Cardiac-specific GCN5L1 deficiency promotes MASLD in HFpEF

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Abstract

The prevalence of cardiometabolic heart failure with preserved ejection fraction (HFpEF) continues to grow, representing over half of heart failure cases in the United States. As no specific medication for HFpEF exists, treatment guidelines focus on the management of comorbidities related to metabolic syndrome (e.g. obesity, diabetes, hypertension) that promote the disease1. These same comorbidities also drive pathology in non-cardiac tissues, and the links between cardiometabolic disease presentations in different organs are increasingly being recognized. Preclinical studies on the potential crosstalk between HFpEF and metabolic disease in the liver (e.g. metabolic dysfunction-associated liver disease; MASLD) have focused on how liver dysfunction may affect the heart, particularly through the release of secreted liver proteins. This may reflect the situation in the clinic, where incident MASLD is a risk factor for future HFpEF development. Here, in contrast to this developing paradigm of liver-initiated cardiac disease, we report for the first time a defect in cardiac metabolism related to the mitochondrial metabolic protein GCN5L1 that drives hepatic steatosis and MASLD in HFpEF.

Treatment guidelines for cardiometabolic heart failure with preserved ejection fraction (HFpEF) focus on the management of comorbidities related to metabolic syndrome (*e.g.* obesity, hypertension) that promote disease progression[1]. These comorbidities also drive dysfunction in non-cardiac tissues, and the links between cardiometabolic disease presentations in different organs are increasingly being recognized. Preclinical studies on the potential crosstalk between HFpEF and metabolic disease in the liver (*e.g.* metabolic dysfunction-associated steatotic liver disease; MASLD) have largely focused on how liver dysfunction may affect the heart[2]. This may reflect clinical situations, where incident MASLD is a risk factor for future HFpEF development[3], and the general rarity of cardiac metabolic defects driving pathophysiology in other organs[4]. Here, in contrast to this paradigm, we report for the first time a cardiac-specific gene deletion that promotes MASLD in mice with HFpEF.

GCN5L1, a ubiquitously expressed protein, modulates cellular metabolism via interactions with mitochondrial and endo-lysosomal protein regulatory complexes[5]. To examine its potential function as a metabolic regulator in HFpEF, young adult C57BL/6NJ male wildtype (WT) and inducible, cardiomyocyte-specific GCN5L1 knockout (cKO) mice were placed on either a chow or HFpEF diet (60% HFD + 0.5 g/L L-NAME)[2] for 15 weeks (**Figures 1A-B**). All studies were carried out with approval from the University of Pittsburgh IACUC. HFpEF cKO mice displayed significantly worse diastolic dysfunction relative to WT mice, along with elevated body weight and fasting blood glucose levels (**Figures 1C-F**). Necropsy analysis following these unexpected findings demonstrated that liver steatosis (including histology-quantified MASLD) was a contributor to the increased body weight observed (**Figures 1G-I, S1**). Biochemical and chromatography analysis confirmed that significantly elevated liver triglycerides and cholesterol levels were the basis of the observed steatosis in cKO HFpEF livers (**Figures 1J-L**).

Bulk RNA-seq of HFpEF mouse livers demonstrated that cholesterol metabolism pathways were the most highly dysregulated in cKO mice. This manifested as a downregulation of genes involved in

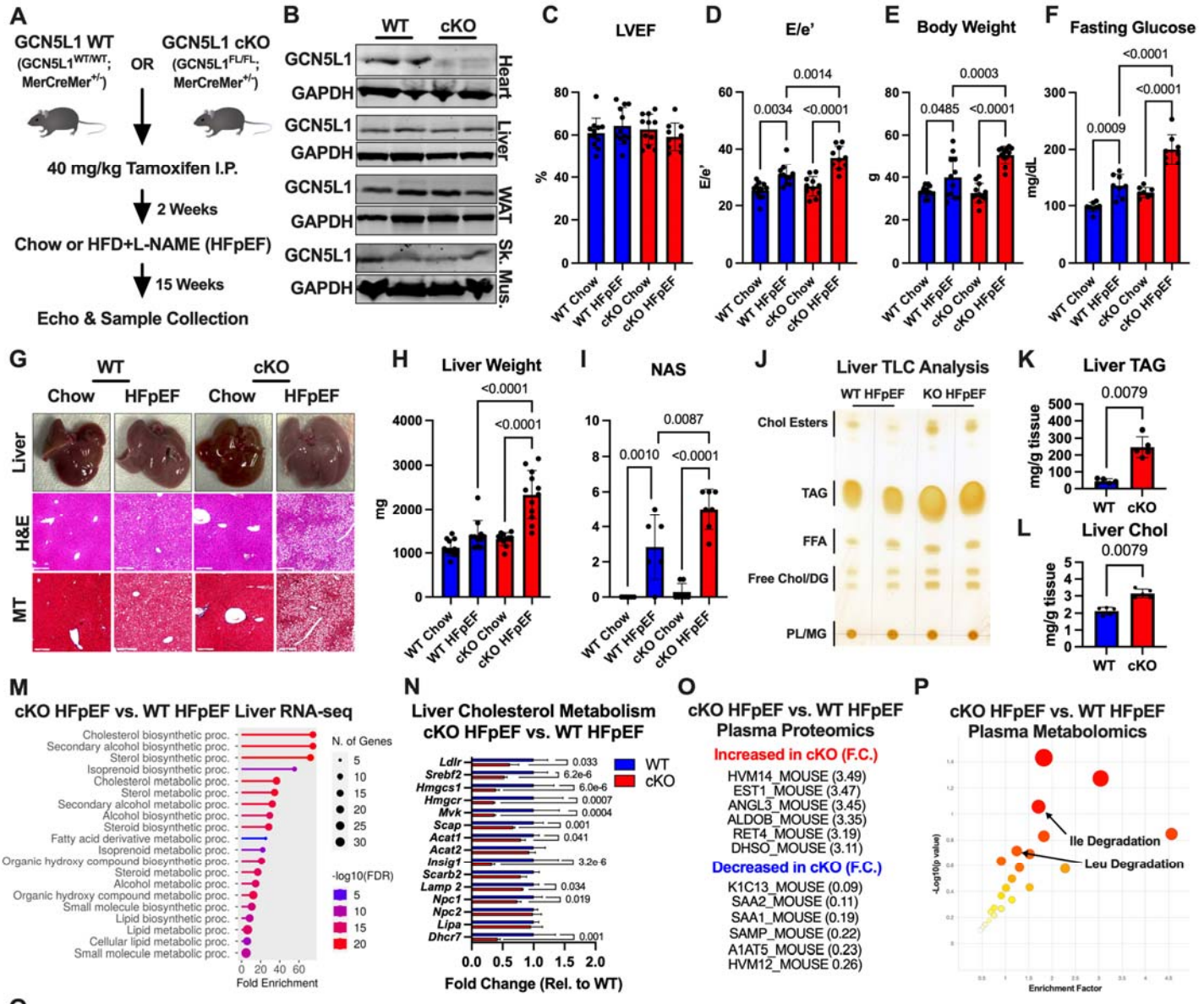
lysosomal cholesterol trafficking (*e.g. Npc1*), with the subsequent accumulation of unprocessed cholesterol likely suppressing hepatic cholesterol synthesis pathway genes (*e.g. Hmgcr*) (**Figures 1M-N**). Combined with an increased abundance of unprocessed cholesterol esters (**Figure 1J**), these findings suggest that cKO HFpEF mouse livers develop steatosis partly via the abnormal accumulation of cholesterol in lysosomes.

We next attempted to identify the cardiac-derived factor driving liver disease in cKO mice. Unbiased proteomic detection of pooled WT and cKO plasma samples did not detect an obvious candidate. Of note, serum amyloid proteins (*e.g.* SAA1, SAA2), previously identified as potential liver-secreted factors driving heart fibrosis in HFpEF[2], were unexpectedly decreased in cKO HFpEF mice (**Figure 1O, Table S1**). Untargeted metabolomic analysis showed that the branched-chain amino acids (BCAAs) leucine and isoleucine, and their branched-chain keto acid (BCKA) metabolites, were significantly upregulated in the plasma of cKO HFpEF mice (**Figure 1P, Table S2**). Concordantly, genes involved in BCAA/BCKA metabolism (*e.g. Klf15, Bckdha*) were significantly downregulated in the hearts, but not livers, of cKO HFpEF mice (**Figures 1Q-R**). Furthermore, there was decreased expression of cardiac genes involved in the metabolism of leucine/isoleucine BCKA derivatives (*Ivd* and *Acadsb*, respectively), but not valine (*Acad8*), which matched the plasma accumulation of their respective BCAAs and BCKAs (KIC and KMV) (**Figures 1Q-S**). In the liver, WT mice showed a significant decline in leucine in HFpEF, while cKO mice maintained hepatic levels of this BCAA under these conditions (**Figure 1U**). Combined, these data suggest that reduced BCAA/BCKA metabolism in cKO mouse hearts, and the subsequent release of cardiac BCAAs/BCKAs into the circulation, may be the heart-derived factor driving MASLD in cKO HFpEF mice.

Finally, we examined how BCAAs/BCKAs may negatively impact liver metabolism and drive MASLD. Impaired cholesterol trafficking in endothelial cells inhibits the activation of mTOR[6], a master regulator of autophagy and lysosomal function. We hypothesized that excess circulating BCAAs/BCKAs may

impede hepatic cholesterol trafficking, leading to mTOR-mediated lysosomal dysfunction and liver cholesterol accumulation. In agreement with this hypothesis, mTOR activation (measured by p-mTOR and p-P70S6K immunoblotting) was reduced in cKO livers relative to WT mice (**Figures 1V, S2A**). Concordantly, treatment of liver HepG2 cells with the leucine-derived BCKA, KIC, resulted in a similar reduction in mTOR activity (**Figures 1W, S2B**)**.** Combined, these data suggest that elevated cardiac-derived BCAA/BCKAs in the circulation negatively impact liver cholesterol metabolism via the mTOR pathway.

In summary, we provide the first example of a cardiac-specific gene deletion driving fatty liver disease in HFpEF. Loss of GCN5L1 in the heart during HFpEF led to disrupted cardiac BCAA/BCKA metabolism, which resulted in hepatic cholesterol accumulation. These findings suggest that the heart-liver signaling axis in HFpEF is bidirectional, and should not be viewed solely as a liver-initiated process in this disease.


A
GCN5L1 WT
(GCN5L1WT/WT; MerCreMer+/-)
OR
GCN5L1 cKO
(GCN5L1FL/FL; MerCreMer+/-)
40 mg/kg Tamoxifen I.P.
2 Weeks
Chow or HFD+L-NAME (HFpEF)
15 Weeks
Echo & Sample Collection
B
WT
cKO
GCN5L1
GAPDH
Heart
Liver
WAT
Sk. Mus.
C
LVEF
D
E/e'
E
Body Weight
F
Fasting Glucose
WT Chow
WT HFpEF
cKO Chow
cKO HFpEF
G
Chow
HFpEF
Liver
H&E
MT
H
Liver Weight
I
NAS
J
Liver TLC Analysis
WT HFpEF
KO HFpEF
Chol Esters
TAG
FFA
Free Chol/DG
PL/MG
K
Liver TAG
mg/g tissue
L
Liver Chol
M
cKO HFpEF vs. WT HFpEF Liver RNA-seq
Cholesterol biosynthetic proc.
Secondary alcohol biosynthetic proc.
Sterol biosynthetic proc.
Isoprenoid biosynthetic proc.
Cholesterol metabolic proc.
Sterol metabolic proc.
Secondary alcohol metabolic proc.
Alcohol biosynthetic proc.
Steroid biosynthetic proc.
Fatty acid derivative metabolic proc.
Isoprenoid metabolic proc.
Organic hydroxy compound biosynthetic proc.
Steroid metabolic proc.
Alcohol metabolic proc.
Organic hydroxy compound metabolic proc.
Small molecule biosynthetic proc.
Lipid biosynthetic proc.
Lipid metabolic proc.
Cellular lipid metabolic proc.
Small molecule metabolic proc.
N. of Genes
-log10(FDR)
Fold Enrichment
N
Liver Cholesterol Metabolism
cKO HFpEF vs. WT HFpEF
Ldlr
Srebf2
Hmgcs1
Hmgcr
Mvk
Scap
Acat1
Acat2
Insig1
Scarb2
Lamp 2
Npc1
Npc2
Lipa
Dhcr7
WT
cKO
Fold Change (Rel. to WT)
O
cKO HFpEF vs. WT HFpEF
Plasma Proteomics
Increased in cKO (F.C.)
HVM14_MOUSE (3.49)
EST1_MOUSE (3.47)
ANGL3_MOUSE (3.45)
ALDOB_MOUSE (3.35)
RET4_MOUSE (3.19)
DHSO_MOUSE (3.11)
Decreased in cKO (F.C.)
K1C13_MOUSE (0.09)
SAA2_MOUSE (0.11)
SAA1_MOUSE (0.19)
SAMP_MOUSE (0.22)
A1AT5_MOUSE (0.23)
HVM12_MOUSE 0.26)
P
cKO HFpEF vs. WT HFpEF
Plasma Metabolomics
Ile Degradation
Leu Degradation
Enrichment Factor
Q
Heart BCAA/BCKA genes
WT HFpEF
cKO HFpEF
Normalized Counts
Klf15
Bcat2
Bckdk
Bckdha
Bckdhb
Ivd
Acadsb
Acad8
S
Plasma BCAAs
Fold Change (Rel. to WT)
Leu
Ile
Val
T
Plasma KIC & KMV
Area
U
Liver Leucine
Area/ISTD
V
Liver mTOR activity
Relative to GAPDH
p-mTOR
p-p70S6K
R
Liver BCAA/BCKA genes
W
HepG2 mTOR activity
Vehicle
KIC
X
BCAT
BCKDH
BCAAs
BCKAs
Ac/Suc-CoA
TCA
Cardiac KIC & KMV
GCN5L1cKO +HFpEF
mTOR
Cholesterol
Autophagy

)1

FIGURE 1 – Cardiac-specific GCN5L1 deficiency promotes MASLD in HFpEF.

A. Experimental protocol. **B.** Confirmation of cardiac-specific GCN5L1 deletion in cKO hearts. **C.** Left ventricle ejection fraction. **D.** Left ventricle E/e' ratio. **E.** Body weight. **F.** Fasting blood glucose. **G.** Gross anatomy and histological staining (H&E and Masson's Trichrome; MT) of livers. **H.** Liver weight **I.** NAFLD activity score (NAS). **J-L.** Liver triglycerides (TAG) and cholesterol (Chol). **M.** ShinyGO transcriptome analysis. **N.** Liver cholesterol metabolism genes. **O-P.** Plasma proteomics (pooled samples) and untargeted plasma metabolomics (BioCyc pathway analysis). **Q-R.** BCAA and BCKA metabolism genes in heart and liver **S.** Plasma BCAAs **T.** Plasma KIC and KMV. **U.** Liver leucine levels. **V.** mTOR and p70S6K phosphorylation in HFpEF livers. **W.** mTOR and p70S6K phosphorylation after KIC treatment in liver HepG2 cells. **X.** Proposed model. All graphs represent mean ± SD. Mann-Whitney U-test or Student's T-test was used for comparisons between two groups, one-way ANOVA was used for comparisons between multiple groups.

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