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Proteomic Biomarkers of Sacubitril/Valsartan Treatment Response in Heart Failure with Preserved Ejection Fraction: Molecular Insights into Sex Differences

Thanh Thanh L. Nguyen, BA^{1,2}, Min Wang, MD³, Duan Liu, PhD¹, Seethalakshmi Iyer, MS³, Hilda Mariana Gonzalez Bonilla, MD³, Nancy Acker, RN³, Vishakantha Murthy, PhD, MBA, CCRP^{1,3}, Sanskriti Shrivastava, MD^{3,4}, Viral Desai, MD⁵, John C. Burnett Jr., MD³, Margaret Redfield, MD³, Kent R. Bailey, PhD⁶, Richard M. Weinshilboum, MD¹, Naveen L. Pereira, MD^{1,3}

¹Department of Molecular Pharmacology and Experimental Therapeutics, Mayo Clinic, Rochester, MN, USA;

²Graduate School of Biomedical Sciences, Mayo Clinic, Rochester, MN, USA;

³Department of Cardiovascular Medicine, Mayo Clinic, Rochester, MN, USA;

⁴Internal Medicine, The Wright Center, Scranton, PA, USA;

⁵Division of General Internal Medicine, Palliative Medicine and Medical Education, University of Louisville, Louisville, KY, USA;

⁶Divisions of Clinical Trials and Biostatistics, Department of Quantitative Health Sciences, Mayo Clinic, Rochester, Minnesota

Sacubitril/valsartan was recently approved for the treatment of Heart Failure with Preserved Ejection Fraction (HFpEF). Although sacubitril/valsartan did not significantly reduce HF hospitalizations or deaths overall, a significant benefit in women was observed¹. Characterization of sacubitril/valsartan response molecular biomarkers in patients with HFpEF and the mechanism underlying sex-biased response has not been described.

To address these knowledge gaps, we performed proteomic profiling for 369 plasma proteins using the Olink Cardiometabolic platform in 37 patients (males=18; females=19) prior to and 5 weeks after sacubitril/valsartan treatment. Patients' age was 74 ± 7.6 years (males 74.1 ± 7.6 ; females 74.2 ± 7.7), mean left ventricular ejection fraction (LVEF) $59.7 \pm 5.7\%$ with no significant difference between males ($58.3 \pm 6.0\%$) and females ($61.1 \pm 4.8\%$) ($P=0.16$). Co-morbidities included hypertension, diabetes mellitus, atrial fibrillation, stroke, ischemic heart disease, and 12-month prior HF hospitalization in 89%, 43%, 73%, 8%, 27%, and 54% of patients, respectively. This study was approved by the Mayo Clinic Institutional Review Board. Data is available upon reasonable request to the corresponding author.

Corresponding Author: Naveen L. Pereira, MD, Pereira.Naveen@mayo.edu, Department of Cardiovascular Medicine, Mayo Clinic, 200 First Street SW, Rochester, MN 55905, Tel: +1 507-284-4441 | Fax: 507-266-0228.

Disclosure

Richard Weinshilboum is co-founder of and stockholder in OneOme, LLC. Other authors declared no conflict of interest

We performed a clinical assay (Electrochemiluminescence Immunoassay) for plasma NT-proBNP and found that sacubitril/valsartan treatment in the overall cohort resulted in a significant reduction in NT-proBNP ($P=0.0013$, $N=37$, average reduction=26%) (Figure A). We then obtained Normalized Protein Expression (NPX), a relative protein quantification logarithmically transformed for a more normal distribution, for 369 proteins from Olink. Fourteen proteins significantly changed after drug treatment based on paired student's t-test at a 5% false discovery rate (Figure B). Nine of these 14 molecules were involved in the TGF- β signaling pathway. The most significantly upregulated molecule, CHRD2, is an antagonist of TGF- β signaling, while the most significantly down-regulated molecule, ENG, and 7 other down-regulated molecules, IGFBP7, TGFBI, CD16, CDH6, ICAM5, NT-proBNP, and ST2, were activating factors of or molecules induced by TGF- β . This observation suggested that sacubitril/valsartan systematically dampened TGF- β signaling in our overall HFpEF cohort.

We further stratified the samples by sex and conducted paired t-tests separately in men and women. There was no statistically significant difference ($P=0.1445$) between the downregulation of plasma NT-proBNP observed in women (-35%, $N=19$) and that observed in men (-16%, $N=18$) even though the downregulation was qualitatively more significant in women (Figure C). Additionally, soluble ICAM-5 and KITLG were more significantly down-regulated in women than in men (Figure D&E). Because females had larger and more consistent NT-proBNP reductions than males in our cohort, the differentially expressed proteins observed could be associated with NT-proBNP rather than sex. To test for sex-specific effect directly for these molecules, we ran a generalized linear model for post-treatment NPX with pre-treatment NPX, sex and NTproBNP level as predictor variables. Post-treatment NT-proBNP was not significantly associated with neither ICAM5 ($P=0.0971$) nor KITLG ($P=0.793$). Sex was a significant predictor for post-treatment ICAM5 levels ($P=0.0314$) but was not for KITLG levels ($P=0.649$), suggesting that the difference in statistical significance between men and women of the KITLG change is hypothesis-generating and would require validation in a larger sample. ICAM5 is highly expressed in brain and lung tissue, while KITLG is highly expressed in fibroblasts, gut and lung tissue (see <https://gtexportal.org/>). ICAM5 increases TGF- β 1 expression in activated T-cells², while KITLG is a cytokine that is upregulated in adverse left ventricular remodeling³. The mechanisms underlying the downregulation of these molecules in women but not in men and its association with therapeutic benefits in HFpEF with sacubitril/valsartan treatment remain to be determined.

To our knowledge this is the first reported plasma proteomic profiling of sacubitril/valsartan response in patients with HFpEF. Our findings indicate that sacubitril/valsartan's mechanism of action in HFpEF may be mediated primarily via the TGF- β pathway. Of interest, TGF- β is a biomarker that differentiates HFpEF from other types of HF⁴, further supporting the observation that molecular mechanisms affected by sacubitril/valsartan are possibly targets of known HFpEF pathophysiology. Additionally, we observed that NT-proBNP was reduced in the overall cohort but more significantly in female than in male patients. The extent to which NT-proBNP decreases after sacubitril/valsartan treatment has been shown to predict risk for subsequent HF hospitalization and cardiovascular death⁵ and therefore our findings may provide a potential explanation for the beneficial effect of sacubitril/valsartan

in females but not in males when treated for HFpEF. Correlated with this sex-mediated response, ICAM5, and possibly KITLG were identified to be downregulated by sacubitril/valsartan more significantly in females than males, possibly explaining its beneficial mechanism of action in females. Our findings provide new insights into mechanisms underlying sacubitril/valsartan action in HFpEF. Validation in larger sample sizes especially to detect smaller changes in protein markers, untargeted proteome analysis and mechanistic functional work are needed to determine if knowledge of the reported molecules and/or pathways could contribute to the precision treatment of HFpEF, particularly for women who are at higher risk for this disease.

Sources of Funding

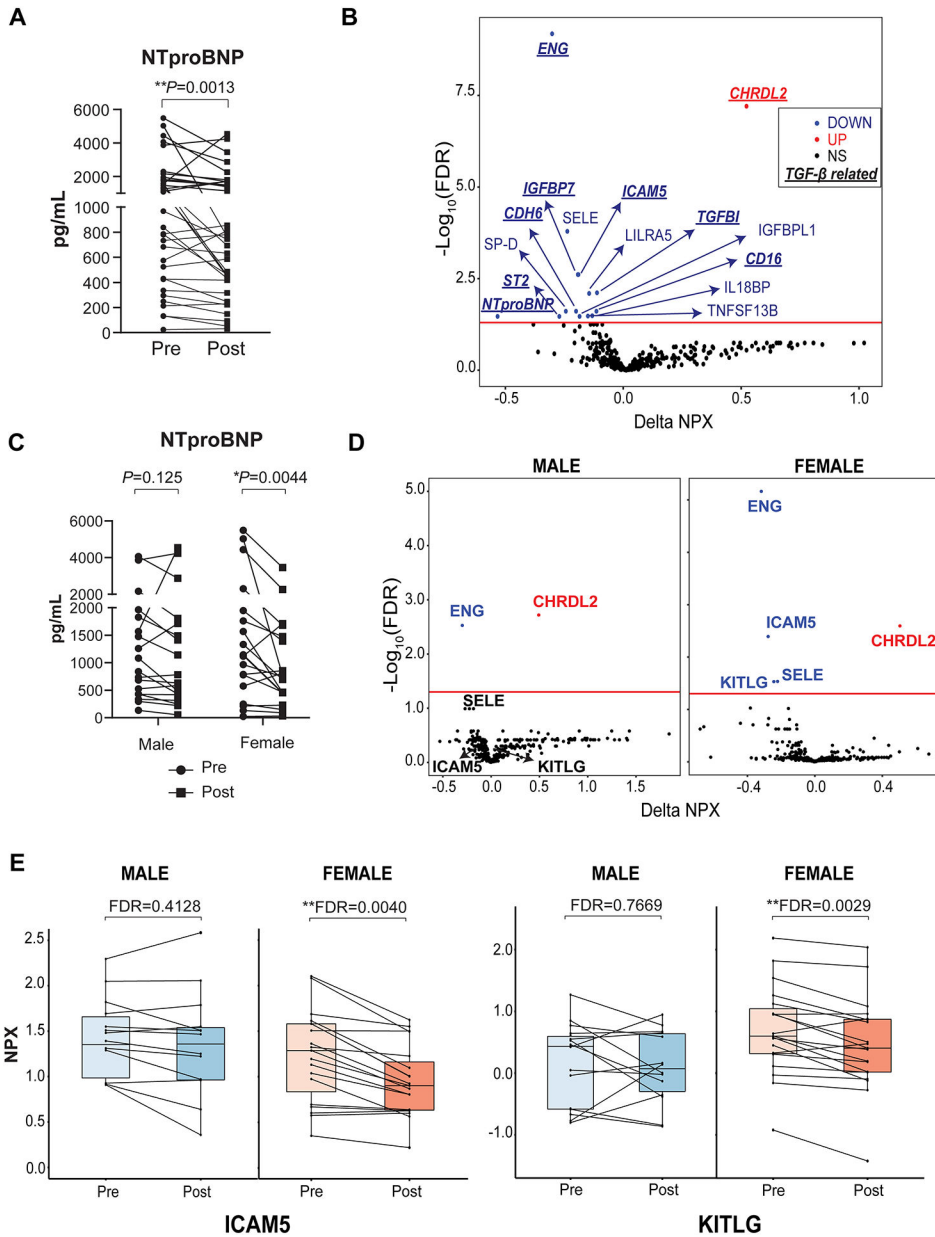
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Abbreviations:

HFpEF	Heart Failure with Preserved Ejection Fraction
LVEF	Left Ventricular Ejection Fraction
NPX	Normalized Protein Expression

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Proteomic profiling of HFpEF patients prior to and after 5 weeks of sacubitril/valsartan treatment.
 (A) Plasma level of NTproBNP in all patients before and after drug treatment measured by standard clinical electrochemiluminescence immunoassay. *P*-value reflected paired t-test of log₂-transformed values. (B) Volcano plot of proteomic changes before and after 5 weeks of sacubitril/valsartan treatment (paired student’s t-test with Benjamini–Hochberg multiple comparison correction. All proteomic analyses were done with R version 4.1.1). Y axis represents -log₁₀ of false discovery rate (FDR). X axis represents Normalized Protein Expression (NPX) change. Red line in the plot represents significant cutoff at 5% FDR. (C) Plasma level of NTproBNP in female and male patients before and after drug treatment measured by standard clinical electrochemiluminescence immunoassay (*P*-value reflected paired t-test of log₂-transformed values). (D) Volcano plot of changes before and

after 5 weeks of sacubitril/valsartan treatment stratified by sex (paired student's t-test with Benjamini–Hochberg multiple comparison correction). Y axis represents $-\log_{10}$ of FDR. X axis represents NPX change. Red line in the plot represents significant cutoff at 5% FDR. Because SELE showed a trend of being changed by sacubitril/valsartan in male patients, it was conservatively not considered female-specific response and not discussed in the main text. **(E)** Two proteins changed after sacubitril/valsartan treatment that were female specific. Each dot represents a patient.

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