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Peer Review File

Rare variant contribution to the heritability of coronary artery disease

REVIEWER COMMENTS

Reviewer #1 (Remarks to the Author):

Rocheleau et al. present a partitioned SNP-based heritability analysis of CAD, finding that ~50% of the heritability is due to ultra-rare SNVs in low LD. They further analyze multiple annotations, including evolutionary constraint measures, functional annotation measures, cell type annotations, and other biological functional annotations. Given the large contribution of very rare variants, most of which are non-coding, I appreciate their efforts to dig deeper into the heritability analyses in this way. I was particularly interested in the enrichment of many of the annotations on a per-variant basis, even when they accounted for less heritability overall, such as the evolutionarily constrained sites that account for less overall, but much higher per-variant or in terms of log impact ratio (Fig. 2). I have no comments about their methods or specific steps, which I think were described well and are all reasonable. Overall, it is an interesting and well-written paper that I think is of interest.

I have four main points/questions:

1. There could be more biological interpretation based on the observed enrichment. The evolutionary constraint, cell type enrichment, and functional annotation enrichment would be a fascinating story to expand on, specifically in regard to CAD. Can the authors leverage any of their findings to explore an evolutionary understanding of why CAD risk alleles are observed? The pairing of very rare, low-LD with constrained sites would be interesting to explore from a population genetics and evolutionary perspective. What do the functional annotation findings mean for possible treatment? Other than listing these findings (pg 11-13), the authors don't go into what they could mean in a deeper way, but that would be a good addition to the manuscript.

2. Perhaps somewhat related to (1) above, could the authors place their findings in the context of the several recent papers (they cited some) that have evaluated rare & ultra-rare variant heritability? Several have made similar findings (substantial rare variant contribution) across the same or different traits, while others seem to be skeptical of these. The second paragraph of the Discussion does this specifically in comparison to another CAD paper, and the fourth paragraph of the discussion discusses their findings related to the burden heritability regression method, but only to say they are somewhat supportive. Can they go a step or two further to describe what they think the overall role of very rare variants is in explaining the variation in complex traits? Do the authors think this is a more general finding than CAD alone?

3. The authors give a nice summary of how their estimates are very similar to the reported estimates of Tcheandjieu 2022. They also note that the SEs of their h2SNP estimates are large, particularly at the low & rare MAF bins. I believe the MAF bin ranges of Tcheandjieu are somewhat different than those evaluated here, but it provides an opportunity to approximately double the sample size if the authors were to bin their data in the same way, then meta-analyze with the published estimates from Tcheandjieu et al., thus reducing the SEs. I'm not suggesting the authors change their core analyses, or that they redo any of the analyses in which they estimated enrichment for various functional annotations, which would be a huge task. But, with the chance to drastically increase the N, even for the European-ancestry subset, by meta-analyzing across the reported Tcheandjieu estimates and theirs, they could presumably reduce the SEs and put better bounds on the estimated contribution of very rare variants, which would be a substantial step forward. There are of course caveats to this suggestion, as the published estimates from Tcheandjieu were based on imputed data, the phenotypic definitions could be slightly different (I'm not a CAD researcher and can't speak to those aspects), one would have to assume no overlap of individuals between the two datasets (I think this is the case, but I'm not certain), and there are certainly study specific differences, but it would be interesting to see how a meta-analyzed estimate compares. Given the qualitative comparisons already in the manuscript, I imagine it would align well.

4. I appreciate why the authors restricted their analyses to genetically homogeneous groupings, and therefore the most homogeneous with the largest sample size. However, I would ask the authors to reconsider their removal of all non-European ancestry groupings from any analysis. As has been argued elsewhere (https://www.nature.com/articles/d41586-020-02547-3), there is utility in analyzing all the sample, even when they are smaller, and providing the results. With heritability analyses, the authors are cautious because of admixture, and that is a concern, but within the groupings they show in their supplemental figures, there are still substantial numbers very close to the 1000 Genomes continental clusters. Their ADMIXTURE analysis also shows an EAS grouping that is very homogeneous. While subsetting to clusters with lower levels of admixture does reduce sample sizes, those could still be analyzed, at least for a core set of analyses, partitioning h2SNP into a smaller number of bins. The rarest MAF bins would have to be removed I imagine, but common, low-frequency, and rare MAF bins and LD halves could still be utilized. It's worth noting, too, that some of the studies referenced by the authors did just that - Jang 2022 Nat. Hum. Behav. included both EUR and African ancestry samples using the same TOPMed study, and Tcheandjieu 2022 evaluated multiple groupings. Both studies acknowledged the smaller sample sizes is not optimal, but that the benefit of improving our understanding of the traits in these other groups is important as well.

Following the spirit of Ben-Eghan referenced above, building resources across groupings would help the overall fairness of the study, and, following point (3) above, the authors could use similar groupings to Tcheandjieu and meta-analyze, thus potentially bringing the sample sizes analyzed up to substantial numbers, which really would make an impact.

Reviewer #2 (Remarks to the Author):

The authors use a previously published approach, GREML-LDMS-I, to estimate CAD heritability in European ancestry individuals from the NHLBI TOPMed program - whole genome sequence data are used to assess contributions from ultra-rare variants. They also use different sets of functional annotations to investigate CAD heritability enrichment, highlighting several functional processes as key drivers of CAD risk. A sense of replication in their results is provided since a similar CAD observed heritability was previously reported in non-Hispanic White participants of the Million Veteran Program. In addition, using WGS data, they confirmed a disproportionate contribution to CAD heritability originating from rare SNVs in low LD, which was previously reported for genotyped and imputed data.

This is a clearly written paper with sound, detailed methodology. I only have minor comments.

1. Considering that they identify ultra-rare variants that have large contributions to CAD heritability in genetically inferred European participants, the authors should investigate the MAF distribution of these variants in other genetic ancestry population groups. Likewise for rare variants.
2. Related to the above comment, what proportion of the ultra-rare variants are specific to European-like populations? Likewise for rare variants.

Authors reply to all Reviewers: We sincerely thank both Reviewers and the Editors for their helpful comments. We have addressed the comments and provide our responses and manuscript changes below.

Reviewer #1 (Remarks to the Author):

Rocheleau et al. present a partitioned SNP-based heritability analysis of CAD, finding that ~50% of the heritability is due to ultra-rare SNVs in low LD. They further analyze multiple annotations, including evolutionary constraint measures, functional annotation measures, cell type annotations, and other biological functional annotations. Given the large contribution of very rare variants, most of which are non-coding, I appreciate their efforts to dig deeper into the heritability analyses in this way. I was particularly interested in the enrichment of many of the annotations on a per-variant basis, even when they accounted for less heritability overall, such as the evolutionarily constrained sites that account for less overall, but much higher per-variant or in terms of log impact ratio (Fig. 2). I have no comments about their methods or specific steps, which I think were described well and are all reasonable. Overall, it is an interesting and well-written paper that I think is of interest.

Authors reply: We thank the Reviewer for the positive comments regarding the appropriateness of the methods, the quality of the writing and the overall interest in our paper.

I have four main points/questions:

1. There could be more biological interpretation based on the observed enrichment. The evolutionary constraint, cell type enrichment, and functional annotation enrichment would be a fascinating story to expand on, specifically in regard to CAD. Can the authors leverage any of their findings to explore an evolutionary understanding of why CAD risk alleles are observed? The pairing of very rare, low-LD with constrained sites would be interesting to explore from a population genetics and evolutionary perspective. What do the functional annotation findings mean for possible treatment? Other than listing these findings (pg 11-13), the authors don't go into what they could mean in a deeper way, but that would be a good addition to the manuscript.

Authors reply: We agree with the Reviewer that a population genetics perspective would be fascinating as to why and when CAD risk alleles appear. Some evolutionary models suggest

that negative selection or balancing selection might explain the allele frequency spectrum reported in many GWAS/exome studies. Although very interesting, we did not want to expand too much further on the subject as it would require substantial analytic efforts that we believe is somewhat outside the scope of the current work and would be better served in a separate follow-up paper. Instead, we added a paragraph in the Discussion explaining what recent studies have shown regarding functional convergence of common and rare variants, and their potential implication for drug discovery and treatment.

"A recent study identified rare and ultra-rare coding variants in 17 genes associated with CAD, 14 of which showed at least moderate prior genetic, biological and/or clinical evidence [31]. It revealed an excess of ultrarare coding variants in 321 known CAD genes, demonstrating that many rare and ultrarare coding variants in additional CAD genes await discovery. For most complex diseases, these rare variants have been hypothesized to be under negative (or purifying) selection, eliminating large-effect mutations and leaving behind common-variant associations in thousands of less essential loci [32,33]. Exome studies reported that most rare coding variants have been previously identified in loci overlapping those detected by GWAS of common variants [34,35], suggesting some level of functional convergence across the allelic frequency spectrum [36,37]. Studies have shown that this convergence signature may guide future fine-mapping studies and reveal potential drug targets [38,39]."

2. Perhaps somewhat related to (1) above, could the authors place their findings in the context of the several recent papers (they cited some) that have evaluated rare & ultra-rare variant heritability? Several have made similar findings (substantial rare variant contribution) across the same or different traits, while others seem to be skeptical of these. The second paragraph of the Discussion does this specifically in comparison to another CAD paper, and the fourth paragraph of the discussion discusses their findings related to the burden heritability regression method, but only to say they are somewhat supportive. Can they go a step or two further to describe what they think the overall role of very rare variants is in explaining the variation in complex traits? Do the authors think this is a more general finding than CAD alone?

Authors reply: As the Reviewer notes, our study supports the idea that rare and ultra-rare variants contribute to CAD risk, and that this finding is supported by other studies focused on other traits and diseases which also show substantial contribution of rare and ultra-rare variants.

We have modified the Discussion to describe an overall role of very rare variants in explaining complex traits and diseases.

“In line with other recent studies, our results suggest that ultra-rare variants contribute a substantial proportion of missing heritability in CAD and that rare-variant associations remain to be identified by large well-powered whole-genome sequencing studies. Functional studies are also needed to establish a better understanding of the role of rare variants on complex traits and diseases in general.”

3. The authors give a nice summary of how their estimates are very similar to the reported estimates of Tcheandjieu 2022. They also note that the SEs of their h2SNP estimates are large, particularly at the low & rare MAF bins. I believe the MAF bin ranges of Tcheandjieu are somewhat different than those evaluated here, but it provides an opportunity to approximately double the sample size if the authors were to bin their data in the same way, then meta-analyze with the published estimates from Tcheandjieu et al., thus reducing the SEs. I'm not suggesting the authors change their core analyses, or that they redo any of the analyses in which they estimated enrichment for various functional annotations, which would be a huge task. But, with the chance to drastically increase the N, even for the European-ancestry subset, by meta-analyzing across the reported Tcheandjieu estimates and theirs, they could presumably reduce the SEs and put better bounds on the estimated contribution of very rare variants, which would be a substantial step forward. There are of course caveats to this suggestion, as the published estimates from Tcheandjieu were based on imputed data, the phenotypic definitions could be slightly different (I'm not a CAD researcher and can't speak to those aspects), one would have to assume no overlap of individuals between the two datasets (I think this is the case, but I'm not certain), and there are certainly study specific differences, but it would be interesting to see how a meta-analyzed estimate compares. Given the qualitative comparisons already in the manuscript, I imagine it would align well.

Authors reply: We thank the Reviewer for this comment. We agree with the Reviewer that meta-analyzing our results with Tcheandjieu’s results could increase the sample size and potentially reduce the uncertainty of the rare variant contribution to CAD heritability. We also agree with the Reviewer regarding the caveats to this suggestion (imputed vs. whole genome sequencing, different binning of variants, different phenotypic definitions). We have added a new section “*Comparison with previously published CAD heritability estimate”* in the Results:

"We compared the CAD heritability estimate in our inferred European sample with the most recent estimate reported in 19,392 non-Hispanic White participants of the Million Veteran Program (MVP) [7]. Tcheandjieu et al. applied the same GREML-LDMS-I approach but binned the variants differently: quartiles of LD scores and six MAF bins were used (0.1% < MAF ≤ 1%, 1% < MAF ≤ 10%, 10% < MAF ≤ 20%, 20% < MAF ≤ 30%, 30% < MAF ≤ 40%, 40% < MAF ≤ 50%). Using the GCTA REML EM algorithm, we estimated a total observed heritability h^2_{obs}= 14.1% (SE = 5.5%), which is considerably less than their estimated h^2_{obs}= 24.4% (SE = 4.7%). The major discrepancy came from SNVs in the lowest LD score quartile (Q1) of the lowest MAF bin (0.1% < MAF ≤ 1%) (**Supplementary Table 6**, MVP estimates were provided by A. Hilliard and T. Assimes, personal communication). However, when we added the ultra-rare variants (MAF ≤ 0.1%), which were not included in the MVP heritability analyses, the missing gap was more than closed with h^2_{obs}= 26.9% (SE = 10.7%) (**Supplementary Fig. 19**). We note that there are differences that may contribute to discrepancies in CAD heritability estimates between our study and Tcheandjieu et al. including whole-genome sequencing vs. imputed data, different binning of variants and phenotypic definitions."

Because of the differences in the two studies, we chose not to include the meta-analysis results in the manuscript. However, to satisfy the Reviewer's curiosity, we provide a Table below (which is an extension of Supplementary Table 6) showing a fixed-effects inverse-variance weighted meta-analysis in each LD score-MAF bin. If the Reviewers or Editors feel strongly about including the meta-analysis in the main manuscript, we are happy to include the meta-analysis results in the Supplement.

		TOPMed		**Million Veteran Program**		**Meta-analysis**	
MAF	**LD score**	**Variance**	**SE**	**Variance**	**SE**	**Variance**	**SE**
0.1% < MAF < 1%	Q1	0.014844	0.042349	0.121141	0.041483	0.0691	0.0296
0.1% < MAF < 1%	Q2	0.025535	0.034602	0.027394	0.023343	0.0268	0.0194
0.1% < MAF < 1%	Q3	0.005106	0.023598	0.006894	0.006736	0.0068	0.0065
0.1% < MAF < 1%	Q4	0.002371	0.004330	0.000138	0.002530	0.0007	0.0022

1% < MAF < 10%	Q1	0.005249	0.013299	0.019395	0.015377	0.0113	0.0101
1% < MAF < 10%	Q2	0.002954	0.016745	0.012908	0.017468	0.0077	0.0121
1% < MAF < 10%	Q3	0.026154	0.019866	0.000001	0.009228	0.0046	0.0084
1% < MAF < 10%	Q4	0.007064	0.008263	0.000001	0.003669	0.0012	0.0034
10% < MAF < 20%	Q1	0.001473	0.003035	0.011873	0.006152	0.0035	0.0027
10% < MAF < 20%	Q2	0.001031	0.004534	0.000727	0.010227	0.0010	0.0041
10% < MAF < 20%	Q3	0.002718	0.011754	0.012089	0.007290	0.0095	0.0062
10% < MAF < 20%	Q4	0.003671	0.008562	0.000001	0.003027	0.0004	0.0029
20% < MAF < 30%	Q1	0.000602	0.002303	0.000001	0.004878	0.0005	0.0021
20% < MAF < 30%	Q2	0.001059	0.003323	0.000094	0.008361	0.0009	0.0031
20% < MAF < 30%	Q3	0.001625	0.010038	0.007114	0.006202	0.0056	0.0053
20% < MAF < 30%	Q4	0.004332	0.008243	0.001297	0.002215	0.0015	0.0021
30% < MAF < 40%	Q1	0.000538	0.002157	0.000001	0.004503	0.0004	0.0019
30% < MAF < 40%	Q2	0.000792	0.002835	0.008559	0.007725	0.0017	0.0027
30% < MAF < 40%	Q3	0.002900	0.009171	0.004073	0.005752	0.0037	0.0049
30% < MAF < 40%	Q4	0.013732	0.008485	0.000104	0.002354	0.0011	0.0023
40% < MAF < 50%	Q1	0.001723	0.002002	0.000001	0.004184	0.0014	0.0018
40% < MAF < 50%	Q2	0.000615	0.002556	0.000001	0.007019	0.0005	0.0024
40% < MAF < 50%	Q3	0.004418	0.008133	0.010146	0.005291	0.0084	0.0044
40% < MAF < 50%	Q4	0.010645	0.007443	0.000001	0.002189	0.0008	0.0021
	h2 =	0.141151	0.055455	0.243954	0.047000	0.1693	0.0359

Despite the differences, we want to emphasize the fact that both total CAD heritability estimates in TOPMed and in MVP demonstrate an important contribution from rare SNVs in low LD.

Hence, we modified the 2nd paragraph of the Discussion:

“However, their estimation did not include ultra-rare variants (MAF ≤ 0.1%) and was based on genotyped and imputed instead of WGS data. When we added the ultra-rare variants (MAF ≤ 0.1%), our heritability estimate was comparable to theirs (h^2_{obs}= 26.9%, **Supplementary Fig 19**). One possible explanation might lie in the differences of allelic frequencies between the TOPMed and the MVP sample: many variants in TOPMed placed in the ultra-rare bin were included in the rare bin of MVP. An alternative explanation could be that their imputed dataset captured ultra-rare variants in LD with rare variants, hence inflating the contribution from their rare variants bin, while our WGS dataset really classified these ultra-rare variants in their appropriate MAF bin. Nonetheless, both analyses point to a disproportionate contribution to CAD heritability originating from rare SNVs in low LD.”

4. I appreciate why the authors restricted their analyses to genetically homogeneous groupings, and therefore the most homogeneous with the largest sample size. However, I would ask the authors to reconsider their removal of all non-European ancestry groupings from any analysis. As has been argued elsewhere (https://www.nature.com/articles/d41586-020-02547-3), there is utility in analyzing all the sample, even when they are smaller, and providing the results. With heritability analyses, the authors are cautious because of admixture, and that is a concern, but within the groupings they show in their supplemental figures, there are still substantial numbers very close to the 1000 Genomes continental clusters. Their ADMIXTURE analysis also shows an EAS grouping that is very homogeneous. While subsetting to clusters with lower levels of admixture does reduce sample sizes, those could still be analyzed, at least for a core set of analyses, partitioning h2SNP into a smaller number of bins. The rarest MAF bins would have to be removed I imagine, but common, low-frequency, and rare MAF bins and LD halves could still be utilized. It's worth noting, too, that some of the studies referenced by the authors did just that - Jang 2022 Nat. Hum. Behav. included both EUR and African ancestry samples using the same TOPMed study, and Tcheandjieu 2022 evaluated multiple groupings. Both studies acknowledged the smaller sample sizes is not optimal, but that the benefit of improving our understanding of the traits in these other groups is important as well.

Following the spirit of Ben-Eghan referenced above, building resources across groupings would help the overall fairness of the study, and, following point (3) above, the authors could use similar groupings to Tcheandjieu and meta-analyze, thus potentially bringing the sample sizes analyzed up to substantial numbers, which really would make an impact.

Authors reply: We thank the Reviewer for this comment. We would like to reassure the Reviewer that we strongly agree with the importance of including non-European populations in genetic studies. Our main concern with presenting heritability estimates from other non-European populations (Black/African American, Hispanic/Latino, East Asian, South Asian) was not only low sample size in these groups, but mostly admixture (see Supplementary Fig 3). It is true that the East Asian group (EAS) in the bottom panel is quite homogeneous but, unfortunately, less than 1,000 EAS participants were available for analysis. As we indicated in the Discussion, "the sampling variance of the variance estimate in each LD score-MAF bin is proportional to the effective number of independent variants, but the corresponding standard error is approximately inversely proportional to the sample size". As seen in Fig 1 for the TOPMed genetically inferred European group, the error bars are already quite large in the ultra-rare and rare variant bins and that, for a sample size which is more than 22 times the TOPMed EAS sample size.

Following the suggestion of the Reviewer, and to ensure fairness in underrepresented populations, we added a full analysis in a sample of inferred African ancestry. This analysis showed the limitations of the GREML-LDMS-I approach when dealing with a low sample size. We included a new section "*CAD heritability estimation in the African ancestry sample"* in the Results:

"We also estimated CAD heritability in a restricted sample of 1,733 cases and 7,783 controls of inferred African genetic ancestry (see **Supplementary methods** for details). The total observed heritability h^2_{obs}= 25.5% (SE = 18.1%), although none of the LD score-MAF bin contributions were significant within one SE (**Supplementary Table 7**, **Supplementary Fig. 20**). Using a prevalence of 6.5% in the U.S. Black population as in Tcheandjieu et al. [7], heritability on the liability scale h^2_{liab} = 39.3% (SE = 27.9%). This estimate is larger than the one in our TOPMed European sample, and also larger than the one reported in MVP (h^2_{liab} = 30.0%). In the African ancestry sample, the contribution from ultra-rare variants with low LD score is not as high as in our European sample (0.037/0.255 ≈ 14.6%). This might be explained by the fact that, of the 28.1 million SNVs in Europeans and 35.7 million SNVs in Africans used in GCTA computations, only 12.9 million SNVs are shared, and the distribution of these variants across MAF bins is quite different (**Supplementary Table 8**, **Supplementary Fig. 21**). Owing to the large SEs observed in the African sample, all the subsequent analyses presented in this paper are restricted to the European sample."

A new section describing the QCs in the African sample was added in the Supplementary methods, along with Supplementary Figs 8-11, 20-21, and Supplementary Tables 7-8. We also added the following sentences in the 6th paragraph of the Discussion:

"However, to promote fairness and transparency in genomic research [30], we estimated CAD heritability in a much smaller sample of inferred African genetic ancestry. The observed heritability in the African sample (h^2_{obs}= 25.5%) was comparable to the observed heritability (h^2_{obs}= 23.9%) in the European sample, although the contribution from ultra-rare variants with low LD score was not as important (~15% versus ~50%). Yet caution is required since all LD score-MAF bin contributions in the African sample showed large SEs."

Reviewer #2 (Remarks to the Author):

The authors use a previously published approach, GREML-LDMS-I, to estimate CAD heritability in European ancestry individuals from the NHLBI TOPMed program - whole genome sequence data are used to assess contributions from ultra-rare variants. They also use different sets of functional annotations to investigate CAD heritability enrichment, highlighting several functional processes as key drivers of CAD risk. A sense of replication in their results is provided since a similar CAD observed heritability was previously reported in non-Hispanic White participants of the Million Veteran Program. In addition, using WGS data, they confirmed a disproportionate contribution to CAD heritability originating from rare SNVs in low LD, which was previously reported for genotyped and imputed data.

This is a clearly written paper with sound, detailed methodology. I only have minor comments.

Authors reply: We thank the Reviewer for the positive comment regarding our choice of the methodology and the quality of our writing.

1. Considering that they identify ultra-rare variants that have large contributions to CAD heritability in genetically inferred European participants, the authors should investigate the MAF distribution of these variants in other genetic ancestry population groups. Likewise for rare variants.

Authors reply: Following the Reviewer's suggestion, we compared the shared variants which contributed to CAD heritability in European and African genetic ancestry groups. The results are shown in the new Supplementary Table 8 and Supplementary Fig. 21. Even though the African sample size is 2.3 times smaller than the European one, there are 11,105,820 + 7,985,019 (ultra-rare + rare) specific to African, compared to 13,790,692 + 1,259,314 (ultra-rare + rare) specific to European, reflecting the increased genetic diversity found in Africans. The shared variants in the European and African samples (~12.9 million) do not distribute in the same MAF bins except, to some extent, the common variants (Supplementary Fig. 21).

2. Related to the above comment, what proportion of the ultra-rare variants are specific to European-like populations? Likewise for rare variants.

Authors reply: To compare the allele frequencies with another database, we downloaded the Genome Aggregation Database (gnomAD) v4.1.0 joint allele frequency dataset. We added a new section "*Allele frequency comparison with gnomAD"* in the Results, along with the corresponding section in the Methods, and the new Supplementary Table 9.

"In general, if the effect sizes from the same set of variants were similar across different ancestry, we should expect to observe similar contribution from this set of variants to heritability estimates across ancestry groups. We compared the overlap of each MAF bin variant set in our European sample with genetically inferred groups from the latest Genome Aggregation Database (gnomAD) (see **Methods)** [26]. As expected, the proportion of SNVs shared with the gnomAD non-Finnish European group was very high in all four MAF bins (ranging from 93% to 99%, **Supplementary Table 9**), meaning that almost all SNVs display the same allele frequency. Unsurprisingly, apart from the non-Finnish European group, the ultra-rare bin ($0 < MAF \leq 0.1\%$) showed a moderate overlap only with the gnomAD African/African American group, and to some extent with the Admixed American group."

REVIEWERS' COMMENTS

Reviewer #1 (Remarks to the Author):

Rocheleau et al. present an updated and revised version of their manuscript on the partitioned heritability of CAD, finding contributions of very rare variants.

As before, I have no major comments, and believe the methods are done well and written clearly. I appreciate the authors' efforts to address my prior comments, which they have done, particularly in regards to meta-analyzing with other published results and including a section focused on the non-European ancestry samples.

I have no further comments.

Reviewer #2 (Remarks to the Author):

This is a well-written paper and the authors have addressed my comments.