DNA methylation profiling of circulating CD4+ T cells and network analysis reveal that JUNB, SETD7, and MEF2D genes predict patients with heart failure preserved vs. reduced ejection fraction


1University of Campania Luigi Vanvitelli, Naples, Italy
2Brigham And Women’S Hospital, Harvard Medical School, Boston, United States of America
3Federico II University Hospital, Naples, Italy
4AO dei Colli - Monaldi Hospital, Naples, Italy
5San Giovanni di Dio and Ruggi d’Aragona University Hospital, Salerno, Italy

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Background: Heart failure (HF) with preserved ejection fraction (HFpEF) represents about 50% of new HF diagnosis but still lacks specific biomarkers and effective therapy. Epigenetic-sensitive changes accumulated over time, mainly guided by DNA methylation and demethylation, can lead to chromatin remodelling and affect transcriptional molecular networks underlying microvascular inflammation, oxidative stress, and maladaptive cardiac remodelling which predispose synergistically to HFpEF.

Purpose: We hypothesize that circulating genome-wide DNA methylation profiles may identify novel biomarkers for discriminating HFpEF vs. HF with reduced EF (HFrEF) and provide insight into the underlying pathophysiology of disease.

Methods: A total of n=47 subjects were enrolled including patients with HFpEF (n=22) and HFrEF (n=13) as well as healthy subjects (n=12) serving as control group (CON). Starting from peripheral blood biospecimens, we isolated CD4+ T cells and extracted genomic DNA for genome-wide methylene analysis and total RNA for experimental validation set.

Results: The reduced representation bisulfite sequencing (RRBS) revealed that patients with HFpEF had unique variations in DNA methylation profiles as compared to HFrEF and CON groups and reflected distinct pathogenic mechanisms. Using the list of differentially methylated CpG sites in HFpEF, we performed a complex network analysis to indentify the "HFpEF interactome" (Fig.1 A-B). Then, using the DisGeNET database we extracted n=12 candidate genes which were already associated with diastolic dysfunction, cardiac hypertrophy, cardiac fibrosis, inflammation, obesity, and hypertension, as key pathophenotypes of HFpEF (Fig.1 C). The hub genes included the ACTB, VAV2, JUNB, HOOK2, SETD7, PNKP, ASAP1, PAICS, DNM1L, HIF1AN, MEF2D, and NRG1. Validation experiment set by qRT-PCR revealed that elevated mRNA levels of the JUNB, SETD7, and MEF2D hypermethylated genes significantly discriminated HFpEF patients vs. CON (p<0.01, p<0.01, p<0.001, respectively) and vs. HFrEF patients (p<0.05, p<0.01, p<0.001, respectively) (Fig. 2A). Receiver operating characteristic (ROC) curve showed that network-oriented JUNB (AUC: 1, p<0.001), SETD7 (AUC: 0.97, p<0.01), and MEF2D (AUC: 1, p<0.001) genes have a high diagnostic accuracy in discriminating HFpEF vs. CON as well as HFpEF vs. HFrEF (AUC: 1, p<0.01, AUC: 0.97, p<0.01, AUC: 1, p<0.001, respectively) (Fig. 2B).

Conclusions: Circulating CD4+ T cell-derived hypermethylation of JUNB, SETD7, and MEF2D may reveal novel molecular drivers of HFpEF and suggest possible biomarkers to optimize patient phenotyping.
Figure 1: Network analysis. A) Clustering heatmap shows the mean methylation levels of the differentially methylated CpG sites (dmCpGs)-associated genes in CD4+ T cells isolated from HFpEF patients vs. healthy subjects (CON). The colour indicates DNA methylation levels where blue represents hypomethylation and orange represents hypermethylation. B) The list of dmCpGs was mapped to the left-ventricle protein-protein interaction network to identify the "HFpEF interactome". C) Using DEGNet database, we extracted n=12 HFpEF candidate genes including ACTB, VAV2, HOXC5, SETD7, PNKP, ASAP1, PAICS, DNMT1, HIF1AN, MEF2D, NPHST1, and JUNB.