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Identification of DNA Damage Repair Enzyme *Ascc2* as Causal for Heart Failure with Preserved Ejection Fraction (HFpEF)

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HFpEF is an increasingly prevalent syndrome characterized by diastolic dysfunction and preserved ejection fraction, which is distinct from heart failure with reduced ejection fraction (HFrEF) in terms of pathogenesis and effective therapeutic management[1]. HFpEF is highly heterogeneous and involves multiple genetic and environmental factors, complicating the dissection of genetic mechanisms of the disease. Here we employ systems genetics approaches involving both mouse and human datasets to identify genetic determinants that play causal roles in diastolic function, a prominent feature of HFpEF.

We initially examined isoproterenol-induced cardiomyopathy in 105 inbred strains of mice constituting the Hybrid Mouse Diversity Panel (HMDP), a systems genetics resource that enables genome-wide association studies (GWAS) in mice[2]. Cardiac function was determined weekly using echocardiography, and global transcriptomic profiling of left ventricles was performed after 21 days of isoproterenol infusion (Fig. Ai). We used E/A ratio (the ratio of peak velocity blood flow in early diastole to peak velocity flow in late diastole) as a surrogate for cardiac diastolic function. First, to identify candidate causal genes affecting diastolic function we used genetic association to map both local loci (within 10 Mb of the gene being regulated) and distal loci controlling gene expression. Those genes whose local expression is correlated with diastolic function are likely to be causal since local variation is largely regulated in *cis* by neighboring regulatory elements rather than in

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Disclosure Statement

None.

Animals

All animal experiments were approved by the University of California Los Angeles (UCLA) Animal Care and Use Committee, in accordance with Public Health Service guidelines.

trans by factors downstream of diastolic function[3]. A total of 274 such genes ($p<0.01$) were identified and are available upon request. Second, to rule out false positives and to identify the most promising genes, we asked if any of these genes mapped to GWAS loci associated with E/A ratio in the isoproterenol HMDP (Fig. Aii) as well as loci identified in human GWAS for traits associated with HFpEF. The gene *Ascc2* was one of 6 candidates whose *cis* component was associated with E/A ratio and was also located in both mouse (Chr 11, peak E/A ratio SNP rs29436095, p-value = 1.65E-06) (Fig. Aiii) and human GWAS loci (Fig. B). Human ASCC2 was originally identified as a component of the activating signal cointegrator 1 (ASC-1) complex. The ubiquitin-binding activity of ASCC2 functions in DNA alkylation damage repair and ribosome quality control pathways[4]. In the HMDP, *Ascc2* is regulated locally (peak SNP rs3698210) (Fig. Ci), with the G allele being associated with higher *Ascc2* expression and lower E/A ratios (Fig. Cii). Strains with lower *Ascc2* expression exhibited higher E/A ratio (Fig. Ciii). In addition to E/A ratio, local genetic variation in *Ascc2* expression was significantly correlated with several key traits including lung function (Fig. Civ). Consistently, genes that were significantly associated with *Ascc2* in the HMDP were enriched in pathways related to DNA damage and repair (Fig. Cv).

We further validated *Ascc2* using *in vitro* and *in vivo* models. In neonatal rat ventricular myocytes (NRVMs), *Ascc2* knockdown by siRNAs caused an increase of *Nppa* and *Nppb* expression (Fig. Di), genes whose expression is increased in HFpEF patients. The levels of ASCC2 protein localized to the nucleus were increased in NRVMs following treatment with phenylephrine (PE) (Fig. Dii)[5]. To determine if HFpEF is associated with an induction of the DNA damage response (DDR) we examined ataxia telangiectasia mutated (ATM) kinase, one of the best characterized DDR markers, in a “two-hit” HFpEF model induced by feeding a high fat diet and the nitric oxide inhibitor l-NAME for 7 weeks[5]. Indeed, phosphorylated ATM was increased in the heart in the HFpEF mouse model (Fig. Diii).

We validated the role of *Ascc2 in vivo* using cardiac-specific knockout (KO) mice generated by breeding *Ascc2* flox mouse (C57BL/6N-A^{tm1Brd} Ascc2$^{tm1a(EUCOMM)Wtsi}$/Bay Mmucd) with αMHC-Mer-Cre-Mer. Control and *Ascc2* heterozygous (het) knockout mice were studied in the “two-hit” HFpEF model[5]. Compared with control mice, *Ascc2*-het mice exhibited reduced *Ascc2* levels in the heart but not in other tissues. In common with the NRVM studies above, ASCC2 levels in the nucleus increased following feeding the HFpEF diet (Fig. Ei). *Ascc2*-het mice exhibited greater diastolic dysfunction and impaired exercise tolerance but retained normal left ventricular ejection fraction (LVEF) and glucose tolerance (Fig. Eii). Factors associated with heart failure (*Nppa* and *Nppb*), DNA damage response (p-ATM) and inflammation (*IL6* and *Tnf*) were increased in heart tissue of *Ascc2*-het mice (Fig. F).

Our results reveal, for the first time, a role of the DDR in HFpEF. ASCC2 is a component of the ASC-1 complex which plays essential roles in transcriptional regulation, DNA damage repair and ribosome quality control pathways[4]. We hypothesize that ASCC2 translocates into the nucleus in HFpEF and regulates heart function by mediating DNA damage response. The effect appears to occur in cardiomyocytes given our findings in NRVMs and cardiomyocyte specific knockouts. The fact that the *ASCC2* locus is associated with traits relevant to HFpEF in human GWAS suggests that it may play a role in the human disease.

Acknowledgments

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Data Availability

The data that support the findings of this study, including reagents used, statistical methods, gene expression and mapping data are available from the corresponding author upon request.

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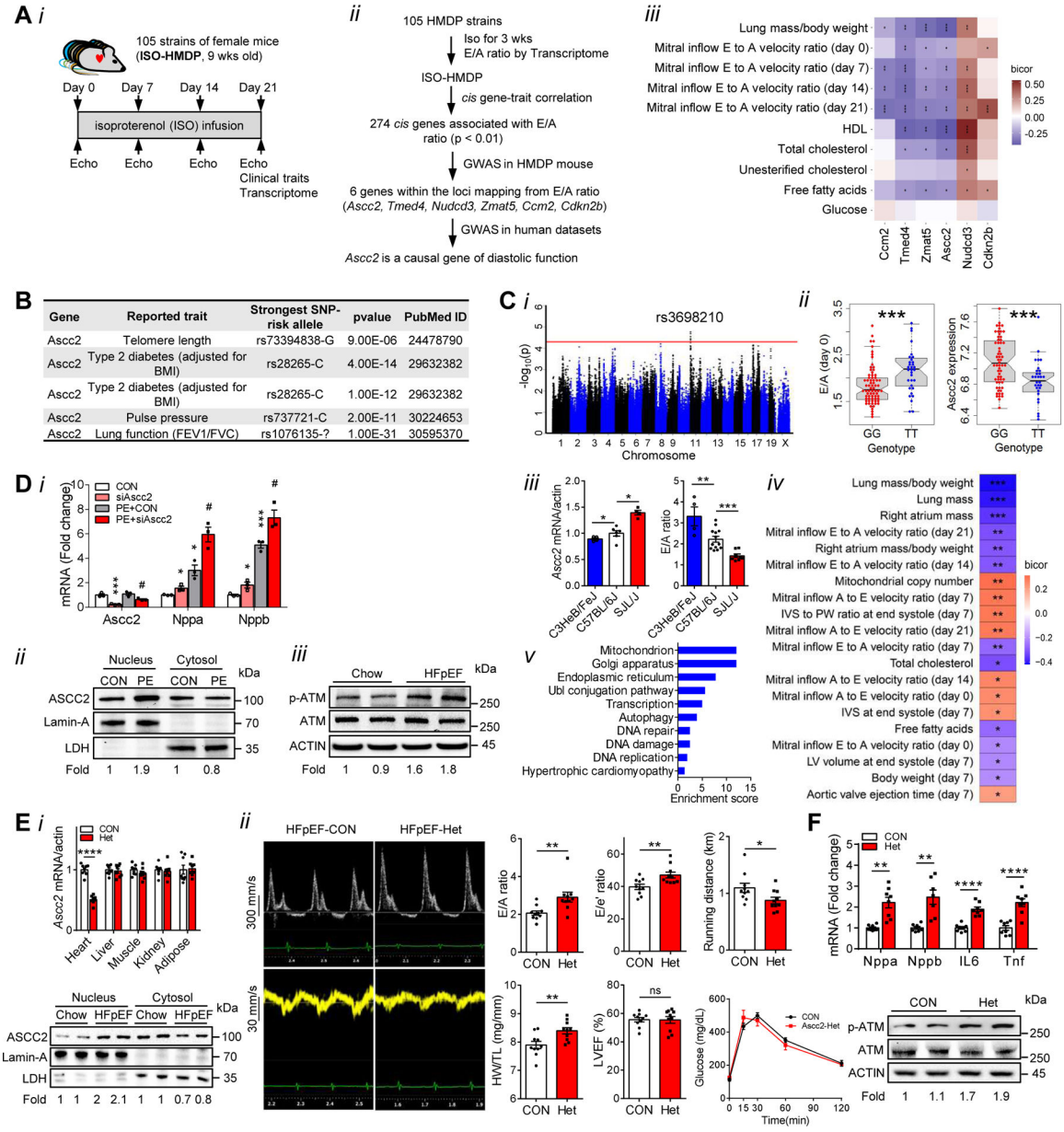

Figure. Identification of *Ascc2* as a Causal Gene for HFpEF.

A. Identification of *Ascc2* as a *cis*-regulator of diastolic function. (**A**i) Experimental design for the development of isoproterenol (ISO) induced cardiomyopathy across HMDP strains. 9 week old female mice from 105 inbred strains were treated with ISO (30 mg/kg/day) for 21 days. Heart function was examined weekly with echocardiography (Echo). After 21 days of ISO infusion, mice were sacrificed and left ventricle transcriptome was determined. N= 3–12 for each strain. (**A**ii) Schematic overview showing identification of causal genes for diastolic function. Local (*cis*) expression quantitative trait loci were determined for a total of 25697 genes using a 5% false discovery rate (FDR) and about 200,000 SNPs as described[2]. (**A**iii) Heatmap showing the associations between top candidate genes and diastolic traits. **B.** Human GWAS loci encompassing the *ASCC2* gene were significantly associated with clinical traits related to HFpEF (https://www.ebi.ac.uk/gwas/genes/ASCC2). The human

ASCC2 gene exhibits significant local genetic regulation (https://gtexportal.org/home/gene/ASCC2).

C. *Ascc2* local eQTL maps to a GWAS locus for E/A ratio in the ISO-HMDP and is inversely correlated with diastolic dysfunction. (**C**i) Manhattan plot showing the significance (−log10 of p) of all SNPs and eQTL of *Ascc2* in ISO-HMDP. The peak local eQTL SNP for *Assc2* expression maps within 10kb of the gene and is within the Chromosome 11 GWAS locus for E/A ratio[2]. The threshold of significance is p < 4e-3. (**C**ii) Correlation of E/A ratio and *Ascc2* heart expression with genotype at peak SNP associated with *Ascc2* on chromosome 11 (rs3698210). (**C**iii) *Ascc2* expression and E/A ratio in indicated strains. (**C**iv) Heatmap showing the correlation between local genetic variation in *Ascc2* expression and parameters of diastolic function in the ISO-HMDP. (**C**v) Enriched pathways of *Ascc2* associated genes (p < 0.001) in ISO-HMDP. The enrichment analysis was performed by The Database for Annotation, Visualization and Integrated Discovery (DAVID).

D. (Di) *Nppa* and *Nppb* expression in NRVMs treated with phenylephrine (PE, 100uM) and siRNAs targeting *Ascc2* for 24h (* as compared to control (CON) and # as compared to PE+CON). (**D**ii) Nuclear and cytoplasmic fraction of ASCC2 in NRVMs after PE treatment for 48h. (**D**iii) Heart phosphorylated ATM level after HFD + l-NAME feeding for 7 weeks. The “two-hit” HFpEF model was originally developed by the Hill group[5] and our results were consistent with the phenotypes they reported.

E. (**E**i) qRT-PCR showing *Ascc2* mRNA level in heart and other tissues of CON and *Ascc2*-het mice (upper). Nuclear and cytoplasmic fraction of ASCC2 in the heart after HFD + l-NAME feeding for 7 weeks (lower). (**E**ii) Representative images of echocardiography (E/A and E/e’ waves), diastolic function, running distance, and glucose tolerance test (GTT) of CON and *Ascc2*-het mice. Procedures for measuring HFpEF phenotypes were essentially identical to those in Schiattarella et. al.[5].

F. Gene expression and p-ATM levels in the heart of CON and *Ascc2*-het mice after HFD + l-NAME feeding.

Each point represents a mouse. All data are presented as the mean ± SEM. *P < 0.05, **P < 0.01, ***P < 0.001, and ****p < 0.0001, by 1-way ANOVA, 2-way ANOVA, or by Student’s t test.