nature portfolio

Peer Review File

Genome-wide analysis of heart failure yields insights into disease heterogeneity and enables prognostic prediction in the Japanese population

Corresponding Author: Dr Kaoru Ito

This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
This study explores the genetic basis of heart failure (HF) and its subtypes in the Japanese population using a large-scale GWAS. By analyzing over 16,000 HF cases and 213,000 controls, the authors identified 19 novel loci, including Japanese-specific variants, and highlighted differences between HF subtypes like HFrEF and HFpEF. Cross-ancestry meta-analyses revealed shared and population-specific genetic risks, such as a TTN variant with stronger effects in Japanese individuals. Functional analyses linked genetic findings to pathways like Notch signaling. The authors also developed a polygenic risk score (PRS) that predicts early-onset HF and stratifies mortality risk, providing new insights into HF genetics and supporting its potential for precision medicine applications.

1. The manuscript presents a thorough analysis of genetic loci associated with heart failure, including differences between Japanese and European populations. These findings are intriguing, particularly the identification of loci with population-specific effects, such as the TTN variant. The manuscript need to further explore the clinical implications of these genetic differences to provide a comprehensive understanding of their potential impact on heart failure treatment and management. The authors should consider discussing specific ways in which these population-specific genetic insights could inform heart failure prevention, diagnosis, or management strategies in Japanese individuals, such as through targeted screening programs or personalized treatment plans. Including a discussion on how these genetic findings could be integrated into precision medicine approaches, such as through genetic screening or personalized treatment strategies, would enhance the translational impact of the study.

2. While the development of PRS is a significant contribution, the limitations of applying PRS in clinical settings, especially across different populations, should be considered and discussed.

3. The discovery of novel loci significantly advances our understanding of the genetic basis of HF. The manuscript mentions functional annotations such as eQTL and sQTL analyses, but these results are not deeply integrated into the discussion of biological mechanisms. Could the authors provide a more detailed interpretation of how the identified loci influence HF pathophysiology?

4. The manuscript emphasizes the Japanese-specific findings, without inclusion of other East Asian datasets. This is a limitation acknowledged by the authors. Could the authors suggest potential collaborations or strategies to validate these findings further in larger East Asian cohorts?

Minor
1. All abbreviations in Figures should be spelled out in footnote
2. In Figure 1, the numbers of cases for different types of HF were provided for Japanese population but not for European population. It will be helpful to provide such information.
3. It is hard to summarize the information in Figure 3. It might be helpful to summarize the same/similar pathways identified

by GO, KEGG, etc.

Reviewer #2

(Remarks to the Author)
Review_ NCOMMS-24-76119-T

Comments

The current study sought to analyze the contribution of common genetic variation in patients with heart failure in the Japanese population.
First, the authors performed a Japanese GWAS of all-cause HF, HFrEF, HFpEF and non-ischemic HF. Thereby, 18 genome-wide significant loci were identified, with 5 novel loci (most of them for all-cause HF and HFrEF, similar to previous GWAS (PMID: 36517512). Then using a cross-ancestry approach and multi-trait analysis the authors performed a meta-analysis and overall identified 19 novel loci associated with HF or HF associated traits.
Second, the authors used several gene prioritization methos to identify causal genes at the reported loci including the evaluation of known cardiomyopathy genes and gene-drug interactions relevant for HF. Finally, the authors calculated a polygenic risk score, based on the results of the cross-ancestry analysis and tested its ability for age at onset of HF and cardiovascular/HF mortality prediction.

Overall, the study included the first stand-alone GWAS of the Japanese population and provides a large cross-ancestry analysis in HF. In addition, the authors nicely used several bioinformatic analyses to clarify the role of specific high-risk genes and drug targets. However, we have some major concerns:

1. Presentation of the identified and replicated loci in the initial GWAS is hard to follow. I recommend including parts of Supplementary Figure 1 (mainly Miami plot) in the main manuscript as it is an essential part of the results. The authors separated their cohort into HFrEF and HFpEF, as previously performed in a GWAS by Joseph et al (PMID: 36517512). Thereby, the authors identified only a small number of loci associated with HFpEF, reflecting the heterogeneity of the phenotype. As the estimated heritability for each HF type is clinically relevant (in particular the low number of identified loci in HFpEF and NIHF) this should be included and discussed in the manuscript.

2. To increase statistical power the authors performed a multi-trait analysis using MRI data from UK Biobank. Thereby, the highest association with HF was seen for left ventricular mass. This is surprising as previous studies have shown that LV volume was mainly associated with HF. However, this association could be caused by arterial hypertension as strong risk factor for LVH and HF. In contrast, the authors show that the lead variant for HFrEF in the Japanese population is protective for arterial hypertension? Are other explanations possible for the association with a lower blood pressure?

3. The authors state that in particular in HFrEF variance in sarcomeric genes is of importance. Can the authors comment on the role of Omecamtiv as treatment in HFrEF in this context?

4. The authors identify a common variant in the TTN gene and describe it as a prognostic marker for HF. Regarding the minor allele frequency this variant was described more often in Europeans than in the Japanese population. However, in Fig. 4a the risk allele frequency is described higher in Europeans than in the East Asian population? Also, in which Biobank did the authors test for the association with heart failure death?

5. The authors calculated a PRS and tested its performance in mortality and HF prediction. However, the PRS included several HF subtypes and “meta-traits”. From the reviewers point of view this does not present a specific polygenic risk score for heart failure but pools many genetically affected cardiovascular traits. Thus, the authors should build and test a PRS for HF as well as such meta-trait PRS and compare both for their predictive value. Moreover, it would be interesting to see how strong these PRSs perform in prediction of heart failure, when in comparison to clinically determined cardiovascular risk factors such as hypertension or diabetes or traits such as atrial fibrillation or CAD. Finally, is there a benefit in adding a “HF PRS” to clinical prediction tools?

6. Minor comments:

- In the meantime, the SUMMIT trial has been published with positive outcome for tirzepatide in HFpEF (PMID: 39555826). This should be cited and added to the discussion.

- I would recommend including arterial hypertension to the baseline characteristics (Suppl. Table 1) -

In the PDF Suppl. File only Suppl. Table 1 is displayed.

- The authors further analyzed common variants of known cardiomyopathy genes. Overall, 108 genes were evaluated, of which only one common variant in the Titin gene was associated with worse outcome. Please include the overall number of

genes analyzed in the main manuscript.

Reviewer #3

(Remarks to the Author)
The authors present a GWAS of heart failure (HF) and two subtypes (HFpEF and HFrEF) in a Japanese population (Biobank Japan). Previous GWAS of HF and subtypes have predominantly been in European individuals and transferability of risk loci and polygenic risk scores are not well understood for the Japanese population. The authors therefore sought to conduct a GWAS of HF/HF subtypes in BBJ and compare this to European ancestry GWAS, as well as a meta-analysis to identify novel loci.

1) Some of the main results are not explicitly stated in the text, and the reader has to look through many supplementary tables and figures to find the relevant information. Could the authors add some of the main results into the main text. For example:
- Pg 7 - the authors state that the estimated SNP heritability of each HF phenotype is described in Supplementary Notes. These should be reported in the main text as they provide information on the heterogeneity across the different phenotypes. It would also be useful to provide a comparison with previously reported SNP-h2 for HF subtypes and whether they are similar or different to previous studies, and what could explain any differences.

- Pg 7 “two of the novel loci were specific to the Japanese population and were not found in Europeans (Supplementary Notes 3, Supplementary Fig. 3a, b, c, d and Supplementary Table 4, 5)”. Given these are novel finings, the text could be changed to:
“...two of the novel HF loci were specific to the Japanese population and have not been observed in Europeans - rs76704104, found in the intronic region of SPRED2 was associated with HFrEF, and rs78228190 in intronic region of CSMD1 and associated with HFpEF”

- “We assessed 62 previously reported HF loci and confirmed that their effects were mostly concordant with our Japanese GWAS (Suppl Notes 2, Suppl Fig 2, Suppl Table 3). This can be changed to “We assessed 62 previously reported HF loci and confirmed that their effects were mostly concordant with our Japanese GWAS, with a Pearson’s correlation between effect size of 0.76 for all-cause HF and 0.81 for HFrEF (Suppl Fig 2, Suppl Table 3).

2) Given the overall good concordance in effect size between previous European and BBJ but some novel loci, a more suitable subheading instead of ‘GWAS revealed a Japanese-specific genetic architecture’ could be ‘GWAS revealed Japanese-specific HF risk loci’.

3) It would be useful to clearly distinguish novel variants from novel disease genes identified from the Japanese GWAS as well as the cross-ancestry meta-analysis and MTAG analyses. There may be different variants that could be mediating risk through the same gene. For example, the authors mention 5 previously unreported loci (Pg 7). Are any of these variants in genes where genetic variants have previously been reported to be associated with HF/subtypes?

4) In addition, information on pleiotropic effects of novel variants/novel genes would be useful for understanding mediating risk factors. The Accelerating Medicines Partnership® Program for Common Metabolic Diseases provides a great resource to investigate loci:

For example, common variants in the SPRED2 gene have also previously been associated with T2D. See
https://hugeamp.org/gene.html?gene=SPRED2

Common variants in CAND2 have been associated with atrial fibrillation, DCM, and HF and subtypes, which is not mentioned.
https://hugeamp.org/gene.html?gene=CAND2

Common variants in DPY19L4 are also associated with Hba1c, glucose and T2D, which is not mentioned.
https://hugeamp.org/gene.html?gene=DPY19L4

Common variants in CACNA1D are associated with blood pressure, PR interval and Hba1c
https://hugeamp.org/gene.html?gene=CACNA1D

5) What is the genetic correlation between the HF subtypes?

6) The authors conducted MTAG analyses using only LV mass as it was genetically corelated with all HF phenotypes. We know there is heterogeneity in disease aetiology across different subtypes, and it seems like a missed opportunity not to conduct genetic correlation analyses with a broader range of risk factor traits. Genetic correlation between each subtype and other risk factor traits would be a useful main figure to understand mediating risk factors for each subtype and would be a good way to inform which traits to combine in MTAG analyses to identify sub-type specific loci.

7) There is a known difference in prevalence of the two subtypes in males and females. Are there sex-differences in the

association for the identified loci?

8) Suppl table 1 gives the mean LVEF in each phenotype. The niHF encompasses both niHFrEF and niHFpEF, and this is reflected in the large SE for LVEF in the niHF group. Henry et al 2023 (preprint 10.1101/2023.10.01.23296379) report distinct aetiology and non-cardiac tissue enrichment for niHFrEF and niHFpEF. This would be important to discuss.

9) The authors refer to model J in Fig 5a on page 18 but the models are not labelled in Fig 5a.

0) Pg 18 Pseudo R2 values. Should these be 0.0709 and 0.0737 instead of 0.709 and 0.737?

1) Though the R2 are similar, what is the correlation between the PRS?

2) In Pg 16 the authors mention drug effects and make the statement that all the drug effects were concordant with their genetic analyses. It doesn't seem like the authors investigated whether the risk allele identified in GWAS is a proxy for drug inhibition through formal analyses? For example, one way to do is a drug-Mendelian randomisation analyses where gene/protein expression or a mediating phenotype is used as a proxy for drug effects. I note the lack of East Asian QTL data, but risk factor data can also be used (e.g. blood pressure, the allele associated with lower BP would be considered the proxy for nifepidine exposure and therefore should be associated with increase risk of HF). If no formal analyses have been done to determine the direction of effect, the authors should remove the statement. Instead they could say that several identified risk loci are targets of drugs that are known to have cardiotoxic side-effects e.g. CACNA1D is a known target of nifedipine, a calcium channel blocker used to treat angina, but is known to worsen HF etc.

The authors mention potential drug effects in the discussion as well, but no formal analyses such as drug-Mendelian randomisation has been conducted to determine direction of effect.

3) Apologies if I have missed this, but if not in the manuscript, please add a description of how phenotypes were ascertained.

4) Pg 8 Pleiotropic effects – please state in main text how many traits were tested for each SNP, what was the multiple-testing p-value threshold used for statistical significance.

5) Typo Suppl note pg 4 line 76: rs35593046 'was' successfully replicated with nominal association ($P < 0.05$) with the same effect direction.

6) It would be useful to have a table of contents in the excel sheet

7) Discussion, the authors state the Japanese GWAS identified 20 gwas significant loci. but on Pg 7 the authors state "total number of HF-associated signals to 21"

8) The discussion fails to mention common variant associations in CACNA1D with blood pressure, PR interval and Hba1c https://hugeamp.org/gene.html?gene=CACNA1D

9) Discussion Pg 20 Line 403-404 wording: This includes five new loci, where variants 'have' significantly 'higher' prevalence in East Asians.

Reviewer #4

(Remarks to the Author)
I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
The authors have addressed all the questions. Thank you.

Reviewer #2

(Remarks to the Author)
The authors have incorporated several comments raised by the reviewers which lead to a substantial improvement in the manuscript.

I only have minor comments:

1) I recommend mentioning in the abstract that the majority of identified loci were associated with the HFrEF subtype.

2) By now, more than 60.000 Titin missense variants haven been reported (PMID: 38438525). In the discussion the authors hypothesise that the common variant described here may directly affect cardiac function and prognosis. Given the large difference in allele frequency and effect size of the TTN variant between the UKBB and the BBJ, it seems likely that the presence of this variant is rather part of a multi-hit model or provides an additive effect to a different risk factor (PMID: 39959973) than directly causing a clinically relevant phenotype. In particular given the high association of Titin variants with atrial fibrillation (PMID: 31691645). Therefore, until experimental data is available this should be discussed.

Reviewer #3

(Remarks to the Author)
I appreciate the effort taken by the authors to address all my comments.

Response to Comment 1
I appreciate the authors providing this information. However, for readability, could authors please present this as table (if editors allow) for easier comparison.

The authors state there was overall in confidence intervals indicating concordance between Eur and Japanese. The Japanese cohort is much smaller, so we expect bigger CI for these estimates. The difference in point estimates is quite striking (12.6% vs 6.4% for HFrEF), despite the significantly smaller sample size (almost a fifth of Eur N). It would perhaps be good to highlight this difference in point estimate for HFrEF.

Finally could the authors rationalise why for all HF subtypes in Eur the population prevalence was considered the same?

	European			Japanese		
	Pop prev	SNP h2	Liab h2	Pop prev	SNP h2	Liab h2
All HF	2.4	2.5 (0.2)	6.5	1.5 (0.3)	4.8 (1.0)	
HFreEF	1.2	6.4 (0.5)	2.4	1.5 (0.3)	12.6 (2.9)	
HFpEF	1.2	4.0 (0.4)	2.9	0.5 (0.3)	2.8 (1.7)	
NIHF	1.2	4.0 (0.7)	1.7	1.1 (0.3)	3.1 (1.0)	

Response to Comment 2: Thank you for clarifying the correlation in effect size between BBJ and previous analyses.

Response to Comment 3: Thank you for clarifying novel genes

Response to Comment 4: Thank you for looking into pleiotropic associations.

For CACNA1D, the authors have added:
The alternate allele (T) was associated with protection against HF (Supplementary Table 2 and 24) and high blood pressure (Supplementary Table 12). The Accelerating Medicines Partnership Program for Common Metabolic Diseases also suggested that common variants in CACNA1D are associated with blood pressure. Taken together, rs35593046 could protect against HF by lowering blood pressure.

The authors first state that the allele that protects against HF is associated with high blood pressure (which is counterintuitive), but then say it could protect against HF by lowering BP. Could you clarify whether the HF risk allele s associated with higher or lower blood pressure.

Response to Comment 5:
Thank you for adding the figure showing rg.

Response to Comment 6: Thank you for showing the rg with risk factor traits. These results are very interesting and as the authors say, they highlight some ancestry differences in risk factor contribution, and as such I strongly recommend for this to be included as a main figure and be discussed.

As the authors state, CAD is less strongly associated in BBJ, apart from with HFrEF, for which we know ischemic disease had higher prevalence. The lack of association rg between BMI with any of the traits in Eur but strong association in BBJ is intriguing indeed, as is the -ve rg between TC and LDL-C in BBJ. I think these are important results to discuss.

Response to Comment 7: Thank you for presenting the sex-stratified results.
Could you please clarify in text that the rs11066015 showed a significant sex-difference in HFpEF. For Suppl fig 1b, I'm assuming you are plotting the effect sizes between males and females for HFpEF? Could you clarify in the legend?

Response to Comment 8-17: Thank you for sufficiently addressing my comments

Reviewer #4

(Remarks to the Author)
I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

Version 2:

Reviewer comments:

Reviewer #3

(Remarks to the Author)
Thank you to the authors for addressing all my comments. I have no further comments.

We are grateful for the opportunity to submit our revised manuscript for publication in *Nature Communications* as an original article. We appreciate the reviewers for providing us with such high-quality review comments and a chance for revision. According to the comprehensive and constructive feedback, we substantially revised our manuscript. We believe these changes will make it more robust and informative. We hope you will find our revised manuscript suitable for publication in *Nature Communications*. The major changes in the revised manuscript and point-by-point response to comments from the reviewers follow.

Major changes

1. Moving important findings from the Supplementary Materials to the Main text

As per the reviewer's suggestion, we moved several items from the Supplementary Materials to the Main text. These include the Miami plot of the Japanese GWASs and heritability estimates, as they present essential components of our study. We also discussed the differences between HFrEF and HFpEF. For example, the GWAS of HFrEF identified more significant loci compared to HFpEF despite its smaller sample size, and showed greater enrichment in specific pathways, including those related to the sarcomere. These results highlight the importance of genetic analyses of HF subtypes.

2. Genetic correlation between HF and cardiovascular/metabolic traits

We performed genetic correlation analyses between HF and a broad range of cardiovascular/metabolic traits in both BBJ and EUR. These analyses revealed AF was the most strongly correlated phenotype in both populations. Interestingly, CAD showed a stronger correlation in EUR while blood pressure and BMI showed stronger correlations in BBJ. These findings suggest population-specific differences in the genetic underpinnings of HF and its subtypes.

3. Supporting the evidence of newly emerging medications

The pathway analysis of HFrEF revealed enrichment in sarcomere-related pathways, which may support the beneficial effects of the selective cardiac myosin activator, Omecamtiv Mecarbil, in patients with HFrEF. Additionally, cross-ancestry meta-analysis linked HF with *GIPR*. An agonist of *GIPR*, Tirzepatide, was recently reported to reduce cardiovascular events and HF hospitalization compared to placebo in a randomized controlled trial, and our results are consistent with these findings. Of note, we did not perform Mendelian randomization analyses, as not all pQTLs were available even in the latest and largest proteome-wide association study, which limited the interpretation of our findings.

4. Enhanced discussion of clinical implications

We added discussions on how genetic findings could inform heart failure prevention, diagnosis, and management strategies for Japanese individuals. Specifically, our study demonstrated that using information on the lead variant at the *TTN* locus successfully identified patients with HF who were at risk for cardiovascular death. Additionally, HF-PRS could stratify individuals at risk for developing HF. These findings indicate that genetic information has the potential to identify individuals at risk, facilitating earlier screening, timely diagnosis, and early interventions.

5. PRS limitations

We discussed the limitations of applying PRS across different populations, particularly their low transferability between ancestries. As per the reviewer's suggestion, we included a comparison of clinical scores with and without PRS, which showed a modest improvement in predictive ability. As shown in the previous studies, PRS may be especially useful for younger individuals with fewer comorbidities, where clinical scores alone may be less effective.

6. Detailed explanations for functional annotations

We added a section, "Detailed explanations for novel loci," in the Supplementary Notes to provide a more in-depth interpretation of how the identified loci are associated with the pathophysiology of HF.

Other changes

1. Acknowledgement of limitations

We addressed the lack of other large East Asian cohorts for replication analysis in the Limitations section.

2. Discussion on subtype analysis

We expanded the discussion on the importance of analyzing heart failure subtypes, highlighting differences in genetic associations between HFpEF and HFrEF.

3. Implications *of TTN* Variant

We added a discussion on the potential use of the identified *TTN* variant for heart failure screening and risk stratification.

Summary

These changes were made in response to reviewer comments to enhance the manuscript's validity, readability, and clinical relevance.

Reviewers' Comments:

Reviewer #1:

Remarks to the Author:

This study explores the genetic basis of heart failure (HF) and its subtypes in the Japanese population using a large-scale GWAS. By analyzing over 16,000 HF cases and 213,000 controls, the authors identified 19 novel loci, including Japanese-specific variants, and highlighted differences between HF subtypes like HFrEF and HFpEF. Cross-ancestry meta-analyses revealed shared and population-specific genetic risks, such as a *TTN* variant with stronger effects in Japanese individuals. Functional analyses linked genetic findings to pathways like Notch signaling. The authors also developed a polygenic risk score (PRS) that predicts early-onset HF and stratifies mortality risk, providing new insights into HF genetics and supporting its potential for precision medicine applications.

We appreciate the reviewer's careful assessment of our manuscript and are grateful for the numerous constructive comments. Accordingly, we revised our manuscript to enhance its validity and readability. The following is a point-by-point response to each comment.

Reviewer #1, Major comment #1

The manuscript presents a thorough analysis of genetic loci associated with heart failure, including differences between Japanese and European populations. These findings are intriguing, particularly the identification of loci with population-specific effects, such as the *TTN* variant. The manuscript needs to further explore the clinical implications of these genetic differences to provide a comprehensive understanding of their potential impact on heart failure treatment and management. The authors should consider discussing specific ways in which these population-specific genetic insights could inform heart failure prevention, diagnosis, or management strategies in Japanese individuals, such as through targeted screening programs or personalized treatment plans. Including a discussion on how these genetic findings could be integrated into precision medicine approaches, such as through genetic screening or personalized treatment strategies, would enhance the translational impact of the study.

We appreciate the constructive comments. HF is a heterogeneous syndrome, and the usefulness of genetic testing depends on the HF subtypes. As we showed in the manuscript, genetic testing is likely to be more useful for HFrEF than HFpEF. Given that a single variant could discriminate patients with HF at risk for high mortality, this lead variant can be a potential biomarker for HF patients. We discussed the genetic differences between HF subtypes in the Discussion section. Also, as per Reviewer #2's suggestion (Major comment 5), we compared the clinical scores with the clinical scores + PRS. AUC improved from 0.769 to 0.773 with NRI 0.152 (SE 0.027) and IDI 0.0039 (0.0006). It means that there is a benefit in adding HF-PRS to clinical prediction tools, although the benefit is modest. However, we still believe that HF-PRS is useful, particularly for younger individuals with few comorbidities where clinical risk scores do not work well. We added these methods, results, and interpretations in the main text as follows.

Below is the corresponding text (track changes are highlighted in red).

(Main text Lines 471 - 477)

In our HF-PRS, integrating HFrEF, HFpEF, AF, CAD, and left ventricular mass improved the performance and enabled the detection of early-onset HF cases. Moreover, we demonstrated that HF-PRS could predict HF death in subjects who had not yet been diagnosed as having HF. These findings indicate that HF-PRS has the potential to serve as a tool for identifying individuals predisposed to developing heart failure, thereby facilitating earlier screening, more timely diagnosis, and the implementation of preventive or therapeutic interventions before the disease manifests clinically.

(Main text Lines 466 – 469)

Incorporating the *TTN* common variant screening into clinical practice could lead to earlier interventions and improved outcomes by identifying high-risk individuals, who might otherwise be overlooked by genetic testing methods focused solely on rare *TTN* variants.

(Main text Lines 493 – 499)

Fourth, our research showed the modest benefit of adding HF-PRS to a clinical score. Previous research showed that the addition of genetic information to clinical risk assessment was more significant among younger individuals[44], partly because younger individuals do not have comorbidities and the clinical risk assessment does not work well for them[45]. The mean age of our study was 66 years (Supplementary Table 1), potentially reducing PRS's effectiveness. Further research is necessary to assess the PRS in this important population.

(Main text Lines 700 – 702)

We compared the AUC, NRI, and IDI of a clinical score based on systolic blood pressure, diabetes mellitus, AF, CAD, and dyslipidemia with the clinical score + HF-PRS using the test dataset.

Reviewer #1, Major comment #2

While the development of PRS is a significant contribution, the limitations of applying PRS in clinical settings, especially across different populations, should be considered and discussed.

We agreed with the Reviewer's comment that PRS has low transferability between ancestries. Since we tested our HF-PRS only in the Japanese population, additional work is necessary to recalibrate the PRS before applying it to other ancestries. We have discussed this in the Discussion section.

Below is the corresponding text (track changes are highlighted in red).

(Main text Lines 489 – 493)

Third, since our HF-PRS was specifically developed and calibrated for the Japanese population, applying it to a population of other ancestry may result in reduced performance due to differences in genetic architectures across populations. Therefore, recalibrating the HF-PRS for each target population is essential, as we did in the "PRS derivation and performance" section.

Reviewer #1, Major comment #3

The discovery of novel loci significantly advances our understanding of the genetic basis of HF. The manuscript mentions functional annotations such as eQTL and sQTL analyses, but these results are not deeply integrated into the discussion of biological mechanisms. Could the authors provide a more detailed interpretation of how the identified loci influence HF pathophysiology?

Thank you for the constructive comment. In the present study, eQTL and sQTL provided a link between a lead variant to genes for *DPY19L4*, *CARM1*, *EDNRA*, *ZNF397*, *PLPP3*, *SLC4A7*, *ILRUN,* and *BDNF*, and are consistent with the results derived from other prioritizing methods. For example, rs6471480 was an eQTL in the tibial artery for *DPY19L4*. *DPY19L4* was also found to be significant in the aorta in H-MAGMA. Additionally, our previous study showed it was associated with coronary artery disease.

Due to the word limits in the main text, we added the "Detailed explanations for novel loci" section in the Supplementary Notes and described the biological mechanisms for each locus there.

Below is the corresponding revised text (track changes are highlighted in red).

(Supplementary Note Lines 137 – 145)

DPY19L4

This locus was found in a Japanese GWAS for all-cause HF, and the lead variant for this locus was rs6471480 (Supplementary Table 2). rs6471480 is an eQTL in the subcutaneous adipose tissue and tibial artery (Supplementary Table 14). *DPY19L4* was significant in the aorta and adrenal gland H-MAGMA. According to the Accelerating Medicines Partnership Program for Common Metabolic Diseases, common variants in *DPY19L4* are associated with HbA1c, glucose, and type 2 diabetes. Given that *DPY19L4* was also associated with coronary artery disease in our previous study[8], this locus was likely to contribute to HF development through ischemic heart disease.

(Supplementary Note Lines 169 – 180)

CARM1

This locus was found in a cross-ancestry meta-analysis for non-ischemic HF. *CARM1* knockout mice showed congenital heart disease (Supplementary Table 33). The splicing-TWAS suggested that *CARM1* splicing in the heart was associated with HF (Supplementary Table 29), which was consistent with the knockout mouse results.

EDNRA

This locus was found in a cross-ancestry meta-analysis for HFrEF. The lead SNP was rs7659823. *EDNRA* knockout mice showed congenital arterial diseases (patent ductus arteriosus and overriding aortic valve) and heart diseases (ventricular septal defect; Supplementary Table 33). The splicing-TWAS suggested that *EDNRA* splicing in the heart was associated with HF (Supplementary Table 29). Combined with knockout mouse results and the previous literature, *EDNRA* is supposedly involved in the development of the heart and artery[11].

11. Asai R, et al. Endothelin receptor type A expression defines a distinct cardiac subdomain within the heart field and is later implicated in chamber myocardium formation. *Development* 137, 3823-3833 (2010).

(Supplementary Note Lines 185 – 203)

ZNF397

This locus was found in a cross-ancestry meta-analysis for HFrEF. The lead variant was rs5823966. TWAS showed that *ZNF397* in the tibial artery was associated with HF (Supplementary Table 28), and the splicing-TWAS suggested that *ZNF397* splicing in the heart was also associated with HF (Supplementary Table 29). According to the Accelerating Medicines Partnership Program for Common Metabolic Diseases, *ZNF397* is associated with left ventricular wall thickness.

PLPP3

This locus was found in MTAG for all-cause HF. The lead variant was rs10888955. TWAS suggested that *PLPP3* in fibroblasts was associated with HF (Supplementary Table 39). According to the Accelerating Medicines Partnership Program for Common Metabolic Diseases, *PLPP3* is associated with hypertension and coronary artery disease, which is consistent with the TWAS results.

SLC4A7

This locus was found in MTAG for all-cause HF. The lead SNP was rs582505. The splicing-TWAS suggested that *SLC4A7* splicing in fibroblasts was associated with HF (Supplementary Table 40). *SLC4A7* knockdown mice showed abnormal blood pressure and abnormal vasculature morphology (Supplementary Table 44). A previous report showed that this variant was associated with hypertension[7], which was consistent with the knockout mouse results.

(Supplementary Note Lines 209 – 224)

ILRUN

This locus was found in MTAG for all-cause HF. The lead SNP was rs2492863. TWAS suggested that *ILRUN* was associated with HF in various tissues, including the left atrium, left ventricle, blood, kidney, subcutaneous fat, and kidney (Supplementary Table 39). *ILRUN* deficiency in mice resulted in abnormal glucose and lipid metabolism (Supplementary Table 44). Since tagged variants with rs2492863 were associated with metabolic phenotypes, such as body mass index[13] and lipids[14], *ILRUN* is likely to contribute to HF development through metabolic disorders.

BDNF

This locus was found in MTAG for all-cause HF and HFpEF. The lead SNPs were rs10501087 and rs35038967, respectively. The splicing-TWAS suggested that *BDNF* splicing in adipose tissue was associated with HF development (Supplementary Table 40), and H-MAGMA using the adipose tissue dataset showed a significant association between *BDNF* and HF (Supplementary Table 42). Also, *BDNF* has been reported to be responsible for obesity (Supplementary Table 43), and *BDNF* knockout mice showed obesity as well (Supplementary Table 44).

Reviewer #1, Major comment #4

The manuscript emphasizes the Japanese-specific findings, without inclusion of other East Asian datasets. This is a limitation acknowledged by the authors. Could the authors suggest potential collaborations or strategies to validate these findings further in larger East Asian cohorts?

Thank you for pointing this out. The China Kadoorie Biobank (CKB), the Korean Genome and Epidemiology Study (KoGES), and the Taiwan Precision Medicine Initiative (TPMI) offer the PheWeb (https://pheweb.ckbiobank.org/, https://koges.leelabsg.org/, and https://pheweb.ibms.sinica.edu.tw/, respectively). However, summary statistics are not available, and no results were obtained when we searched for the Japanese-specific variants. Moreover, although HF-GWAS typically shows a strong signal in the *PITX2* locus (chromosome 4)[1], we found no signal in this region in the CKB "i50 heart failure" (https://pheweb.ckbiobank.org/pheno/i50) and the TPMI "428.1: Congestive heart failure (CHF) NOS" (https://pheweb.ibms.sinica.edu.tw/pheno/428.1) PheWeb. The KoGES PheWeb has no heart failure phenotype. Therefore, to expand genetic analyses among East Asian populations, collaboration with these biobanks will likely become essential. Hence, we acknowledged this in the limitations section.

Below is the corresponding text.

(Main text Lines 478 – 485)

Our study has several limitations. First, we did not have other large cohorts for the replication analysis. We did not replicate Japanese-specific HF loci using other East Asian cohorts since there were no other large East Asian biobanks available with enough of these patients. Instead, we assessed these variants with European data, showing the same effect direction. As for cross-ancestry meta-analyses, we did not have a non-overlapping corresponding cohort for all-cause HF and NIHF. Hence, we used European all-cause HF data for the replication of HFrEF and HFpEF cross-ancestry meta-analyses, showing almost the same effect direction.

Reviewer #1, Minor comment #1

All abbreviations in Figures should be spelled out in footnote

Thank you for pointing this out. We added all abbreviations in the footnotes.

Below is the excerpt from the Figure 1 legend. There are many changes, so we did not list all changes here, but all changes are highlighted in red in the main text and the Supplementary Material.

(Main text Figure 1 legend Lines 951 – 958)

AFR, African; AMR, American; EAS, East Asian; GBMI, Global Biobank Meta-analysis Initiative; GWAS, genome-wide association study; HF, heart failure; HFpEF, heart failure with preserved ejection fraction; HFrEF, heart failure with reduced ejection fraction; H-MAGMA, Hi-C-coupled MAGMA; MAGMA, Multi-marker Analysis of GenoMic Annotation; MIGWAS, miRNA–target gene networks; MRI, magnetic resonance imaging; MVP, Million Veteran Program; NIHF; non-ischemic heart failure; scDRS, single-cell disease relevance score; TWAS, transcriptome-wide association study; UKBB, UK Biobank; VEGAS, versatile gene-based association study.

Reviewer #1, Minor comment #2

In Figure 1, the numbers of cases for different types of HF were provided for Japanese population but not for European population. It will be helpful to provide such information.

We agree about the number of cases and have replaced Figure 1 as follows.

[Figure 1]

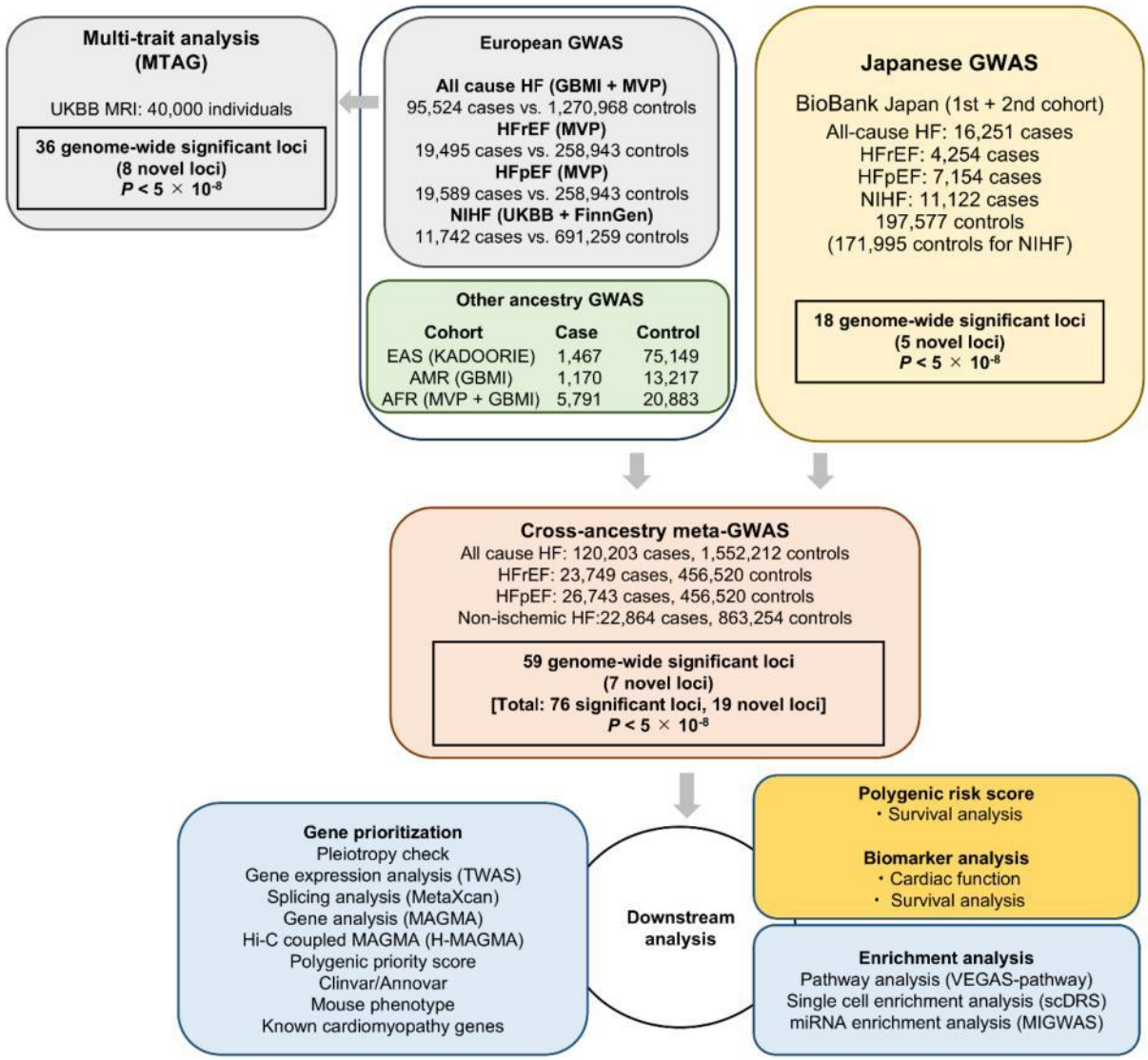


Fig. 1 | Overview of the study design. Flowchart of the study, which encompasses the Japanese GWAS with the BioBank Japan, a trans-ancestry meta-analysis with large-scale European and other population GWAS followed by the downstream analyses. AFR, African; AMR, American; EAS, East Asian; GBMI, Global Biobank Meta-analysis Initiative; GWAS, genome-wide association study; HF, heart failure; HFpEF, heart failure with preserved ejection fraction; HFrEF, heart failure with reduced ejection fraction; H-MAGMA, Hi-C-coupled MAGMA; MAGMA, Multi-marker Analysis of GenoMic Annotation; MIGWAS, miRNA–target gene networks; MRI, magnetic resonance imaging; MVP, Million Veteran Program; NIHF; non-ischemic heart failure; scDRS, single-cell disease relevance score;

TWAS, transcriptome-wide association study; UKBB, UK Biobank; VEGAS, versatile gene-based association study.

Reviewer #1, Minor comment #3

It is hard to summarize the information in Figure 3. It might be helpful to summarize the same/similar pathways identified by GO, KEGG, etc.

We are sorry for the confusing presentations. We colored each dot based on pathway types, namely cell cycle, contractile unit, development/differentiation, senescence, and others.

Below is the corresponding figure.

[Figure 4]

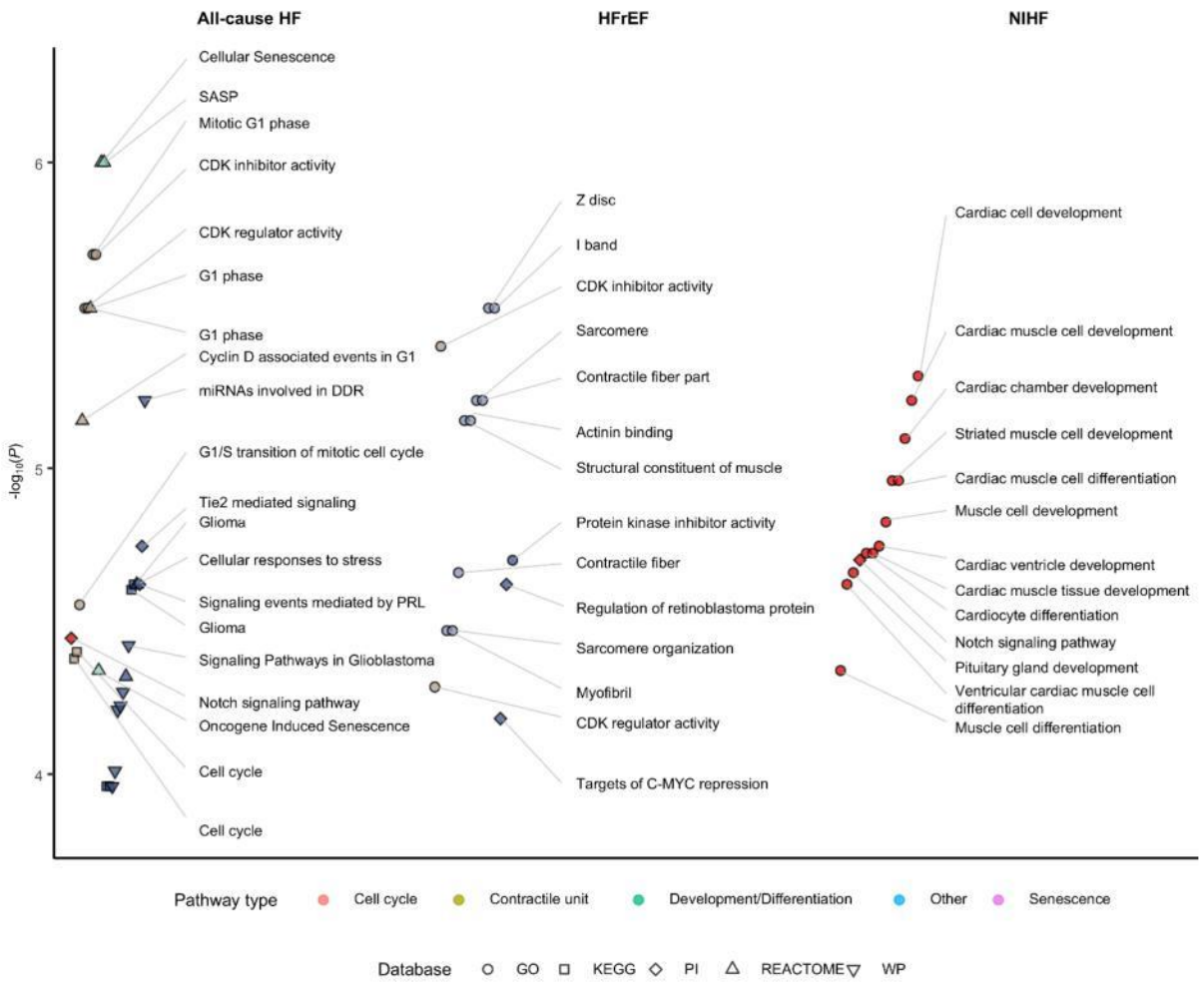


Fig. 4 | Pathway analysis for each HF phenotype. Enriched terms (FDR < 0.05) for each HF phenotype from GO, KEGG, PI, Reactome, and WP. Up to the top 20 enriched terms in each HF phenotype gene set are labeled. The color of the dots indicates the pathway. There was no enriched pathway for HFpEF. CDK, Cyclin-dependent kinases; DDR, DNA damage response; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; PI, Pathway Interaction database; PRL, prolactin; SASP, Senescence-Associated Secretory Phenotype; WP, Wiki Pathways.

Reviewer #2:

Remarks to the Author:

The current study sought to analyze the contribution of common genetic variation in patients with heart failure in the Japanese population. First, the authors performed a Japanese GWAS of all-cause HF, HFrEF, HFpEF and non-ischemic HF. Thereby, 18 genome-wide significant loci were identified, with 5 novel loci (most of them for all-cause HF and HFrEF, similar to previous GWAS (PMID: 36517512). Then using a cross-ancestry approach and multi-trait analysis the authors performed a meta-analysis and overall identified 19 novel loci associated with HF or HF associated traits. Second, the authors used several gene prioritization methos to identify causal genes at the reported loci including the evaluation of known cardiomyopathy genes and gene-drug interactions relevant for HF. Finally, the authors calculated a polygenic risk score, based on the results of the cross-ancestry analysis and tested its ability for age at onset of HF and cardiovascular/HF mortality prediction. Overall, the study included the first stand-alone GWAS of the Japanese population and provides a large cross-ancestry analysis in HF. In addition, the authors nicely used several bioinformatic analyses to clarify the role of specific high-risk genes and drug targets. However, we have some major concerns:

Thank you for reading our manuscript carefully, raising important points, and providing practical advice. The following are the point-by-point responses to each of your comments.

Reviewer #2, Major Comment #1

Presentation of the identified and replicated loci in the initial GWAS is hard to follow. I recommend including parts of Supplementary Figure 1 (mainly Miami plot) in the main manuscript as it is an essential part of the results. The authors separated their cohort into HFrEF and HFpEF, as previously performed in a GWAS by Joseph et al (PMID: 36517512). Thereby, the authors identified only a small number of loci associated with HFpEF, reflecting the heterogeneity of the phenotype. As the estimated heritability for each HF type is clinically relevant (in particular the low number of identified loci in HFpEF and NIHF) this should be included and discussed in the manuscript.

We appreciate the comments to improve our manuscript. We have moved the Miami plot of Japanese GWAS into the main Figure 2, recognizing its importance as a central visual summary of the primary GWAS findings. Also, we have revised the main text to incorporate a discussion of heritability estimates for each HF subtype, which were previously reported in the Supplementary Notes. The results now appear in the main Results section, where we presented both SNP heritability and liability-scale heritability values for all-cause HF, HFrEF, HFpEF, and NIHF. Our findings demonstrated that HFpEF exhibited the lowest SNP heritability among the subtypes, a pattern consistent with its clinical heterogeneity and higher comorbidity burden. In contrast, the higher heritability observed in HFrEF was accompanied by the identification of several novel loci, some of which were enriched in the sarcomere genes. These observations are further discussed in detail in the Discussion section. Moreover, the enrichment of HFrEF-associated loci in the sarcomere genes

may help explain the observed clinical benefit of Omecamtiv Mecarbil, a selective cardiac myosin activator, in HFrEF patients, as Reviewer #2, Major Comment #3 suggested.

Below is the corresponding text. Track changes are highlighted in red.

[Figure 2]

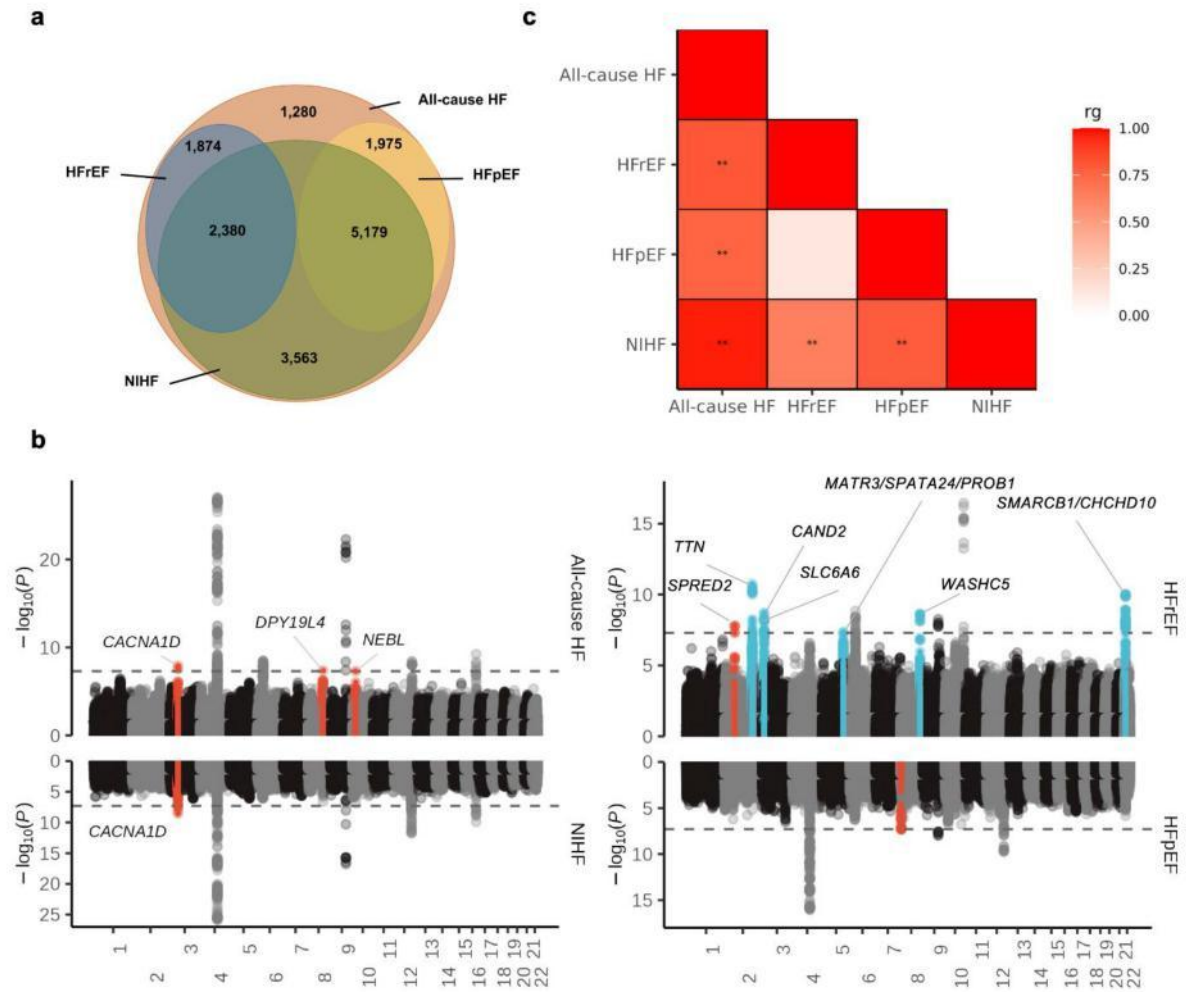


Fig. 2 | Results of the Japanese GWAS. (a) Sample overlap between each HF phenotype in the Japanese GWAS. **(b)** Miami plot of Japanese GWAS. -log$_{10}$(*P* value) on the *y*-axis is shown against the genomic positions (hg19) on the *x*-axis. Association signals that reached a genome-wide significance level ($P < 5 \times 10^{-8}$) are shown in red if they are new loci and in blue if previously reported only in MTAG but not in GWAS. Two-sided *P* values were calculated using a logistic regression model. (**c**) Genetic correlation between HF GWAS. * indicates $P < 0.05$, and ** $P < 0.0083$ (0.05/6). GWAS, genome-wide association study; HF, heart failure; HFrEF, heart failure with reduced ejection fraction; HFpEF, heart failure with preserved ejection fraction; MTAG, multi-trait analysis of GWAS.

(Main text Lines 129 - 135)

The proportion of the variation in HF phenotypes (the single-nucleotide polymorphism (SNP) heritability; h^2) was estimated to be 1.5% (s.e.m 0.3%) for all-cause HF, 1.5% (0.3%) for HFrEF, 0.5% (0.3%) for HFpEF, and 1.1% (0.3%) for NIHF using linkage disequilibrium (LD)-score regression

(LDSC) (Supplementary Table 6). The liability-scale h^2 was estimated at 4.8% (s.e.m. 1.0%) for all-cause HF, 12.6% (2.9%) for HFrEF, 2.8% (1.7%) for HFpEF, and 3.1% (1.0%) for NIHF, suggesting the heterogeneous heritability in each HF phenotype.

(Main text Lines 449 - 457)

As shown in the previous research[5], our Japanese GWAS also revealed fewer genome-wide significant loci in HFpEF compared to HFrEF, even though the sample size was larger in HFpEF. HFpEF has been reported to be a heterogeneous syndrome characterized by a high comorbidity burden[41], which could lead to less heritability. In contrast, HFrEF GWAS revealed novel loci that have not been found in all-cause HF and showed enrichment in the sarcomere genes. These results may support the finding that Omecamtiv Mecarbil, the selective cardiac myosin activator, improved the prognosis of patients with HFrEF[42]. Our research suggested the importance of analyses of HF subsets, and future research could reveal more genes related to contractile functions by increasing the sample size of HFrEF.

5. Joseph J, et al. Genetic architecture of heart failure with preserved versus reduced ejection fraction. *Nat Commun* 13, 7753 (2022).

41. Roh J, Hill JA, Singh A, Valero-Munoz M, Sam F. Heart Failure With Preserved Ejection Fraction: Heterogeneous Syndrome, Diverse Preclinical Models. *Circ Res* 130, 1906-1925 (2022).

42. Teerlink JR, et al. Cardiac Myosin Activation with Omecamtiv Mecarbil in Systolic Heart Failure. *N Engl J Med* 384, 105-116 (2021).

Reviewer #2, Major Comment #2

To increase statistical power the authors performed a multi-trait analysis using MRI data from UK Biobank. Thereby, the highest association with HF was seen for left ventricular mass. This is surprising as previous studies have shown that LV volume was mainly associated with HF. However, this association could be caused by arterial hypertension as strong risk factor for LVH and HF. In contrast, the authors show that the lead variant for HFrEF in the Japanese population is protective for arterial hypertension? Are other explanations possible for the association with a lower blood pressure?

We are sorry for the confusing presentations. We assume that the reviewer referred to rs35593046. rs35593046 was more frequently observed in East Asians than Europeans and was associated with protection against both HF development (β = -0.0630 in all-cause HF, shown in the Results section) ***and*** high blood pressure (Supplementary Fig. 4a and Supplementary Table 12). Hence, it is likely that rs35593046 offers protection against HF by lowering blood pressure. We have included this statement in the “8. Detailed explanations for novel loci” section in the Supplementary Notes.

Below is the corresponding text. Track changes are highlighted in red.

(Supplementary Note Lines 128 - 136)

CACNA1D

This locus was found in a Japanese GWAS for all-cause HF and non-ischemic HF, and cross-ancestry meta-analysis for all-cause HF. The lead SNP is rs35593046 in both the Japanese GWAS

and the cross-ancestry meta-analysis. rs35593046 is more frequent in East Asians (53.8%) than in Europeans (26.8%). The alternate allele (T) was associated with protection against HF (Supplementary Tables 2 and 24) and high blood pressure (Supplementary Table 12). The Accelerating Medicines Partnership Program for Common Metabolic Diseases also suggested that common variants in *CACNA1D* are associated with blood pressure. Taken together, rs35593046 could protect against HF by lowering blood pressure.

Reviewer #2, Major Comment #3

The authors state that in particular in HFrEF variance in sarcomeric genes is of importance. Can the authors comment on the role of Omecamtiv as treatment in HFrEF in this context?

Thank you for raising an important point. Omecamtiv mecarbil is a selective cardiac myosin activator designed to enhance cardiac contractility by directly targeting the sarcomere and has been proven to improve the prognosis of patients with HFrEF. Given the importance of sarcomere gene variants in HFrEF GWAS, our results support this evidence. We discuss the role of Omecamtiv in the Discussion section.

Below is the corresponding text. Track changes are highlighted in red.

(Main text Lines 452 – 455)

In contrast, HFrEF GWAS revealed novel loci that have not been found in all-cause HF and showed enrichment in the sarcomere genes. These results may support the finding that Omecamtiv Mecarbil, the selective cardiac myosin activator, improved the prognosis of patients with HFrEF[42].

42. Teerlink JR, et al. Cardiac Myosin Activation with Omecamtiv Mecarbil in Systolic Heart Failure. *N Engl J Med* 384, 105-116 (2021).

Reviewer #2, Major Comment #4

The authors identify a common variant in the *TTN* gene and describe it as a prognostic marker for HF. Regarding the minor allele frequency this variant was described more often in Europeans than in the Japanese population. However, in Fig. 4a the risk allele frequency is described higher in Europeans than in the East Asian population? Also, in which Biobank did the authors test for the association with heart failure death?

We apologize for the confusing presentation. Among the genome-wide significant variants with $r^2 >$ 0.8 with the lead variant (rs1484116) in the *TTN* locus, we found a missense variant rs2627043. The alternate allele frequency of rs2627043 was higher in East Asians compared to Europeans (64.7% vs. 20.1%). Conversely, when reporting the risk allele frequency, it was found to be higher in Europeans than in East Asians (79.9% vs. 35.3%). Additionally, the minor allele frequency was higher in Japanese individuals than in Europeans (35.3% vs. 20.1%), which partly explains why this locus was able to be detected in the Japanese GWAS. In Fig. 5, we employed lead variants in *TTN* (rs1484116), *NEBL* (rs61870083), and *BAG3* (rs7075837) because there is no tagged missense variant in the *NEBL* and *BAG3* loci, aiming to analyze these three loci in a consistent manner. Therefore, we presented the allele frequency and effect sizes of the risk allele (Fig. 5a and b), not the alternate

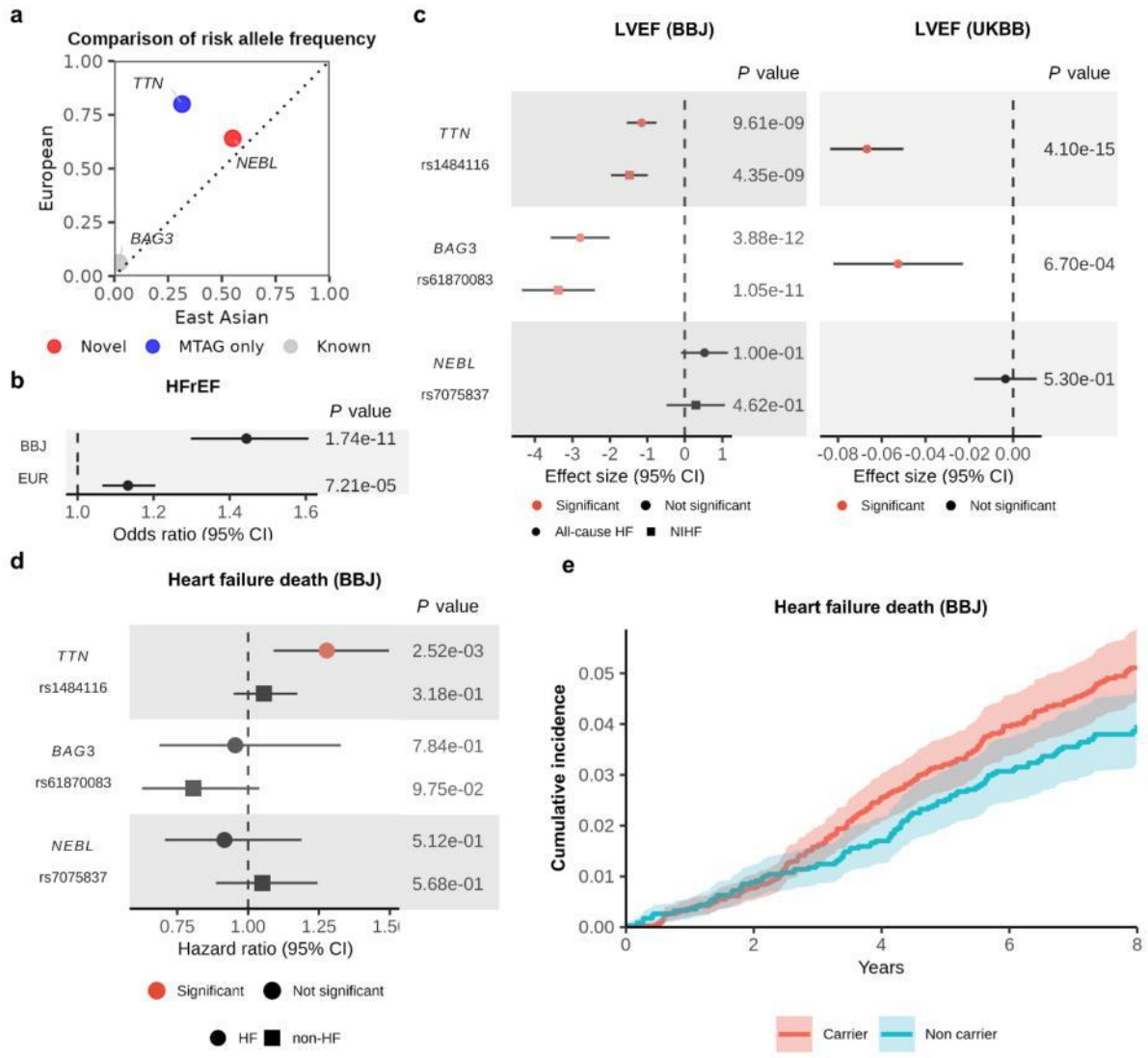
allele, as our focus was on risk variants. We then used BBJ to assess the association between rs1484116 and heart failure mortality (Fig.5d).

Below is the corresponding text. Track changes are highlighted in red.

(Main text Lines 712 - 718)

For survival analysis, the Cox proportional hazards model was used to assess the association between HF-PRS and long-term mortality and to evaluate the impact of lead variants in cardiomyopathy genes on long-term mortality. We obtained survival follow-up data with the ICD-10 code for 132,737 individuals from the BBJ dataset[48]. The causes of death were classified into two categories according to ICD-10 codes: cardiovascular death (I00–I99) and HF death (I50). For lead variants in cardiomyopathy gene analyses, we split individuals into HF cases and controls, followed by performing the survival analysis separately.

[Figure 5]



Reviewer #2, Major Comment #5

The authors calculated a PRS and tested its performance in mortality and HF prediction. However, the PRS included several HF subtypes and "meta-traits". From the reviewer's point of view this does not present a specific polygenic risk score for heart failure but pools many genetically affected cardiovascular traits. Thus, the authors should build and test a PRS for HF as well as such meta-trait PRS and compare both for their predictive value. Moreover, it would be interesting to see how strong these PRSs perform in prediction of heart failure, when in comparison to clinically determined cardiovascular risk factors such as hypertension or diabetes or traits such as atrial fibrillation or CAD. Finally, is there a benefit in adding a "HF PRS" to clinical prediction tools?

In response to the reviewer's insightful suggestions, we undertook a comprehensive analysis to evaluate the predictive utility of PRSs for HF in the Japanese population. Specifically, we constructed and assessed PRSs derived from single-population HF-GWAS, meta-GWAS across populations, and meta-trait PRSs that encompassed genetically influenced cardiovascular traits. Our comparative analysis revealed that, among PRSs derived from single ancestry GWAS, the PRS derived from EAS All-cause HF GWAS exhibited the highest performance due to the population specificity. Additionally, the cross-ancestry meta-PRS demonstrated superior performance compared to PRS derived from single-ancestry GWASs. Furthermore, utilizing a multi-trait PRS approach led to even greater performance enhancement. (Fig. 6a and Supplementary Fig. 15b).

To further contextualize the performance of HF-PRS, we compared the multi-trait PRS for HF against clinical risk factors including systolic blood pressure, diabetes, atrial fibrillation, coronary artery disease, and dyslipidemia. When integrating a clinical risk score including these clinical variables with the HF-PRS, we observed a modest improvement in predictive accuracy. The AUC increased from 0.769 to 0.773, accompanied by a net reclassification improvement (NRI) of 0.152 (standard error [SE] 0.027) and an integrated discrimination improvement (IDI) of 0.0039 (SE 0.0006) (Please refer to the Results "Development of a HF-PRS and its performance" section). These metrics suggest that, although the additive effect is modest, incorporating the HF-PRS into clinical prediction models can enhance risk stratification.

Importantly, we recognize the potential value of HF-PRS in younger individuals who have not yet developed comorbid conditions. In such populations, traditional clinical risk scores do not work well, as shown previously, whereas HF-PRS can provide early indicators of susceptibility. Therefore, our study population, with a mean age of 66.3 years, predominantly consists of older individuals, which may limit the generalizability of our findings to younger demographics. In light of these considerations, we have revised the manuscript to include detailed descriptions of our methodologies, results, and interpretations related to the construction and evaluation of the HF-PRS and meta-trait PRS. We added the following description to the manuscript. Track changes are highlighted in red.

(Main text Lines 399 – 401)

As suggested previously, cross-ancestry PRS showed higher performance than those from single population-derived PRS (Fig. 6a and Supplementary Fig. 15b). Additionally, combining HF- related phenotypes further improved the performance (Fig. 6a and Supplementary Fig. 15b).

[Fig. 6a and Supplementary Fig. 15b]

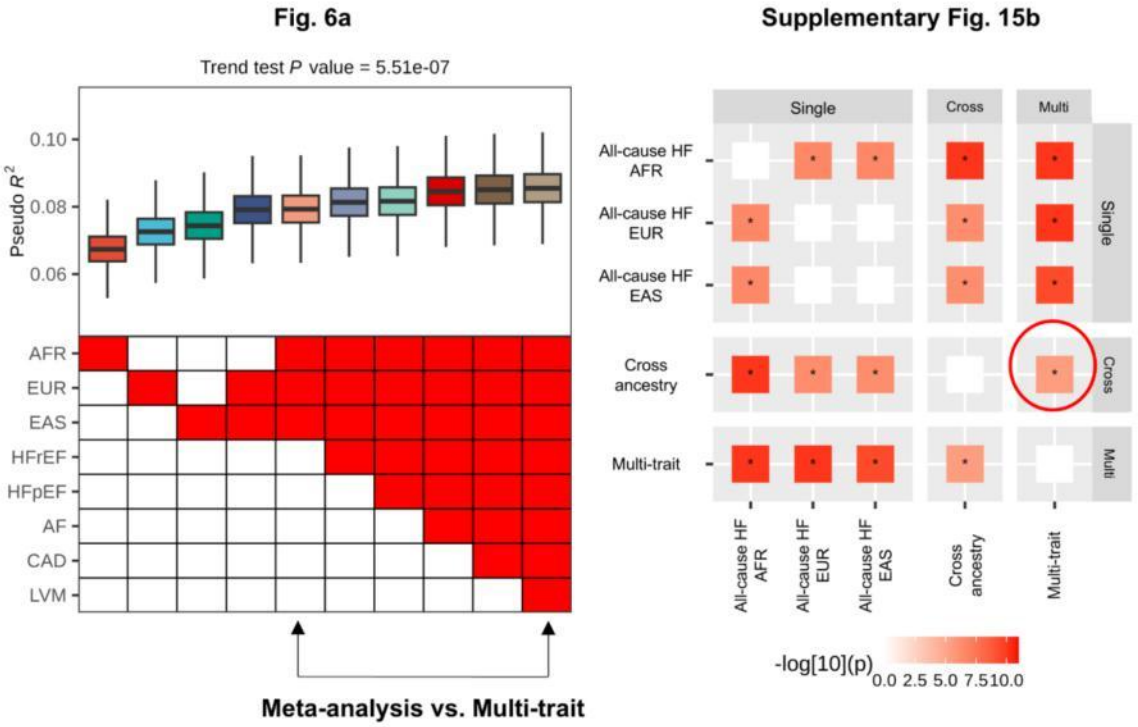


Fig. 6 | Performance and clinical impact of the HF-PRS. (a) Each point indicates Nagelkerke's pseudo R2 for all-cause HF case-control status in the Japanese independent cohort (1,837 cases and 11,319 controls). PRS-derivation cohorts are indicated on the x-axis. (b) Association between HF-PRS and onset age of HF. The onset age of HF in individuals with data available (n = 10,810) is shown based on the HF-PRS tertiles. The number of individuals in each tertile is 3,603 to 3,604. The center line of the box plot indicates the median, the bounds represent the first and third quartile, and the whiskers reach to 1.5 times the interquartile range. (c) Kaplan-Meier estimates of cumulative events from cardiovascular mortality (left) and HF death (right) are shown with a band of 95%CI. Individuals are classified into high (top tertile), intermediate (middle tertile), and low PRS (bottom tertile). P values were calculated for PRS by Cox proportional hazard analysis and the significance was set at P = 8.3 × 10−3 (0.05/6). AF, atrial fibrillation; AFR, African; CAD, coronary artery disease; EAS, East Asian; EUR, European; HF, heart failure; HFpEF, heart failure with preserved ejection fraction; HFrEF, heart failure with reduced ejection fraction; HR, hazard ratio; LVM, left ventricular mass; PRS, polygenic risk score; SE, standard error.

Supplementary Fig. 15 | PRS performance. (a) Correlation between PRS. * indicated P < 0.05, ** indicated P < 1.79 × 10-3 (0.05/24). (b) Pairwise comparison of PRS performance. Distribution of the pairwise difference of Nagelkerke's pseudo-R2 (Δ pseudo-R2: Pseudo R2Score Y – Pseudo R2Score X, X, and Y are found at the axis) between each pair of PRS models. The distributions were obtained by bootstrapping 5.0 × 104 times. Two-sided bootstrap P values were calculated by counting the

number of Δ pseudo-R2 ≤ 0 or Δ pseudo-R2 > 0 and then multiplying the lower value by the minimum estimated P value (2 * 1 / (5.0 × 104) = 4 × 10−5: two-sided). The significance was set at P = 5.0 × 10−3 (0.05/10). (c) PRS distribution and HF prevalence. Prevalence of HF based on the HF-PRS deciles in each combination of GWAS. The number of individuals in each decile is 2,631-2,632. Data are presented as medians and 95% CI. AF, atrial fibrillation; AFR, African; CAD, coronary artery disease; CI, confidence interval; Cross, cross ancestry PRS; EAS, East Asian; EUR, European; HF, heart failure; HFpEF, heart failure with preserved ejection fraction; HFrEF, heart failure with reduced ejection fraction; LVM, left ventricular mass; Multi, multi-trait PRS; PRS, polygenic risk score; Single, single ancestry PRS.

(Main text Lines 405 – 409)

We compared the predictive ability of a clinical score based on systolic blood pressure, diabetes, AF, CAD, and dyslipidemia with that of the clinical score + PRS. The AUC was improved from 0.769 to 0.773 by adding HF-PRS to the clinical score. The net reclassification index (NRI) and the integrated discrimination improvement (IDI) were 0.152 (s.e.m. 0.002) and 0.0039 (s.e.m. 0.0006).

(Main text Lines 493 – 499)

Fourth, our research showed the modest benefit of adding HF-PRS to a clinical score. Previous research showed that the addition of genetic information to clinical risk assessment was more significant among younger individuals[44], partly because younger individuals do not have comorbidities and the clinical risk assessment does not work well for them[45]. The mean age of our study was 66 years (Supplementary Table 1), potentially reducing PRS's effectiveness. Further research is necessary to assess the PRS in this important population.

44. Samani NJ, et al. Polygenic risk score adds to a clinical risk score in the prediction of cardiovascular disease in a clinical setting. *Eur Heart J* 45, 3152-3160 (2024).

45. Marston NA, et al. Predictive Utility of a Coronary Artery Disease Polygenic Risk Score in Primary Prevention. *JAMA Cardiol* 8, 130-137 (2023).

(Main text Lines 700 – 702)

We compared the AUC, NRI, and IDI of a clinical score based on systolic blood pressure, diabetes mellitus, AF, CAD, and dyslipidemia with the clinical score + HF-PRS using the test dataset.

Reviewer #2, Minor Comment #1

In the meantime, the SUMMIT trial has been published with positive outcome for tirzepatide in HFpEF (PMID: 39555826). This should be cited and added to the discussion.

Thank you for bringing up this important research. Our research linked the tirzepatide to HF through the *GIPR* locus, which supports the result of the SUMMIT trial.

Below is the corresponding text and table.

(Main text Lines 352 – 356)

Tirzepatide, one of the emerging antidiabetics[34, 35], could be a potential drug repositioning candidate through the *GIPR* locus, which had a protective role against HF. A recent randomized controlled trial

showed that treatment with tirzepatide led to a lower risk of a composite of cardiovascular death or worsening HF than placebo in HFpEF[36], and our findings supported this evidence.

34. Frias JP, et al. Tirzepatide versus Semaglutide Once Weekly in Patients with Type 2 Diabetes. *N Engl J Med* 385, 503-515 (2021).

35. Rosenstock J, et al. Efficacy and safety of a novel dual GIP and GLP-1 receptor agonist tirzepatide in patients with type 2 diabetes (SURPASS-1): a double-blind, randomised, phase 3 trial. *Lancet* 398, 143-155 (2021).

36. Tirzepatide for Heart Failure with Preserved Ejection Fraction and Obesity. *N Engl J Med* 392, 427-437 (2025).

Reviewer #2, Minor Comment #2

I would recommend including arterial hypertension to the baseline characteristics (Suppl. Table 1)

We agreed with the Reviewer, and Supplementary Table 1 now includes systolic and diastolic blood pressure.

[Supplementary Table 1]

Supplementary Table 1. Patient characteristics

	All-cause HF	HFrEF	HFpEF	NIHF	Control	Control (NIHF)
n	16,251	4,254	7,154	11,122	197,577	171,995
Age, years	66.37 (13.49)	63.52 (13.09)	66.65 (13.78)	65.61 (14.15)	62.69 (13.98)	61.99 (14.33)
Male	10369 (63.8)	3266 (76.8)	4058 (56.7)	6643 (59.7)	104084 (52.7)	84911 (49.4)
SBP, mmHg	138.1 (20.2)	135.2 (20.1)	139.1 (20.0)	137.5 (20.2)	133.9 (19.6)	132.7 (19.6)
DBP, mmHg	79.3 (12.6)	79.3 (12.9)	79.2 (12.4)	79.2 (12.8)	78.9 (12.1)	78.4 (12.2)
Coronary artery disease	5129 (31.6)	1874 (44.1)	1975 (27.6)	0 (0.0)	25582 (12.9)	0 (0.0)
Atrial fibrillation	4737 (29.1)	1163 (27.3)	2527 (35.3)	3661 (32.9)	397 (0.2)	163 (0.1)
Diabetes mellitus	4988 (30.7)	1483 (34.9)	2065 (28.9)	2770 (24.9)	45312 (22.9)	37307 (21.7)
Dyslipidemia	5420 (33.4)	1626 (38.2)	2445 (34.2)	2922 (26.3)	51582 (26.1)	38753 (22.5)
Stroke	1944 (12.0)	411 (9.7)	902 (12.6)	1272 (11.4)	14611 (7.4)	12814 (7.5)
Ejection fraction, %	48.94 (16.31)	28.74 (7.44)	62.08 (6.91)	50.86 (16.39)	NA	NA

Data are shown as mean (standard deviation) for continuous variables, number (%) for categorical variables.
HF: Heart failure
HFrEF: Heart failure with reduced ejection fraction
HFpEF: Heart failure with preserved ejection fraction
NIHF: Non ischemic heart failure
SBP: Systolic blood pressure
DBP: Diastolic blood pressure

Reviewer #2, Minor Comment #3

In the PDF Suppl. File only Suppl. Table 1 is displayed.

We appreciate this comment. We provided Supplementary Tables as an Excel file, where each Supplementary Table can be viewed by switching between the tabs in the Excel sheet. We referred to Supplementary Table 1 in the main manuscript as follows.

(Main text Lines 114 – 116)

The mean LV ejection fraction (LVEF) was 48.94% for all-cause HF, 28.74% for HFrEF, 62.08% for HFpEF, and 50.86% for NIHF (Supplementary Table 1).

Reviewer #2, Minor Comment #4

The authors further analyzed common variants of known cardiomyopathy genes. Overall, 108 genes were evaluated, of which only one common variant in the Titin gene was associated with worse outcome. Please include the overall number of genes analyzed in the main manuscript.

In the main manuscript, we examined the overlap between all loci that reached genome-wide significance in the HF-GWASs (Japanese GWAS: Supplementary Table 2, Cross-ancestry GWAS: Supplementary Table 24) and known cardiomyopathy genes (Supplementary Table 19). Among the 108 known cardiomyopathy genes, only three reached genome-wide significance. Hence, we tested just these three variants in this analysis.

We modified the description as follows. Track changes are highlighted in red.

(Main text Lines 359 – 363)

From the Japanese GWAS (Supplementary Table 2) and cross-ancestry meta-analyses (Supplementary Table 24), we found three genome-wide significant loci in *TTN*, *NEBL*, and *BAG3* among 108 known cardiomyopathy genes (Supplementary Table 19). Given the nature of cardiomyopathy genes, we hypothesized that the lead variants of these loci by themselves could affect cardiac function and prognosis.

Reviewer #3:

Remarks to the Author:

The authors present a GWAS of heart failure (HF) and two subtypes (HFpEF and HFrEF) in a Japanese population (Biobank Japan). Previous GWAS of HF and subtypes have predominantly been in European individuals and transferability of risk loci and polygenic risk scores are not well understood for the Japanese population. The authors therefore sought to conduct a GWAS of HF/HF subtypes in BBJ and compare this to European ancestry GWAS, as well as a meta-analysis to identify novel loci.

We sincerely appreciate the reviewer's thoughtful feedback, which has significantly contributed to enhancing the quality and clarity of our manuscript. In response, we have undertaken a thorough revision process, carefully addressing each point raised. Our detailed, point-by-point responses to the reviewer's comments are provided below, outlining the specific changes made and the rationale behind them.

Reviewer #3, Comment #1

Some of the main results are not explicitly stated in the text, and the reader has to look through many supplementary tables and figures to find the relevant information. Could the authors add some of the main results into the main text. For example:

-Pg 7- the authors state that the estimated SNP heritability of each HF phenotype is described in Supplementary Notes. These should be reported in the main text as they provide information on the heterogeneity across the different phenotypes. It would also be useful to provide a comparison with previously reported SNP-h2 for HF subtypes and whether they are similar or different to previous studies, and what could explain any differences.

- Pg 7 "two of the novel loci were specific to the Japanese population and were not found in Europeans (Supplementary Notes 3, Supplementary Fig. 3a, b, c, d and Supplementary Table 4, 5)". Given these are novel finings, the text could be changed to: "...two of the novel HF loci were specific to the Japanese population and have not been observed in Europeans - rs76704104, found in the intronic region of SPRED2 was associated with HFrEF, and rs78228190 in intronic region of CSMD1 and associated with HFpEF"

- "We assessed 62 previously reported HF loci and confirmed that their effects were mostly concordant with our Japanese GWAS (Suppl Notes 2, Suppl Fig 2, Suppl Table 3). This can be changed to "We assessed 62 previously reported HF loci and confirmed that their effects were mostly concordant with our Japanese GWAS, with a Pearson's correlation between effect size of 0.76 for all-cause HF and 0.81 for HFrEF (Suppl Fig 2, Suppl Table 3).

We appreciate the reviewer's important comments regarding the presentation of our main findings. In response, we have revised the manuscript to incorporate key results directly into the main text, thereby enhancing clarity and accessibility for readers.

.(Heritability) Regarding the estimated SNP heritability (h^2) of each HF phenotype, we agree that this information is crucial for understanding the genetic architecture and heterogeneity across different

HF subtypes. Consequently, we have included these estimates in the main text. Our analysis revealed that the 95% confidence intervals of the liability-scale h^2 overlapped between the BBJ cohort and European populations for each HF subtype, indicating concordance with previous research. This suggests that the genetic contributions to HF are consistent across these populations.

(Japanese-specific loci) In addressing the identification of novel loci specific to the Japanese population, we have revised the main text to explicitly describe these findings. Specifically, we now state that two novel HF loci were identified as unique to the Japanese population and have not been observed in European populations. The first, rs76704104, located in the intronic region of the *SPRED2* gene, was associated with HFrEF. The second, rs78228190, found in the intronic region of the *CSMD1* gene, was associated with HFpEF. These findings underscore the importance of conducting genetic studies in diverse populations to uncover population-specific genetic risk factors.

(Replication of previously reported loci) In evaluating the replication of previously reported HF loci, we agree with the reviewer's suggestion to provide quantitative measures of concordance. Accordingly, we have added information on Pearson's correlation coefficients between effect sizes observed in our Japanese GWAS and those reported in prior studies. The correlation coefficient was 0.76 for all-cause HF, and it was 0.81 for HFrEF. These values indicate a substantial agreement in genetic associations between our study and previous research, reinforcing the validity of our findings.

All these revisions have been incorporated into the manuscript, with changes highlighted in red.

(Main text Lines 129 – 138)

The proportion of the variation in HF phenotypes (the single nucleotide polymorphism (SNP) heritability; h^2) was estimated to be 1.5% (s.e.m 0.3%) for all-cause HF, 1.5% (0.3%) for HFrEF, 0.5% (0.3%) for HFpEF, and 1.1% (0.3%) for NIHF using linkage disequilibrium (LD)-score regression (LDSC) (Supplementary Table 6). The liability-scale h^2 was estimated at 4.8% (s.e.m. 1.0%) for all-cause HF, 12.6% (2.9%) for HFrEF, 2.8% (1.7%) for HFpEF, and 3.1% (1.0%) for NIHF, suggesting the heterogeneous heritability in each HF phenotype. The liability-scale h^2 in Europeans was 2.5% (0.2%) for all-cause HF, 6.4% (0.5%) for HFrEF, 4.0% (0.4%) for HFpEF, and 4.0% (0.7%) for NIHF. The 95% confidence intervals of the liability-scale h^2 were overlapped between BBJ and EUR in each HF subtype.

(Main text Lines 578 – 580)

The all-cause HF population prevalence was set at 2.4% for EUR[54]. The prevalence for HFrEF, HFpEF and NIHF were set at 1.2%, half of all-cause HF.

54. Martin SS, et al. 2024 Heart Disease and Stroke Statistics: A Report of US and Global Data From the American Heart Association. Circulation 149, e347-e913 (2024).

(Main text Lines 142 – 147)

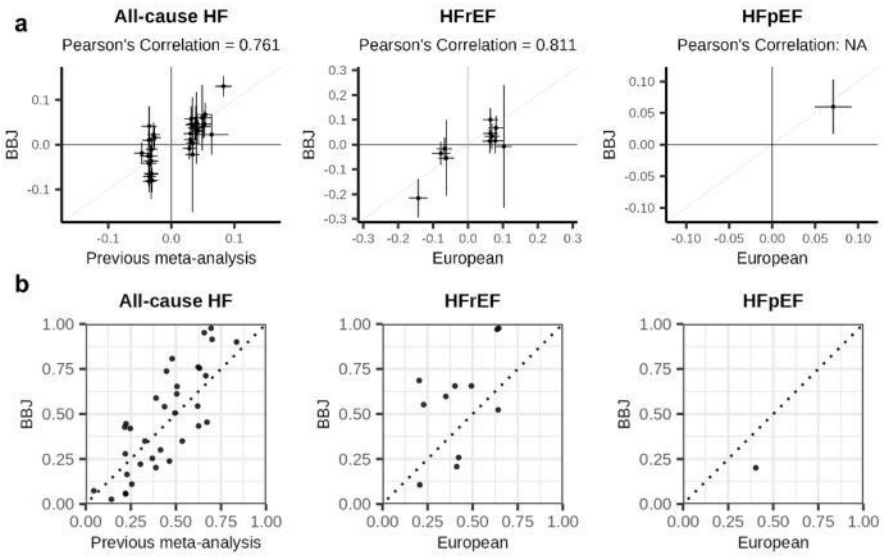
Additionally, while the reproducibility of the novel loci was obtained in both internal and external replications, two of the novel HF loci were specific to the Japanese population and have not been observed in Europeans - the lead variant rs76704104 found in the intronic region of *SPRED2* was associated with HFrEF, and the lead variant rs78228190 located in the intronic region of *CSMD1* was

associated with HFpEF (Supplementary Notes 2, Supplementary Fig. 3a, b, c, d and Supplementary Table 8, 9).

(Main text Lines 139 - 142)

We assessed the 62 previously reported HF loci and confirmed that their effects were mostly concordant with our Japanese GWAS, with a Pearson's correlation between effect size of 0.76 for all-cause HF and 0.81 for HFrEF (Supplementary Notes 1, Supplementary Fig. 2, Supplementary Table 7).

[Supplementary Fig. 2]



Reviewer #3, Comment #2

Given the overall good concordance in effect size between previous European and BBJ but some novel loci, a more suitable subheading instead of 'GWAS revealed a Japanese-specific genetic architecture' could be 'GWAS revealed Japanese-specific HF risk loci'.

Thank you for your thoughtful comments. As suggested, we acknowledged that the overall good concordance with previous studies demonstrates the reliability of our novel loci. And we agree that the suggested subheading is more suitable for the manuscript.

In line with the reviewer's recommendation, we have adopted the proposed subheading, which more accurately reflects the content of that section.

(Main text Lines 107 – 107)

GWAS revealed Japanese-specific HF risk loci

Reviewer #3, Comment #3

It would be useful to clearly distinguish novel variants from novel disease genes identified from the Japanese GWAS as well as the cross-ancestry meta-analysis and MTAG analyses. There may be different variants that could be mediating risk through the same gene. For example, the authors mention 5 previously unreported loci (Pg 7). Are any of these variants in genes where genetic variants have previously been reported to be associated with HF/subtypes?

We appreciate this comment. We searched for these 5 novel genes (*CACNA1D*, *DPY19L4*, *NEBL*, *SPRED2*, and *CSMD1*) using The Accelerating Medicines Partnership Program, Open Targets, and Genebass, and these genes and their variants have not been reported to be associated with HF, at least in the context of population genetics. Only *NEBL* is one of the known cardiomyopathy genes, encompassing several pathogenic variants, as discussed in the main text.

We added the following description to the manuscript regarding this point.

(Main text Lines 206 – 212)

Taken together, *DPY19L4*, *CSMD1*, *SPRED2*, and *NEBL* were prioritized by at least three out of 10 predictors as Japanese-specific HF-related genes, and *CACNA1D* was prioritized in both Japanese-GWAS and cross-ancestry meta-analysis (Fig. 3a, 3b, and Supplementary Table 22). None of these 5 genes has been reported to be associated with HF according to the Accelerating Medicines Partnership Program (https://www.nih.gov/research-training/accelerating-medicines-partnership-amp), Open Targets (https://www.opentargets.org/) and Genebass (https://app.genebass.org/).

Reviewer #3, Comment #4

In addition, information on pleiotropic effects of novel variants/novel genes would be useful for understanding mediating risk factors. The Accelerating Medicines Partnership® Program for Common Metabolic Diseases provides a great resource to investigate loci:

For example, common variants in the SPRED2 gene have also previously been associated with T2D. See https://hugeamp.org/gene.html?gene=SPRED2

Common variants in CAND2 have been associated with atrial fibrillation, DCM, and HF and subtypes, which is not mentioned. https://hugeamp.org/gene.html?gene=CAND2

Common variants in DPY19L4 are also associated with Hba1c, glucose and T2D, which is not mentioned. https://hugeamp.org/gene.html?gene=DPY19L4

Common variants in CACNA1D are associated with blood pressure, PR interval and Hba1c. https://hugeamp.org/gene.html?gene=CACNA1D

How are gene names selected for locus identification in Tables 1 and 2 and Figure 1? More

generally, the authors should consider using opentarget to map locus to genes in addition to the described annotation.

Thank you very much for your valuable comment.

(Pleiotropic effects) We agree that incorporating pleiotropic associations of novel variants and genes can offer important insights into their potential biological roles and mediating risk factors. In response,

we have now included a description of the pleiotropic effects for *SPRED2*, *DPY19L4*, and *CACNA1D* in the "8. Detailed explanations for novel loci" section of the Supplementary Notes. As for *CAND2*, while we recognize its known association with AF, DCM, and HF, this locus had already been reported in the previous HF MTAG GWAS. As such, we did not include it in the section specifically focused on novel loci, which was intended to highlight findings not previously described in the literature.

(Gene names) Concerning the gene names in Tables 2, 24, 37, and Figure 3, the selection of genes was guided by the combination of several gene-prioritizing methods shown in Figure 3 of the revised manuscript. Prioritized genes are listed in Supplementary Tables 22, 35, and 46 for loci identified in the BBJ GWAS (Supplementary Table 2), cross-ancestry meta-analysis (Supplementary Table 24), and MTAG (Supplementary Table 37), respectively.

In addition, following your suggestion, we integrated Open Targets-based prioritized genes into these supplementary tables (2, 24, and 37) to provide an external comparative framework. This resource indeed adds valuable information, while we note certain limitations: We noted that several lead variants identified in our Japanese GWAS, such as those at the *NEBL* and *AOPEP* loci, were not represented in the Open Targets database. Furthermore, Open Targets prioritization, which relies on eQTL and other functional annotation data, may not always capture the established causal gene at a given locus. For example, the top-prioritized gene in Open Targets did not necessarily link variants to the known cardiomyopathy genes (*TTN* and *BAG3* in BBJ [Supplementary Table 2] and *MYBPC3* in MTAG [Supplementary Table 37]), even though these were supported by the broader literature and genetic architecture. Previous research suggested that prioritization based on QTL may occasionally identify incorrect genes[2]. Hence, to ensure transparency and rigor, we have listed up to three genes prioritized by Open Targets for each lead variant and compared them with our prioritized method. Notably, all prioritized genes in novel loci were prioritized by Open Targets except for *CARM1* in the cross-ancestry meta-analysis for NICM (Supplementary Table 24). Given these considerations, we have continued to use our previously validated prioritization framework (*Nat Genet*. 2022;54:1803-1815), while also presenting Open Targets results as a complementary reference.

We added the following descriptions to the revised manuscript. The track changes are highlighted in red.

(Supplementary Note Lines 128 – 144)

CACNA1D

This locus was found in Japanese GWAS for all-cause HF and non-ischemic HF, and cross-ancestry meta-analysis for all-cause HF. The lead SNP is rs35593046 in both the Japanese GWAS and the cross-ancestry meta-analysis. rs35593046 is more frequent in East Asians (53.8%) than in Europeans (26.8%). The alternate allele (T) was associated with protection against HF (Supplementary Table 2 and 24) and high blood pressure (Supplementary Table 12). The Accelerating Medicines Partnership Program for Common Metabolic Diseases also suggested that common variants in *CACNA1D* are associated with blood pressure. Taken together, rs35593046 could protect against HF by lowering blood pressure.

DPY19L4

This locus was found in Japanese GWAS for all-cause HF, and the lead variant for this locus was rs6471480 (Supplementary Table 2). rs6471480 is an eQTL in the subcutaneous adipose tissue and tibial artery (Supplementary Table 14). *DPY19L4* was significant in the aorta and adrenal gland H-MAGMA. According to the Accelerating Medicines Partnership Program for Common Metabolic Diseases, common variants in *DPY19L4* are associated with HbA1c, glucose and type 2 diabetes. Given that *DPY19L4* was also associated with coronary artery disease in our previous research[8], this locus was likely to contribute to HF development through ischemic heart disease.

(Supplementary Note Lines 150 – 159)

SPRED2

This locus was found in Japanese GWAS for HFrEF and the lead variant was rs76704104 (Supplementary Table 2). The allele frequency of rs76704104 in East Asians is 1.6% and rs76704104 has not been reported in other ancestries. *SPRED2* is one of the monogenic genes responsible for Noonan syndrome, which causes hypertrophic cardiomyopathy (Supplementary Table 18). *SPRED2* knockout mice showed abnormal heart morphology, which is consistent with Noonan syndrome in humans (Supplementary Table 20). These data indicated that this locus contributed to HF development through cardiomyopathy. Aside from cardiomyopathy, common variants in *SPRED2* are associated with type 2 diabetes according to the Accelerating Medicines Partnership Program for Common Metabolic Diseases.

[Supplementary Table 2 Caption]

Prioritized genes were chosen based on "Supplementary Table 22. Prioritized genes in Biobank Japan cohorts"

[Supplementary Table 24 Caption]

Prioritized genes were chosen based on "Supplementary Table 35. Prioritized genes in the cross-ancestry meta-analysis"

[Supplementary Table 37 Caption]

Prioritized genes were chosen based on "Supplementary Table 46. Prioritized genes in MTAG"

[Supplementary Table 2 (partially extracted)]

Supplementary Table 2. Novel loci identified in the Japanese GWAS

All-cause HF

rsID	Chr	Pos	Ref	Alt	Freq	Beta	SE	*P*	N	Nearest gene	Prioritized gene	Open Targets
Novel												
rs35593046	3	53553923	G	T	0.563	-0.069	0.012	1.203E-08	206494	*CACNA1D*	*CACNA1D*	*TKT, CACNA1D, ACTR8*
rs6471480	8	95734714	C	T	0.344	0.069	0.013	4.193E-08	206495	*DPY19L4*	*DPY19L4*	*DPY19L4, VIRMA, RAD54B*
rs7075837	10	21026889	G	A	0.458	-0.066	0.012	4.890E-08	206494	*NEBL*	*NEBL*	*No data*

[Supplementary Table 24 (partially extracted)]

Supplementary Table 24. Novel loci identified in the cross-ancestry meta-analysis

All-cause HF

rsID	Chr	Pos	Ref	Alt	Freq	Beta	SE	*P*	N	Nearest gene	Prioritized gene	Open Targets
Novel												
rs35593046	3	53553923	G	T	0.329	-0.032	0.006	1.048E-08	1667020	*CACNA1D*	*CACNA1D*	*TKT, CACNA1D, ACTR8*
rs10851802	15	69596441	A	G	0.699	-0.029	0.005	2.839E-08	1676240	*PAQR5*	*GLCE*	*GLCE, PAQR5, KIF23*
Previously reported only in MTAG												
rs7650482	3	12841804	A	G	0.580	0.030	0.005	6.791E-09	1667910	*CAND2*	*CAND2*	*CAND2, TSEN2, RAF1*

[Supplementary Table 37 (partially extracted)]

Supplementary Table 37. Novel loci identified in multi trait analysis

All-cause HF

rsID	Chr	Pos	Ref	Alt	Freq	Beta	SE	P	N	Nearest gene	Prioritized gene	Open Targets
Novel												
rs10888955	1	56574356	T	C	0.564	0.012	0.002	1.099E-08	210216	*ENSG00000235612*	*PLPP3*	*No data*
rs582505	3	27510737	C	A	0.349	-0.007	0.001	4.517E-08	980383	*SLC4A7*	*SLC4A7*	*SLC4A7, NEK10, EOMES*
rs4860810	4	67889229	C	T	0.778	-0.008	0.001	3.798E-08	1350140	*RNU6-699P*	*CENPC/RNU6-699P*	*No data*

Reviewer #3, Comment #5

What is the genetic correlation between the HF subtypes?

Thank you for your insightful comment regarding the genetic correlation between HF subtypes. In our analysis, we found that the genetic correlation between HFrEF and HFpEF was quite low (rg 0.124, SE 0.121), suggesting a limited shared genetic architecture between these two subtypes. In contrast, other pairs of HF subtypes demonstrated substantially higher genetic correlations, indicating more overlap in their underlying genetic determinants. We also note that there is no case sample overlap between HFrEF and HFpEF in the Japanese GWAS (Figure 2a). This separation further reinforces the observed low genetic correlation and supports the rationale for treating these two subtypes as genetically distinct entities, rationalizing that analyzing HFrEF and HFpEF separately contributes to improved subtype-specific discovery and interpretation.

Regarding these points, we have added the following descriptions, figures, and tables to the manuscript. Track changes are highlighted in red.

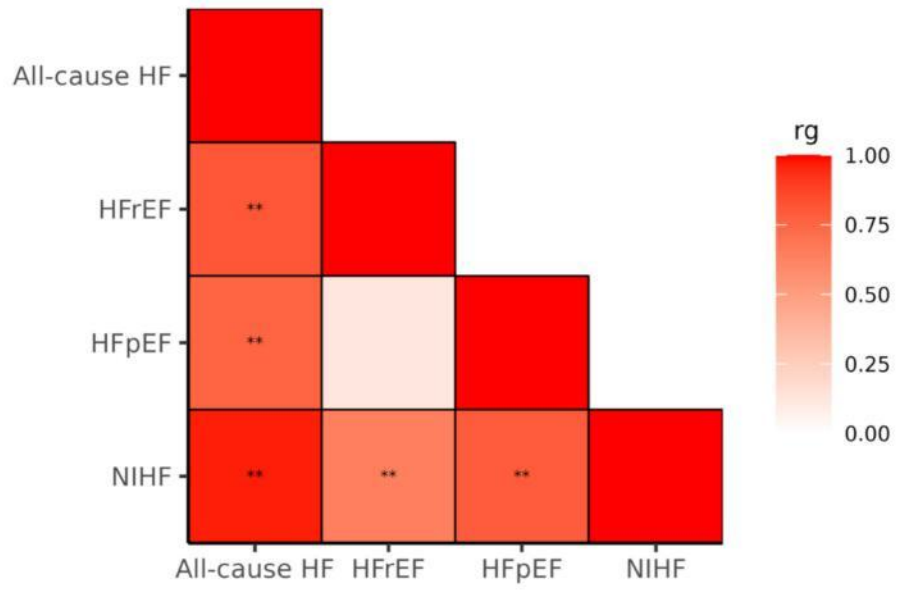
(Main text Lines 125 – 126)

The genetic correlation between HFrEF and HFpEF was low (0.124; s.e.m. 0.121; Fig. 2c and Supplementary Table 4).

(Main text Lines 610 – 611

Cross-trait LDSC was performed to estimate genetic correlation (rg) between HF and cardiac MRI traits, between HF subtypes, and between HF subtypes and risk factors.

[Fig.2c]



Japanese GWAS. (b) Miami plot of Japanese GWAS. -$\log_{10}$(*P* value) on the *y*-axis is shown against the genomic positions (hg19) on the *x*-axis. Association signals that reached a genome-wide significance level ($P < 5 \times 10^{-8}$) are shown in red if new loci and in blue if previously reported only in MTAG but not in GWAS. Two-sided *P* values were calculated using a logistic regression model. (c) Genetic correlation between HF GWAS. * indicates $P < 0.05$, and ** $P < 0.0083$ (0.05/6). GWAS, genome-wide association study; HF, heart failure; HFrEF, heart failure with reduced ejection fraction; HFpEF, heart failure with preserved ejection fraction; MTAG, multi-trait analysis of GWAS.

[Supplementary Table 4]

Supplementary Table 4. Genetic correlation of HF subtypes

pheno1	pheno2	rg	se	z	p	h2_obs	h2_obs_se	h2_int	h2_int_se	gcov_int	gcov_int_se
All-cause HF	HFrEF	0.81	0.087	9.3679	7.4008e-21	0.0139	0.003	1.041	0.008	0.5419	0.0065
All-cause HF	HFpEF	0.761	0.083	9.1492	5.7348e-20	0.0069	0.003	1.035	0.008	0.7083	0.0066
All-cause HF	NIHF	0.968	0.03	32.118	2.4376e-226	0.0134	0.0029	1.045	0.0068	0.878	0.0065
HFrEF	HFpEF	0.124	0.183	0.6784	0.4975	0.0069	0.003	1.034	0.008	0.0391	0.0051
HFrEF	NIHF	0.649	0.121	5.3691	7.9127e-08	0.0134	0.0029	1.045	0.0068	0.3955	0.0062
HFpEF	NIHF	0.789	0.111	7.101	1.2383e-12	0.0134	0.0029	1.045	0.0068	0.6177	0.006

HF: Heart failure
HFrEF: Heart failure with reduced ejection fraction
HFpEF: Heart failure with preserved ejection fraction
NIHF: Non-ischemic heart failure

Reviewer #3, Comment #6

The authors conducted MTAG analyses using only LV mass as it was genetically corelated with all HF phenotypes. We know there is heterogeneity in disease etiology across different subtypes, and it seems like a missed opportunity not to conduct genetic correlation analyses with a broader range of risk factor traits. Genetic correlation between each subtype and other risk factor traits would be a useful main figure to understand mediating risk factors for each subtype and would be a good way to inform which traits to combine in MTAG analyses to identify sub-type specific loci.

Thank you for this valuable and insightful suggestion. In response, we conducted a comprehensive analysis of genetic correlations between each HF subtype and a broad set of cardiovascular and metabolic risk factor traits across both BBJ and EUR (Supplementary Figure 1c). These analyses revealed that AF emerged as the phenotype most strongly correlated with all-cause HF across both populations. Interestingly, we observed that in BBJ, CAD showed a weaker genetic correlation with HF compared to what we found in EUR, while traits such as blood pressure and body mass index demonstrated relatively stronger correlations in BBJ. These findings suggest potential ancestry-specific differences in the genetic underpinnings of HF and its subtypes.

We agree that this analysis provides meaningful insight into the heterogeneity of HF etiology and offers an important guide for the selection of appropriate traits in subtype-specific MTAG. In light of your suggestion, we have included these genetic correlation results as a new figure in the revised manuscript to more clearly illustrate the shared and distinct genetic risk profiles of each HF subtype.

All corresponding descriptions and figure references have been added to the manuscript, with revisions highlighted in red.

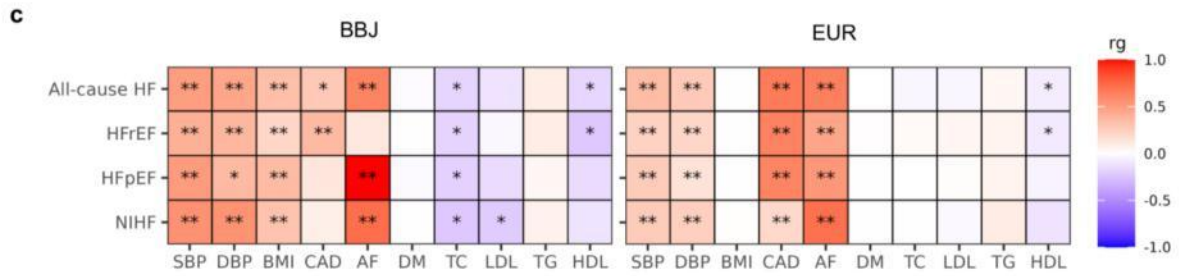
(Main text Lines 125 ─ 129)

The genetic correlation between HFrEF and HFpEF was low (0.124; s.e.m. 0.121; Fig. 2c and Supplementary Table 4). AF was correlated with all-cause HF in both BBJ (0.62 [s.e.m. 0.07]) and EUR (0.64 [0.03]; Supplementary Fig. 1c and Supplementary Table 5). While the genetic correlation between CAD and HF was stronger in EUR, blood pressure and body mass index showed stronger correlations with HF in BBJ.

(Main text Lines 610 ─ 611)

Cross-trait LDSC was performed to estimate genetic correlation (rg) between HF and cardiac MRI traits, between HF subtypes, and between HF subtypes and risk factors.

[Supplementary Figure 1c]



Supplementary Fig. 1 | Results of Japanese GWAS. … (c) Genetic correlation between HF and risk factors. * indicates P < 0.05 and ** indicates P < 0.00125 (0.05/40 tested pairs). AF, atrial fibrillation; BBJ, Biobank Japan; BMI, body mass index; CAD, coronary artery disease; DBP, diastolic blood pressure; DM, diabetes mellitus; EUR, European; GWAS, genome-wide association study; HDL, high-density lipoprotein cholesterol; HF, heart failure; HFpEF, heart failure with preserved ejection fraction; HFrEF, heart failure with reduced ejection fraction; LDL, low-density lipoprotein cholesterol; LDSC, Linkage disequilibrium score regression; MTAG, multi-trait analysis of GWAS; NIHF; non-ischemic heart failure; SBP, systolic blood pressure; TC, total cholesterol; TG, triglyceride.

[Supplementary Table 5 (partially extracted)]

Supplementary Table 5. Genetic correlation between HF and risk factors

East Asian

Phenotype1	Phenotype2	rg	se	z	p	h2_obs	h2_obs_se	h2_int	h2_int_se	gcov_int	gcov_int_se
All-cause HF	BMI	0.3317	0.0597	5.5576	2.7349e-08	0.1753	0.0074	1.1089	0.015	0.0132	0.007
All-cause HF	DBP	0.4599	0.0741	6.2026	5.5541e-10	0.0577	0.0045	1.0687	0.0108	0.0106	0.006
All-cause HF	HDL	-0.1762	0.0852	-2.0686	0.0386	0.1378	0.0335	1.2237	0.0954	-0.0193	0.0052
All-cause HF	LDL	-0.1207	0.0778	-1.5512	0.1209	0.0723	0.0159	1.0987	0.044	-0.0171	0.006
All-cause HF	SBP	0.5013	0.0712	7.0368	1.9677e-12	0.0717	0.0051	1.0663	0.0122	0.0123	0.0063
All-cause HF	TC	-0.1808	0.067	-2.7004	0.0069	0.0843	0.0145	1.1123	0.0415	-0.0171	0.006
All-cause HF	TG	0.0965	0.0654	1.4763	0.1399	0.1367	0.0305	1.0972	0.0373	0.0038	0.0056
All-cause HF	AF	0.6253	0.0698	8.9589	3.2785e-19	0.0556	0.0109	1.0355	0.0147	0.2453	0.007
All-cause HF	CAD	0.2798	0.092	3.0401	0.0024	0.0733	0.0092	1.0714	0.0131	0.0916	0.0053
All-cause HF	DM	-0.0159	0.0148	-1.0754	0.2822	0.0864	0.0069	1.0542	0.0364	0.0066	0.0029

European

Phenotype1	Phenotype2	rg	se	z	p	h2_obs	h2_obs_se	h2_int	h2_int_se	gcov_int	gcov_int_se
All-cause HF	BMI	0.004	0.0082	0.4839	0.6285	0.2117	0.0067	1.0304	0.0278	0.0144	0.0053
All-cause HF	DBP	0.2743	0.0229	11.9586	5.8518e-33	0.1127	0.0047	1.1148	0.0328	0.0118	0.0088
All-cause HF	HDL	-0.0772	0.027	-2.855	0.0043	0.1139	0.014	1.3238	0.2228	-0.0614	0.0128
All-cause HF	LDL	-0.0371	0.0284	-1.3024	0.1928	0.0807	0.0087	1.0784	0.0794	0.0259	0.0124
All-cause HF	SBP	0.3496	0.0204	17.1473	6.583e-66	0.1227	0.0049	1.1168	0.0348	0.0262	0.0096
All-cause HF	TC	-0.0376	0.0292	-1.2885	0.1976	0.0962	0.0125	1.1333	0.1146	0.0204	0.013
All-cause HF	TG	0.0442	0.03	1.4754	0.1401	0.0917	0.0112	1.1727	0.1329	0.0577	0.0137
All-cause HF	AF	0.6407	0.0261	24.5485	4.4869e-133	0.0328	0.0022	1.1014	0.0268	0.2239	0.0088
All-cause HF	CAD	0.6627	0.0216	30.6545	2.3042e-206	0.14	0.0084	1.0915	0.0263	0.1731	0.008
All-cause HF	DM	5e-04	0.0079	0.062	0.9506	0.0581	0.0027	1.1808	0.0515	0.0129	0.0046

Reviewer #3, Comment #7

There is a known difference in prevalence of the two subtypes in males and females. Are there sex-differences in the association for the identified loci?

Thank you for your important comment regarding potential sex-specific differences in the genetic associations with HF. Given the well-documented differences in the prevalence and clinical presentation of HF subtypes between males and females, we conducted sex-stratified analyses to test for interaction effects between sex and identified loci. Specifically, we evaluated 27 variant-HF pairs that were significant in BBJ GWAS and set the significance threshold as interaction P < 1.85 × 10^{-3} (0.05/27). Among these, rs11066015 showed a significantly stronger effect in males (13 -0.17, s.e.m 0.03) compared to females (13 -0.06, s.e.m 0.03), with an interaction $P = 1.5 \times 10^{-3}$). This result suggests the possibility of sex-specific genetic effects at this locus, potentially contributing to observed differences in disease susceptibility or expression between sexes.

In response to your comment, we have added the following description to the revised manuscript, along with the relevant results. Track changes are highlighted in red.

(Main text Lines 119 – 124)

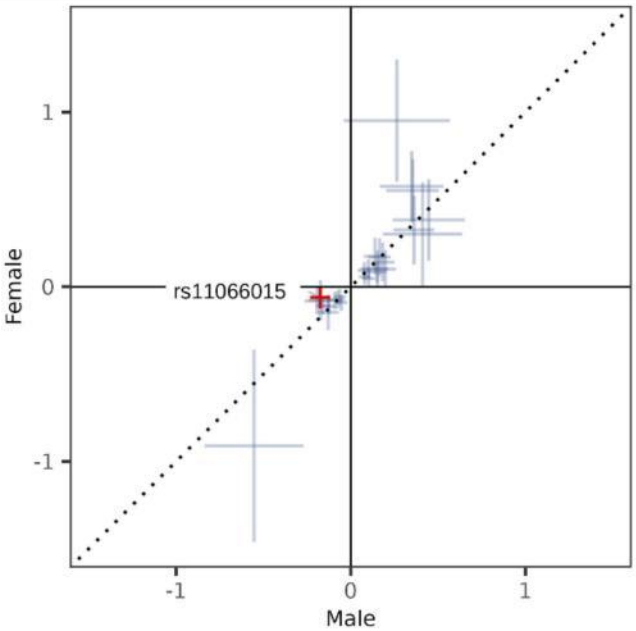
Additionally, we performed sex-stratified analyses for significant 27 variant-HF phenotype pairs among 18 genome-wide significant loci (Supplementary Table 2). Among these, rs11066015 showed a significantly stronger effect in males (β -0.17 [standard error of the mean [s.e.m.0.03]) than compared to females (β -0.06 [0.03]), along with interaction *P* = 1.5 × 10-3; Bonferroni adjusted significance threshold: *P* < 0.05/27 variants (Supplementary Fig.1b and Supplementary Table 3).

(Supplementary Note Lines 253 – 257)

Sex stratified analysis

We conducted sex-stratified analyses for 27 variant-HF phenotype pairs among 18 genome-wide significant loci. We performed the Cochran's Q test and showed P value for the Cochran's Q test as well as the I2 statistic. We also assessed P value for interaction term for sex, applying a Bonferroni-corrected significance threshold as interaction P value < 1.85 × 10-3 (0.05/27).

[Supplementary Figure 1b]



Supplementary Fig. 1 | Results of Japanese GWAS. (a) QQ plot for each HF phenotype in Japanese GWAS. (b) The result of the sex-stratified analyses for 27 variant-HF phenotype pairs. The statistically significant variant ($P < 1.85 \times 10^{-3}$) was highlighted in red.

[Supplementary Table 3 (partially extracted)]

Supplementary Table 3. Sex stratified analysis of variants identified in BBJ GWAS

	SNP						Interaction			
rsID	BETA	SE	P	BETA	SE	P	FREQ	BETA	SE	P
All-cause HF										
rs35593046	-0.0639	0.01777	0.000327386	-0.0575	0.02254	0.01072	0.56179	-0.0112	0.0284	0.69271
rs1906595	-0.125	0.018	1.87975e-12	-0.1121	0.02226	08019e-0	0.54907	-0.0121	0.02872	0.67277
rs6457934	0.079	0.018	7.83556e-06	0.05694	0.02244	0.01118	0.47467	0.02794	0.02864	0.32927
rs6471480	0.103	0.018	1.67517e-08	0.04621	0.02343	0.04857	0.34405	0.05514	0.02951	0.0617
rs147288039	0.354	0.076	8.19125e-06	0.55147	0.09096	18899e-0	0.01274	-0.1484	0.12009	0.2167
rs7075837	-0.055	0.018	1.932E-03	-0.0912	0.02262	4508e-0	0.45798	0.03863	0.02873	0.17872
rs35429	-0.092	0.020	6.4186e-06	-0.0782	0.02588	0.00238	0.25673	-0.0151	0.03275	0.64456
rs2359171	0.078	0.019	4.44519e-05	0.09355	0.02396	0.0001	0.30114	-0.0098	0.03047	0.74726
HFrEF										
rs76704104	-0.554	0.144	3.61185e-05	-0.9118	0.28061	0.00024	0.02328	0.34665	0.32226	0.28207
rs1484116	0.197	0.032	7.29777e-10	0.10141	0.05524	0.06642	0.34181	0.09571	0.06309	0.12925
rs2305398	0.164	0.033	7.33939e-07	*0.1696*	*0.0547*	*0.0022*	0.30169	-0.009	0.06412	0.88827
rs73031103	-0.174	0.033	2.16676e-07	-0.0772	0.05822	0.18502	0.70892	-0.0899	0.06663	0.17739
rs10052399	0.138	0.032	1.94451e-05	0.17477	0.05398	0.00134	0.38182	-0.0161	0.06264	0.79746
rs4711456	0.1823	0.03406	7.1123e-08	0.14144	0.05626	0.01194	0.67266	0.04428	0.06745	0.51147
rs34866937	-0.1295	0.03105	3.159e-05	-0.1458	0.05201	0.00519	0.50733	0.01634	0.06194	0.79187
rs147288039	0.26354	0.15493	0.0889406	0.95096	0.17784	35977e-0	0.01247	-0.6563	0.23201	0.00467
rs61870083	0.36133	0.05906	5.02574e-09	0.32523	0.10064	0.002	0.05475	0.04105	0.11788	0.72767
rs2267039	0.15258	0.03238	2.27992e-06	0.10295	0.05425	0.05772	0.60603	0.04226	0.06319	0.50368
HFpEF										
rs12646754	0.15067	0.02732	3.99549e-08	0.08502	0.03031	0.00513	0.44304	0.06256	0.04112	0.12821
rs78228190	0.44637	0.10514	6.61333e-05	0.38307	0.11821	0.00214	0.01198	0.07677	0.15857	0.62831
rs147288039	0.41114	0.11515	0.000712279	0.30219	0.1515	0.04609	0.01249	0.11013	0.18057	0.54194
rs11066001	-0.193	0.03593	5.7609e-08	-0.0805	0.03937	0.04076	0.2502	-0.1481	0.05298	0.00517
NIHF										
rs35593046	-0.0766	0.02171	0.000424698	-0.0736	0.02558	0.00408	0.5624	-0.0128	0.03379	0.70569
rs1906595	-0.1581	0.02151	2.43332e-13	-0.1085	0.02537	97984e-0	0.54872	-0.0515	0.03413	0.13118
rs147288039	0.34858	0.09283	0.000306119	0.57416	0.10309	81113e-0	0.01263	-0.1654	0.14324	0.24812
rs11066015	-0.1751	0.0289	9.4591e-10	-0.0601	0.03342	0.07197	0.2432	-0.1413	0.04474	0.00159
rs2359171	0.10067	0.02293	1.2775e-05	0.10361	0.02718	0.00015	0.30169	0.00709	0.03611	0.84423

HF: Heart failure
HFrEF: Heart failure with reduced ejection fraction
HFpEF: Heart failure with preserved ejection fraction
NIHF: Non-ischemic heart failure

Reviewer #3, Comment #8

Suppl table 1 gives the mean LVEF in each phenotype. The niHF encompasses both niHFrEF and niHFpEF, and this is reflected in the large SE for LVEF in the niHF group. Henry et al 2023 (preprint 10.1101/2023.10.01.23296379) report distinct aetiology and non-cardiac tissue enrichment for niHFrEF and niHFpEF. This would be important to discuss.

We appreciate your bringing up this important point. We also observed that NIHF exhibited lower heritability compared to HFrEF in our analysis. As the reviewer suggested, this may indicate that NIHF is still a heterogeneous syndrome, suggesting that stratifying NIHF into its two major subtypes, non-ischemic HFrEF and non-ischemic HFpEF, could enhance genetic signal detection and increase statistical power. This could explain the lower heritability in NIHF.

We have incorporated this discussion into the revised manuscript to better contextualize the observed differences in heritability and to acknowledge the need for more refined phenotypic resolution in future studies. The corresponding additions are highlighted in red.

(Main text Lines 500 – 504)

Fifth, NIHF showed fewer significant signals compared to HFrEF even though the sample size of NIHF was larger than that of HFrEF. Given the differential risk factor effects on non-ischemic HFrEF and non-ischemic HFpEF[46], NIHF appears to be a more heterogeneous syndrome than HFrEF. Future research is warranted to investigate these subtypes of NIHF.

46. Henry A, et al. Mapping the aetiological foundations of the heart failure spectrum using human genetics. *medRxiv*, 2023.2010.2001.23296379 (2023).

Reviewer #3, Comment #9

The authors refer to model J in Fig 5a on page 18 but the models are not labelled in Fig 5a.

Thank you for pointing this out to improve the clarity of our manuscript.

We revised the manuscript accordingly. Track changes are highlighted in red.

(Main text Lines 403 – 403)

Hereafter, we used the best model (the all-included model in Fig. 6a)

Reviewer #3, Comment #10

Pg 18 Pseudo R2 values. Should these be 0.0709 and 0.0737 instead of 0.709 and 0.737?

We appreciate the reviewer's careful attention to detail in identifying the typographical error concerning the pseudo-R^2 values. The values were mistakenly presented, whereas the correct values were 0.0709 and 0.0737, as the reviewer indicated.

We revised the manuscript accordingly.

(Main text Lines 397 – 399)

the PRS derived from BBJ showed higher performance in the Japanese population than those from European (pseudo R^2 = 0.0709 in European versus 0.0737 in BBJ; Fig. 6a)

Reviewer #3, Comment #11

Though the R2 are similar, what is the correlation between the PRS?

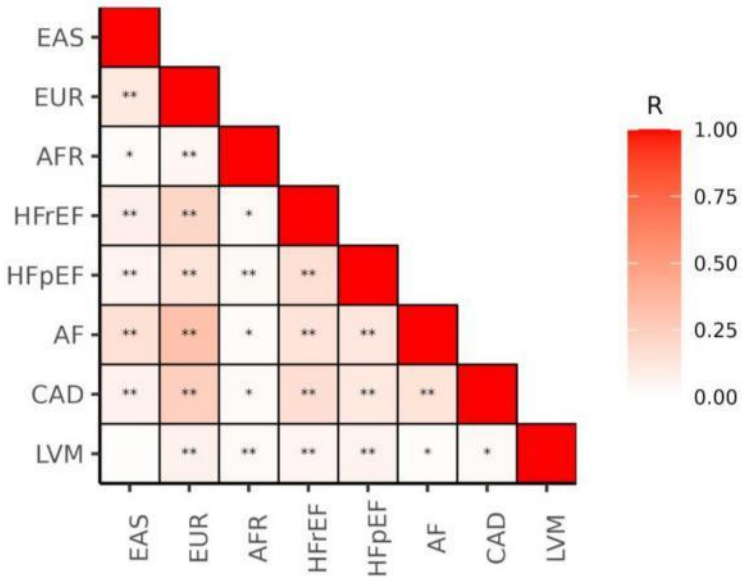
Thank you for your insightful question regarding the correlation between the PRSs. Upon analysis, we observed that the correlations between the PRSs were generally not high. The highest correlation was found between the European-derived HF PRS and AF PRS, with an R^2 value of 0.32. This modest correlation indicates that, while there is some shared genetic architecture between these conditions, each PRS may capture distinct genetic risk factors. Furthermore, a recent study demonstrated that coronary artery disease PRSs with similar predictive performance at the population level yielded divergent risk estimates at the individual level. It could also partly explain the low correlation between PRSs (*JAMA*. 2025;333(1):60-70). This variability emphasizes the necessity of integrating multiple PRSs to enhance the accuracy and reliability of overall PRS performance for individual risk assessments.

We have incorporated these findings into the revised manuscript.

(Main text Lines 394 ─ 396)

Correlations between PRSs were not high, and the most correlated PRS-pair was EUR HF and AF (R^2 = 0.32; Supplementary Fig. 15a and Supplementary Table 51).

[Supplementary Figure 15a)



Supplementary Fig. 15 | PRS performance. (a) Correlation between PRS. * indicated P < 0.05, ** indicated P < 1.79 × 10-3 (0.05/24). (b) … AF, atrial fibrillation; AFR, African; CAD, coronary artery disease; CI, confidence interval; Cross, cross ancestry PRS; EAS, East Asian; EUR, European; HF, heart failure; HFpEF, heart failure with preserved ejection fraction; HFrEF, heart failure with reduced ejection fraction; LVM, left ventricular mass; Multi, multi-trait PRS; PRS, polygenic risk score; Single, single ancestry PRS.

[Supplementary Table 51]

Supplementary Table 51. Correlation between PRSs

Phenotype1	Phenotype2	r	se	p
AF	EUR	0.31984	0.00826	1.22E-310
CAD	EUR	0.24618	0.00845	8E-181
HFrEF	EUR	0.20881	0.00853	2E-129
CAD	HFrEF	0.16657	0.0086	1.8E-82
HFpEF	HFrEF	0.16213	0.0086	3.5E-78
AF	EAS	0.15727	0.00861	1.3E-73
CAD	AF	0.14396	0.00863	7.3E-62
AF	HFrEF	0.14134	0.00863	1.1E-59
HFpEF	EUR	0.14121	0.00863	1.4E-59
AF	HFpEF	0.12423	0.00865	2.1E-46
CAD	HFpEF	0.1161	0.00866	1E-40
EAS	EUR	0.11157	0.00866	1E-37
HFrEF	EAS	0.08236	0.00869	3E-21
LVM	EUR	0.06798	0.0087	5.9E-15
CAD	EAS	0.06571	0.0087	4.5E-14
LVM	HFpEF	0.06379	0.0087	2.4E-13
HFpEF	EAS	0.0558	0.00871	1.5E-10
LVM	HFrEF	0.04483	0.00871	2.7E-07
AFR	EUR	0.04318	0.00871	7.2E-07
HFpEF	AFR	0.03374	0.00871	0.00011
LVM	AFR	0.03004	0.00872	0.00057
LVM	CAD	0.02713	0.00872	0.00186
CAD	AFR	0.02701	0.00872	0.00195
HFrEF	AFR	0.02542	0.00872	0.00355
AF	AFR	0.02252	0.00872	0.0098
AFR	EAS	0.02132	0.00872	0.01446
LVM	AF	0.01733	0.00872	0.04683
LVM	EAS	0.00968	0.00872	0.26668

Reviewer #3, Comment #12

In Pg 16 the authors mention drug effects and make the statement that all the drug effects were concordant with their genetic analyses. It doesn't seem like the authors investigated whether the risk allele identified in GWAS is a proxy for drug inhibition through formal analyses? For example, one way to do is a drug-Mendelian randomisation analyses where gene/protein expression or a mediating phenotype is used as a proxy for drug effects. I note the lack of East Asian QTL data, but risk factor data can also be used (e.g. blood pressure, the allele associated with lower BP would be considered the proxy for Nifepidine exposure and therefore should be associated with increased risk of HF). If no formal analyses have been done to determine the direction of effect, the authors should remove the statement. Instead, they could say that several identified risk loci are targets of drugs that are known to have cardiotoxic side-effects e.g. CACNA1D is a known target of nifedipine, a calcium channel blocker used to treat angina, but is known to worsen HF etc. The authors mention potential drug effects in the discussion as well, but no formal analyses such as drug-Mendelian randomisation has been conducted to determine direction of effect.

We appreciate the reviewer's thoughtful comments concerning the interpretation of drug effects in our manuscript. In our study, we linked candidate medications to diseases through *EDNRA*, *CACNA1D*, *CDK6,* and *GIPR*. While these genes are known targets of a pharmacological agent, we

acknowledge that we did not perform formal analyses, such as drug-target MR, to establish causal relationships between genetic variants and drug effects on HF risk. Unfortunately, the absence of comprehensive proteomic quantitative trait loci (pQTL) data for *EDNRA*, *CACNA1D,* and *CDK6*, even in the latest largest proteome data (Nature 622, 329–338 (2023)), limited our ability to conduct robust MR analyses for these targets. Additionally, as for using mediating phenotypes, there is no such corresponding phenotype for *EDNRA*, *CDK6,* and *GIPR*.

Hence, as suggested by the reviewer, we have deleted the sentence "All these outcomes were concordant with our genetic analyses". We have also included a discussion of these limitations in the revised manuscript, in which we stated that we were not able to perform drug-Mendelian randomization analysis due to the limited availability of summary statistics.

We added the following description to the revised manuscript.

(Main text Lines 504 – 507)

Finally, we did not perform a drug-Mendelian randomization analysis to elucidate the causal pathways linking genetic variants, drug targets, and HF risk, since GWAS summary statistics for *EDNRA*, *CACNA1D* and *CDK6* were not available even in the latest largest proteome GWAS[47].

47. Sun BB, et al. Plasma proteomic associations with genetics and health in the UK Biobank. *Nature* 622, 329-338 (2023).

Reviewer #3, Comment #13

Apologies if I have missed this, but if not in the manuscript, please add a description of how phenotypes were ascertained.

We appreciate the reviewer's attention to the methodology of phenotype ascertainment in our study. In BBJ, disease phenotypes were determined based on diagnoses made by physicians at participating hospitals, adhering to relevant clinical guidelines. BBJ chose this approach to ensure the accuracy and reliability of disease classification, as it leveraged the clinical expertise of healthcare professionals and standardized diagnostic criteria. We added the following description to the revised manuscript.

(Main text Lines 526 – 528)

The disease phenotypes were determined by the physician's diagnosis based on clinical symptoms and diagnostic test(s), under general medical practices and relevant clinical guidelines.

Reviewer #3, Comment #14

Pg 8 Pleiotropic effects – please state in main text how many traits were tested for each SNP, what was the multiple-testing p-value threshold used for statistical significance.

Thank you for the reviewer's careful attention to this important statistical aspect. We added a 'Pleiotropy check' section to the Supplementary Methods in the Supplementary Note, in which we provided detailed methods. We examined the pleiotropic effects of 18 lead variants identified as

991 genome-wide significant in BBJ GWAS with 41 cardiovascular-related phenotypes. We reported the Z
992 scores when $P < 5\times 10^{-8}$ in each phenotype, which is widely accepted in GWAS.

(Main text Lines 155 – 157)

To assess the pleiotropic effects, we used the 18 lead variant in each locus as a proxy and assessed its effect sizes in Japanese GWAS of 41 cardiovascular-related phenotypes (Supplementary Table 11)[6].

(Supplementary Note Lines 259 – 264)

Pleiotropy check

Among genome-wide significant lead variants in each HF subtype, we assessed their effect sizes using previously reported Japanese GWAS data[12]. From a total of 220 phenotypes, we selected 41 cardiovascular-related phenotypes (heart-, vascular-, blood pressure-, diabetes-, lipid-related traits) after excluding medications and quantitative traits. We reported effect sizes for associated phenotypes when $P < 5.0 \times 10^{-8}$ in each phenotype.

[Supplementary Table 11 (partially extracted)]

Supplementary Table 11. Summary statistics used to assess pleiotropic effects

Category	Phenotype	file_name
Blood pressure	Diastolic_blood_pressure	hum0197.v3.BBJ.DBP.v1
Blood pressure	Mean_arterial_pressure	hum0197.v3.BBJ.MAP.v1
Blood pressure	Pulse_pressure	hum0197.v3.BBJ.PP.v1
Lipid	HDL-cholesterol	hum0197.v3.BBJ.HDLC.v1
Lipid	LDL-cholesterol	hum0197.v3.BBJ.LDLC.v1

993 We added the following description to the revised manuscript.
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Reviewer #3, Comment #15

Typo Suppl note pg 4 line 76: rs35593046 'was' successfully replicated with nominal association (P < 0.05) with the same effect direction.

We appreciate the reviewer's attention to the typographical error. We revised the supplementary notes accordingly.

(Supplementary Note Lines 67 –67)

rs35593046 was successfully replicated with nominal association

Reviewer #3, Comment #16

It would be useful to have a table of contents in the excel sheet

Thank you for your constructive comments. We added a table of contents to the first tab of the Supplementary Tables.

[Supplementary Tables (partially extracted)]

Supplementary Tables
Table of contents

Reviewer #3, Comment #17

Discussion, the authors state the Japanese GWAS identified 20 gwas significant loci. but on Pg 7 the authors state "total number of HF-associated signals to 21"

Thank you for pointing out the discrepancy in the reported number of significant loci. The number of genome-wide significant loci in the Japanese GWAS was 18, as we delineated in lines 116-118. To further investigate potential independent signals within these loci, we performed a conditional analysis and identified three independent variants, thereby increasing the total number of HF-associated signals to 21.

We recognize that the mention of "20 genome-wide significant loci" in the Discussion section was inconsistent with the results presented earlier. Therefore, we have corrected this to accurately reflect the identification of 18 genome-wide significant "loci" in the Japanese GWAS.

(Main text Lines 434 – 434)

The Japanese GWAS identified 18 genome-wide significant loci associated with HF.

Reviewer #3, Comment #18

The discussion fails to mention common variant associations in CACNA1D with blood pressure, PR interval and Hba1c https://hugeamp.org/gene.html?gene=CACNA1D

We appreciate the reviewer's constructive comments. As per "Reviewer #3, Comment #4", we added the "8. Detailed explanations for novel loci" section in the Supplementary Notes. This addition provides a more comprehensive understanding of the pleiotropic effects of *CACNA1D* and its relevance to cardiovascular and metabolic traits. Please also refer to that reply.

Reviewer #3, Comment #19

Discussion Pg 20 Line 403-404 wording: This includes five new loci, where variants 'have' significantly 'higher' prevalence in East Asians.

We appreciate the reviewer's attention to this wording issue and have revised the manuscript accordingly.

(Main text Lines 434 – 435)

This includes five new loci, where lead variants have a significantly higher prevalence in East Asians.

Reviewer #4:

Remarks to the Author:

I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

We sincerely appreciate the reviewer's great help. We are grateful for the constructive feedback provided, which has been instrumental in enhancing the quality and clarity of our manuscript. All responses to the specific comments and suggestions can be found in the detailed replies above. Thank you once again for your thoughtful review and contribution to improving our manuscript.

References

1. Levin MG, Tsao NL, Singhal P, Liu C, Vy HMT, Paranjpe I, Backman JD, Bellomo TR, Bone WP, Biddinger KJ, Hui Q, Dikilitas O, Satterfield BA, Yang Y, Morley MP, Bradford Y, Burke M, Reza N, Charest B, Regeneron Genetics C, Judy RL, Puckelwartz MJ, Hakonarson H, Khan A, Kottyan LC, Kullo I, Luo Y, McNally EM, Rasmussen-Torvik LJ, Day SM, Do R, Phillips LS, Ellinor PT, Nadkarni GN, Ritchie MD, Arany Z, Cappola TP, Margulies KB, Aragam KG, Haggerty CM, Joseph J, Sun YV, Voight BF and Damrauer SM. Genome-wide association and multi-trait analyses characterize the common genetic architecture of heart failure. *Nat Commun*. 2022;13:6914.

2. Selewa A, Luo K, Wasney M, Smith L, Sun X, Tang C, Eckart H, Moskowitz IP, Basu A, He X and Pott S. Single-cell genomics improves the discovery of risk variants and genes of atrial fibrillation. *Nat Commun*. 2023;14:4999.

Summary to the Reviewers:

We appreciate the comments from the reviewers on our revised manuscript. We are also grateful for your evaluation that our study has been substantially improved, and for the opportunity to further revise and resubmit our manuscript.

In response to the reviewers' feedback, we have made additional corrections and improvements to the manuscript. Especially, we expanded the discussion on the potential mechanisms of the *TTN* common variant, rs2627043, as suggested by Reviewer #2, and enhanced the genetic interpretation accordingly. Furthermore, to address Reviewer #3's concern, we moved Supplementary Figure 1c (Genetic correlation between HF and risk factors) to the main Figure 2d, as it represents an essential component of our study. We have also added a discussion on the differences in genetic correlations between East Asian (EAS) and European (EUR) populations. For example, coronary artery disease showed a stronger correlation in EUR, whereas body mass index was more strongly correlated in EAS. These findings underscore the importance of cross-ancestry genetic analyses. We hope you will find that these revisions have made our manuscript worth considering for publication in *Nature Communications*. The point-by-point responses to all comments from the reviewers can be found below.

Reviewers' Comments:

Reviewer #1:

Remarks to the Author:

The authors have addressed all the questions. Thank you.

We sincerely thank the reviewer for providing valuable and constructive advice, which was of great help to improve our manuscript.

Reviewer #2:

Remarks to the Author:

The authors have incorporated several comments raised by the reviewers which lead to a substantial improvement in the manuscript. I only have minor comments:

We are grateful for your positive feedback. We also appreciate your additional advice and comments to enhance the clarity and validity of our study. The following is a point-by-point response to each of your comments.

Reviewer #2, Minor comment #1

I recommend mentioning in the abstract that the majority of identified loci were associated with the HFrEF subtype.

We appreciate your constructive suggestion. While the majority of the genome-wide significant loci were identified in all-cause HF, this was driven by the larger sample size. Among the 76 genome-wide significant loci, 51 were associated with all-cause HF, 31 with HFrEF, 8 with HFpEF, and 13 with non-ischemic HF, with some overlaps between all-cause HF and HF subtypes, and between HF subtypes. As the reviewer rightly pointed out, the GWAS for HFrEF yielded the greatest number of significant loci among the HF subtypes despite its smaller sample size. We have revised the abstract accordingly to mention this key point. The revised text is highlighted in red.

(Main text Lines 67 - 79) [Abstract]

To understand the genetic basis of heart failure (HF) in the Japanese population, we performed genome-wide association studies (GWASs) comprising 16,251 all-cause HF cases, 4,254 HF with reduced ejection fraction (HFrEF) cases, 7,154 HF with preserved ejection fraction cases, and 11,122 non-ischemic HF cases among 213,828 individuals and identified five novel loci. A subsequent cross-ancestry meta-analysis and multi-trait analysis of the GWAS data identified 19 novel loci in total, with 31 out of the 76 genome-wide significant loci associated with HFrEF despite its smaller sample size. Among these susceptibility loci, a common non-coding variant in TTN (rs1484116) was associated with reduced cardiac function and worse long-term mortality. We leveraged the HF meta-GWASs along with cardiac function-related GWASs to develop a polygenic risk score (PRS) for HF. The PRS successfully identified early-onset HF and those with an increased risk of long-term HF mortality. Our results shed light on the shared and distinct genetic basis of HF between Japanese and European populations and improve the clinical value of HF genetics.

Reviewer #2, Minor comment #2

By now, more than 60.000 Titin missense variants haven been reported (PMID: 38438525). In the discussion the authors hypothesise that the common variant described here may directly affect cardiac function and prognosis. Given the large difference in allele frequency and effect size of the *TTN* variant between the UKBB and the BBJ, it seems likely that the presence of this variant is rather part of a multi-hit model or provides an additive effect to a different risk factor (PMID: 39959973) than directly causing a clinically relevant phenotype. In particular given the high association of Titin variants with atrial fibrillation (PMID: 31691645). Therefore, until experimental data is available this should be discussed.

Thank you for raising this important point. We fully agree with the Reviewer's opinion that the interpretation of *TTN* variants remains complex, in part due to the exceptionally large encoded protein and the diverse functional areas. Therefore, we have expanded our discussion to mention these complexities. Specifically, we now consider the possibility that the identified variant may act as part of a multi-hit model, and the potential for this variant to exert an additive effect in combination with other risk factors. These considerations have been incorporated into the Discussion section, and the revised text is provided below.

(Main text Lines 503 – 509)

Third, even though our analysis identified a missense variant, rs2627043, in the *TTN* locus, the precise causal mechanism underlying HF development remains unclear. In the context of AF, a previous study has demonstrated that the presence of a *TTN* truncating variant can act additively with polygenic risk, resulting in higher penetrance of AF[46]. This finding suggests that the *TTN* variant could function as part of a two- or multi-hit model in which other risk factors exert additive effects in combination with the variant. Further experimental studies are required to elucidate the causal mechanisms involved.

Reviewer #3:

Remarks to the Author:

I appreciate the effort taken by the authors to address all my comments.

We are grateful for your positive comment. We also appreciate your additional suggestions to improve our manuscript. Accordingly, the following modifications have been made in our manuscript.

Reviewer #3, Major Comment #1

I appreciate the authors providing this information. However, for readability, could authors please present this as table (if editors allow) for easier comparison. The authors state there was overall in confidence intervals indicating concordance between Eur and Japanese. The Japanese cohort is much smaller, so we expect bigger CI for these estimates. The difference in point estimates is quite striking (12.6% vs 6.4% for HFrEF), despite the significantly smaller sample size (almost a fifth of Eur N). It would perhaps be good to highlight this difference in point estimate for HFrEF. Finally could the authors rationalise why for all HF subtypes in Eur the population prevalence was considered the same?

European Japanese

Pop prev SNP h2 Liab h2 Pop prev SNP h2 Liab h2

HFreEF 1.2 6.4 (0.5) 2.4 1.5 (0.3)12.6 (2.9)

HFpEF 1.2 4.0 (0.4) 2.9 0.5 (0.3)2.8 (1.7)

All HF 2.4 2.5 (0.2) 6.5 1.5 (0.3) 4.8 (1.0)

NIHF 1.2 4.0 (0.7) 1.7 1.1 (0.3) 3.1 (1.0)

We appreciate the reviewer's thoughtful comments to improve our manuscript. First, we have provided all the necessary information in Supplementary Table 6 to make it easy for readers to compare the results. As the reviewer pointed out, whereas the confidence intervals in the heritability estimation were larger in the Japanese population (BBJ) compared to those of Europeans (EUR) due to the smaller sample size, the difference in point estimates was especially noticeable in HFrEF. Therefore, we have mentioned this in the main text.

Regarding the population prevalence of HF subtypes in EUR, we set the prevalence of HFrEF, HFpEF, and NIHF as 1.2%, which is half of all-cause HF prevalence based on the previous reports (PMID: 37295177 and 28492288). We have added these reports to the References. We also added a "Population prevalence" column in the Supplementary Table 6 as per the reviewer's suggestion.

The revised text and table are shown below.

(Main text Lines 131 – 140)

The proportion of the variation in HF phenotypes (the single-nucleotide polymorphism (SNP) heritability; h2) was estimated to be 1.5% (s.e.m. 0.3%) for all-cause HF, 1.5% (0.3%) for HFrEF, 0.5% (0.3%) for HFpEF, and 1.1% (0.3%) for NIHF using linkage disequilibrium (LD)-score regression (LDSC) (**Supplementary Table 6**). The liability-scale h2 was estimated at 4.8% (s.e.m. 1.0%) for all-cause HF, 12.6% (2.9%) for HFrEF, 2.8% (1.7%) for HFpEF, and 3.1% (1.0%) for NIHF, suggesting the heterogeneous heritability in each HF phenotype. The liability-scale h2 in Europeans was 2.5% (0.2%) for all-cause HF, 6.4% (0.5%) for HFrEF, 4.0% (0.4%) for HFpEF, and 4.0% (0.7%) for NIHF.

While the 95% confidence intervals of the liability-scale h2 overlapped between BBJ and EUR in each HF subtype, the heritability for HFrEF tended to be higher in BBJ than in EUR.

(Main text Lines 598 - 599)

The prevalence of HFrEF, HFpEF[57], and NIHF[58] was set at 1.2%, half of all-cause HF.

(References)

57. Dunlay SM, Roger VL, Redfield MM. Epidemiology of heart failure with preserved ejection fraction. Nat Rev Cardiol 14, 591-602 (2017).

58. Akhtar KH, et al. An Individual Patient-Level Meta-Analysis of Ischemic Versus Nonischemic Cardiomyopathy and Trajectory of Decongestion in Patients With Acute Decompensated Heart Failure. Am J Cardiol 200, 32-39 (2023).

[Supplementary Table 6]

	BBJ					EUR				
	Observed scale		Liability scale			Observed scale		Liability scale		
HF subtype	h^2	SE	Population prevalence	h^2	SE	h^2	SE	Population prevalence	h^2	SE
All-cause HF	1.5	0.3	6.5	4.8	1.0	1.0	0.1	2.4	2.5	0.2
HFrEF	1.5	0.3	2.4	12.6	2.9	3.1	0.3	1.2	6.4	0.5
HFpEF	0.5	0.3	2.9	2.8	1.7	2	0.2	1.2	4.0	0.4
NIHF	1.1	0.3	1.7	3.1	1.0	0.5	0.1	1.2	4.0	0.7

BBJ: Biobank Japan
EUR: European
SE: standard error
HF: Heart failure
HFrEF: Heart failure with reduced ejection fraction
HFpEF: Heart failure with preserved ejection fraction
NIHF: Non-ischemic heart failure

Reviewer #3, Major Comment #2

Thank you for clarifying the correlation in effect size between BBJ and previous analyses.

Reviewer #3, Major Comment #3

Thank you for clarifying novel genes.

We sincerely appreciate your helpful comments, especially regarding the clarification of effect size correlation and novel gene identification.

Reviewer #3, Major Comment #4

Thank you for looking into pleiotropic associations. For *CACNA1D*, the authors have added: *The alternate allele (T) was associated with protection against HF (Supplementary Table 2 and 24) and high blood pressure (Supplementary Table 12). The Accelerating Medicines Partnership Program for Common Metabolic Diseases also suggested that common variants in CACNA1D are associated with blood pressure. Taken together, rs35593046 could protect against HF by lowering blood pressure.*

The authors first state that the allele that protects against HF is associated with high blood pressure (which is counterintuitive), but then say it could protect against HF by lowering BP. Could you clarify whether the HF risk allele is associated with higher or lower blood pressure.

We apologize for the confusing description. By "The alternate allele (T) was associated with protection against HF and high blood pressure", we meant that "the alternate allele was associated with reduced risk of HF and lower blood pressure", as shown in Supplementary Tables 2 and 12. Hence, the HF risk allele (G) was associated with higher blood pressure. To make this clearer, we revised the manuscript accordingly.

(Supplementary Notes 7 Lines 133 – 135)

The alternate allele (T) was associated with protection against both HF (**Supplementary Tables 2** and **24**) and high blood pressure (**Supplementary Table 12**).

[Supplementary Table 2 and 12] (The effect direction is highlighted in yellow)

Supplementary Table 2. Novel loci identified in the Japanese GWAS
All-cause HF

rsID	Chr	Pos	Ref	Alt	Freq	Beta	SE	*P*	N	Nearest gene	Prioritized gene
Novel											
rs35593046	3	53553923	G	T	0.563	-0.069	0.012	1.203E-08	206494	*CACNA1D*	*CACNA1D*

Supplementary Table 12. Pleiotropic effects of significant loci
All-cause HF

SNP	Category	Phenotype	Chr	Pos	Ref	Alt	Beta	SE	*P*
Novel									
rs35593046	Blood pressure	Diastolic blood pressure	3	53553923	G	T	-0.022	0.004	5.400E-10
rs35593046	Blood pressure	Mean arterial pressure	3	53553923	G	T	-0.022	0.003	1.700E-10
rs35593046	Blood pressure	Systolic blood pressure	3	53553923	G	T	-0.019	0.003	4.400E-08

Reviewer #3, Major Comment #5

Thank you for adding the figure showing rg.

Thank you for your positive feedback on the updated figure showing rg.

Reviewer #3, Major Comment #6

Thank you for showing the rg with risk factor traits. These results are very interesting and as the authors say, they highlight some ancestry differences in risk factor contribution, and as such I strongly recommend for this to be included as a main figure and be discussed. As the authors state, CAD is less strongly associated in BBJ, apart from with HFrEF, for which we know ischemic disease had higher prevalence. The lack of association rg between BMI with any of the traits in Eur but strong association in BBJ is intriguing indeed, as is the -ve rg between TC and LDL-C in BBJ. I think these are important results to discuss.

Thank you for this important suggestion. We agree with the Reviewer and have moved Supplementary Figure 1c to the main Figure 2d. We previously reported a Japanese nationwide HF registry, in which ischemic heart failure was less common in Japan than in other countries. This difference may partly explain the observed differences in genetic correlation between CAD and HF across ancestries.

A possible mechanism underlying the difference in genetic correlation for BMI is that the impact of BMI on cardiovascular outcomes might differ across populations. As previously shown, Asians have more visceral adiposity, which is more metabolically adverse, at any given BMI than Europeans.

Regarding the association between TC/ LDLC and HF, it has been reported that low cholesterol levels are associated with poor prognosis in heart failure (PMID: 19033015), while there is little direct evidence showing ancestry differences in these associations. Thus, the differences in genetic associations that we demonstrated will require further validation through larger multi-ancestry studies in the future.

We discussed these points in the Discussion section. The revised text and figure are shown below.

(Main text Lines 127 - 131)

The genetic correlation between HFrEF and HFpEF was low (0.124 [s.e.m. 0.121]; Fig. 2c and Supplementary Table 4). AF was correlated with all-cause HF in both BBJ (0.62 [s.e.m. 0.07]) and EUR (0.64 [0.03]; **Fig. 2d** and **Supplementary Table 5**). While the genetic correlation between CAD and HF was stronger in EUR, blood pressure and body mass indices showed stronger correlations with HF in BBJ.

(Main text Line 443 - 454)

Genetic correlation analyses between HF and risk factors showed differences between Japanese and Europeans. AF was strongly associated with HF subtypes in both Japanese and Europeans, except for Japanese HFrEF (**Fig. 2d**). BMI was genetically correlated with HF only in Japanese, while CAD was more correlated with HF in Europeans. A previous study demonstrated that ischemic heart disease was less common in patients with HF in Japanese than in Europeans[41], which may partly explain the difference in genetic correlation between CAD and HF across ancestries. Asians have more visceral adiposity, which is more metabolically adverse, at any given BMI compared to Europeans[42]. This suggests that the increase in BMI may have a greater impact on cardiovascular diseases in East Asians than in Europeans. On the other hand, due to limited direct evidence showing

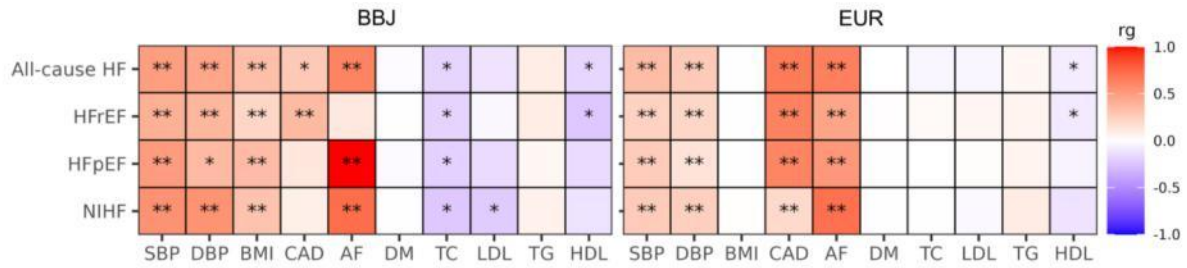
196 ancestry differences in the association between TC and LDL-C with HF, further validation through
197 larger multi-ancestry studies will be warranted in the future to confirm these findings.

198 (References)

199 41. Ide T, et al. Clinical Characteristics and Outcomes of Hospitalized Patients With Heart Failure
200 From the Large-Scale Japanese Registry Of Acute Decompensated Heart Failure (JROADHF). Circ J
201 85, 1438-1450 (2021).

202 42. Fujimoto WY, et al. Risk Factors for Type 2 Diabetes: Lessons Learned from Japanese Americans
203 in Seattle. J Diabetes Investig 3, 212-224 (2012).

204 [Figure 2d]



205

206 Fig. 2 | Results of the Japanese GWAS. (a) … (d) Genetic correlation between HF and risk factors. *
207 indicates P < 0.05 and ** indicates P < 0.00125 (0.05/40 tested pairs). AF, atrial fibrillation; BBJ,
208 Biobank Japan; BMI, body mass index; CAD, coronary artery disease; DBP, diastolic blood pressure;
209 DM, diabetes mellitus; EUR, European; GWAS, genome-wide association study; HDL, high-density
210 lipoprotein cholesterol; HF, heart failure; HFpEF, heart failure with preserved ejection fraction; HFrEF,
211 heart failure with reduced ejection fraction; LDL, low-density lipoprotein cholesterol; NIHF; non-
212 ischemic heart failure; SBP, systolic blood pressure; TC, total cholesterol; TG, triglyceride.

213

Reviewer #3, Major Comment #7

214 **Thank you for presenting the sex-stratified results. Could you please clarify in text that the**
215 **rs11066015 showed a significant sex-difference in HFpEF. For Suppl fig 1b, I'm assuming you**
216 **are plotting the effect sizes between males and females for HFpEF? Could you clarify in the**
217 **legend?**

218 We apologize for our unclear presentation and thank you for your careful review, which has greatly
219 improved our manuscript. We performed sex-stratified analyses for 27 variant-HF phenotype pairs
220 (Supplementary Table 2 and Supplementary Fig.1b). Among these, rs11066015 reached statistical
221 significance in both the GWAS and sex-stratified analysis for NIHF (Supplementary Table 3). We
222 have now clarified this point in the main text. Additionally, as you correctly assumed, Supplementary
223 Fig.1b and Supplementary Table 3 display the effect estimates in males and females separately. To
224 improve clarity, we have revised the manuscript, the figure legends, and the supplementary methods
225 accordingly. Below are the corresponding text, figure, and table.

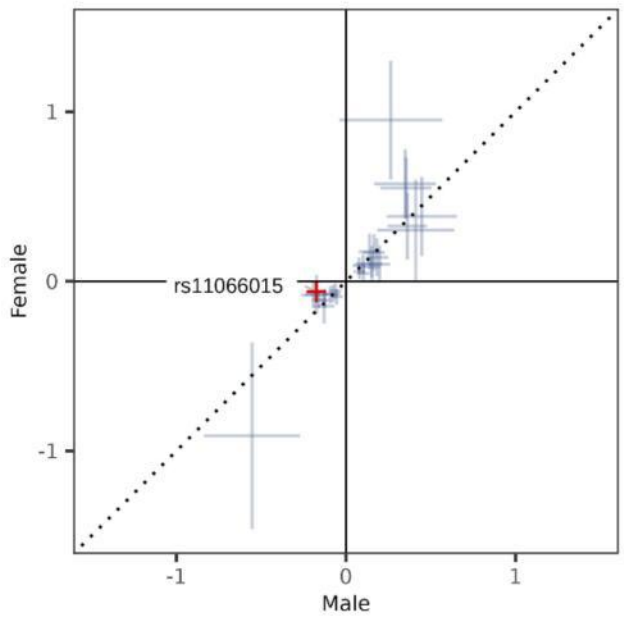
226 (Main text Lines 121 ― 126)

Additionally, we performed sex-stratified analyses for significant 27 variant-HF phenotype pairs among 18 genome-wide significant loci (Supplementary Table 2). Among these, rs11066015 for NIHF showed a significantly stronger effect in males (β -0.17 [standard error of the mean [s.e.m. 0.03]) compared to females (β -0.06 [0.03]), along with interaction P = 1.5 × 10-3; Bonferroni adjusted significance threshold: P < 0.05/27 variants (**Supplementary Fig.1b** and **Supplementary Table 3**). (Supplementary methods, Lines 255 - 261)

Sex-stratified analysis

We conducted sex-stratified analyses for 27 variant-HF phenotype pairs among 18 genome-wide significant loci. We performed Cochran's Q test and showed the P value for the Cochran's Q test as well as the I^2 statistic. We also assessed P value for the interaction term for sex, applying a Bonferroni-corrected significance threshold as interaction P value < 1.85 × 10-3 (0.05/27). In addition to the heterogeneity and P value for the interaction term, we showed effect estimates in males and females separately (**Supplementary Fig.1b** and **Supplementary Table 3**).

[Supplementary Fig. 1b]



Supplementary Fig. 1 | Results of Japanese GWAS. (a) QQ plot for each HF phenotype in Japanese GWAS. (b) The result of the sex-stratified analysis for 27 variant-HF phenotype pairs. Among these pairs, rs11066015 showed a significant sex difference in NIHF. Effect sizes with their 95% CI for males are shown on the *x*-axis, and those for females are shown on the *y*-axis. The statistically significant variant (P < 1.85 × 10-3) is highlighted in red.

[Supplementary Table 3 (partially extracted)] (rs11066015 was highlighted in yellow)

SNP	Male				Female				Interaction			
rsID	FREQ	BETA	SE	*P*	FREQ	BETA	SE	*P*	FREQ	BETA	SE	*P*
NIHF												
rs35593046	0.562723	-0.0766373	0.0217079	0.000424698	0.562082	-0.0735941	0.0255837	0.0040847	0.562395	-0.0127603	0.0337887	0.70569006
rs1906595	0.548669	-0.158055	0.0215054	2.43332e-13	0.548768	-0.108501	0.0253668	1.97984e-05	0.54872	-0.0515234	0.0341331	0.13117528
rs147288039	0.0127643	0.348577	0.0928317	0.000306119	0.0125007	0.57416	0.103091	1.81113e-07	0.0126293	-0.165435	0.143243	0.24812013
rs11066015	0.243141	-0.175137	0.0288988	9.4591e-10	0.243252	-0.0601407	0.0334242	0.0719681	0.243197	-0.141308	0.0447381	0.001585514
rs2359171	0.3014	0.100669	0.0229333	1.2775e-05	0.301964	0.103612	0.0271804	0.000151897	0.301689	0.00709445	0.0361074	0.844232953

Reviewer #3, Major Comment #8-17

Thank you for sufficiently addressing my comments.

We appreciate your confirmation that our responses have sufficiently addressed your comments.

Reviewer #4:

Remarks to the Author:

I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

We again thank all the reviewers for their evaluation and comments on our revised manuscript.