**Differential biomarker expression in heart failure patients with and without mitral regurgitation: insights from BIOSTAT-CHF**

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**ABSTRACT (240 words)**

**Background:** Mitral regurgitation (MR) frequently coexists with heart failure (HF).

**Objectives:** To better understand potential pathophysiological differences between patients with HF with or without moderate-severe MR, we compared differentially expressed circulating biomarkers between these two groups.

**Methods:** The Olink Proteomics® Multiplex Cardiovascular (CVD) -II, CVD-III, Immune Response and Oncology-II panels of 363 unique proteins from different pathophysiological domains were used to investigate the biomarker profiles of HF patients from index and validation cohorts of the BIOSTAT-CHF study stratified according to the presence of moderate-to-severe MR or no-mild MR.

**Results:** The index cohort included 888 patients (46%) with moderate-to-severe MR and 1029 (54%) with no-mild MR at baseline. The validation cohort included 522 patients (33%) with moderate-to-severe MR and 1076 (66%) with no-mild MR at baseline. Compared to patients with no-mild MR, those with moderate-to-severe MR had lower body mass index, higher comorbidity burden, signs and symptoms of more severe HF, lower systolic blood pressure, and larger left atrial and ventricular dimensions, in both cohorts. NT-proBNP, CA125, fibroblast growth factor 23 (FGF23) and growth hormone 1 (GH1) were up-regulated, whereas leptin (LEP) was down-regulated in patients with moderate-severe MR versus no-mild MR, in both index and validation cohorts.

**Conclusion:** Circulating biomarkers differently expressed in HF patients with moderate-severe MR versus no-mild MR were related to congestion, lipid and mineral metabolism and oxidative stress. These findings may be of value for the development of novel treatment targets in HF patients with MR.

**Key words:** Heart Failure; Biomarkers; Mitral regurgitation

**INTRODUCTION**

Analysis of circulating biomarkers is a well-established method to identify pathophysiological pathways in specific heart failure (HF) phenotype and potentially may identify new therapeutic targets. Evidence-based pharmacological treatments for HF (i.e. neurohormonal antagonists) target circulating biomarkers, such as catecholamines, angiotensin, and aldosterone, which were shown to be over expressed in HF patients, and associated with HF severity and progression (1). Furthermore, several studies have shown that patients characteristics (i.e. age) and HF etiology (i.e. ischaemic versus non-ischaemic) significantly influence pathway over- or under-expression, unveiling potentially different and specific biomarkers and therapeutic targets (2,3)

Mitral regurgitation (MR) is a frequent cardiovascular comorbidity in patients with heart HF, is associated with a poor prognosis and has recently been identified as a possible therapeutic target in selected HF patients (4-8). A few studies evaluated circulating plasma proteins in patients with MR by a proteomic approach. They included small cohorts of patients with degenerative mitral valve disease, and used healthy individuals as control (9,10). However, there is no evidence regarding pathophysiological pathways in patients with symptomatic HF and MR.

The aim of this study was to compare differentially expressed circulating biomarkers in HF patients with or without moderate/severe MR included in two independent large cohorts.

**METHODS**

**Patient population**

A Systems Biology Study to Tailored Treatment in Chronic Heart Failure (BIOSTAT-CHF) was a European project, including two prospective, observational, multinational cohorts. The design and primary results of BIOSTAT-CHF have been published elsewhere (11). Briefly, the index cohort included a total of 2516 patients from eleven centers in Europe, with signs and symptoms of worsening HF and either a left ventricular ejection fraction (LVEF) ≤40%, or plasma concentrations of N-terminal pro-B-type natriuretic peptide (NT-proBNP) >2000 ng/L. Included patients were either treatment naïve or receiving <50% of the target dose of angiotensin-converting enzyme inhibitors (ACEi) or angiotensin receptor blockers (ARB) and beta-blockers at the time of inclusion. Patients were enrolled from December 2010 to December 2012. Median follow-up was 21 months. A second independent cohort from six centers in Scotland, the validation cohort, included 1738 patients with HF and a previous documented HF admission requiring diuretic treatment, not previously treated or receiving ≤50% of target doses of ACEi/ARBs and/or beta-blockers. Patients were recruited from October 2010 until April 2014. Median follow-up was 21 months. This study complies with the Declaration of Helsinki, and medical ethics committees of participating centers approved the study. All patients provided written informed consent.

**Mitral regurgitation assessment**

Patients underwent two-dimensional transthoracic echocardiography at inclusion using a commercially available echocardiography (3.5 MHz probe). MR was identified and evaluated using two-dimensional and color Doppler echocardiography. According to the study protocol, only the presence of moderate or severe MR at baseline echocardiography (as compared to no or mild MR) was recorded. Both patients with primary and secondary MR were enrolled, but detailed data on MR mechanism or etiology were not collected.

**Biomarkers**

The Olink Proteomics® Multiplex Cardiovascular (CVD) -II, CVD-III, Immune Response and Oncology-II panels of 363 unique proteins from different pathophysiological domains were used to investigate the biomarker profiles in both BIOSTAT-CHF index and validation cohorts. Complete data about both biomarkers and mitral regurgitation were available in 1917/2516 and 1598/1738 patients, from the index and validation cohorts respectively. NT-proBNP is named natriuretic peptide B (NPPB), cancer antigen (CA125) is named mucin 16 cell surface associated (MUC16).

**Statistical analyses**

The normal distribution of continuous variables was explored with Kolmogorov-Smirnov and the Shapiro-Wilk tests. Continuous variables following a normal distribution are reported as mean ± standard deviation (SD) and were compared using the Student t test, whereas those not following a normal distribution are presented as median and interquartile range (IQR) and were compared with the Mann-Whitney U test. Categorical variables are reported as counts and percentages and were compared using the χ2 or Fisher exact tests, as appropriate.

Differences in expression of biomarkers in HF patients with or without MR were tested using Linear Models for Microarray data analysis (Limma) software (version 3.34.9), using a fold change cut-off of 1.15 (equal to a log2 fold change cut-off of 0.2), and a false discovery rate <0.05 according to the Benjamini-Hochberg method.

The impact of MR on the effect of the differentially expressed biomarkers on clinical outcome were investigated using a backward stepwise Cox regression. A composite end-point including HF hospitalization and all-cause death was used. Forrest plots and spline curves were obtained. Data were reported as hazard ratio and 95% confidence interval.

All tests were performed two-sided, and a p-value of less than 0.05 was considered statistically significant. Statistical analyses were performed using R, A Language and Environment for Statistical Computing, version 3.5.1 (R Foundation for Statistical Computing, Vienna, Austria), IBM SPSS Statistics for Windows, version 23.0.0.3 (Armonk, NY: IBM Corp.), and Cytoscape (version 3.8.0) with plugin ClueGO (version 2.5.7) and CluePedia (version 1.5.7) 24, 25.

**RESULTS**

**Baseline characteristics**

*Index cohort*

Out of the 1917 patients of the index cohort included in this analysis 888 (46%) were affected by moderate-to-severe MR, while 1029 (54%) had no/mild MR at baseline. Compared to patients with no/mild MR, those with moderate-to-severe MR had lower body mass index and were less likely to have a history of previous cardiac surgery for valvular heart disease. They were also more likely to have chronic kidney disease, advanced symptoms and signs of heart failure (higher New York Heart Association [NYHA] class and hepatomegaly), lower levels of systolic blood pressure and to receive mineralocorticoid receptor antagonist (MRA) and digoxin **(Table 1)**. Finally, larger left ventricular and atrial dimensions were observed in patients with moderate-to-severe MR compared to those with no-mild MR **(Table 2).**

*Validation cohort*

Out of the 1598 patients of the validation cohort included in this analysis 522 (33%) were affected by moderate-to-severe MR, while 1076 (66%) had no/mild MR at baseline. Compared to patients with no/mild MR, those with moderate-to-severe MR were less likely to be males, and, as in the index cohort, had lower body mass index (BMI). They also had a lower prevalence of ischaemic HF etiology and peripheral arterial disease, whereas atrial fibrillation and chronic kidney disease were more common in this group compared to that including patients with no/mild MR. Moreover, they had lower systolic blood pressure levels and were more likely to receive beta-blockers, MRA and digoxin **(Table 3).** Finally, echocardiographic features distinguishing the two groups in the index cohort were similar to the validation cohort **(Table 4).**

**Biomarker profiles**

*Index cohort*

In the index cohort 5 proteins (fibroblast growth factor 21 [FGF21], fibroblast growth factor 23 [FGF23], growth hormone 1 [GH1], MUC16, and NPPB) were up-regulated, whereas 2 proteins were down-regulated (leptin [LEP] and acid phosphatase 5 tartrate resistant [ACP5]) in patients with moderate-to-severe MR compared to those with no/mild MR (**Figures 1 and 2)**. Biomarkers’ expression in the index cohort stratified by left ventricular ejection fraction (> or = versus <50%) and history of atrial fibrillation is reported in **Supplementary Figure 1.**

Among these biomarkers, 3/5 of up-regulated (FGF23, MUC16 and NPPB) and 1/2 of down-regulated (LEP) were significantly correlated with hospitalization for heart failure and/or death, as displayed in **Supplementary Figure 2**. The impact of these biomarkers on outcome was not affected by MR status **(Supplementary Table 1).**

*Validation cohort*

In the validation cohort 9 proteins were up-regulated in patients with moderate-to-severe MR, with 4 (FGF23, NPPB, GH1 and MUC16) overlapping with the 5 biomarkers found to be up-regulated in the index cohort. On the other hand, LEP was the only down-regulated biomarker in patients with moderate-to-severe versus no-mild MR **(Figure 1 and 3)**. Biomarkers’ expression in the validation cohort stratified by left ventricular ejection fraction (> or = versus <50%) and history of atrial fibrillation is reported in **Supplementary Figure 1.**

FGF23, MUC16 and NPPB over-regulation was significantly associated with clinical outcome in the validation cohort, coherently with results of the index cohort, whereas LEP did not show significant prognostic value **(Supplementary Figure 3).**

There was a significant interaction between MR and the impact of ACP5 on hospitalization for HF or all-cause death (**Supplementary Table 2 and Supplementary Figure 4**).

**DISCUSSION**

Using a panel of biomarkers from different biological domains in two large, independent cohorts of patients with HF, we identified multiple differences in protein expression between patients with moderate-to-severe MR versus those with no or mild MR. In particular, in both the validation and index cohort we found a significant over-expression of NPPB, MUC16, FGF23 and GH1 and under-expression of LEP in patients with moderate-to-severe MR compared to those with no-mild MR.

*Markers of congestion and heart failure severity*

NPPB, released by cardiomyocytes in response to LV stress, is, together with BNP, the most validated biomarker for HF diagnosis and risk prognostication.12 Higher NPPB values in patients with HF and moderate-to-severe MR, compared to those with no-mild MR, and their strong association with unfavorable outcome, were already observed in previous studies. Successful correction of MR, when associated with a positive reverse remodeling, leads to a reduction in natriuretic peptides circulating levels (12). High NPPB levels are associated with poor prognosis in HF patients undergoing percutaneous treatment of secondary MR (13).

MUC16, also called serum carbohydrate antigen 125 (Ca125), is produced by serous epithelial cells and mesothelial cells as a response to congestion and inflammation, and elevated circulating levels has been associated with adverse outcome in heart failure (14). Previous analyses have investigated the predictive role MUC16 in the BIOSTAT-CHF index cohort, showing, consistently with our results, a strong association with adverse prognosis and NPPB levels (15). High levels of MUC16 were observed in patients with functional MR in a small study including 77 patients with non-ischaemic dilated cardiomyopathy (16). Over-expression of this protein in patients with significant MR was confirmed in our larger population.

FGF23 is a phosphaturic hormone primarily secreted by osteocytes to maintain phosphate and mineral homeostasis. In patients with chronic kidney disease elevation of circulating FGF23 has been linked with cardiac remodeling leading to left ventricle hypertrophy, with strong correlation with increased with HF events and cardiovascular mortality (17,18). Subsequent studies have shown that FGF23 over expression is common and predicts severe prognosis in patients with acute and chronic HF even without renal disfunction (33,34). In the BIOSTAT-CHF index cohort higher plasma FGF23 levels were independently associated with volume overload, less successful up-titration of ACE inhibitors (or angiotensin receptor blockers) and an increased risk of all-cause mortality and HF hospitalization (21).

Altogether these findings appear to be in line with current evidence that considers NPPB, MUC16 and FGF23 as reliable biomarkers of HF severity with a strong relation to congestion status, and further stress the role of MR in the pathological cascade of HF.

*FGF23 and mitral annular calcification*

FGF23 has been proposed as a potential biomarker for vascular and cardiac calcifications, coherently with its role in mineral metabolism. Silva et al. have demonstrated that high FGF23 levels were independent predictors of mitral annular calcification (MAC) in a study population of 150 diabetic patients with chronic kidney disease (22). In 3585 patients included in the Cardiovascular Health Study FGF23 and fetuin-A were found to be significantly associated with higher MAC rates (23). Serum FGF23 levels were associated to accelerated annual MAC progression, assessed by repeated computed tomography, in 6547 patients enrolled in the Multi-Ethnic Study of Atherosclerosis study (24).

As our echocardiographic data did not include MAC, we were not able to test its association with MR severity or FGF23 levels. Nevertheless, the over expression of FGF23 in patients with moderate-to-severe MR may be partly explained by the possible presence of MAC in a population with high rates of chronic kidney disease. As MAC is emerging as a negative prognostic factor in MR, especially when associated with trans-mitral pressure gradient (25), and also represents a limit to both surgical and transcatheter correction approaches (26), further research to clarify the pathway leading to its development are needed.

*Dysregulation of growth hormone cascade*

GH1, secreted by the pituitary gland, stimulates through its specific receptors the activation of a complex signaling cascade that leads to hepatic production of IGF1. IGF1 is an anabolic hormone, pivotal for increasing bone length and density and muscle mass during growth. IGF1 acts on the cardiovascular system in different ways. It enhances the production of nitric oxide reducing systemic vascular resistance, induces the reuptake of calcium by the sarcoplasmic reticulum by regulating sarco/endoplasmic reticulum Ca2+-ATPase (SERCA2) and attenuates left ventricular remodeling in experimental models of HF (27).

Patients with cachexia and advanced HF presented increased GH1 but lower IGF1 levels compared with non-cachectic individuals. As the IGF-1/GH ratio in non-cachectic was 12-fold higher in non-cachectic patients than in cachectic patients, GH resistance (defined as high GH levels and low IGF-1 levels) is thought to be associated with advanced HF (28). We could speculate that a dysregulation of GH axis may induce oxidative stress, left ventricular remodeling and cardiac cachexia.

The over expression of GH in patients with significant MR in our cohort is coherent with their status of “sicker” patients with advanced HF.

*Leptin and cachexia in heart failure*

Leptin is a cell-signaling molecule associated with central obesity and visceral fat; it is released by adipocytes, especially by visceral fat, and acts on the hypothalamus to promote satiety, reduce food intake and increase energy expenditure. Its peripheral effects are thought to be pivotal in obesity associated HF. Maladaptive effects of hyperleptinemia in obesity encompass stimulation of sympathetic nervous and renin-angiotensin systems, enhancing aldosterone secretion, sodium retention and heart remodeling (29). In HF patients circulating leptin levels are markedly elevated and associated with adverse prognosis (30). However, studies have shown that plasma leptin concentration are low in HF patient with cachexia compared to non-cachectic patient, even after correction for body fat content (31,32).

In our study leptin was under expressed in patients with moderate-to-severe MR both in the index and validation cohort. Cachexia incidence in our study populations was not available, but BMI was significantly lower in patients with MR compared to those without in both cohorts. As patients with MR had more advanced HF, a higher incidence of cachexia in this group was likely, and may account for leptin under expression.

**LIMITATIONS**

We have to recognize several limitations of the present study. Firstly, the results of this study were based on a post-hoc analysis. Moreover, echocardiographic images at baseline were not evaluated by a Core Laboratory and echocardiographic data, including MR, are site-reported without specific protocol or information regarding loading conditions provided. Notably, details about MR degree (i.e. differentiation between none versus mild versus moderate versus severe MR) and etiology (i.e. differentiation between primary and secondary MR) are not available. Finally, populations included are not contemporary heart failure populations since enrolment period was 2010-2014. Nevertheless, our findings will likely inform future studies aimed at better evaluate diagnostic and therapeutic tools in patients with MR and HF.

**CONCLUSIONS**

In two large independent HF populations, biomarkers associated with congestion, HF severity (i.e. NPPB, MUC16, FGF23) and mineral metabolism (i.e. FGF23), were up-regulated in patients with moderate-to-severe MR compared to those with no-mild MR. GH1 over-expression, associated with oxidative stress and left ventricular remodeling, and LEP under-expression, associated with lipid metabolism and cachexia, were also observed in patients with moderate-to-severe MR versus no-mild MR. These findings provide new insights regarding possible new therapeutic targets in patients with HF and MR.

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**FIGURE LEGENDS**

**Figure 1.** Venn diagram of number of differentially expressed proteins in patients with moderate-severe mitral regurgitation (MR) versus no-mild MR in index cohort and validation cohort.

**Figure 2.** Volcano plots of differential protein expression in patients with moderate-severe MR versus no-mild MR in the index cohort

**Figure 3.** Volcano plots of differential protein expression in patients with moderate-severe MR versus no-mild MR in the validation cohort

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| --- | --- | --- | --- | --- |
| **Table 1 Baseline clinical characteristics of the index cohort stratified by presence of moderate to severe mitral regurgitation** | | | | |
|  | **Overall**  **(n = 1917)** | **Moderate or severe MR (n = 888)** | **No or mild MR**  **(n = 1029)** | **P-value** |
| Age (years) | 68.8 ± 12.1 | 68.6 ± 11.8 | 68.9 ± 12.3 | 0.538 |
| Male sex | 1411 (73.6) | 656 (73.9) | 755 (73.4) | 0.804 |
| BMI (kg/m2) | 27.8 ± 5.4 | 27.3 ± 5.2 | 28.1 ± 5.6 | **0.001** |
| Race |  |  |  | 0.126 |
| Caucasian | 1894 (98.8) | 877 (98.8) | 1017 (98.8) |  |
| Asian | 10 (0.5) | 3 (0.3) | 7 (0.7) |  |
| Black | 4 (0.2) | 4 (0.4) | 0 (0) |  |
| Other | 9 (0.5) | 4 (0.4) | 5 (0.49) |  |
| Previous HF hospitalization(s) in last year | 589 (30.7) | 280 (31.5) | 309 (30.3) | 0.477 |
| Primary ischaemic HF aetiology | 844 (44.9) | 395 (45.4) | 449 (44.5) | 0.73 |
| Smoking |  |  |  | 0.203 |
| Past | 919 (48) | 420 (47.5) | 499 (48.5) |  |
| Current | 283 (14.8) | 120 (13.6) | 163 (15.8) |  |
| Medical history |  |  |  |  |
| Hypertension | 1183 (61.7) | 538 (60.6) | 645 (62.7) | 0.346 |
| Diabetes | 606 (31.6) | 273 (30.7) | 333 (32.4) | 0.447 |
| Myocardial infarction | 707 (36.9) | 315 (35.5) | 392 (38.1) | 0.235 |
| CABG | 327 (17.1) | 147 (16.5) | 180 (17.5) | 0.586 |
| Previous valvular surgery | 138 (7.2) | 51 (5.7) | 87 (8.45) | **0.022** |
| Previous PCI | 388 (20.2) | 163 (18.4) | 225 (21.9) | 0.056 |
| Atrial fibrillation | 870 (45.4) | 407 (45.8) | 463 (45) | 0.713 |
| Stroke | 183 (9.5) | 84 (9.5) | 99 (9.6) | 0.904 |
| Peripheral arterial disease | 205 (10.7) | 93 (10.5) | 112 (10.9) | 0.771 |
| Chronic kidney disease | 539 (28.1) | 274 (30.9) | 265 (25.7) | **0.013** |
| Current malignancy | 71 (3.7) | 30 (3.4) | 41 (4) | 0.484 |
| Chronic obstructive pulmonary disease | 328 (17.1) | 144 (16.2) | 184 (17.9) | 0.334 |
| Device therapy |  |  |  | 0.31 |
| Pacemaker | 138 (7.2) | 59 (6.6) | 79 (7.7) |  |
| ICD | 134 (7) | 69 (7.8) | 65 (6.3) |  |
| CRT-P | 33 (1.7) | 18 (2) | 15 (1.5) |  |
| CRT-D | 118 (6.1) | 58 (6.5) | 60 (5.8) |  |
| NYHA functional class | |  |  | **0.002** |
| I | 40 (2.1) | 18 (2.1) | 22 (2.2) |  |
| II | 690 (37) | 291 (33.5) | 399 (40.1) |  |
| III | 902 (48.4) | 429 (49.4) | 473 (47.5) |  |
| IV | 231 (12.4) | 130 (15) | 101 (10.1) |  |
| Clinical profile |  |  |  |  |
| Peripheral oedema | 458 (28.9) | 226 (30) | 232(27.8) | 0.335 |
| Hepatomegaly | 263 (13.8) | 153 (17.3) | 110 (10.7) | **< 0.001** |
| SBP (mmHg) | 125 ± 22 | 123 ± 22 | 126 ± 23 | **< 0.001** |
| DBP (mmHg) | 75 ± 13 | 75 ± 13 | 75 ± 14 | 0.329 |
| HR (bpm) | 80 ± 19 | 81 ± 19 | 79 ± 20 | 0.068 |
| Type of visit |  |  |  | 0.059 |
| Inpatient hospitalization | 1356 (70.7) | 642 (72.3) | 714 (69.4) |  |
| Outpatient (scheduled) | 439 (25) | 202 (22.7) | 277 (26.9) |  |
| Outpatient (unscheduled) | 82 (4.3) | 44 (4.9) | 38 (3.7) |  |
| HF therapy |  |  |  |  |
| ACE/ARB use | 1388 (72.4) | 627 (70.6) | 761 (74) | 0.102 |
| B-blocker use | 1597 (83.3) | 731 (82.3) | 866 (84.1) | 0.281 |
| MRA use | 1007 (52.5) | 511 (57.5) | 496 (48.2) | **< 0.001** |
| Loop diuretic use | 1907 (99.5) | 884 (99.5) | 1023 (99.4) | 0.688 |
| Digoxin use | 358 (18.7) | 195 (22) | 163 (15.8) | **0.001** |
| Data are presented as n (%) and mean ± standard deviation.  ACEi, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; BMI, body mass index; CABG, coronary artery bypass graft; CRT-D, cardiac resynchronization therapy with defibrillator; CRT-P, cardiac resynchronization therapy with pacemaker; DBP, diastolic blood pressure; HF, heart failure; HR, heart rate; ICD, implantable cardioverter-defibrillator; MR, mitral regurgitation; MRA, mineralocorticoid receptor antagonist; NYHA, New York Heart Association; PCI, percutaneous coronary intervention; SBP, systolic blood pressure. | | | | |

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| **Table 2:** **Baseline echocardiographic data and laboratory characteristics of the index cohort stratified by presence of moderate to severe mitral regurgitation.** | | | | | |
|  | **Overall**  **(n = 1917)** | **Moderate or severe MR (n = 888)** | **No or mild MR**  **(n = 1029)** | **P-value** | |
| **Echocardiographic data** |  |  |  |  |
| LVEF (%) | 30 (25-36) | 30 (24-35) | 30 (25-38) | **< 0.001** |
| LVEF categories |  |  |  | **< 0.001** |
| HFrEF (LVEF < 40%) | 1440 (80.8) | 718 (84.8) | 722 (77.1) |  |
| HFmrEF (LVEF 40-49%) | 226 (12.7) | 84 (9.9) | 142 (15.2) |  |
| HFpEF (LVEF ≥50%) | 117 (6.6) | 45 (5.3) | 72 (7.7) |  |
| LV remodelling |  |  |  | **< 0.001** |
| Normal geometry | 285 (21.1) | 121 (18.2) | 164 (24) |  |
| Concentric remodelling | 47 (3.5) | 17 (2.6) | 30 (4.4) |  |
| Concentric hypertrophy | 202 (15) | 75 (11.3) | 127 (18.5) |  |
| Eccentric hypertrophy | 816 (60.4) | 452 (68) | 364 (53.1) |  |
| LVEDD (mm) | 61 (55-67) | 63 (57-69) | 59 (53-65) | **< 0.001** |
| LVESD (mm) | 50 (43-57) | 52 (45-59) | 48 (41-55) | **< 0.001** |
| Left atrium diameter (mm) | 47 (43-52) | 49 (44-54) | 46 (41-50) | **< 0.001** |
| **Laboratory data** |  |  |  |  |
| Haemoglobin (g/dL) | 13.3 (11.9-14.5) | 13.3 (11.9-14.5) | 13.3 (11.9-14.5) | 0.852 |
| Creatinine (μmol/L) | 102 (84-131) | 106 (85-132) | 100 (84-126) | **0.024** |
| eGFR CKD-EPI (mL/min/1.73 m2) | 59 (44-77) | 59 (43-76) | 60 (45-79) | **0.05** |
| Urea (mmol/L) | 11 (7.4-17.8) | 11.5 (7.7 19.4) | 10.5 (7.3-16.4) | **0.002** |
| Sodium (mmol/L) | 140 (137-142) | 140 (137-142) | 140 (137-142) | 0.645 |
| NT-proBNP (ng/L) | 2677 (1204-5587) | 3088 (1400-6573) | 2433 (1051-5014) | **< 0.001** |
| Data are presented as n (%) and median (Q25–Q75).  CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; eGFR, estimated glomerular filtration rate; HFmrEF, heart failure with mid-range ejection fraction; HFpEF, heart failure with preserved ejection fraction; HFrEF, heart failure with reduced ejection fraction; LV, left ventricle; LVEDD, left ventricular end-diastolic diameter; LVEF, left ventricular ejection fraction; LVESD, left ventricular end-systolic diameter; MR, mitral regurgitation; NT-proBNP, N-terminal pro-B-type natriuretic peptide, | | | | |

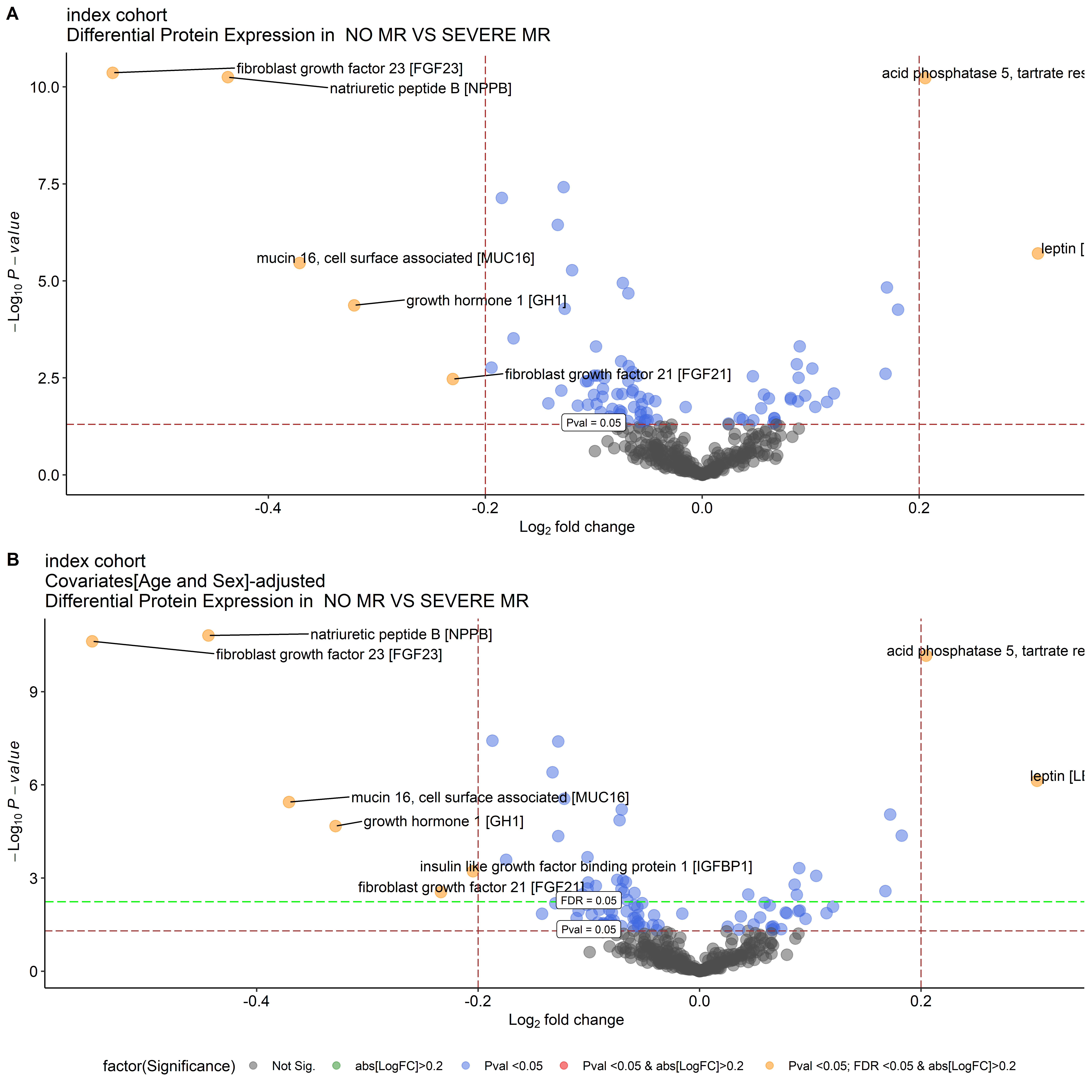
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| **Table 3. Baseline clinical characteristics of the validation cohort stratified by presence of moderate to severe mitral regurgitation** | | | | |
|  | **Overall (n = 1598)** | **Moderate or severe MR (n = 522)** | **No or mild MR (n = 1076)** | **P-value** |
| Age (years) | 73.8 ± 10.6 | 74.5 ± 10.9 | 73.4 ± 10.5 | 0.056 |
| Male sex | 1063 (66.5) | 315 (60.3) | 748 (69.5) | **< 0.001** |
| BMI (kg/m2) | 28.9 ± 6.3 | 27.5 ± 6.1 | 29.6 ± 6.3 | **< 0.001** |
| Race |  |  |  | 0.155 |
| Caucasian | 1589 (99.4) | 520 (99.6) | 1069 (99.3) |  |
| Asian | 6 (0.4) | 0 (0) | 6 (0.6) |  |
| Black | 1 (0.06) | 1 (0.2) | 0 (0) |  |
| Other | 2 (0.13) | 1 (0.2) | 1 (0.1) |  |
| Previous HF hospitalization(s) in last year | 423 (26.9) | 147 (28.6) | 276 (26) | 0.282 |
| Primary ischaemic HF aetiology | 1035 (93.8) | 323 (91) | 712 (95.2) | **0.002** |
| Smoking |  |  |  | **0.048** |
| Past | 552 (34.7) | 167 (32.2) | 385 (36) |  |
| Current | 215 (13.5) | 85 (16.4) | 130 (12.1) |  |
| Medical history |  |  |  |  |
| Hypertension | 932 (58.5) | 288 (55.6) | 644 (59.9) | 0.102 |
| Diabetes | 518 (32.6) | 150 (28.8) | 368 (34.4) | 0.024 |
| Myocardial infarction | 783 (49.1) | 241 (46.3) | 542 (50.5) | 0.115 |
| CABG | 288 (18) | 87 (16.7) | 201 (18.7) | 0.322 |
| Previous valvular surgery | 102 (6.4) | 25 (4.8) | 77 (7.2) | 0.069 |
| Previous PCI | 293 (18.5) | 83 (16) | 210 (19.8) | 0.068 |
| Atrial fibrillation | 713 (45) | 272 (52.5) | 441 (41.3) | **< 0.001** |
| Stroke | 293 (18.5) | 95 (18.4) | 198 (18.6) | 0.938 |
| Peripheral arterial disease | 354 (22.7) | 99 (19.4) | 255 (24.4) | **0.026** |
| Chronic kidney disease | 722 (45.7) | 272 (52.4) | 450 (42.5) | **< 0.001** |
| Current malignancy | 68 (4.3) | 18 (3.5) | 50 (4.7) | 0.267 |
| Chronic obstructive pulmonary disease | 294 (18.5) | 85 (16.5) | 209 (19.6) | 0.14 |
| Device therapy |  |  |  | **< 0.001** |
| Pacemaker | 107 (6.7) | 32 (6.1) | 75 (7) |  |
| ICD | 64 (4) | 34 (6.5) | 30 (2.8) |  |
| CRT-P | 23 (1.4) | 8 (1.5) | 15 (1.4) |  |
| CRT-D | 49 (3.1) | 27 (5.2) | 22 (2) |  |
| NYHA functional class | |  |  | 0.697 |
| I | 16 (1) | 3 (0.57) | 13 (1.2) |  |
| II | 663 (41.5) | 217 (41.6) | 446 (41.5) |  |
| III | 707 (44.3) | 233 (44.6) | 474 (44.1) |  |
| IV | 211 (13.2) | 69 (13.2) | 142 (13.2) |  |
| Clinical profile |  |  |  |  |
| Peripheral oedema | 431 (30.1) | 151 (32.4) | 280 (28.9) | 0.179 |
| Haepatomegaly | 57 (3.9) | 19 (4) | 38 (3.9) | 0.872 |
| SBP (mmHg) | 126 ± 22 | 124 ± 22 | 127 ± 23 | **0.004** |
| DBP (mmHg) | 69 ± 13 | 69 ± 12 | 69 ± 13 | 0.851 |
| HR (bpm) | 74 ± 16 | 75 ± 17 | 74 ± 16 | 0.212 |
| Patient recruited from |  |  |  | **0.003** |
| Clinic | 737 (46.1) | 257 (49.2) | 480 (44.6) |  |
| General Practitioner | 31 (1.9) | 2 (0.38) | 29 (2.7) |  |
| Ward | 830 (51.9) | 263 (50.4) | 567 (52.7) |  |
| HF therapy |  |  |  |  |
| ACE/ARB use | 1127 (70.8) | 370 (71.4) | 757 (70.6) | 0.718 |
| B-blocker use | 1155 (72.6) | 397 (76.6) | 758 (70.6) | **0.012** |
| MRA use | 516 (32.4) | 189 (36.5) | 327 (30.5) | **0.016** |
| Loop diuretic use | 1575 (99) | 516 (99.6) | 1059 (98.7) | 0.085 |
| Digoxin use | 288 (18.1) | 110 (21.2) | 178 (16.6) | **0.024** |
| Data are presented as n (%) and mean±standard deviation.  ACEi, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; BMI, body mass index; CABG, coronary artery bypass graft; CRT-D, cardiac resynchronization therapy with defibrillator; CRT-P, cardiac resynchronization therapy with pacemaker; DBP, diastolic blood pressure; HF, heart failure; HR, heart rate; ICD, implantable cardioverter-defibrillator; MR, mitral regurgitation; MRA, mineralocorticoid receptor antagonist; NYHA, New York Heart Association; PCI, percutaneous coronary intervention; SBP, systolic blood pressure. | | | | |

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| **Table 4. Baseline echocardiographic data and laboratory characteristics of the validation cohort stratified by presence of moderate to severe mitral regurgitation.** | | | | |
|  | **Overall**  **(n = 1598)** | **Moderate or severe MR (n = 522)** | **No or mild MR**  **(n = 1076)** | **P-value** |
| **Echocardiographic data** |  |  |  |  |
| LVEF (%) | 40 (35-50) | 35 (28-45) | 43 (35-52) | **< 0.001** |
| LVEF categories |  |  |  | **< 0.001** |
| HFrEF (LVEF < 40%) | 702 (46.2) | 310 (62.3) | 392 (38.1) |  |
| HFmrEF (LVEF 40-49%) | 389 (25.6) | 106 (21.6) | 283 (27.5) |  |
| HFpEF (LVEF ≥50%) | 427 (28.1) | 74 (15.1) | 353 (34.3) |  |
| LV remodeling |  |  |  | **< 0.001** |
| Normal geometry | 104 (14.7) | 32 (11.8) | 72 (16.5) |  |
| Concentric remodeling | 83 (11.7) | 17 (6.3) | 66 (15.1) |  |
| Concentric hypertrophy | 242 (34.2) | 80 (29.5) | 162 (37.2) |  |
| Eccentric hypertrophy | 278 (39.3) | 142 (52.4) | 136 (31.2) |  |
| LVEDD (mm) | 55 (48-61) | 58 (52-63) | 53 (47-59) | **< 0.001** |
| LVESD (mm) | 44 (36-54) | 50 (41-56) | 41 )33-49) | **< 0.001** |
| Left atrium diameter (mm) | 45 (41-50) | 47 (44-52) | 44 (40-48) | **< 0.001** |
| **Laboratory data** |  |  |  |  |
| Haemoglobin (g/dL) | 13.2 (11.8-14.5) | 13 (11.8-14.3) | 13.3 (11.8-14.7) | **0.047** |
| Creatinine (μmol/L) | 97 (89-124) | 102 (82-133) | 95 (79-122) | **< 0.001** |
| eGFR CKD-EPI (mL/min/1.73 m2) | 60 (43-78) | 56 (39-74) | 62 (45-79) | **< 0.001** |
| Urea (mmol/L) | 8.6 (6.5-12) | 9.1 (6.9-13.4) | 8.4 (6.4-11.3) | **< 0.001** |
| Sodium (mmol/L) | 139 (137-141) | 139 (137-141) | 139 (137-141) | 0.985 |
| NT-proBNP (ng/L) | 1356 (510-3531) | 2270 (900-5319) | 1078 (399-2810) | **< 0.001** |
| Data are presented as n (%) and median (Q25–Q75).  CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; eGFR, estimated glomerular filtration rate; HFmrEF, heart failure with mid-range ejection fraction; HFpEF, heart failure with preserved ejection fraction; HFrEF, heart failure with reduced ejection fraction; LV, left ventricle; LVEDD, left ventricular end-diastolic diameter; LVEF, left ventricular ejection fraction; LVESD, left ventricular end-systolic diameter; MR, mitral regurgitation; NT-proBNP, N-terminal pro-B-type natriuretic peptide | | | | |

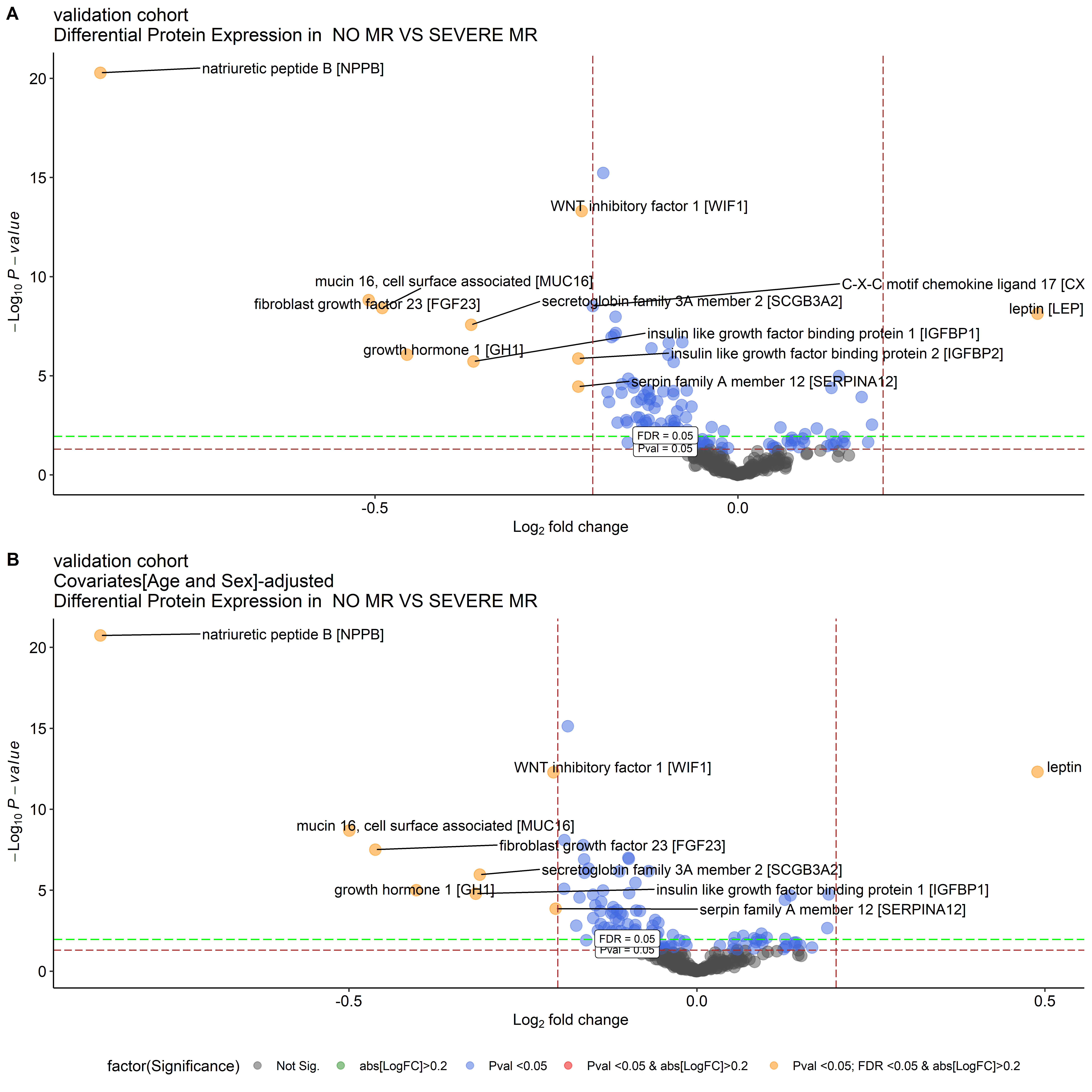
**Figure 1.**



**Figure 2.**

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**Figure 3.**

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