Cardiac-specific deletion of GCN5L1 promotes fatty liver disease in HFpEF

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Word Count: 800

Keywords: GCN5L1, HFpEF, BCAAs, Heart, Liver, Steatosis, MAFLD

Running Title: Heart-to-Liver Crosstalk in HFpEF

The authors have declared that no conflict of interest exists.

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 disease. 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-associated fatty liver disease; MAFLD) 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 MAFLD 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 MAFLD in HFpEF.

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[1]. 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 disease[1]. These same comorbidities also drive pathology in non-cardiac tissues, and the links between cardiometabolic disease presentations in different organs are increasingly being recognized[2].

Preclinical studies on the potential crosstalk between HFpEF and metabolic disease in the liver (*e.g.* metabolic-associated fatty liver disease; MAFLD) have focused on how liver dysfunction may affect the heart, particularly through the release of secreted liver proteins[2]. This may reflect the situation in the clinic, where incident MAFLD is a risk factor for future HFpEF development[3]. Here, in contrast to this developing paradigm of liver-initiated cardiac disease, we report for the first time a defect in cardiac metabolism that drives hepatic steatosis and MAFLD in HFpEF.

Young adult C57BL/6NJ male wildtype (WT) and cardiomyocyte-specific knockout mice of the mitochondrial metabolic regulatory protein GCN5L1 (GCN5L1 cKO)[4] were placed on either a chow or HFpEF diet (60% high fat diet + 0.5 g/L L-NAME)[2] for 15 weeks (**Figure [A]**). All studies were carried out with approval from the University of Pittsburgh IACUC and followed NIH guidelines. While both WT and GCN5L1 cKO HFpEF mice developed diastolic dysfunction in the absence of systolic impairment as expected, GCN5L1 cKO animals displayed significantly elevated body weight and fasting blood glucose levels relative to WT mice (**Figure [A]**). Necropsy analysis following this unexpected finding demonstrated that GCN5L1 cKO mice on a HFpEF diet had significantly increased liver weights and histology-quantified MAFLD (NAS; **Figure [B]**). Biochemical and chromatography analysis showed that significantly elevated liver triglycerides and cholesterol levels were the basis for the observed hepatic steatosis in GCN5L1 cKO HFpEF mice (**Figure [B]**).

Bulk RNA-seq on HFpEF mouse livers demonstrated that transcriptional pathways related to cholesterol were highly dysregulated in GCN5L1 cKO mice. This manifested as a downregulation of genes involved in lysosomal cholesterol processing (*e.g. Npc1*, *Lamp2*), along with the suppression of hepatic cholesterol synthesis pathways, in GCN5L1 cKO mice (**Figure [C]**). Combined with the increased abundance of unprocessed cholesterol esters in chromatography analysis (**Figure [B]**), these findings suggest that the livers of GCN5L1 cKO HFpEF mice develop steatosis partly from an abnormal accumulation of cholesterol in lysosomes.

We next attempted to determine the cardiac-derived factor that was driving liver disease in GCN5L1 cKO mice. Unbiased proteomic detection of pooled WT and GCN5L1 cKO plasma samples did not detect an obvious protein signal. Of note, serum amyloid proteins (*e.g.* SAA1, SAA2, SAMP), previously identified as potential liver-secreted factors driving heart fibrosis in HFpEF[2], were significantly decreased in GCN5L1 cKO HFpEF mice (**Figure [D]**). Untargeted metabolomic analysis of plasma samples showed that branched-chain amino acids (BCAAs) and their metabolites were significantly upregulated in GCN5L1 cKO HFpEF mice (**Figure [D]**). We examined BCAA metabolic gene expression in WT and GCN5L1 cKO HFpEF mice by bulk RNA-seq, and found that genes involved in BCAA and branched-chain ketoacid (BCKA) metabolism (*e.g. Klf15, Bckdha, Bckdhb*) were significantly downregulated in the hearts, but not livers, of GCN5L1 cKO HFpEF mice (**Figure [E]**). Furthermore, there was a downregulation of cardiac genes involved in the metabolism of leucine and isoleucine BCKA derivatives (*Ivd* and *Acadsb*, respectively), but not valine (*Acad8*), which matched the plasma accumulation of their BCAA parent metabolites (**Figure [E]**). Lastly, while the livers of WT mice showed a significant decline in leucine after HFpEF diet exposure, GCN5L1 cKO mice maintained their tissue levels of this BCAA under HFpEF conditions (**Figure [E]**), potentially via uptake from the circulation. Combined, these data suggest that reduced cardiac BCAA/BCKA metabolism in GCN5L1 cKO mice, and the subsequent release of cardiac BCAAs/BCKAs into the circulation, may be the factor driving the observed hepatic steatosis.

Finally, as lysosomal processes are involved in cholesterol handling, and BCAAs are known to inhibit hepatic autophagy[5], we hypothesized there may be a defect in these pathways in GCN5L1 cKO mouse livers. As predicted, immunoblotting for the key autophagosome regulatory protein LC3 demonstrated a reduction in both total and active (LC3-II) forms of this protein (**Figure [F]**), suggesting an overall suppression of autophagy/lysosomal processes in the livers of cardiac-specific GCN5L1 cKO HFpEF mice.

In summary, we provide the first example of a cardiac-specific gene deletion driving metabolic dysfunction and liver disease progression in HFpEF. Loss of GCN5L1 in the heart during HFpEF led to disrupted cardiac BCAA metabolism and subsequent elevation of plasma BCAA/BCKA metabolites, which promoted lipid accumulation and autophagy dysfunction in the liver. These findings suggest that the heart-liver signaling axis in HFpEF is bidirectional, and that future studies of interorgan crosstalk will require sophisticated mechanisms to determine the tissue source of the signal.

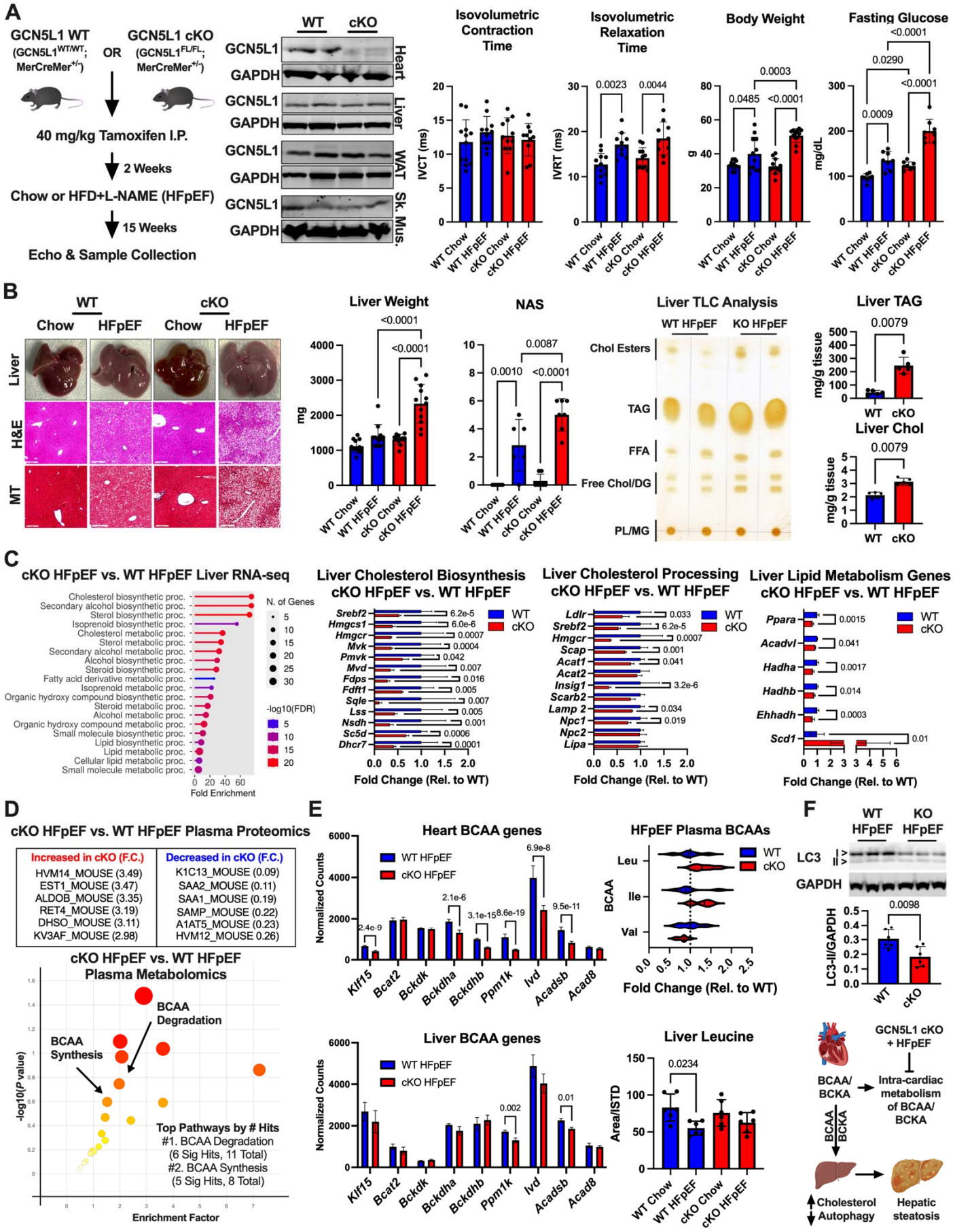
A
GCN5L1 WT (GCN5L1WT/WT; MerCreMer+/-)
OR
GCN5L1 cKO (GCN5L1FL/FL; MerCreMer+/-)
40 mg/kg Tamoxifen I.P.
2 Weeks
Chow or HFD+L-NAME (HFpEF)
15 Weeks
Echo & Sample Collection
WT
cKO
GCN5L1
GAPDH
Heart
Liver
WAT
Sk. Mus.
Isovolumetric Contraction Time
Isovolumetric Relaxation Time
Body Weight
Fasting Glucose
IVCT (ms)
IVRT (ms)
g
mg/dL
WT Chow
WT HFpEF
cKO Chow
cKO HFpEF
B
Chow
HFpEF
Liver
H&E
MT
Liver Weight
NAS
Liver TLC Analysis
Liver TAG
Liver Chol
mg
mg/g tissue
Chol Esters
TAG
FFA
Free Chol/DG
PL/MG
C
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
Liver Cholesterol Biosynthesis cKO HFpEF vs. WT HFpEF
Liver Cholesterol Processing cKO HFpEF vs. WT HFpEF
Liver Lipid Metabolism Genes cKO HFpEF vs. WT HFpEF
Srebf2
Hmgcs1
Hmgcr
Mvk
Pmvk
Mvd
Fdps
Fdft1
Sqle
Lss
Nsdh
Sc5d
Dhcr7
Ldlr
Scap
Acat1
Acat2
Insig1
Scarb2
Lamp 2
Npc1
Npc2
Lipa
Ppara
Acadvl
Hadha
Hadhb
Ehhadh
Scd1
Fold Change (Rel. to WT)
D
cKO HFpEF vs. WT HFpEF Plasma Proteomics
Increased in cKO (F.C.)
HVM14_MOUSE (3.49)
EST1_MOUSE (3.47)
ALDOB_MOUSE (3.35)
RET4_MOUSE (3.19)
DHSO_MOUSE (3.11)
KV3AF_MOUSE (2.98)
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)
cKO HFpEF vs. WT HFpEF Plasma Metabolomics
BCAA Degradation
BCAA Synthesis
Top Pathways by # Hits
#1. BCAA Degradation (6 Sig Hits, 11 Total)
#2. BCAA Synthesis (5 Sig Hits, 8 Total)
-log10(P value)
Enrichment Factor
E
Heart BCAA genes
HFpEF Plasma BCAAs
Liver BCAA genes
Liver Leucine
Normalized Counts
Klf15
Bcat2
Bckdk
Bckdha
Bckdhb
Ppm1k
Ivd
Acadsb
Acad8
Leu
Ile
Val
BCAA
Area/ISTD
F
WT HFpEF
KO HFpEF
LC3
GAPDH
LC3-II/GAPDH
GCN5L1 cKO + HFpEF
BCAA/BCKA
Intra-cardiac metabolism of BCAA/BCKA
Cholesterol
Autophagy
Hepatic steatosis

FIGURE – Cardiac-specific deletion of GCN5L1 promotes the development of liver steatosis.

A. Wildtype (WT) and cardiomyocyte-specific GCN5L1 knockout (cKO) mice displayed normal systolic function (ICVT) and impaired diastolic function (IVRT) after 15 weeks of HFpEF diet (60% HFD + 0.5 g/L L-NAME in drinking water). GCN5L1 cKO mice displayed significantly increased body weight and fasting blood glucose. N = 8-12, One-way ANOVA. **B.** GCN5L1 cKO mice displayed significantly increased liver steatosis, liver weights, and NAFLD activity score (NAS). N = 7-12, One-way ANOVA. Liver triglycerides (TAG) and cholesterol (Chol) were increased in GCN5L1 cKO mice by thin-layer chromatography (TLC) and biochemical quantification. N = 5, Mann-Whitney. **C.** KEGG pathway analysis using ShinyGO demonstrated that cholesterol and lipid pathways were dysregulated in GCN5L1 cKO mice. N = 4, Adjusted *P* values. **D.** Plasma proteomics (pooled samples, N = 5 per group) and untargeted plasma metabolomics (N = 6, KEGG pathway analysis using Metaboanalyst) showed that branched-chain amino acids (BCAAs) were among the top modified pathways in GCN5L1 cKO mice. **E.** BCAA metabolism genes were downregulated in GCN5L1 cKO hearts, with minimal changes observed in liver. N = 4, Adjusted *P* values. Targeted metabolomics demonstrated ~25% higher leucine and isoleucine in plasma of GCN5L1 cKO mice. N = 6, Fold change). WT HFpEF mice displayed a significant decrease in liver leucine levels that was absent in GCN5L1 cKO HFpEF mice. N = 6, One-way ANOVA. **F.** Autophagosome LC3 levels are significantly reduced in GCN5L1 cKO mouse livers. N = 6, Two-tailed T-test.

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ACKNOWLEDGEMENTS

This work was supported by: National Institutes of Health Fellowships (F31DK134089 and T32HL110849) to B.A.S.M; National Institutes of Health Shared Instrument Grants (S10OD023402, S10OD032141) to S.L.G.; National Institutes of Health Research Grants (R01HL147861, R0HL156874) and American Heart Association Established Investigator Award (23EIA1037834) to I.S. The University of Pittsburgh Center for Metabolism and Mitochondrial Medicine is supported by the Pittsburgh Foundation (MR2020 109502) grant to M.J.J. This project used the University of Pittsburgh Medical Center (UPMC) Hillman Cancer Center and Tissue and Research Pathology/Pitt Biospecimen Core shared resource, which is supported in part by National Institutes of Health Center Granty P30CA047904. Echocardiography was carried out by the University of Pittsburgh Rodent Ultrasonography Core, which received funding from the NIH Shared Instrumentation Grant Program (S10OD023684).

AUTHOR CONTRIBUTIONS

P.B. and I.S. conceived the study and designed experiments. P.B., B.A.S.M., M.W.S., J.R.M., N.B., M.S-S., A.M.V., R.G.R.M, C.L.H, and S.J.M. performed experiments. P.B., C.L.H., S.J.M., R.G.R.M., and I.S. analyzed data. P.B. and I.S. prepared figures. S.S.S., B.A.K., S.K.R., S.L.G, and M.J.J. provided critical input and expertise. I.S. drafted the manuscript. P.B. and I.S. edited and revised the manuscript.