactosidase [OS=Homo sapiens]	9.76E+07		1.66E+08	3.36E+07	1.9E+08	1.57E+08	4.03E+07	4.33E+06	4.54E+06
P19835-1	Bile salt-activated lipase [OS=Homo sapiens]	3.64E+07	2E+06	2.89E+07	1.06E+08	9.78E+07	1.12E+08	1E+07	3.47E+06	
P30530	Tyrosine-protein kinase receptor UFO [OS=Homo sapiens]	1.06E+08		1.7E+08	1.25E+08	2.34E+08	3.63E+08	3.8E+07	3.89E+07	1.39E+07
P35555	Fibrillin-1 [OS=Homo sapiens]	7.29E+06	2.68E+06	1.31E+07	4.11E+07	1.98E+07	3.84E+07	6.08E+06	4.92E+06	
Q8NFZ8	Cell adhesion molecule 4 [OS=Homo sapiens]	7.75E+07		6.08E+07	1.69E+08	2.39E+08	2.33E+08	4.39E+07	1.63E+07	2.96E+06

Q8WVN6	Secreted and transmembrane protein 1 [OS=Homo sapiens]	9.21E+07	2.91E+07	7.99E+07	1.49E+09	1.04E+09	1.27E+09	3.87E+07	6.2E+07	
Q8WZ75-1	Roundabout homolog 4 [OS=Homo sapiens]	5.24E+07		7.78E+07	2.7E+08	2.77E+08	5.61E+07	6.33E+06	1.35E+07	1.07E+07
Q9HCU0	Endosialin [OS=Homo sapiens]	6.58E+07	1.01E+07	4.59E+07	2.48E+08	3.7E+08	2.15E+08	3.73E+07	4.16E+07	
A0A0B4J1X5	Immunoglobulin heavy variable 3-74 [OS=Homo sapiens]	3.93E+06		1.49E+07	1.58E+07	3.07E+07	2.07E+07	1.14E+07	4.15E+06	
O95336	6-phosphogluconolactonase [OS=Homo sapiens]	1.75E+07		2.56E+07	1.15E+08	9.59E+07	8.71E+07	3.77E+06	4.14E+06	
P00558	Phosphoglycerate kinase 1 [OS=Homo sapiens]	8.67E+06	1.39E+08		3.53E+07	6.07E+07	2.51E+07	6.28E+06	1.89E+06	
P00738	Haptoglobin [OS=Homo sapiens]		1.13E+08	6.68E+09	6.7E+08	4.65E+08	1.01E+08	1.33E+07		8.53E+08
P01034	Cystatin-C [OS=Homo sapiens]	3.75E+07		2.07E+07	5.31E+07	3.65E+07	4.83E+07	3.16E+07	6.03E+06	
P01614	Immunoglobulin kappa variable 2D-40 [OS=Homo sapiens]		6.76E+07	2.14E+08	5.65E+08	2.1E+08	6.64E+08		7E+07	1.86E+08
P04004	Vitronectin [OS=Homo sapiens]	4.04E+07		3.03E+07	8.03E+07	1.36E+08	9.7E+07	1.17E+07	3.9E+06	
P07288-1	Prostate-specific antigen [OS=Homo sapiens]	2.36E+07		1.76E+08	1.47E+08	5.32E+08	1.2E+08		2.62E+07	1.06E+07
P09603	Macrophage colony-stimulating factor 1 [OS=Homo sapiens]	1.13E+07		2.09E+07	8.05E+07	2.9E+07	2.73E+07	5E+06	2.68E+06	
P10451-1	Osteopontin [OS=Homo sapiens]	7.1E+07		3.4E+07	8.82E+07	3.98E+07	5.55E+08	1.31E+07	1.32E+08	

P14543-1	Nidogen-1 [OS=Homo sapiens]	3.94E+07	2.54E+07	2.65E+08	8.14E+07	1.72E+08	1.12E+07	2.32E+06	
P15151-1	Poliovirus receptor [OS=Homo sapiens]	1.53E+07	1.12E+08	2.89E+07	3.38E+07	5.77E+07	1.83E+06	3.5E+06	
P15328	Folate receptor alpha [OS=Homo sapiens]	1.4E+07	6.54E+06	6.44E+07	6.23E+07	3.51E+07	6.45E+06	3.34E+06	
P22891-2	Isoform 2 of Vitamin K-dependent protein Z [OS=Homo sapiens]	9.84E+07	6.4E+07	1.2E+08	5.9E+07	5.39E+07		1.81E+07	5.43E+06
P39059	Collagen alpha-1(XV) chain [OS=Homo sapiens]	4.99E+07	9.58E+07	2.37E+08	1.49E+08	1.11E+08	1.76E+07	1.04E+07	
Q03403	Trefoil factor 2 [OS=Homo sapiens]	3.94E+07	5.24E+07	1.48E+08	2.58E+07	9.85E+07	1.67E+07	1.09E+07	
Q6UVK1	Chondroitin sulfate proteoglycan 4 [OS=Homo sapiens]	1.99E+07	7.41E+06	5.3E+07	3.54E+07	1.58E+07	7.85E+06	3.1E+06	
Q8IYS5-7	Isoform 7 of Osteoclast-associated immunoglobulin-like receptor [OS=Homo sapiens]	6.11E+07	2.67E+07	9.83E+07	6.61E+07	7.59E+07	9.85E+06	6.46E+06	
Q92820	Gamma-glutamyl hydrolase [OS=Homo sapiens]	1.61E+07	1.48E+07	1.71E+07	5.16E+07	1.53E+07	3.07E+06		5.5E+06
Q93088	Betaine--homocysteine S-methyltransferase 1 [OS=Homo sapiens]	3.17E+07	1.91E+07	2.87E+07	6.07E+07	2.7E+07	2.48E+07	5.37E+06	
Q96RW7-1	Hemicentin-1 [OS=Homo sapiens]	2.4E+07	1.12E+07	1.55E+08	7.85E+07	1.26E+08	1.1E+07		6.58E+06
Q9BRK3-2	Isoform 2 of Matrix remodeling-associated protein 8 [OS=Homo sapiens]	6.67E+06	9.68E+06	7.27E+07	4.22E+08	5.53E+07	4.96E+06	6.92E+06	

Q9H3G5	Probable serine carboxypeptidase CPVL [OS=Homo sapiens]	2.36E+07		2.79E+07	5.3E+07	1.27E+08	1.29E+08	5.49E+06	1.04E+07	
Q9HAT2	Sialate O-acetylesterase [OS=Homo sapiens]	1.91E+07		3.06E+07	4.26E+07	3.81E+07	9.94E+07	1.15E+07		1.22E+06
Q9UKU9	Angiopoietin-related protein 2 [OS=Homo sapiens]	1.58E+07		1.74E+06	1.53E+08	8.25E+07	1.79E+08	1.81E+06	6.96E+06	
Q9Y646	Carboxypeptidase Q [OS=Homo sapiens]	3.63E+07		7.38E+07	3.5E+07	6.55E+07	7.15E+07	1.87E+07		8.16E+06

Table S1. Continued.

		Abundances								
Accession	**Description**	**Control woman**	**Control woman**	**Control woman**	**Control woman**	**HFrEF woman**	**HFrEF woman**	**HFrEF woman**	**HFrEF woman**	**HFrEF woman**
A0A0C4DH25	Immunoglobulin kappa variable 3D-20 [OS=Homo sapiens]	4.3E+07	1.6E+07	1.34E+07	1.39E+07	2.98E+07	1.31E+08	4.2E+08	2.59E+07	2.28E+07
O14773	Tripeptidyl-peptidase 1 [OS=Homo sapiens]	8.56E+07	6.16E+07	8.4E+07	8.07E+07	1.45E+07	6.86E+07	1.21E+08	4E+07	4.49E+07
O60494	Cubilin [OS=Homo sapiens]	2.72E+08	4.02E+08	1.85E+08	9.84E+07	5.02E+06	2.26E+08	5.2E+07	3.99E+07	9E+07
O75594	Peptidoglycan recognition protein 1 [OS=Homo sapiens]	3.57E+08	6.65E+07	1.62E+07	3.88E+07	7.12E+07	1.02E+09	9.16E+08	8.21E+07	1.11E+08
O94919	Endonuclease domain-containing 1 protein [OS=Homo sapiens]	5.55E+07	1.2E+07	2.29E+07	5.19E+07	1.12E+06	1.07E+08	2.5E+08	8.79E+06	1.28E+07
P00450	Ceruloplasmin [OS=Homo sapiens]	5.29E+08	3.2E+08	7.11E+07	6.28E+08	3.28E+08	9.1E+08	2.04E+08	2.28E+08	5.29E+08

P00734	Prothrombin [OS=Homo sapiens]	1.63E+08	1.95E+08	2.17E+08	1.5E+08	1.09E+08	2.18E+08	3.39E+08	1E+08	2.25E+08
P01008	Antithrombin-III [OS=Homo sapiens]	2.24E+07	6.64E+07	4.89E+06	9.48E+06	4.82E+07	6.68E+07	7.65E+07	1.95E+07	2.97E+07
P01009-1	Alpha-1-antitrypsin [OS=Homo sapiens]	9.26E+08	9.41E+08	9.2E+07	4.73E+08	8.16E+08	1.12E+09	3.79E+08	8.87E+07	1.05E+09
P01011-1	Alpha-1-antichymotrypsin [OS=Homo sapiens]	8.99E+08	3.08E+08	2.9E+08	3.17E+08	2.29E+08	1.45E+09	1.72E+09	3.67E+08	5.18E+08
P01133-1	Pro-epidermal growth factor [OS=Homo sapiens]	1.11E+09	9.45E+08	3.66E+08	1.43E+08	5.04E+07	1.01E+09	1.43E+08	1.96E+08	5.64E+08
P01619	Immunoglobulin kappa variable 3-20 [OS=Homo sapiens]	3.2E+08	1.17E+08	1.52E+08	1.1E+08	5.14E+08	1.13E+09	4.93E+09	2.39E+08	1.63E+08
P01833	Polymeric immunoglobulin receptor [OS=Homo sapiens]	8.18E+08	9.63E+08	6.32E+08	3.39E+08	7.51E+07	2.26E+09	1.41E+09	2.97E+08	1.86E+09
P01834	Immunoglobulin kappa constant [OS=Homo sapiens]	7.53E+09	2.66E+09	2.87E+09	2.73E+09	3.01E+09	2.71E+10	2.15E+10	4.73E+09	2.5E+10
P01859	Immunoglobulin heavy constant gamma 2 [OS=Homo sapiens]	2.51E+09	1.13E+09	1.26E+09	5.16E+08	6.92E+08	2.67E+09	3.16E+09	5.84E+08	2.2E+10
P01876	Immunoglobulin heavy constant alpha 1 [OS=Homo sapiens]	1.1E+09	1.77E+09	5.19E+08	6.82E+08	8.05E+08	4.2E+09	7.17E+08	1.14E+09	1.11E+07
P02533	Keratin, type I cytoskeletal 14 [OS=Homo sapiens]	2.83E+07	2.86E+07	1.21E+07	5.69E+06	1.36E+07	2.66E+07	3.67E+06	4.35E+07	4.33E+06
P02671-1	Fibrinogen alpha chain [OS=Homo sapiens]	1.21E+08	2.52E+07	2.64E+07	1.97E+07	1.63E+08	3.6E+08	2.76E+08	2.68E+07	1.4E+08

P02749	Beta-2-glycoprotein 1 [OS=Homo sapiens]	8.6E+07	1.11E+08	1.1E+08	2E+07	2.72E+07	1.07E+08	2.99E+08	2.93E+07	7E+07
P02750	Leucine-rich alpha-2-glycoprotein [OS=Homo sapiens]	2.24E+08	6.27E+07	6.25E+07	7.26E+08	6.2E+07	1.12E+09	1.69E+09	1.14E+08	2.73E+07
P02751-15	Isoform 15 of Fibronectin [OS=Homo sapiens]	8.8E+07	4.08E+07	3.16E+07	1.52E+07	1.43E+07	1.48E+08	8.78E+07	3.63E+07	4.51E+07
P02753	Retinol-binding protein 4 [OS=Homo sapiens]	1.61E+08	6.95E+07	7.71E+07	4.3E+06	4.39E+08	1.32E+08	9.92E+08	2.98E+07	3.14E+07
P02760	Protein AMBP [OS=Homo sapiens]	8.75E+09	4.69E+09	2.1E+10	9.26E+09	7.32E+09	3.44E+10	7.04E+10	1.12E+10	3.32E+09
P02763	Alpha-1-acid glycoprotein 1 [OS=Homo sapiens]	2.87E+08	3.33E+08	2.87E+08	2.11E+09	7.57E+07	7.38E+08	2.25E+09	3.24E+08	3.02E+08
P02765	Alpha-2-HS-glycoprotein [OS=Homo sapiens]	2.47E+08	1.04E+08	1.14E+09	3.09E+07	2.52E+08	3.68E+08	1.07E+09	7.94E+07	4.71E+07
P02768-1	Serum albumin [OS=Homo sapiens]	2.68E+10	6.63E+10	5.58E+10	2.79E+10	4.19E+10	5.32E+10	6.15E+10	3.67E+10	8.26E+10
P02774-3	Isoform 3 of Vitamin D-binding protein [OS=Homo sapiens]	1.34E+08	2.08E+08	1.27E+08	2.88E+07	2.6E+08	1.27E+08	2.75E+08	7.54E+07	2.08E+08
P02787	Serotransferrin [OS=Homo sapiens]	6.46E+08	2.67E+09	1.52E+09	2.43E+09	2.74E+09	1.64E+09	3.92E+09	1.35E+09	3.75E+09
P02790	Hemopexin [OS=Homo sapiens]	2.69E+08	3.85E+08	2.41E+08	1.37E+08	4.16E+08	6.01E+08	6.96E+08	1.63E+08	6.71E+08
P04004	Vitronectin [OS=Homo sapiens]	6.93E+07	5E+07	7.81E+06	1.46E+07	6.58E+07	6.76E+07	1.63E+07	2.25E+07	4.22E+07
P04083	Annexin A1 [OS=Homo sapiens]	2.26E+08	2.24E+08	6.01E+07	3.82E+08	3.83E+07	1.11E+09	2.46E+08	1.19E+08	3.06E+08
P04180	Phosphatidylcholine-sterol acyltransferase [OS=Homo sapiens]	3.01E+07	1.31E+07	1.68E+07	7.18E+05	8.28E+05	2.72E+07	2.56E+07	9.1E+06	1.03E+07

P04217	Alpha-1B-glycoprotein [OS=Homo sapiens]	6.52E+08	3.39E+08	9.28E+07	1.18E+09	2.97E+08	1.54E+09	3.59E+08	1.58E+08	1.76E+08
P04264	Keratin, type II cytoskeletal 1 [OS=Homo sapiens]	1.19E+09	2.51E+09	7.19E+08	4.32E+08	5.29E+08	6.36E+08	4.37E+08	2.22E+08	5.34E+08
P04406	Glyceraldehyde-3-phosphate dehydrogenase [OS=Homo sapiens]	7.61E+07	4.4E+07	1.81E+07	5.36E+07	9.62E+06	1.05E+08	3.26E+07	1.93E+07	9.22E+07
P04746	Pancreatic alpha-amylase [OS=Homo sapiens]	1.08E+09	7.98E+08	5.73E+08	4.21E+07	7.38E+07	5.48E+09	1.85E+09	7.75E+08	8.23E+08
P05090	Apolipoprotein D [OS=Homo sapiens]	2.13E+09	3.54E+08	3.25E+08	9.4E+08	2.36E+08	2.38E+09	4.05E+09	5.45E+08	7.9E+08
P05154	Plasma serine protease inhibitor [OS=Homo sapiens]	1.22E+09	8.3E+08	7.25E+07	4.58E+08	7.88E+07	1.88E+09	1.42E+08	3.21E+08	1.45E+09
P05155-3	Isoform 3 of Plasma protease C1 inhibitor [OS=Homo sapiens]	4.81E+08	3.69E+08	3.65E+07	9.53E+07	8.13E+06	5.05E+08	1.74E+08	1.89E+07	1.89E+08
P05543	Thyroxine-binding globulin [OS=Homo sapiens]	2.34E+08	2.9E+07	6.7E+07	7.04E+07	2.06E+07	2.97E+08	1.37E+08	2.8E+07	3.22E+07
P06396	Gelsolin [OS=Homo sapiens]	1.6E+08	6.78E+07	2.06E+08	1.14E+08	9.84E+07	1.34E+09	1.95E+09	1.68E+08	7.37E+07
P06702	Protein S100-A9 [OS=Homo sapiens]	9.12E+07	1.33E+07	3.31E+07	5.72E+07	1.9E+07	1.17E+09	8.44E+06	5.5E+06	2.24E+08
P06733-1	Alpha-enolase [OS=Homo sapiens]	4.05E+07	2.01E+07	1.58E+07	2.52E+07	1.24E+06	1.06E+08	1.22E+07	5.61E+06	5.23E+07
P06870-1	Kallikrein-1 [OS=Homo sapiens]	2.89E+08	1.12E+08	5.29E+08	4.44E+07	2.97E+07	1.77E+08	1.19E+08	7.64E+06	1.1E+08
P07339	Cathepsin D [OS=Homo sapiens]	1.58E+08	1.16E+08	1.16E+09	1.58E+08	2.39E+07	6.42E+08	1.75E+09	1.22E+08	1.5E+08
P07858	Cathepsin B [OS=Homo sapiens]	1.35E+07	1.96E+07	3.53E+07	1.97E+07	6.91E+06	8.66E+07	3.19E+08	4.24E+06	1.59E+07

P07911-5	Isoform 5 of Uromodulin [OS=Homo sapiens]	5.06E+10	3.04E+09	2.24E+09	1.59E+10	8.43E+08	6.69E+10	4.14E+09	1.02E+09	1.97E+09
P07998	Ribonuclease pancreatic [OS=Homo sapiens]	3.52E+08	2.11E+08	5.25E+08	1.01E+08	6.52E+07	2.93E+08	5.46E+08	4.79E+07	1.62E+08
P08174-7	Isoform 7 of Complement decay-accelerating factor [OS=Homo sapiens]	2.97E+08	3.92E+07	1.17E+08	1.19E+08	1.7E+07	5.24E+08	3.84E+08	6.95E+07	5.01E+07
P08571	Monocyte differentiation antigen CD14 [OS=Homo sapiens]	8.4E+08	1.49E+08	4.63E+08	2.17E+08	7.86E+07	1.08E+09	2.95E+09	1.17E+08	4.4E+07
P09211	Glutathione S-transferase P [OS=Homo sapiens]	3.01E+07	1.87E+07	1.7E+07	3.38E+07	6.32E+06	6.08E+07	3.5E+07	1.28E+07	2.68E+07
P0DOX2	Immunoglobulin alpha-2 heavy chain [OS=Homo sapiens]	6.72E+07	8.12E+07	3.57E+07	2E+07	4.72E+07	2.53E+08	1.16E+08	6.99E+07	4.23E+07
P0DOX5	Immunoglobulin gamma-1 heavy chain [OS=Homo sapiens]	1.53E+09	1.99E+09	3.95E+08	8.39E+08	6.97E+08	4.75E+09	3.36E+09	8.6E+08	4.42E+09
P0DOY2	Immunoglobulin lambda constant 2 [OS=Homo sapiens]	5.86E+08	2.93E+08	4.4E+08	4.9E+08	8.6E+08	3.9E+09	4.58E+09	6.17E+08	5.15E+08
P10153	Non-secretory ribonuclease [OS=Homo sapiens]	7.72E+08	4.75E+08	3.66E+08	1.26E+08	8.01E+07	7.37E+08	1E+09	1.59E+08	3.86E+08
P10253	Lysosomal alpha-glucosidase [OS=Homo sapiens]	6.69E+08	4.65E+08	5.72E+08	3.02E+08	1.61E+07	4.85E+08	6.49E+08	1.77E+08	9.27E+07
P10909-2	Isoform 2 of Clusterin [OS=Homo sapiens]	1.29E+08	1.27E+08	5.73E+07	7.87E+07	3.81E+07	1.75E+08	8.94E+07	4.06E+07	1.61E+08

P12109	Collagen alpha-1(VI) chain [OS=Homo sapiens]	4.04E+08	2.19E+08	5.68E+07	9.38E+07	1.32E+07	4.43E+08	1.31E+08	3.25E+07	1.68E+08
P12830	Cadherin-1 [OS=Homo sapiens]	3.04E+08	8.1E+07	4.44E+08	1.3E+08	4.52E+07	8.26E+08	1.19E+09	9.89E+07	2.46E+07
P13645	Keratin, type I cytoskeletal 10 [OS=Homo sapiens]	3.6E+08	7.35E+08	2.93E+08	1.92E+08	2.81E+08	2.66E+08	3.37E+08	7.67E+07	2.39E+08
P13647	Keratin, type II cytoskeletal 5 [OS=Homo sapiens]	1.52E+07	1.76E+07	1.84E+07	8.34E+06	1.1E+07	1.34E+07	4.31E+06	1.99E+06	5.88E+06
P13987	CD59 glycoprotein [OS=Homo sapiens]	5.81E+08	2.21E+08	2.62E+08	2.37E+08	1.12E+08	4.99E+08	1.1E+09	1.46E+08	6.08E+08
P15586	N-acetylglucosamine-6-sulfatase [OS=Homo sapiens]	1.32E+08	4.85E+07	1.37E+08	8.53E+07	3.09E+06	2.45E+08	5.95E+08	3.43E+07	4.17E+07
P16070	CD44 antigen [OS=Homo sapiens]	5.85E+08	3.71E+08	3.74E+08	2.53E+08	3.21E+07	6.52E+08	5.2E+08	1.61E+08	3.71E+08
P16444	Dipeptidase 1 [OS=Homo sapiens]	7.54E+07	7.59E+06	2.83E+07	1.44E+07	2.45E+06	1.01E+08	2.04E+07	7.67E+06	7.65E+07
P19652	Alpha-1-acid glycoprotein 2 [OS=Homo sapiens]	1.54E+08	1.35E+08	3.04E+08	5.26E+08	6.61E+07	2.53E+08	1.35E+09	6.92E+07	7.68E+07
P19961	Alpha-amylase 2B [OS=Homo sapiens]	7.36E+07	1.57E+07	2.79E+07	3.74E+06	4.59E+06	7.26E+07	5.94E+07	3.93E+07	7.5E+07
P25311	Zinc-alpha-2-glycoprotein [OS=Homo sapiens]	7.37E+08	6.98E+08	1.49E+09	4.83E+09	4.65E+08	2.75E+09	9.15E+09	1.23E+09	3.93E+08
P29508	Serpin B3 [OS=Homo sapiens]	6.7E+08	2.71E+08	9.86E+07	1.52E+08	9.57E+07	1.39E+09	4.04E+07	1.51E+07	4.97E+08
P30086	Phosphatidylethanolamine-binding protein 1 [OS=Homo sapiens]	1.86E+07	7.55E+06	4.75E+06	4.06E+06	1.04E+06	2.56E+07	1.81E+08	3.94E+06	1.26E+07
P30740	Leukocyte elastase inhibitor [OS=Homo sapiens]	6.24E+07	3.68E+07	1.93E+07	4.45E+07	2.93E+07	6.12E+08	9.45E+06	7.29E+06	2.45E+08

P35527	Keratin, type I cytoskeletal 9 [OS=Homo sapiens]	6.41E+08	1.2E+09	2.92E+08	1.48E+08	1.7E+08	1.7E+08	7.48E+07	3.79E+07	1.2E+08
P35908	Keratin, type II cytoskeletal 2 epidermal [OS=Homo sapiens]	7.14E+07	3.36E+08	8.94E+07	9.86E+07	8.22E+07	9.51E+07	1.32E+08	1.41E+07	6.89E+07
P41222	Prostaglandin-H2 D-isomerase [OS=Homo sapiens]	2.09E+09	4.25E+08	2.1E+09	1.33E+09	1.17E+09	7.51E+09	1.24E+10	1.6E+09	5.46E+08
P42785-2	Isoform 2 of Lysosomal Pro-X carboxypeptidase [OS=Homo sapiens]	3.26E+07	1.53E+07	1.89E+07	7.53E+06	1.57E+06	4.47E+07	3.08E+07	7.31E+06	7.79E+06
P54802	Alpha-N-acetylglucosaminidase [OS=Homo sapiens]	1.9E+08	6.99E+07	2.14E+08	1.42E+08	5.56E+06	2.99E+08	2.65E+08	3.29E+07	4.58E+07
P55290-4	Isoform 4 of Cadherin-13 [OS=Homo sapiens]	3.38E+07	6.63E+06	2.52E+07	1.15E+07	1.23E+07	3.75E+07	9.08E+07	4.11E+06	4.82E+06
P80188	Neutrophil gelatinase-associated lipocalin [OS=Homo sapiens]	7.36E+07	1.55E+07	7.7E+07	1.3E+07	1.47E+08	3.72E+08	5.73E+07	1.86E+07	3.95E+08
P98160	Basement membrane-specific heparan sulfate proteoglycan core protein [OS=Homo sapiens]	1.01E+09	2.82E+08	7.62E+08	1.3E+08	5.64E+08	2.7E+09	9.4E+09	4.1E+08	4.01E+08
P98164	Low-density lipoprotein receptor-related protein 2 [OS=Homo sapiens]	1.42E+08	4.55E+08	2.1E+08	6.6E+07	3.41E+06	2.81E+08	5.74E+07	3.83E+07	2.5E+07
Q08380	Galectin-3-binding protein [OS=Homo sapiens]	7.58E+08	8.56E+08	2.07E+08	2.91E+08	3.63E+07	1.1E+09	2.6E+08	2.34E+08	5.68E+08

Q12805	EGF-containing fibulin-like extracellular matrix protein 1 [OS=Homo sapiens]	1.92E+08	1.36E+08	4.66E+07	2.91E+07	5.35E+07	8.8E+08	4.14E+08	1.49E+08	1.13E+08
Q12907	Vesicular integral-membrane protein VIP36 [OS=Homo sapiens]	1.04E+09	2.12E+08	4.34E+07	3.14E+08	1.76E+08	2.71E+09	1.6E+09	5.88E+07	1.83E+08
Q14624-1	Inter-alpha-trypsin inhibitor heavy chain H4 [OS=Homo sapiens]	1.18E+09	8.84E+08	4.56E+08	6.1E+08	4.99E+08	3.68E+09	2.83E+09	5.68E+08	1.39E+09
Q16270-1	Insulin-like growth factor-binding protein 7 [OS=Homo sapiens]	3.4E+08	3.42E+08	3.35E+07	9.55E+07	2.42E+07	4.09E+08	6.39E+07	1.09E+08	3.78E+08
Q16769	Glutaminyl-peptide cyclotransferase [OS=Homo sapiens]	6.03E+08	1.05E+08	1.84E+08	1.04E+08	5.2E+07	5.94E+08	5.39E+08	5.9E+07	6.92E+08
Q6EMK4	Vasorin [OS=Homo sapiens]	8.63E+08	7.16E+08	5.22E+08	3.6E+08	5.82E+07	1.24E+09	9.73E+08	2.67E+08	5.62E+08
Q8NFZ8	Cell adhesion molecule 4 [OS=Homo sapiens]	1.02E+08	2.89E+07	5.26E+07	2.99E+07	5.27E+06	8.22E+07	1.38E+08	2.11E+07	1.2E+08
Q8WVN6	Secreted and transmembrane protein 1 [OS=Homo sapiens]	3.6E+08	5.49E+07	7.84E+07	3.11E+07	9.83E+07	5.63E+08	1.48E+09	3.6E+07	3.81E+08
Q8WZ75-1	Roundabout homolog 4 [OS=Homo sapiens]	3.78E+08	1.32E+08	5.34E+06	7.22E+07	4.3E+06	1.17E+08	1.47E+07	3.89E+06	2.92E+07
Q96PD5-2	Isoform 2 of N-acetylmuramoyl-L-alanine amidase [OS=Homo sapiens]	4.82E+07	8.62E+07	1.23E+08	5.58E+07	4.61E+06	8.39E+07	4.17E+08	9.76E+06	3.57E+07

Q9HCU0	Endosialin [OS=Homo sapiens]	7.24E+07	8.61E+07	1.35E+08	7.04E+07	1.1E+07	2.1E+08	2.76E+08	6.85E+07	1.09E+08
Q9UKU9	Angiopoietin-related protein 2 [OS=Homo sapiens]	8.11E+07	2.03E+06	1.45E+06	6.03E+06	2.48E+06	2.44E+08	2.06E+07	2.8E+06	1.28E+07
Q9UNN8	Endothelial protein C receptor [OS=Homo sapiens]	7.47E+08	2.11E+08	5.61E+07	1.47E+08	6.48E+06	2.77E+08	3.4E+08	3.35E+07	1.68E+08
A0A0B4J1X5	Immunoglobulin heavy variable 3-74 [OS=Homo sapiens]	1.03E+07		4.84E+06	2.7E+06	1.83E+07	2.5E+07	2.69E+07	4.23E+06	1.37E+07
O00187-1	Mannan-binding lectin serine protease 2 [OS=Homo sapiens]	4.14E+08	5.03E+07	4.74E+07	7.35E+07	1.28E+08	1.83E+08	3.32E+06	6.14E+07	
O14498	Immunoglobulin superfamily containing leucine-rich repeat protein [OS=Homo sapiens]	3.41E+07	1.17E+07	1.04E+07	9.23E+06	3E+06	7.61E+07	4.37E+07		2.93E+06
O43451	Maltase-glucoamylase, intestinal [OS=Homo sapiens]	8.05E+07	7.91E+07	2.02E+08	4.31E+07		3.27E+08	7.45E+07	7.43E+07	2.61E+07
O43653	Prostate stem cell antigen [OS=Homo sapiens]	4.86E+07	3.92E+07	4.13E+07	1.32E+07		7.28E+07	2.39E+08	1.41E+07	4.18E+07
O75882-1	Attractin [OS=Homo sapiens]	9.47E+07	3.26E+07	5.25E+07	1.77E+07		1.04E+08	3.91E+07	5.53E+06	1.31E+07
P00738	Haptoglobin [OS=Homo sapiens]	7.06E+07		3.74E+07	3.65E+08	7.3E+08	3.34E+08	8.76E+08	1.69E+07	3.27E+08
P00747	Plasminogen [OS=Homo sapiens]	3.91E+07	1.54E+07		3.21E+06	2.71E+07	2.26E+07	5.74E+06	1.29E+07	1.25E+07
P00751-1	Complement factor B [OS=Homo sapiens]	2.88E+06	1.29E+07	7.7E+07		9.16E+07	1.57E+08	1.99E+07	4.24E+06	1.94E+07
P01019	Angiotensinogen [OS=Homo sapiens]	2.69E+07	4.2E+07	6.51E+06		9.76E+07	4.13E+07	2.15E+08	3.1E+06	1.2E+07

P01034	Cystatin-C [OS=Homo sapiens]	4.33E+07	2.89E+07	2.69E+07	1.88E+07	1.95E+07		8.86E+07	2.22E+07	4.68E+07
P01042-2	Isoform LMW of Kininogen-1 [OS=Homo sapiens]	3.38E+09	3.67E+09	2.1E+09	1.34E+09		6.31E+09	4.74E+09	1.77E+09	4.33E+09
P01614	Immunoglobulin kappa variable 2D-40 [OS=Homo sapiens]	1.42E+08	4.84E+07	6.66E+07	7.24E+07	1.25E+08	4.54E+08	1.84E+09	2.88E+07	
P01860	Immunoglobulin heavy constant gamma 3 [OS=Homo sapiens]	2.65E+07	1.73E+06	1.19E+08	3.54E+06	1.05E+09	9.51E+06	2.55E+07		6E+07
P01861	Immunoglobulin heavy constant gamma 4 [OS=Homo sapiens]	2.95E+07	2.85E+07		3.98E+06	3.76E+07	3.07E+07	1.37E+08	4.81E+06	6.37E+07
P02788	Lactotransferrin [OS=Homo sapiens]	2.5E+07	2.07E+07		6.86E+07	2.34E+08	1.33E+09	2.05E+06	1.1E+08	2.91E+09
P05062	Fructose-bisphosphate aldolase B [OS=Homo sapiens]	1.32E+08	2E+07	6.55E+06	2.54E+07		2.09E+08	7.81E+07	4.44E+06	7.43E+06
P05109	Protein S100-A8 [OS=Homo sapiens]	1.49E+08	4.51E+07	8.17E+07	1.03E+08	3.96E+07	1.73E+09		3.1E+07	1.84E+08
P05451	Lithostathine-1-alpha [OS=Homo sapiens]	1.61E+08	2.36E+07	2.95E+08	1.38E+07	1.79E+08	6.66E+08	4.77E+09	6.78E+07	
P06310	Immunoglobulin kappa variable 2-30 [OS=Homo sapiens]	1.5E+07	2.77E+07	2.8E+06	1.95E+06	2.18E+08	4.17E+08	1.05E+08	9.26E+06	
P06865	Beta-hexosaminidase subunit alpha [OS=Homo sapiens]	1.91E+07	4.3E+06	7.96E+07	9E+06		6.2E+06	3.43E+07	2.86E+06	1.92E+07
P07195	L-lactate dehydrogenase B chain [OS=Homo sapiens]	5.53E+07	2.09E+07	6.47E+06	1.97E+07		1.81E+08	2.02E+08	1.78E+07	1.18E+07

P09603	Macrophage colony-stimulating factor 1 [OS=Homo sapiens]	1.81E+07		2.99E+07	4.25E+06	2.66E+06	2.94E+07	4.16E+07	3.16E+06	1.15E+07
P0DJD8	Pepsin A-3 [OS=Homo sapiens]	1.24E+09	1.74E+08	7.97E+08	4.56E+08	2.92E+08		3.47E+09	6.27E+08	7.88E+08
P0DMV8	Heat shock 70 kDa protein 1A [OS=Homo sapiens]	1.31E+07	2.75E+06	2.86E+06	7.44E+06		3.99E+06	6.98E+06	7.3E+05	7.83E+06
P10451-5	Isoform 5 of Osteopontin [OS=Homo sapiens]	6.15E+08	7.38E+08	6.92E+08	3.63E+08	1.14E+07		5.92E+08	2.57E+08	2.93E+08
P11117	Lysosomal acid phosphatase [OS=Homo sapiens]	5.52E+07	9.99E+07	1.02E+08	2.91E+07		3.66E+07	1.56E+08	3.41E+07	3.17E+07
P11142-1	Heat shock cognate 71 kDa protein [OS=Homo sapiens]	1.05E+07	1.17E+07	5.83E+06	2.9E+06		3.52E+07	4.06E+06	9.01E+06	1.09E+07
P14543-1	Nidogen-1 [OS=Homo sapiens]	5.86E+07	5.31E+06	1.11E+07	1.76E+07	1.98E+07	1.75E+08	2.03E+08		5.96E+07
P14618	Pyruvate kinase PKM [OS=Homo sapiens]	1.66E+07	5.21E+06	2.94E+07	1.38E+07		1.79E+08	1.68E+07	8.33E+06	2.55E+06
P15144	Aminopeptidase N [OS=Homo sapiens]	2.7E+08	8.2E+07	2.54E+08	7.88E+07		4.17E+08	3.51E+08	4.58E+07	8.29E+07
P15289-1	Arylsulfatase A [OS=Homo sapiens]	1.01E+08	2.79E+07	1.11E+08	5.4E+07		6.62E+07	9.22E+07	2.39E+07	1.63E+07
P15311	Ezrin [OS=Homo sapiens]	3.78E+07	6.4E+06	3.48E+07	2.74E+07		4.28E+06	1.69E+07	1.25E+07	4.24E+07
P16278	Beta-galactosidase [OS=Homo sapiens]	3.77E+07	8.89E+07	4.13E+08	4.62E+07		4.03E+07	1.95E+08	1.63E+07	3.16E+07
P16870	Carboxypeptidase E [OS=Homo sapiens]	1.09E+07	1.53E+07	1.14E+07	3.21E+06		3.86E+07	6.09E+07	6.36E+06	2.97E+07
P17900	Ganglioside GM2 activator [OS=Homo sapiens]	1.1E+08	1.87E+07	9.35E+07	8.64E+07	1.47E+07	7.67E+07	6.99E+08	1.68E+07	
P19835-1	Bile salt-activated lipase [OS=Homo sapiens]	4.58E+07	3.78E+07	1.01E+08	1.71E+07		9.3E+07	2.2E+07	1.09E+07	7.42E+07

P22352	Glutathione peroxidase 3 [OS=Homo sapiens]	2.49E+07	1.26E+07	5.46E+06	7.15E+06	2.45E+06	2.02E+07	1.84E+08		3.76E+06
P22792	Carboxypeptidase n subunit 2 [OS=Homo sapiens]	1.4E+08	3.68E+07	5.75E+06	1.16E+07		7.87E+07	1.61E+07	7.88E+06	7.57E+06
P24855	Deoxyribonuclease-1 [OS=Homo sapiens]	1.89E+08	1.68E+08	3.41E+07	1.25E+07		1.81E+08	3.63E+08	4.13E+07	2.47E+08
P30530	Tyrosine-protein kinase receptor UFO [OS=Homo sapiens]	1.76E+08	1.17E+08	9.47E+07	6.91E+07		1.7E+08	5.71E+07	9.87E+06	5.35E+07
P35555	Fibrillin-1 [OS=Homo sapiens]	1.37E+07	7.46E+06	1.13E+07	1.28E+07		2.57E+07	3.8E+07	6.92E+06	1.68E+07
P43652	Afamin [OS=Homo sapiens]	7.5E+06	2.71E+07	1.95E+07	1.71E+07	4.5E+06	5.87E+06	2.13E+07		4.79E+06
P53634-1	Dipeptidyl peptidase 1 [OS=Homo sapiens]	4.64E+07	2.19E+07	1.07E+08	2.07E+07		6.44E+07	2.63E+08	9.28E+06	1.04E+07
Q01459	Di-N-acetylchitobiase [OS=Homo sapiens]	8.59E+07	3.98E+07	9.47E+07	3.72E+07		1.38E+08	2.11E+08	1.24E+07	6.87E+06
Q01469	Fatty acid-binding protein, epidermal [OS=Homo sapiens]	1.4E+08	5.71E+06	3.22E+07	5.19E+07	4.04E+07	3.01E+08		1.22E+07	2.96E+08
Q02487	Desmocollin-2 [OS=Homo sapiens]	1.49E+07	1.63E+06	1.88E+07	1.61E+07		6.69E+07	7.31E+07	3.04E+06	1.37E+07
Q03403	Trefoil factor 2 [OS=Homo sapiens]	2.53E+07	2.91E+07	2.62E+07		7.12E+07	5E+07	8.72E+07	4.58E+07	8.94E+07
Q07507	Dermatopontin [OS=Homo sapiens]	2.62E+07	1.37E+07	8.83E+06	2.12E+06	1.65E+07	1.29E+08	1.27E+08	2.01E+07	
Q13510-2	Isoform 2 of Acid ceramidase [OS=Homo sapiens]	4.14E+07	4.67E+07	3.75E+08	4.31E+07		7.27E+07	1.39E+08	1.03E+07	1.09E+07
Q8TDQ0	Hepatitis A virus cellular receptor 2 [OS=Homo sapiens]	1.37E+07	1.82E+07	4.14E+07	1.77E+07	5.53E+06	5.04E+07		1.18E+07	3.47E+07
Q96RW7-1	Hemicentin-1 [OS=Homo sapiens]	6.76E+07	4.62E+07	4.93E+07	1.78E+07		1.43E+08	1.79E+08	1.82E+07	2.84E+07

Q9BXP8-1	Pappalysin-2 [OS=Homo sapiens]	5.15E+07	1.57E+07	1.52E+07	6.07E+06		1.95E+08	2.01E+07	3.7E+06	6.74E+06
Q9HAT2	Sialate O-acetylesterase [OS=Homo sapiens]	4.68E+07	3.38E+07	7.92E+06	1.24E+07		5.34E+07	7.19E+07	9.56E+06	3.85E+06
Q9NZP8	Complement C1r subcomponent-like protein [OS=Homo sapiens]	8.21E+07	5.68E+07	2.44E+07	1.49E+07		8.31E+07	6.96E+07	1.18E+07	4.23E+07
Q9Y646	Carboxypeptidase Q [OS=Homo sapiens]	5.05E+07	8.18E+06	2.62E+08	7.82E+07		8.47E+07	1.5E+08	1.01E+07	1.18E+07
P01024	Complement C3 [OS=Homo sapiens]	1.79E+07	1.37E+08		1.02E+07	3.55E+08	6.79E+07		2.66E+07	2.2E+08
P01764	Immunoglobulin heavy variable 3-23 [OS=Homo sapiens]		2.75E+07	7.78E+06	5.23E+06	3.86E+06		7.41E+07	1.4E+07	7.41E+06
P04745	Alpha-amylase 1 [OS=Homo sapiens]	3.43E+09	4.9E+06		3E+08	4.09E+08	1.01E+09	1.69E+08		2.64E+07
P07686	Beta-hexosaminidase subunit beta [OS=Homo sapiens]	8.87E+06		1.83E+08	1.98E+07		3.82E+07	1.43E+08	4.71E+06	3.52E+06
P0DOX7	Immunoglobulin kappa light chain [OS=Homo sapiens]	2.14E+07	4.24E+06	3.43E+06		4.81E+09	1.48E+08	4.81E+10	2.06E+07	
P13727	Bone marrow proteoglycan [OS=Homo sapiens]	4.8E+07	8.08E+06	2.49E+07			1.57E+07	6.75E+07	4.82E+06	3.45E+07
P15328	Folate receptor alpha [OS=Homo sapiens]		2.46E+07	8.33E+06	6.04E+06		2.97E+07	2.83E+07	6.29E+06	2.64E+07
P22891-2	Isoform 2 of Vitamin K-dependent protein Z [OS=Homo sapiens]	4.88E+07	1.05E+08		1.36E+07		3.8E+07	5.42E+07	5.51E+06	4.64E+07
P62979	Ubiquitin-40S ribosomal protein S27a [OS=Homo sapiens]	2.59E+07	1.21E+07	2.1E+07		3.47E+06	1.65E+07		1.14E+07	2.5E+07

P63104	14-3-3 protein zeta/delta [OS=Homo sapiens]	1.14E+07		9.42E+06	7.13E+06		1.6E+07	1.96E+07	7.27E+05	5.96E+06
Q9HCN6-3	Isoform 3 of Platelet glycoprotein VI [OS=Homo sapiens]	2.12E+07	9.22E+06	1.31E+07			1.38E+07	4.73E+07	1.59E+07	1.26E+07