nature portfolio

Peer Review File

Shared Genetic Architecture Contributes to Risk of Major Cardiovascular Diseases

Corresponding Author: Dr Siim Pauklin

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)
Jun Qiao et al have performed a detailed analysis for shared genetic basis of CVD outcomes. They leveraged on extensive datasets totaling over 1 million subjects and 6 CVD outcomes and performed comprehensive analyses using diverse analytical methods. While appreciating the extensive efforts by the authors, I do have some concerns and questions:

1. The employment of diverse analytical methods has made it difficult to follow all the analyses and results. And, the results from various analyses were not always robust, and the consistency among the results were not high. Authors need to take a close look and comment on the “inconsistence” issue. Authors may focus on the results that are robust and consistent across various analyses. By doing so, it will be easier for readers to follow the main results and conclusions.

2. For example, while it is stated that GWAS-PW strengthened LAVA results, there were only 3 genomic regions that were significantly captured by both GWAS-PW and LAVA. Authors need to comment on the low overlapping between the results from LAVA and GWAS-PW analyses. Similarly, do the 38 pleiotropic loci identified from MTAG overlap with the LAVA and GWAS-PW analyses? Actually, the most prominent LPA signal does not seem to be picked up by genetic correlation analyses by LAVA and GWAS-PW. Giving the limited consistence between the various methods employed, how robust would these results be?

3. It is also a bit difficult to assess the shared and novel loci identified in the study through MTAG. Would the novel loci identified be those that do not overlap with lead SNPs from “Results from Original GWAS analysis” column in supp table 9. Was LD between the lead SNP identified from MTAG and lead SNP from the Original GWAS analysis considered?

4. Greater discussion is also required for negatively correlated loci. What would be the biological basis of these loci that increase risk of one CVD but reduce the risk of another CVD? Were all negatively correlated loci observed even after Bonferroni corrections?

5. The HyPrColoc analysis also suggest stronger colocalization of shared causal variants between metabolic traits and the CVD outcomes tested. Was causal inference analysis or MR evaluations performed to assess the metabolic trait associations on the CVD outcomes? Were these colocalization analyses performed for identified shared regions between the 6 CVD outcomes? These results suggest stronger impacts of metabolic traits effects on CVD outcomes tested rather than the effects of one CVD trait on another CVD outcome. Additionally, in the MR analyses there was limited evidence for vertical pleiotropy and much of the shared loci seemed to be due to horizontal pleiotropy and a due to reverse causation.

6. While the proteome analysis identifies some proteins and potential therapeutic targets for specific CVD outcomes, it is highlighted that the 11 proteins were not linked to more than one CVD outcome and provides little evidence to enhance the main question of the manuscript which, was to evaluate for shared basis of major CVD outcomes. Actually, I feel that this part of analyses does not contribute to the main objective of “investigating shared genetic basis of CVD outcomes”. I would suggest for the part of analysis to be removed from the current manuscript, so that the manuscript can be more focused.

7. SNP-based heritability estimates seem rather low – median 1.4%. Would this be typical? Is this because of the SNP filtering process used in the study?

8. “Conversely, PAD displayed superior discoverability while exhibiting less pronounced polygenicity in relation to other CVDs”. It would be good to define discoverability and polygenicity in the manuscript.

Reviewer #2

(Remarks to the Author)
The authors present a comprehensive joint analysis of cardiovascular traits, using almost every tool available currently. The authors have some expectation of shared genetic etiology among CVD phenotypes, and use MTAG to identify novel signals. Further, they explore potential reasons for shared etiology, using functional annotation, colocalization, gene aggregation and so on. It resembles a published manuscript (https://doi.org/10.1093/eurheartj/ehad655.3053), with more phenotypes and analyses.
The manuscript is long and the pieces are not well-tied together. It would benefit from a more concise description of the main results. Even though there may be over 100 novel variants, few of them have any further evidence that justifies a paragraph afterwards. It would be interesting to have a section with the accumulated evidence for the best explained variants, e.g. if a variant has a high coloc score, is a pQTL and is part of a significant pathway, it would deserve highlighting.
I also have a few more points:
- Supplementary Table 1 shows many genes associated with blood pressure/hypertension. It is reasonable to assume that a sizeable portion of these CVD cases is hypertensive, since hypertension is a major risk factor for CVD. How can we be certain that these variants are not simply reflecting the hypertensive status of the individuals?
- I missed a table showing what the original P-value of the "novel" association is in the original GWAS. I would like to see quickly if the P-value went from, say, ~E-7 to ~E-8 or if there was a big increase, which would be unexpected.
- It would also strenghten the paper if there was some sort of replication of the MTAG found variants, using some publicly-available dataset, e.g. MVP or All of US
- Please explain what "opposite" means in the sentence: "two distinct groups of CVDs: those characterized by more genetic signals and shared biological mechanisms (AF, CAD, and VTE), and those exhibiting the opposite (HF, PAD, and Stroke)".
- Sup. Figure 9: there are some variant-phenotype pairs with opposite directions to the others for rs12129500 and rs7528419, so the title of the figure is misleading. Also, there are some arrows missing (e.g. rs12509595 for VTE), what happened to those values?
- Sup. Table 1: Why do tables go from A to D?

Reviewer #3

(Remarks to the Author)
The paper conducted a thorough investigation of cardiovascular diseases using modern genetic methods. The manuscript is overall well-structured and was easy to follow. Especially, the last paragraph of the introductory section was a great overview of the study. My comments can be found below.

Major comments:
- LHC MR paper claims to handle sample overlap, and the consideration is presented in their method derivation. However, the simulation they conducted only includes non-overlapping samples. In this manuscript, GWAS summary statistics frequently include UK Biobank, including the major CVD traits and proteome measurements. Fortunately, the authors can test the their conclusions by including summary statistics without UK Biobank. For example, the arterial fibrillation GWAS (Pubmed ID 30061737) provides UKB-excluded summary statistics (http://csg.sph.umich.edu/willer/public/afib2018/, UKB left-out summary statistics).

- It’s untrustworthy to apply MR to MTAG summary statistics. This type of analysis has not been tested in any methodology/benchmark papers. The authors should conduct MR with the original summary statistics before MTAG and compare to their original MTAG-based MR.

- Several pleiotropy assessment tools have been developed since the introduction of MTAG. One example is PLEIO (https://pubmed.ncbi.nlm.nih.gov/33352115/). It provides a drop-in diagram of Figure 2b. It handles multiple comparison automatically, so post-hoc Bonferroni-correction (which is conservative) can be avoided.

- Considering the pervasive pleiotropy found by the authors, multivariate mendelian randomization (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4325677/) (and the following developments) is likely a better alternative to other MR. Sample overlap should be handled by excluding overlapping samples. Certain arrows in the multivariate MR represent direct/partial causal effects depending on the causal relationship between the traits. Another alternative is to consider genomic structural equation models.

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
Most of my comments have been addressed by providing clarifications and/or additional analyses. However, the responses

to two comments can be further strengthened.

1. Responses to the “overlapping comments” can be strengthened, for example, by 1） providing a clear summary of which results are most robust and consistent across different methods and 2) doing a direct comparison of 38 pleiotropic loci with LAVA/GWAS-PW results.

2. A direct MR analysis of metabolic traits can strengthen the study and thus manuscript.

Reviewer #2

(Remarks to the Author)
The authors answered most of the questions regarding the methods, but did little regarding the writing of the manuscript. A few issues I still encounter:
- The introduction needs to be rewritten and shortened. A few examples that make the introduction wrong:
1) " In detail, heart failure (HF) is a complex clinical syndrome often caused by prior CAD, which represents the final stage of numerous heart diseases." It leads to the interpretation that CAD is the final stage, when heart failure is.
2) It is not true, for instance, that "the concept of meta-analysis has emerged, combining diverse cohorts with similar or genetically correlated traits to amplify the study sample size". The concept introduced seems to be that of multi-trait analysis of GWAS, not that of meta-analysis.

- There are sentences that not reviewed, e.g.:
1) "HF and CAD exhibited augmented estimated higher estimated polygenicity"
2) "1.397K (sd = 0.254K)". Is the K not indicating a multiple of thousand? it is actually simpler to write 1,397 and 254 in this case, or 1.4K and 0.25K. There are other such cases in the text.
3) " were survived after the Bonferroni correction, was also validated by GWAS-PW", that is not standard English.
4) " this was the type of Mendelian randomization (MR) mainly targets" - do you mean "the type THAT MR targets" or do you mean "the type of MR MAIN targets"? The sentence is confusing.
5) The sentence "In conclusion, the observation that more than one CVD showed genetic signals..." is in the Results section. If it is a conclusion, it should not be in Results.
The list is non-exhaustive. I really recommend a thorough review of the writing.

Reviewer #3

(Remarks to the Author)
It feels like a large portion of the rebuttal has been written by a large language model, but I'm happy with the efforts on which the authors put into address my comments. I don't have further concerns.

Version 2:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
My comments have been addressed. No further comments

Re: NCOMMS-24-16271

Title: Shared Genetic Architecture Contributes to Risk of Major Cardiovascular Diseases

We thank the Reviewers and the Editor for the helpful feedback and insightful suggestions. The response from the reviewers is encouraging and constructive. Below, we provide point-by-point responses that address all the comments with additional analyses. By incorporating these new data and findings, our manuscript has been improved substantially.

Reviewer #1 (Remarks to the Author):

Jun Qiao et al have performed a detailed analysis for shared genetic basis of CVD outcomes. They leveraged on extensive datasets totaling over 1 million subjects and 6 CVD outcomes and performed comprehensive analyses using diverse analytical methods. While appreciating the extensive efforts by the authors, I do have some concerns and questions:

1. The employment of diverse analytical methods has made it difficult to follow all the analyses and results. And, the results from various analyses were not always robust, and the consistency among the results were not high. Authors need to take a close look and comment on the "inconsistence" issue. Authors may focus on the results that are robust and consistent across various analyses. By doing so, it will be easier for readers to follow the main results and conclusions.

Re: Thanks for these helpful suggestions. In the present study, we leveraged MTAG to conduct an integrative analysis of the largest available GWAS datasets encompassing six major CVDs in individuals of European ancestry: AF, CAD, VTE, HF, PAD, and

Stroke, which included the identification of novel risk loci and gene prioritization, to characterize the underlying biology of the novel risk loci in the context of CVDs. Furthermore, we explored pleiotropic effects within these extensively expanded datasets generated by MTAG analysis, specifically investigating cross-trait-associated single-nucleotide polymorphisms (SNPs), genes, and biological pathways across a participant count averaging over 1.2 million participants. Specifically, preceding the integration of cardiovascular diseases in a multi-trait analysis, genetic characteristics such as genome-wide and local genetic correlations, and genetic overlap were estimated individually, to identify genome-wide genetic overlap beyond genetic correlation across the six CVDs. The shared genetic basis could be interpreted as genetic variants affecting multiple complex phenotypic traits by vertical and horizontal pleiotropy. Mendelian randomization was applied to elucidate the important role of vertical pleiotropy in CVDs. Subsequently, the outcomes of SNP and genomic loci analyses from the multi-trait assessment, along with cross-trait analysis for replication, were juxtaposed against the results from individual cardiovascular diseases, unearthing many novel pleiotropic loci. To comprehensively characterize the shared genetic mechanisms for each of the six cardiovascular diseases, various methods were employed. Firstly, we explored the convergence of SNPs, genomic loci, and mapped genes and then encompassed biological pathway and functional category analyses.

In all, this study addressed four central inquiries regarding the shared genetic basis of these six CVDs (Fig. 1): i) Can we identify shared genetic architectures amidst the diverse landscape of these clinically distinct CVDs? ii) Can we determine whether the shared genetic architectures related to major CVDs are driven by causal associations (i.e. vertical pleiotropy)? iii) Can we detect additional genomic loci for multiple CVDs (i.e. pleiotropic loci), and whether some of the loci exhibit opposite allelic effects across CVDs? iv) Can we identify functional features of the pleiotropic loci that could account for their widespread impact on cardiovascular pathology? Collectively, these findings will ultimately reshape our understanding of

cardiovascular nosology, spotlight potential cardiovascular physiological mechanisms predisposing to specific clinical presentations, and provide crucial insights into the prevention and treatment of CVDs.

First, different approaches used in this study provide complementary insights, and while inconsistencies between the results may arise, these variations are essential for offering diverse perspectives. For example, when identifying genetic overlap across CVDs in the first section, we emphasized the complementary roles of various methods. One of these methods, genetic correlation (r_g) calculated using LDSC, is a valuable tool for assessing the genetic similarity between two phenotypes. However, it has limitations in capturing all dimensions of genetic overlap. Because rg is a genome-wide summary measure, it cannot differentiate between genetic overlap resulting from a mixture of concordant and discordant effects and the absence of overlap. As a result, r_g can yield an estimated value close to 0, even in cases of substantial overlap. To address this limitation, we used multiple methods with different model assumptions, allowing us to capture the "missing dimension" of genetic overlap. This enabled a more comprehensive description of the shared genetic underpinnings of CVDs, extending beyond what is captured by genetic correlation alone. Among these methods were the bivariate causal mixture model (MiXeR) and local analysis of [co]variant association (LAVA). By employing MiXeR and LAVA, our study revealed a more extensive degree of genetic overlap across all CVDs, encompassing a wide range of both concordant and discordant effect sizes. In particular, MiXeR allowed us to assess polygenicity—the number of genetic variants influencing a trait—across different CVDs. We found that polygenicity varies significantly between CVDs, with AF, VTE, and PAD being substantially less polygenic than other CVD phenotypes, such as CAD, HF, and Stroke. Despite these differences in polygenicity, we observed considerable genetic overlap across all CVDs, as confirmed by local correlations from LAVA. This overlap was evident not only in cases of strong genetic correlations, such as between HF and Stroke, but also in cases with weaker correlations, such as between AF and VTE. Despite weak genome-wide

genetic correlations between AF and VTE, MiXeR indicated a significant overlap in the genetic risk underlying these two conditions. Similarly, LAVA identified a similar number of positively and negatively correlated genomic regions between AF and VTE. These findings suggest that genetic overlap across CVDs has been dramatically underestimated by traditional methods, such as genome-wide genetic correlation, which fail to account for the mixed directions of genetic effects within shared loci.

Then, for other combinations of algorithms aimed at achieving related goals, we place greater emphasis on 'consistent' conclusions across different methods, such as LAVA-GWAS-PW and MTAG-CPASSOC. The latter, in particular, is frequently used to verify the robustness of results. For example, GWAS-PW reinforced the findings from LAVA by providing estimates of the posterior probability for the 2,495 genomic regions defined by LAVA as shared between both traits, which allowed us to identify genomic regions containing variants associated with multiple CVDs based on GWAS results. Another example is that we conducted pairwise cross-trait meta-analyses using the CPASSOC algorithm to investigate whether violations of the assumptions—specifically, equal SNP heritability for each trait and perfect genetic covariance between traits—could bias our MTAG results. In all, we think that aggregating inconsistent conclusions from different algorithms in this case is unnecessary, as discrepancies are likely due to statistical biases introduced by varying algorithmic approaches.

Finally, by integrating the various strategies and methods discussed above, we can more comprehensively characterize the shared genetic mechanisms underlying CVDs.

2.1 For example, while it is stated that GWAS-PW strengthened LAVA results, there were only 3 genomic regions that were significantly captured by both GWAS-PW and LAVA. Authors need to comment on the low overlapping between the results from LAVA and GWAS-PW analyses.

Re: Thank you for your comments. We agree that the low overlap between the results from LAVA and GWAS-PW (with only 3 genomic regions captured by both methods) warrants further clarification. First, it is important to highlight that the algorithmic principles of LAVA and GWAS-PW differ significantly. LAVA utilizes local genetic correlation within a specific locus between paired traits to measure trait associations, while GWAS-PW estimates the posterior probability of colocalization in the same locus. These methodological differences mean that the two approaches may capture distinct aspects of shared genetic architecture. Specifically, only loci showing significant local-r_gs can be analyzed for colocalization to differentiate whether the association between traits is mediated by horizontal and/or vertical pleiotropy, rather than by spurious pleiotropy. Besides, the threshold for GWAS-PW varies across studies, and it is generally accepted that if the posterior probability of colocalization (PPA_3 or PPA_4) exceeds 0.5, this indicates evidence of shared causal variation in the region (Adewuyi et al., 2022). If PPA_3 exceeds 0.9, this suggests high confidence that the locus is associated with both traits via the same causal variant. To explore genetic associations across CVDs more effectively, we initially applied stricter criteria for GWAS-PW, but for better alignment with LAVA, we later used a more lenient threshold for GWAS-PW analysis.

As a result, GWAS-PW analysis identified 2,112 unique genomic regions with variants influencing pairs of CVDs (PPA_3 > 0.5 or PPA_4 > 0.5, Supplementary Table 6), suggesting shared regions and causal variants. Notably, 1,238 unique genomic regions demonstrated robustness for loci shared by more than two trait pairs. Meanwhile, 13 (of 24) unique genomic regions were significantly captured by both bivariate LAVA and GWAS-PW analyses. For example, we observed 6 trait pairs in the locus (LD block 1,398) exhibited strong positive local-r_gs at the nominal significance level ($p < 0.05$), 4 of 6 trait pairs (including AF-CAD, CAD-HF, CAD-PAD, and CAD-Stroke) were survived after the Bonferroni correction, was also validated by GWAS-PW. Further tests in this region using Hypothesis Prioritisation in Multi-trait Colocalization (HyPrColoc) showed this locus exhibited strong

colocalization evidence across all CVDs except VTE and PAD with posterior probability (PP) higher than 0.7, which encompassed the shared causal SNP (rs4977574, an intron of cyclin-dependent protein kinase inhibitors antisense RNA 1 [CDKN2B-AS1] gene on 9q21.3). It has been implied that there was a possible relationship between CDKN2B-AS1 gene rs4977574 A/G polymorphism and coronary heart disease susceptibility, which might modulate the progression and severity of vascular calcification in vascular smooth muscle cells. (From Page 6, line 204 to line 209)

Reference:

Adewuyi EO, Mehta D; International Endogene Consortium (IEC); 23andMe Research Team, Nyholt DR. Genetic overlap analysis of endometriosis and asthma identifies shared loci implicating sex hormones and thyroid signalling pathways. Hum Reprod. 2022;37(2):366-383. doi:10.1093/humrep/deab254

2.2 Similarly, do the 38 pleiotropic loci identified from MTAG overlap with the LAVA and GWAS-PW analyses? Actually, the most prominent LPA signal does not seem to be picked up by genetic correlation analyses by LAVA and GWAS-PW. Giving the limited consistence between the various methods employed, how robust would these results be?

Re: Thank you for your thoughtful comment. To clarify, both LAVA and GWAS-PW analyses were conducted using raw GWAS summary data to determine whether shared genetic architectures exist between CVDs, supporting the subsequent MTAG analysis. These methods provided preliminary insights into the overlap of localized genomic regions. However, the local genetic correlations and colocalization analyses identified regions averaging approximately 1 megabase (Mb) in width, which, given the resolution, requires further refinement to reduce the potential impact of heterogeneous effects on estimation accuracy. To achieve higher resolution and accuracy, we focused on overlapping loci between different CVDs using MTAG

summary data, followed by multi-trait colocalization analysis and Metasoft. This approach yielded higher resolution and more precise results compared to the loci analyzed by LAVA. Given the increase in sample size and the higher precision of the MTAG analysis, the overlap between the pleiotropic loci identified from MTAG and those detected by LAVA and GWAS-PW using raw GWAS summary data cannot be reasonably assessed. The MTAG analysis enabled the identification of additional trait-associated loci that may not have been detected in the original GWAS data. As such, further analyses using LAVA and GWAS-PW based on MTAG summary data would be needed to assess this overlap. However, we believe this is unnecessary, as the current strategy based on MTAG summary data already offers higher resolution and reliability.

3. It is also a bit difficult to assess the shared and novel loci identified in the study through MTAG. Would the novel loci identified be those that do not overlap with lead SNPs from "Results from Original GWAS analysis" column in supp table 9. Was LD between the lead SNP identified from MTAG and lead SNP from the Original GWAS analysis considered?

Re: Thank you for your valuable comment. We would like to clarify the process used to identify novel loci in our study. A locus is classified as novel if it does not overlap with any loci identified in the original GWAS based on their genomic positions. If no overlap is found, the locus is considered novel. For a more detailed discussion of this classification, please refer to Supplementary Note 3 (Supplementary file). As a result of this classification process, the lead SNPs of novel loci identified by MTAG differ by approximately 43.8% from the lead SNPs reported in the original GWAS analysis results, as shown in Supplementary Table 9. Therefore, we then examined the overlap of lead SNPs between MTAG and previously identified GWAS loci, considering linkage disequilibrium (LD). The analysis revealed that 72% (112/155) of the lead SNPs were strongly linked ($R^2 > 0.8$), while only 11% (17/155) showed weak linkage ($R^2 < 0.2$). These results have been added to Supplementary Table 9 to further

strengthen the robustness and reliability of our results.

4. Greater discussion is also required for negatively correlated loci. What would be the biological basis of these loci that increase risk of one CVD but reduce the risk of another CVD? Were all negatively correlated loci observed even after Bonferroni corrections?

Re: Thank you for your helpful suggestions. To complement the results from MiXeR, we applied LAVA to estimate pairwise local genetic correlations (local-$r_g s$) across 2,495 semi-independent genomic regions, each approximately 1 MB in size. This approach allowed us to identify mixed effect directions, even in regions with minimal rg. Only one locus remained significant after Bonferroni correction among the 26 unique genomic regions identified as negatively genetically correlated. This locus, located in the PAD-Stroke trait pair, corresponds to LD block 804 (chr5: 31,679,465–32,727,914). Further investigation of the GWAS-PW results in this region revealed high colocalization between CAD and VTE, particularly in LD block 96 (chr1:109271450–110224230) (PPA_3 > 0.9). To strengthen these findings, we applied HyProcoloc and identified high colocalization between CAD and VTE, including the shared causal SNP rs12740374, located in the 3' UTR region of CELSR2 on 1p13.3. In our multi-trait colocalization of CVD and metabolic traits at overlapping sites based on MTAG summary data before, we identified strong evidence that the shared causal variant, rs12740374, exhibits high colocalization across multiple traits, including MTAG_CVD, MTAG_HF, and four lipid-related traits: low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), triglycerides (TG), and total cholesterol (TC). Then, we repeated the multi-trait colocalization analysis using the original GWAS summary statistics for CAD, HF, VTE, and four lipid-related traits, which showed evidence of high colocalization (PPA > 0.9) with the same shared causal SNP rs12740374. Interestingly, when we examined the Z-values (Beta/se) for rs12740374 across the seven traits, we observed that CAD, HF, LDL-C, TC, and TG all had negative

Z-values, while VTE and HDL-C exhibited positive Z-values. It is worth noting that LAVA did not identify sufficient genetic signals in the HF region, preventing us from calculating the local genetic correlation between CAD and HF. So, we focus our discussion on the negative genetic correlation between CAD and VTE. Previous research has shown that elevated HDL levels negatively correlate with the risk of CAD, while elevated LDL-C levels positively correlate with CAD risk. Although the relationship between VTE and dyslipidemia has been studied in several clinical studies, the results have been inconsistent (Lin *et al.*, 2023). To further explore the genetic relationships between CAD, VTE, and lipid traits, we calculated the local-r_gs in this region. The results revealed that CAD was negatively correlated with HDL-C and positively correlated with LDL-C, TC, and TG, findings that align with previous epidemiological studies. In contrast, VTE exhibited the exact opposite of CAD with these lipids, but the local-r_gs did not reach nominally statistical significance except for VTE-TC ($p < 0.05$). In all, our findings provide new evidence supporting the role of lipid traits in both CAD and VTE, suggesting that lipids may play a completely opposing role in these conditions. Further investigation into the biological mechanisms underlying this negative correlation is needed to explain how this locus contributes to the divergent genetic architecture of these two cardiovascular diseases. (From Supplementary Note 2 in Supplementary file)

Reference:

Lin L, Luo P, Yang M, Wang J, Hou W, Xu P. A bidirectional Mendelian randomized study of classical blood lipids and venous thrombosis. Sci Rep. 2023;13(1):3904. Published 2023 Mar 8. doi:10.1038/s41598-023-31067-z

5. The HyPrColoc analysis also suggest stronger colocalization of shared causal variants between metabolic traits and the CVD outcomes tested. Was causal inference analysis or MR evaluations performed to assess the metabolic trait associations on the CVD outcomes? Were these colocalization analyses performed for identified shared regions between the 6 CVD outcomes? These results suggest stronger impacts of

metabolic traits effects on CVD outcomes tested rather than the effects of one CVD trait on another CVD outcome. Additionally, in the MR analyses there was limited evidence for vertical pleiotropy and much of the shared loci seemed to be due to horizontal pleiotropy and a due to reverse causation.

Re: Thank you for your comments. We would like to clarify the scope and focus of our study. The colocalization analysis of metabolic traits with CVDs was specifically conducted for the shared genomic regions identified between CVDs. Our primary objective was to examine how common metabolic risk factors contribute to the development of shared CVD traits, rather than to assess the relative impacts of metabolic profiles on different CVD outcomes. Regarding causal inference or MR evaluations, we did not prioritize establishing causal relationships between metabolic profiles and CVD outcomes, as this has already been thoroughly investigated in prior research. Instead, our study aimed to explore bidirectional causal relationships between different CVDs to systematically characterize the extent of vertical pleiotropy within CVDs. This approach aligns with our primary focus on understanding shared genetic architectures among CVDs rather than their metabolic risk factors. Importantly, we also emphasize that metabolic risk factors cannot act as mediators between different CVDs, as such a mechanism would contradict established epidemiological evidence. Our analysis revealed limited evidence for vertical pleiotropy, including both forward and reverse causality. This finding suggests that the shared loci observed between CVDs are predominantly driven by horizontal pleiotropy rather than direct causal pathways.

6. While the proteome analysis identifies some proteins and potential therapeutic targets for specific CVD outcomes, it is highlighted that the 11 proteins were not linked to more than one CVD outcome and provides little evidence to enhance the main question of the manuscript which, was to evaluate for shared basis of major CVD outcomes. Actually, I feel that this part of analyses does not contribute to the main objective of “investigating shared genetic basis of CVD outcomes”. I would

suggest for the part of analysis to be removed from the current manuscript, so that the manuscript can be more focused.

Re: We appreciate the reviewer's thoughtful feedback regarding the protein-level analysis. We acknowledge that the findings from this part of the study do not directly address the main objective of investigating the shared genetic basis of major CVD outcomes. This limitation likely stems from several factors, including significant variability in the pQTL data generated by different measurement platforms (e.g., SomaScan and Olink), the relatively small sample size of the pQTL datasets, and some degree of sample overlap between the pQTL data (from UKB and deCODE) and CVD GWAS summary data. These challenges may have impacted the robustness of the results and complicated causal inference. In response, we have moved the protein-level findings to the supplementary materials, ensuring that the main manuscript remains focused on its primary objective. However, the exploration of shared genetic bases for CVD outcomes at the protein level remains a promising area for future research. We hope that larger and more consistent datasets, alongside improved methodologies, will help address these challenges and provide deeper insights into this important aspect of CVD research.

7. SNP-based heritability estimates seem rather low – median 1.4%. Would this be typical? Is this because of the SNP filtering process used in the study?

Re: We thank the reviewer for raising this important point. The SNP-based heritability estimates observed in our study, with a median of 1.4%, are reasonable and reflect the proportion of phenotypic variance explained by all common genetic variants included in the analysis. This is lower than the heritability reported in original GWAS studies for CVDs, which typically incorporate both common and rare variants. Since our analysis focuses only on common variants, such differences are expected. The SNP filtering process used in this study has minimal impact on heritability estimation. Before calculating heritability, LDSC intersects the GWAS summary data with its

reference file (w_hm3.snplist), which includes approximately 1 million common variants. Most of the common variants present in the CVD GWAS summary data were retained during this process. Additionally, previous research (Romero et al., 2022) has shown negligible differences in SNP-based heritability estimates between raw, unfiltered, and filtered datasets using LDSC. To further validate this, we conducted SNP-based heritability calculations for all six CVD traits using both raw (or unfiltered), filtered (aligned with the 1000 Genomes reference), and merged datasets, confirming that the filtering process does not significantly affect the results. We found that SNP-based heritability estimated using LDSC for all CVDs showed little to no sign of variation between unfiltered, filtered, and merged versions in Supplementary Table 2a in Supplementary file.

Reference:

Romero C, Werme J, Jansen PR, et al. Exploring the genetic overlap between twelve psychiatric disorders. Nat Genet. 2022;54(12):1795-1802. doi:10.1038/s41588-022-01245-2

8. “Conversely, PAD displayed superior discoverability while exhibiting less pronounced polygenicity in relation to other CVDs”. It would be good to define discoverability and polygenicity in the manuscript.

Re: Thank you for highlighting this important point. We define "discoverability" and "polygenicity" in the section on MiXeR's methodology. Specifically, "polygenicity" refers to the estimated number of trait-influencing genetic variants ("causal variants"), while "discoverability" reflects the average magnitude of additive genetic associations among these variants. To enhance clarity, we will ensure that these definitions are explicitly reiterated in the results sections. (From Page 4, line 159 to line 162; From Page 17, line 539 to line 542)

Reviewer #2 (Remarks to the Author):

The authors present a comprehensive joint analysis of cardiovascular traits, using almost every tool available currently. The authors have some expectation of shared genetic etiology among CVD phenotypes, and use MTAG to identify novel signals. Further, they explore potential reasons for shared etiology, using functional annotation, colocalization, gene aggregation and so on. It resembles a published manuscript (https://doi.org/10.1093/eurheartj/ehad655.3053), with more phenotypes and analyses.

1. The manuscript is long and the pieces are not well-tied together. It would benefit from a more concise description of the main results. Even though there may be over 100 novel variants, few of them have any further evidence that justifies a paragraph afterwards. It would be interesting to have a section with the accumulated evidence for the best explained variants, e.g. if a variant has a high coloc score, is a pQTL and is part of a significant pathway, it would deserve highlighting.

Re: We appreciate your suggestion to provide a more concise description of the main results and better tie the sections together. To recapitulate, in the present study, we leveraged MTAG to conduct an integrative analysis of the largest available GWAS datasets encompassing six major CVDs in individuals of European ancestry: AF, CAD, VTE, HF, PAD, and Stroke, which included the identification of novel risk loci and gene prioritization, to characterize the underlying biology of the novel risk loci in the context of CVDs. Furthermore, we explored pleiotropic effects within these extensively expanded datasets generated by MTAG analysis, specifically investigating cross-trait-associated single-nucleotide polymorphisms (SNPs), genes, biological pathways, and protein targets across a participant count averaging over 1.2 million participants. This study addressed four central inquiries regarding the shared genetic basis of these six CVDs (Fig. 1): i) Can we identify shared genetic architectures amidst the diverse landscape of these clinically distinct CVDs? ii) Can we determine whether the shared genetic architectures related to major CVDs are driven by causal

associations (i.e. vertical pleiotropy)? iii) Can we detect additional genomic loci for multiple CVDs (i.e. pleiotropic loci), and whether some of the loci exhibit opposite allelic effects across CVDs? iv) Can we identify functional features of the pleiotropic loci that could account for their widespread impact on cardiovascular pathology?

Moreover, in this study, although over 100 novel loci were identified, we specifically focused on loci with strong evidence of colocalization. These loci were prioritized based on several criteria, including colocalization with multiple traits, association with tissue-specific eQTLs, and involvement in significant biological pathways. Due to space limitations, a more detailed discussion of these loci, including additional evidence, is provided in Supplementary Note 2 (Supplementary file).

2. Supplementary Table 1 shows many genes associated with blood pressure/hypertension. It is reasonable to assume that a sizeable portion of these CVD cases is hypertensive, since hypertension is a major risk factor for CVD. How can we be certain that these variants are not simply reflecting the hypertensive status of the individuals?

Re: Thank you for your insightful comment. We agree that a significant portion of the CVD cases included in our study may be hypertensive, as hypertension is a major risk factor for CVD. To address this concern, we conducted colocalization analysis using HyPrColoc to investigate whether there was evidence of colocalization between hypertension and CVDs at the identified overlapping loci. The results did not show any significant colocalization at these loci, suggesting that the identified associations do not reflect the hypertensive status of the individuals. Additionally, we extended our analysis to investigate the relationship between blood pressure traits—systolic blood pressure (SBP), diastolic blood pressure (DBP), and pulse pressure (PP)—and the CVD outcomes. Among these, only two loci (1p34.3 and 7p21.2) exhibited colocalization, specifically between multiple CVDs (CAD, PAD, and Stroke) and blood pressure traits, especially SBP. This observation is biologically plausible, as

CAD, PAD, and Stroke are arterial diseases that are more closely associated with blood pressure traits, leading to some overlap between these conditions and blood pressure-related variants. In conclusion, we believe that the effect of hypertension-related variants on the overlapping loci identified in this study is minimal. However, to further confirm these findings, future studies incorporating larger hypertension GWAS datasets will be necessary.

3. I missed a table showing what the original P-value of the "novel" association is in the original GWAS. I would like to see quickly if the P-value went from, say, ~E-7 to ~E-8 or if there was a big increase, which would be unexpected.

Re: Thank you for your insightful comment. In response, we have included the P-values from both the original GWAS and the CPASSOC analysis in Supplementary Table 9 (Supplementary Table). This additional information will allow you to quickly assess any changes in the significance of the "novel" associations. Specifically, the table compares the P-values from the MTAG with those obtained from the original GWAS and the CPASSOC analysis, allowing for a clear view of whether the P-value changed considerably.

4. It would also strenghten the paper if there was some sort of replication of the MTAG found variants, using some publicly-available dataset, e.g. MVP or All of US

Re: Thank you for the reviewer's suggestion. We agree that replicating the variants in publicly available datasets like MVP or All of Us would enhance the credibility of the findings. However, due to strict data access restrictions, we are unable to access GWAS summary data of European populations from these sources at this time. While the UKB and FinnGen databases provide publicly accessible summary GWAS data for European populations, the datasets used in this study already include data from these sources. Nonetheless, we have replicated the identified shared variants within the meta-analysis or individual analysis of the FinnGen (R12) and UKBB databases.

The results of this replication can be found in Supplementary Table 9 (Supplementary Table). We believe this replication, in part, strengthens the findings, although future work needs to include replication using additional datasets once access becomes possible.

5. Please explain what "opposite" means in the sentence: "two distinct groups of CVDs: those characterized by more genetic signals and shared biological mechanisms (AF, CAD, and VTE), and those exhibiting the opposite (HF, PAD, and Stroke)".

Re: Thanks for these helpful suggestions. To address this question, we clarify that the term “opposite” refers to a group of CVDs (HF, PAD, and Stroke) that exhibit fewer genetic signals and weaker shared biological mechanisms compared to the other group (AF, CAD, and VTE), which is characterized by stronger genetic signals and more prominent shared biological mechanisms. In our study, genetic and functional enrichment analyses revealed that the majority of the shared genetic signals were driven by the stronger genetic signals in AF, CAD, and VTE. Conversely, HF, PAD, and Stroke exhibited fewer genetic signals and shared mechanisms, which supports their classification as a distinct group with weaker associations. We apologize for any confusion caused by the term “opposite” and have revised the manuscript to more clearly describe the distinction between the two groups. (From Page 15, line 476 to line 481)

6. Sup. Figure 9: there are some variant-phenotype pairs with opposite directions to the others for rs12129500 and rs7528419, so the title of the figure is misleading. Also, there are some arrows missing (e.g. rs12509595 for VTE), what happened to those values?

Re: We appreciate the reviewer’s comment. In the rose chart, the dotted circles represent a P-value threshold of 5e-8, which corresponds to specific Z-scores. SNPs with petals extending beyond these circles are considered statistically significant

within the loci, with their colors indicating the direction of the association. We only focus on the direction of significant SNP-trait pairs in this analysis. Additionally, Z-scores are inversely related to P-values, meaning that a smaller Z-value corresponds to a larger P-value. As a result, SNPs such as rs12509595 for VTE have a P-value close to 1, which is why they are not visible in the chart. Similarly, SNPs like rs17465651 and rs2107595 for VTE also show high P-values, which results in their absence from the rose chart.

7. Sup. Table 1: Why do tables go from A to D?

Re: We sincerely apologize for the confusion caused. To clarify, Supplementary Table 1 should only include sections A and B, which present an overview of all cardiovascular diseases and metabolic traits of European ancestry included in this study.

Reviewer #3 (Remarks to the Author):

The paper conducted a thorough investigation of cardiovascular diseases using modern genetic methods. The manuscript is overall well-structured and was easy to follow. Especially, the last paragraph of the introductory section was a great overview of the study. My comments can be found below.

Major comments:

1. LHC MR paper claims to handle sample overlap, and the consideration is presented in their method derivation. However, the simulation they conducted only includes non-overlapping samples. In this manuscript, GWAS summary statistics frequently include UK Biobank, including the major CVD traits and proteome measurements. Fortunately, the authors can test the their conclusions by including summary statistics without UK Biobank. For example, the arterial fibrillation GWAS (Pubmed ID 30061737) provides UKB-excluded summary statistics (http://csg.sph.umich.edu/willer/public/afib2018/, UKB left-out summary statistics).

Re: Thank you for your valuable suggestions. Considering the overlap of samples in our study, we primarily used the LCV method to infer causal relationships between CVDs. The LCV method is particularly robust to sample overlap and is less prone to confounding from horizontal pleiotropy. To validate the reproducibility of the causal associations identified using LCV, we also employed the LHC-MR method. By applying both approaches, we aimed to ensure the robustness of our findings and cross-validate the causal relationships between CVDs. The reviewers have raised concerns about the LHCMR method, which claims to handle sample overlap when inferring causal relationships. However, the original study's simulations only involved non-overlapping samples, and it is uncertain whether the method performs similarly or reliably in the presence of sample overlap. Therefore, to verify the robustness of the LHCMR method, we used non-overlapping GWAS summary statistics (UKB-excluded) for causal inference and compared these results with those from our previous analysis

using overlapping samples. We were able to obtain UKB-excluded summary statistics only for AF and HF. Our analysis revealed that LHCMR performed poorly in inferring causal relationships between AF (UKB-excluded) and other CVDs, with the estimated effect sizes for both positive and negative causal associations inconsistent with prior findings. Similarly, causal inferences between HF (UKB-excluded) and other CVDs also showed poor performance. While the direction of the estimated effect remained broadly consistent with previous results, the interpretation of these results differed.

Therefore, to further address the issue of sample overlap and potential biases, we performed an inverse variance weighting (IVW) MR analysis using the MRlap approach (Mounier et al., 2023). MRlap corrects for biases arising from sample overlap, weak instruments, and winner's curses simultaneously, providing more reliable causal estimates. The MRlap analysis confirmed the previously identified positive causal associations between CAD-HF and VTE-Stroke. However, the AF-HF pair exhibited bias due to reverse causality, which may have skewed the results. Additionally, the VTE-CAD pair suggested a partial causal relationship, but this finding did not survive multiple testing corrections ($P < 0.05$ / no. of independent CVD pairs / no. of MR-tests $= 0.05 / 4 / 2 = 4.17 \times 10^{-3}$, Supplementary Table 8). In all, we found a high degree of agreement between the MRlap and LCV results, with both methods inferring causality that aligns with existing epidemiological evidence. Based on these findings, we considered MRlap conclusions to be the primary outcome of the sensitivity analysis of LCV results. In contrast, LHCMR may have overestimated reverse causality, leading to inconsistent results. (From Page 7, line 239 to line 250)

Furthermore, we also used MRlap to infer causal relationships between AF (UKB-excluded), HF (UKB-excluded), and other CVDs to verify the robustness of the MRlap method. Our analysis revealed that MRlap demonstrated surprising robustness for AF or HF and its causal relationship with other CVDs. Although there were slight differences in the estimates, the overall interpretation of the results remained consistent.

Although sample overlap impacted the point estimates in MRlap analysis, the interpretation of results remained similar.

We previously considered the potential sample overlap between the GWAS summary data of CVDs and the proteomics data from the UK Biobank Pharma Proteomics Project (UKB-PPP). To mitigate this issue, we conducted the primary SMR analysis using the deCODE Health study data and performed sensitivity analyses using UKB-PPP data. However, because UKB-PPP and deCODE utilize different proteomics measurement platforms (SomaScan and Olink, respectively), the reproducibility of our results was limited. As a result, we did not identify any proteins associated with multiple CVDs. In response to reviewer's suggestion, we have moved this section of the results to the supplementary materials.

Reference:

Klarin D, Lynch J, Aragam K, et al. Genome-wide association study of peripheral artery disease in the Million Veteran Program. Nat Med. 2019;25(8):1274-1279. doi:10.1038/s41591-019-0492-5

Lindström S, Wang L, Smith EN, et al. Genomic and transcriptomic association studies identify 16 novel susceptibility loci for venous thromboembolism. Blood. 2019;134(19):1645-1657. doi:10.1182/blood.2019000435

Mounier N, Kutalik Z. Bias correction for inverse variance weighting Mendelian randomization. Genet Epidemiol. 2023;47(4):314-331. doi:10.1002/gepi.22522

2. It’s untrustworthy to apply MR to MTAG summary statistics. This type of analysis has not been tested in any methodology/benchmark papers. The authors should conduct MR with the original summary statistics before MTAG and compare to their original MTAG-based MR.

Re: We apologize for the confusion. We fully agree with the reviewer that applying MR to MTAG-based summary statistics is not methodologically validated. To ensure the

584 robustness and credibility of our findings, we restricted our MR analyses to the original
585 raw GWAS summary statistics, which are methodologically well-established. We
586 acknowledge that certain aspects of our original description may have caused confusion,
587 and we have provided further clarification in the revised manuscript. MTAG-based MR
588 analyses are particularly susceptible to biases introduced by sample overlap, which is a
589 key reason why this approach has not been widely validated in methodological or
590 benchmark studies. Given these limitations, we believe that comparing the results from
591 MTAG-based MR analyses with those derived from the original GWAS-based MR
592 analysis is unnecessary. The latter approach offers greater reliability and
593 methodological rigor, providing a more accurate assessment of causal relationships.
594
595 3. Several pleiotropy assessment tools have been developed since the introduction of
596 MTAG. One example is PLEIO (https://pubmed.ncbi.nlm.nih.gov/33352115/). It
597 provides a drop-in diagram of Figure 2b. It handles multiple comparison automatically,
598 so post-hoc Bonferroni-correction (which is conservative) can be avoided.
599
600 Re: Thank you for your helpful suggestions. To evaluate whether the assumptions of
601 equal SNP heritability across traits and perfect genetic covariance might introduce bias
602 into our MTAG results, we conducted an additional sensitivity analysis using
603 CPASSOC (Zhu *et al*., 2015). CPASSOC is designed to account for heterogeneous
604 genetic effects across traits by computing a cross-trait statistic (SHet) and the
605 corresponding P-value using a sample size-weighted fixed-effect meta-analysis of
606 GWAS summary statistics. This method has been widely used alongside MTAG and
607 has shown strong consistency with MTAG results in previous studies (Bonnemaijer *et*
608 *al*., 2019; Xiong *et al*., 2022; Zheng *et al*., 2024). In response to the reviewer's
609 suggestion, we also performed a PLEIO analysis (Lee *et al*., 2021), which provides an
610 alternative approach to assessing pleiotropy. PLEIO identified 409 genome-wide
611 significant pleiotropic loci, of which 370 (90.4%) overlapped with those identified by
612 CPASSOC, highlighting the strong agreement between the two methods

(Supplementary Table 11). Based on this high degree of consistency, we chose to continue using CPASSOC for validation of the MTAG results in the article.

Reference:

Zhu X, Feng T, Tayo BO, et al. Meta-analysis of correlated traits via summary statistics from GWASs with an application in hypertension. Am J Hum Genet. 2015;96(1):21-36. doi:10.1016/j.ajhg.2014.11.011

Leeuwen EMV, Iglesias AI, et al. Multi-trait genome-wide association study identifies new loci associated with optic disc parameters. Commun Biol. 2019;2:435. Published 2019 Nov 27. doi:10.1038/s42003-019-0634-9

Xiong Z, Gao X, Chen Y, et al. Combining genome-wide association studies highlight novel loci involved in human facial variation. Nat Commun. 2022;13(1):7832. Published 2022 Dec 20. doi:10.1038/s41467-022-35328-9Bonnemaijer PWM,

Zheng S, Tsao PS, Pan C. Abdominal aortic aneurysm and cardiometabolic traits share strong genetic susceptibility to lipid metabolism and inflammation. Nat Commun. 2024;15(1):5652. Published 2024 Jul 5. doi:10.1038/s41467-024-49921-7

Lee CH, Shi H, Pasaniuc B, Eskin E, Han B. PLEIO: a method to map and interpret pleiotropic loci with GWAS summary statistics. Am J Hum Genet. 2021;108(1):36-48. doi:10.1016/j.ajhg.2020.11.017

4. Considering the pervasive pleiotropy found by the authors, multivariate mendelian randomization (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4325677/) (and the following developments) is likely a better alternative to other MR. Sample overlap should be handled by excluding overlapping samples. Certain arrows in the multivariate MR represent direct/partial causal effects depending on the causal relationship between the traits. Another alternative is to consider genomic structural equation models.

Re: We appreciate the reviewer's suggestion to use multivariable Mendelian randomization (MVMR) analyses (Sanderson *et al*., 2021), particularly given the

pervasive pleiotropy observed in our study. MVMR allows for the estimation of causal relationships between multiple traits by using genetic instruments for each trait, which provides a more accurate and aligned causal interpretation compared to genomic structural equation models (genomic SEM) (Grotzinger *et al.*, 2019). While genomic SEM employs all genetic variants to test mediation effects, MVMR focuses specifically on instrument variables (IVs) to estimate causal effects, making it more suitable for this context. We used MVMR to examine the genetic liability to CVDs, adjusting for pleiotropic effects between traits. It is important to note that while MVMR is an effective tool for estimating causal relationships, it does not formally test for mediation. Instead, it adjusts causal estimates to account for potential mediation, as demonstrated by our use of the inverse-variance weighted (IVW) estimate.

Therefore, to address potential sample overlap, we performed MVMR analysis using both raw and UKB-excluded summary data, as recommended by the reviewer. We obtained UKB-excluded summary statistics for HF to minimize potential sample overlap. For Stroke, we used earlier GWAS summary statistics from consortium publications (Malik *et al.*, 2018), which did not include data from the UKB. Our results confirmed that the association between genetic liability to CAD and HF remained statistically significant after adjusting for other CVDs, a pattern also observed for the VTE-Stroke pair. Furthermore, the association between genetic liability to CAD and HF was strengthened in the UKB-excluded summary data, with the odds ratio (OR) increasing from 1.327 (CAD-HF) to 2.718 (CAD-HF_UKB-left-out). In contrast, the association between genetically contributed VTE susceptibility and stroke slightly decreased after adjusting for other CVDs, with the OR changing from 1.093 (VTE-Stroke) to 1.080 (VTE-Stroke_UKB-not-included) (Supplementary Table 8). These findings further support the robust causal relationships of CAD-HF and VTE-Stroke, which align closely with previous observational studies. (From Page 7, line 252 to Page 8, line 263)

Reference:

673 Sanderson E, Spiller W, Bowden J. Testing and correcting for weak and pleiotropic
674 instruments in two-sample multivariable Mendelian randomization. Stat Med.
675 2021;40(25):5434-5452. doi:10.1002/sim.9133
676 Grotzinger AD, Rhemtulla M, de Vlaming R, et al. Genomic structural equation
677 modelling provides insights into the multivariate genetic architecture of complex traits.
678 Nat Hum Behav. 2019;3(5):513-525. doi:10.1038/s41562-019-0566-x
679 Malik R, Chauhan G, Traylor M, et al. Multiancestry genome-wide association study of
680 520,000 subjects identifies 32 loci associated with stroke and stroke subtypes. Nat
681 Genet. 2018;50(4):524-537. doi:10.1038/s41588-018-0058-3

Re: NCOMMS-24-16271A

Title: Shared Genetic Architecture Contributes to Risk of Major Cardiovascular Diseases

We thank the Reviewers and the Editor for the helpful feedback and insightful suggestions. The response from the reviewers is encouraging and constructive. Below, we provide point-by-point responses that address all the comments with additional analyses. By incorporating these new data and findings, our manuscript has been improved substantially.

Reviewer #1 (Remarks to the Author):

Most of my comments have been addressed by providing clarifications and/or additional analyses. However, the responses to two comments can be further strengthened.

1. Responses to the "overlapping comments" can be strengthened, for example, by

1) providing a clear summary of which results are most robust and consistent across different methods.

Re: Thank you for your valuable comment. In the section addressing the shared genetic basis of CVDs, the results from LAVA and MiXeR were highly consistent in identifying genetic overlap beyond genome-wide genetic correlation, providing complementary insights into the genetic architecture of CVDs. In the vertical pleiotropy section, both LCV and MRlap yielded robust causal inferences, further supporting the role of vertical pleiotropy in CVD susceptibility. In the horizontal pleiotropy section, a range of complementary approaches, including MTAG-CPASSOC (for SNP identification), MAGMA-FUMA (for positional mapping), TWAS-FUMA (for eQTL mapping), and MAGMA-Metascape (for

pathway enrichment), consistently identified shared SNPs, susceptibility genes, tissue-specific genes, and pathways. Together, these results together offer a comprehensive understanding of the genetic underpinnings of cardiovascular diseases.

2) doing a direct comparison of 38 pleiotropic loci with LAVA/GWAS-PW results.

Re: Thank you for your valuable comment. We compared the 38 pleiotropic loci identified using MTAG with the 13 unique loci from LAVA and GWAS-PW and found that four loci overlapped: Locus 20 (chr4: 155,106,847–155,678,217), Locus 32 (chr4: 111,283,206–112,163,297), Locus 34 (chr9: 21,683,805–22,138,762), and Locus 38 (chr9: 135,866,940–136,400,566). Notably, within Locus 34 (i.e., LD block 1,398), six trait pairs exhibited strong local r_g at the nominal significance level ($P < 0.05$). Four of these trait pairs—AF-CAD, CAD-HF, CAD-PAD, and CAD-Stroke—remained significant after Bonferroni correction and were validated by GWAS-PW. This locus harbors a shared causal SNP (rs4977574), located within an intron of the *CDKN2B-AS1* gene on chromosome 9q21.3, which was further supported by colocalization analysis of the 38 pleiotropic loci based on MTAG results. Regarding the LPA signals previously mentioned by the reviewers, they were not detected in LAVA and GWAS-PW analyses. This discrepancy arises because LAVA and GWAS-PW rely on the original GWAS datasets, whereas the 38 pleiotropic loci were identified using MTAG, which increases the effective sample size. This expansion allows MTAG to detect additional loci that may not have been captured in the original GWAS analyses. However, the identification of the *CDKN2B-AS1* locus reinforces the idea that MTAG serves as an extension of GWAS findings, enabling the discovery of additional loci through increased statistical power. While the consistency between different methods is limited, the overlap of key loci, such as *CDKN2B-AS1*, supports the robustness of the results (Supplementary Note 2).

2. A direct MR analysis of metabolic traits can strengthen the study and thus manuscript.

Re: Thank you for your comments. We used the MRlap method to perform bidirectional causal inference for 24 metabolic traits/diseases and 6 major CVDs.

After Bonferroni correction (P = 0.05 / 144 combinations = 4.17×10^{-4}), 52 of the 144 trait pairs exhibited a positive causal relationship, suggesting that most metabolic traits or diseases have a causal effect on CVDs. Hypertension emerged as the strongest causal factor for CVD, with significant positive associations with HF (OR, 1.237 [CI, 1.192–1.284]) and Stroke (OR, 1.262 [CI, 1.217–1.308]). Conversely, only two trait pairs exhibited an inverse causal relationship (VTE-LDL and CAD-CIMT), which should be interpreted with caution. Additionally, eight trait pairs showed bidirectional causal relationships, with CAD exhibiting a particularly strong bidirectional relationship with six metabolic traits/diseases, especially hypertension (Supplementary Note 6, Supplementary Table 26).

Reviewer #2 (Remarks to the Author):

The authors answered most of the questions regarding the methods, but did little regarding the writing of the manuscript. A few issues I still encounter:

1. The introduction needs to be rewritten and shortened. A few examples that make the introduction wrong:

1) " In detail, heart failure (HF) is a complex clinical syndrome often caused by prior CAD, which represents the final stage of numerous heart diseases." It leads to the interpretation that CAD is the final stage, when heart failure is.

2) It is not true, for instance, that "the concept of meta-analysis has emerged, combining diverse cohorts with similar or genetically correlated traits to amplify the study sample size". The concept introduced seems to be that of multi-trait analysis of GWAS, not that of meta-analysis.

Re: Thank you for your valuable suggestions. We have revised the introduction accordingly to eliminate the potential misunderstandings. (From Page 2, line 82 to Page 3, line 124)

2. There are sentences that not reviewed, e.g.:

1) "HF and CAD exhibited augmented estimated higher estimated polygenicity"

2) "1.397K (sd = 0.254K)". Is the K not indicating a multiple of thousand? it is actually simpler to write 1,397 and 254 in this case, or 1.4K and 0.25K. There are other such cases in the text.

3) " were survived after the Bonferroni correction, was also validated by GWAS-PW", that is not standard English.

4) " this was the type of Mendelian randomization (MR) mainly targets" - do you mean "the type THAT MR targets" or do you mean "the type of MR MAIN targets"? The sentence is confusing.

5) The sentence "In conclusion, the observation that more than one CVD showed genetic signals..." is in the Results section. If it is a conclusion, it should not be in Results.

The list is non-exhaustive. I really recommend a thorough review of the writing.

Re: We apologize for the confusion. We have conducted a thorough review of the article.

1) “Among the six CVDs, HF and CAD exhibited higher estimated polygenicity, indicative of more extensive involvement of genetically correlated genomic regions compared to other CVDs.”

2) “For example, the genetic overlap between CAD and HF was particularly striking, with 1,397 (sd = 254) shared variants, representing 93.3% of the variants influencing CAD and 60.9% of the variants influencing HF. This mirrored their robust positive genome-wide genetic correlation (r_g = 0.677, se = 0.030) and genetic correlation of shared variants (r_{gs} = 0.699, se = 0.013).”

3) “Four of these six trait pairs (including AF-CAD, CAD-HF, CAD-PAD, and CAD-Stroke) remained significant after Bonferroni correction and were also validated by GWAS-PW.”

4) “However, vertical pleiotropy occurs when genetic variation influences one phenotype, which in turn affects the expression of a second phenotype. This type of pleiotropy is the target of Mendelian randomization (MR) studies.”

5) “In all, the observation that multiple CVDs share genetic signals in a given overlapping region is further validated by direct comparisons of SNP- and gene-based results across the six CVDs, which also supported by strong evidence of

colocalization."

This paragraph summarizes the previous content and should be included in the results section. We have revised it to enhance clarity and avoid confusion.

Reviewer #3 (Remarks to the Author):

It feels like a large portion of the rebuttal has been written by a large language model, but I'm happy with the efforts on which the authors put into address my comments. I don't have further concerns.

Re: Thank you for your valuable suggestions. In our response letter, we only use ChatGPT to enhance readability, style and accuracy, ensuring that the text remains free of grammatical, spelling, punctuation, and tone errors. We also carefully reviewed the general policy on AI, which states, "The use of an LLM (or other AI-tool) for AI assisted copy editing purposes does not need to be declared."