nature portfolio

Peer Review File

The Health for Life in Singapore (HELIOS) Study: delivering Precision Medicine research for Asian populations.

Corresponding Author: Professor John Chambers

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)
In this manuscript, Wang et al describe the HELIOS study, a richly phenotyped (including linkage to health data) prospective study of >10k Asian men and women of Chinese, Indian and Malay ancestry from the Singapore general population. The paper highlights important heterogeneity in health outcomes, lifestyle and omic data amongst the three distinct ancestry groups, and is an important step towards addressing health inequity resulting from a lack of representation of Asian individuals in genomic and health research.

1) PRS analysis:
a. In the methods, the authors state that PRS were based on the study with best possible trans-ancestry base data and validation. However, given that the majority of publicly available PRSs (and many of the PRSs used in this study) are from European-ancestry cohorts, the differences in genetic distance between the study group (Chinese, Malay or Indian) and the discovery group (European) could lead to differences in PRS distributions and prediction accuracy. Could the authors report what proportion of variance in each trait is explained by the PRS that has been used (i.e. R2 for each trait, reported on the liability scale for binary traits) in each of the 3 ancestry groups in the HELIOS study. This information can be added to Suppl Table 3.
b. Though there is likely a difference in GWAS sample size, could the authors perform sensitivity analyses using ancestry-specific PRSs where available (e.g. using the East Asian PRS for CAD, which is available on the PGS catalogue: PGP000289) and also PGP000090 which has been calibrated for the South Asian population)? For the same ancestry population, how different is the PRS distribution and prediction accuracy for PRS derived from difference GWAS discovery cohorts?
c. Could the authors report the proportion of participants in the GWAS from which the PRS have been derived that are of East Asian or South Asian ancestry. I acknowledge these studies are published, but it will make it easier for readers to interpret the results if all this information for each PRS is available in one place. This information can be added to Suppl Table 3.
d. The authors state that PRS was estimated in each ancestry group separately, and then merged and normalised to identify ancestry level differences. It is unclear how the data were normalised. Given the PRS are mostly based on European individuals, with different numbers of East and South Asian individuals included, we expect PRS distributions to be different. To express the trans-ancestry PRS on the same scale across ancestries, several methods have been proposed for ancestry adjustment e.g. https://doi.org/10.1186/s13073-022-01074-2. Could the authors provide methodological details on how this was done for this analysis?

2) Methylation analysis
a. Previous studies have shown that methylation risk scores can predict a trait independently of a PRS of the same trait, suggesting the majority of methylation differences may be related to environmental effects and not genetic effects (Shah et al PMID 26119815). Methylome-wide association studies (MWAS) have been shown to identify different genes to GWAS of the same trait (e.g. the strongest predictor of smoking status based on methylation data is in the AHRR gene which has not been identified in GWAS of smoking). This would suggest methylation may capture consequences of exposure while GWAS captures casual pathways that determine the exposure. In Figure 4d the authors tested PRS for traits such as smoking and alcohol consumption. This likely captures behaviours that drive intake and there the associations with methylation PCs may not be as strong as actual exposure. Could the authors add the association of actual alcohol intake and smoking status with

the different PCs?
b. Given the traits in Fig 4 have different units, have these been standardised for comparison of the b_estimate? Please could this be clarified in the legend and methods.
c. Given rice and chapati intake seem to be strongly associated with PC1 of the methylation data, could the authors add rice intake to the food consumption figure 2d to understand how the intake is different in the 3 groups.

Minor comments
1. Fig 2h has interesting results but does not seem to be discussed at all. There are interesting ethnic differences here which may point towards different effective lifestyle interventions for different groups. For example, physical activity, despite being lowest in the Chinese group, has a significant negative association with DBP, which is not seen in the Indian and Malay groups. Similarly higher DASH scores are associated with lower DBP in Indians but not in the other two groups, suggesting diet as a more effective intervention for reducing blood pressure in Indians. Could the authors please add a discussion around these results.
2. There are examples of differential associations with DBP and SBP. Do DASH scores have similar associations with SBP and DBP?
3. The Chinese groups is reported to have lower self-reported physical activity and lower DASH scores than Indians, yet the prevalence of disease is said to be higher. This is not discussed at all. Can the authors elaborate on this finding.
4. Page 19 – line 626. eQTLGen data were used for the SMR and coloc analysis. eQTLGen data is generated from primarily European cohorts. As both SMR and coloc assume the LD structure to be similar in the two datasets being compared, using eQTL data from European individuals with HELIOS data may lead to inaccurate estimation of the posterior probability (in the case of SMR, the estimation of the HEIDI p-value). This limitation should be discussed.
5. Figure 3 - The authors report much larger numbers of ancestry-unique SNPs and INDELs in Chinese individuals compared to the Malay and Indian cohorts. Is this simply a reflection of sample size? Can the authors provide an interpretation in the Discussion?
6. Page 9, line 274-276. The authors stated that the clinical characteristics of the cohort are “broadly representative of the population from which they were recruited”. However, comparison statistics from the population were not provided. Please provide the population statistics, and perform statistical tests to confirm the similarity between the cohort and the population. In addition, could the authors also discuss the potential “healthy volunteer bias”, which is a problem present in many large Biobanks (e.g. UK Biobank). The assumption is that if there is not significant difference in disease prevalence in HELIOS compared to the general population, there shouldn’t be a healthy volunteer bias. Along the same lines, are disease rates similar to those living in China, Malaysia, India?
7. Extended Table 1 and Extended Figure 1 – the authors mentioned that Indian and Malay participants had a higher prevalence for some conditions. What was the statistical test used to confirm the statistical significance of these differences? It would also be great to provide the percentages of disease prevalence in a table.
8. Page 6 Line 186. ‘Indians and Malays had lower levels of lipid metabolites’. Could the authors discuss if this is related to medication use? Is there greater statin use amongst these two groups?
9. In extended figure 1 – the authors presented the prevalence of diagnosed and undiagnosed cases in each group. However, the same clinical thresholds (e.g. blood pressure of 140/90 and BMI of >=30) were used in all three ethnic groups, despite evidence showing that ethnicity-specific cut-offs are needed to achieve the same level of clinical surveillance (e.g. 10.1016/S2213-8587(21)00088-7). Could the authors clarify which guidelines they are using to determine the thresholds, and highlight the ethnicity-specific cut-offs as a limitation of the paper?
10. Page 4, line 91 – what are the “other ethnicities”?
11. Please standardise the colour scheme used to represent the three different ethnic groups. For example, the colour scheme was not the same between Extended Figure 3c and Figure 4a.
12. Figure 3d, the legend shows Indian as purple, but there are no points of purple colour on the plot
13. Though individual level data is not available, could the authors add a data availability statement for any summary statistics (e.g. metabolite GWAS). Will these be made publicly available through online catalogues?

Reviewer #2

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

Reviewer #3

(Remarks to the Author)
Wang and colleagues describe a unique biobank from Singapore, a country with excellent healthcare system and three major ancestry groups (Chinese, Indian and Malay). Given the scarcity of biobank-grade data on Asian populations other than Chinese and Japanese, this is a welcome contribution. The high quality of data collected on the participants and its linkage to medical records make up for the small cohort size. The paper is very light on discoveries other than comparative analyses between the three groups. It seems that the much anticipated discoveries are reserved for an accompanying submission that this reviewer has no access to. Additionally, there are several concerns that should be addressed:
Major:
1- The RNA-seq data are completely missing, which is not acceptable given that this is one modality of omics that was specifically mentioned by the authors. Needless to say, the transcriptomics data will provide a wealth of information for this comparative analysis. Please refer to PMID: 39383868 as an example of what readers should expect from a study of this

nature.
2- Similarly, the authors should provide data on the genetic determinants of the CpG methylation pattern differences between the three groups.
3- Since this is a precision medicine cohort, one would expect the authors to provide a more medically relevant context for their findings. For example, the content of figure 6 is mentioned almost as a note in passing when highlighting the potential of this biobank to inform a deeper understanding of the pathogenesis of chronic diseases without providing details. More such examples should be provided.
4- In line with point #3 above, it is surprising that the authors did not comment at all about the contribution, or lack thereof, of rare variants to the differences between the three groups in their disposition to a wide range of phenotypes. They simply listed the types of variants identified without any clinical context. This is a major deficiency that must be addressed.
5- The authors assert that the linkage to medical records provide numerous opportunities but the only benefit shown was the demonstration of reproducibility of measurements. For example, could the “undiagnosed” diseases identified by the investigators through the biobanking process have been predicted from the linked medical records?
Minor:
1- Please provide more explanation of Figure S4.
2- There is an error in the color code of Figure 3B.

Reviewer #4

(Remarks to the Author)
To the authors,
The manuscript describes the data collection and first analyses of a novel cohort of 10k individuals of Asian descent, separated into Chinese, Malay and Indian background. Samples were collected by random sampling as much as possible, to represent these general populations, and extensive phenotyping was performed at baseline, enriched with various medical/lifestyle data-sources, and the population is being followed-up. Extensive genomic measurements have been performed, including WGS on all individuals, and RNAseq, methylation, metabolomics, microbiomics on subsets. Initial results are performed to demonstrate the generalizability of the cohort, confirming a number of expected but interesting associations, and zooming in on the differences between the three ancestry groups to highlight the value of this dataset for clinical and fundamental research. The work has been performed in accordance with current state-of-the-art practices and is well written. I agree with the authors that this data-source is a valuable contribution to the scientific field in general, and the alleviate the missing data on Asian population in specific. I have some – mostly clarifying – questions.
1) Recruitment of these samples was done via random sampling as much as possible. The characteristics of the cohort are displayed in table 1. To understand the generalizability of the cohort to the wider Singapore (or Asian) population, it would be helpful to include a paragraph on how these characteristics compare to these generalized populations. For example, what was the participation rate, and do the authors envision any selection biases (e.g., healthier or higher educated participants compared to general population, as is common in population studies) that potential users of this resource should be aware of?
2) Similarly, the authors compare and comment on differences between the three subgroups. It would be useful to either comment on the expected generalizability of these findings to their respective populations, and/or subset the three subgroups to similar phenotypic characteristics and repeat the omics-phenotype associations to allow for more direct comparisons. For example, the authors note that the rate of T2D is higher in the Indian/Malay population, and also note that there are CpGs differential in these populations, and finally that these overlap to some extent. Is the CpG difference caused by the different ancestries, or by the different rate of cases?
Minor comments;
3) It is not explicitly stated if the consent/data access allows for international sharing of this resource, and if so which level of data can be shared (e.g., summary statistics only, vs access to processed omics data and phenotypes). Some more details may be useful.
4) I believe at least some analyses were stratified on sex. It may be nice to illustrate more explicitly if/when some sex-specific results were observed.
5) Of the coding variants, 88,995 are protein altering, 82,831 of those are indels - is that correct? In other databases, such as gnomad, the number of missense single nucleotide variants is much higher than the number of frameshift indels. Perhaps I misunderstood the comparison made here, but then please clarify.
6) P5, line 24 - without the methods it’s a bit hard to place this in context, please add a line stating how many PRS were tested, and perhaps where they came from.
7) P10, line 1, seems to be a typo around “are linked”.
8) The figures 5 and 6 have a partially transparent background, causing them to be displayed incorrectly against dark backgrounds.

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
Thank you the authors for addressing my comments.

I have additional comments on the PRS analysis.

a. The authors make statements such as
“Mean values for PRS vary between populations (Figure 3c). The strongest separation was observed for depression, with higher PRS amongst Indians compared to Chinese and Malays (P=2.8x10-162).”

“Based on the difference in PRS between the populations, and the observed risk ratios for T2D, we estimate that the small increase in PRS observed in Indian and Malay populations accounts for only ~5% of their three-fold excess risk of T2D compared to Chinese participants (Indians: 5.7 [95%CI: -1.3 to 12.6]%; Malay: 3.1 [95%CI: -5.4 to 11.6]%, Supplementary Methods).

The difference in PRS means across populations is confounded by differences in LD and allele frequency across different populations. It is not indicative of a difference in genetic risk burden between populations. Therefore, analyses looking at whether a difference in mean can explain the observed differences in prevalence are not appropriate.

b. On the analysis using ancestry-specific PRS, the authors state “results show that the performance of these ancestry specific PRS as assessed by both odds ratio and R2 values is similar to the trans-ancestry model based PGS Scores”. However, some of the ancestry-specific PRS only have a small number of variants (Suppl Table 4. 10 for EAS CAD PRS and 275 for SAS T2D PRS) compared to >1 million variants in the Euro-centric PRS for these 2 traits. It is well-known that P+T underperforms compared to more recent Bayesian method. To make R2 more comparable, the same method should be applied to the summary statistics from different GWAS, which are publicly available. Bayesian methods like SBayesR, SBayesRC, LDpred2 do not require training/validation sets and therefore can simply be applied to GWAS summary statistics to obtain independent SNP sets and weights that can then be used in the target population.

c. For the Eurocentric PRS, it would help for to put the R2 values in context. For example, the R2 for depression ranges from 0.005 - 0.3%, compared to T2D which range from 5-7%. Is this a reflection of a lower genetic contribution (i.e. SNP-based heritability) in general to depression compared to T2D or a lower generalisability of the European PRS for depression? Could the authors add the R2 reported in published literature for these PRS in Europeans as this would provide a better understanding of the difference in R2 across traits.

d. The response to my comment on PRS recalibration is as follows:
“The aim of this analysis was to compare the contribution of polygenic risk to disease between the three ancestry groups. To achieve this, we calculated the PRS for each cohort participant using the same set of PRS-specific genetic variants. We then applied Z-score standardization across all participants, to enable relationships to disease risk, and differences between ancestral groups, to presented on a per SD scale. This approach facilitates comparisons of effect size and ancestry based risk scores between the PRS evaluated. We have updated the Methods section (page 20) accordingly. In contrast, using methods for ancestry adjustment such as residualizing PCs (https://doi.org/10.1186/s13073-022-01074-2) would remove the difference due to the ethnicity, and thus would not enable us to address our specific scientific question.”

As highlighted above, the difference in PRS distribution across the 3 ancestry groups is reflective of differences in LD and allele frequency in these populations compared to a European population. It does not indicate whether the genetic risk burden is higher in one group compared to another. This is why post-hoc methods for standardising the PRS for genetic ancestry onto a standard scale have been proposed so that OR can be directly compared. In addition, the post-hoc PC adjustment in the referenced paper is done within each ancestry group because genetic ancestry is continuous (see https://www.nature.com/articles/s41586-023-06079-4). The referenced paper uses the method by Wang et al. https://pubmed.ncbi.nlm.nih.gov/32762905/ who use this framework to evaluate a CAD PRS for South Asians.

e. The entry for the South Asian PGS catalog lists 282 variants, whereas Suppl Table 4 lists 275. What is the reason for this discrepancy?

Reviewer #2

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

Reviewer #3

(Remarks to the Author)
I thank the authors for their thoughtful response. The inclusion of transcriptomic data is a welcome change. While I understand the authors' desire to delay a comprehensive rare variant analysis for a subsequent publication, it is still important to highlight at least a few examples based on what they already have.

Reviewer #4

(Remarks to the Author)
Dear Authors,
My questions have been sufficiently answered, I appreciate the extra work that was performed.
Best,

Version 2:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
I thank the authors for their comprehensive response to my comments. I have no further comments.

Reviewer #2

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

Reviewer #3

(Remarks to the Author)
Thank you for addressing all of my concerns and congratulations on a nice paper.

March 17th, 2025

Chief Editor for Molecular Biology & Genetics
Nature Communications

Subject: Submission of revised paper [NCOMMS-24-40335]

Thank you for the opportunity to revise and resubmit out manuscript titled “The Health for Life in Singapore (HELIOS) Study: delivering Precision Medicine research for Asian populations”. We appreciate the time and effort that you and the reviewers have invested in evaluating our work. We have carefully considered all comments and have made revisions accordingly.

Below, we provide our point-by-point responses to each of the reviewers' comments. All page numbers mentioned correspond to the revised manuscript with track changes.

Reviewer #1 (Remarks to the Author):

In this manuscript, Wang et al describe the HELIOS study, a richly phenotyped (including linkage to health data) prospective study of >10k Asian men and women of Chinese, Indian and Malay ancestry from the Singapore general population. The paper highlights important heterogeneity in health outcomes, lifestyle and omic data amongst the three distinct ancestry groups and is an important step towards addressing health inequity resulting from a lack of representation of Asian individuals in genomic and health research.

1) PRS analysis:

a. In the methods, the authors state that PRS were based on the study with best possible trans-ancestry base data and validation. However, given that the majority of publicly available PRSs (and many of the PRSs used in this study) are from European-ancestry cohorts, the differences in genetic distance between the study group (Chinese, Malay or Indian) and the discovery group (European) could lead to differences in PRS distributions and prediction accuracy. Could the authors report what proportion of variance in each trait is explained by the PRS that has been used (i.e. R2 for each trait, reported on the liability scale for binary traits) in each of the 3 ancestry groups in the HELIOS study. This information can be added to Suppl Table 3.

Thank you for the relevant comment. We have calculated the R^2 on the liability scale for the six traits for which we had tested the performance of the PRS derived from the PGS Catalogue. These mirror the disease odds ratios, and the conclusions are unchanged. The information has been added to **Results** section (pages 6-7) and **Supplementary Table 4**.

b. Though there is likely a difference in GWAS sample size, could the authors perform sensitivity analyses using ancestry-specific PRSs where available (e.g. using the East Asian PRS for CAD, which is available on the PGS catalogue: PGP000289) and also PGP000090 which has been

calibrated for the South Asian population)? For the same ancestry population, how different is the PRS distribution and prediction accuracy for PRS derived from difference GWAS discovery cohorts?

As requested, we have carried out a sensitivity analysis using PRS derived from ancestry specific models available in PGS Catalogue. This was limited to Type 2 Diabetes and Coronary Artery Disease as these were the only traits with ancestry specific models available for comparison. The results show that the performance of these ancestry specific PRS as assessed by both odds ratio and R^2 values is similar to the trans-ancestry model based PGS Scores (**Supplementary Table 4**).

c. Could the authors report the proportion of participants in the GWAS from which the PRS have been derived that are of East Asian or South Asian ancestry. I acknowledge these studies are published, but it will make it easier for readers to interpret the results if all this information for each PRS is available in one place. This information can be added to Suppl Table 3.

We have included the ancestry information about the GWAS Variants and development as available in the PGS Catalogue to **Supplementary Table 4**, as suggested by the reviewer.

d. The authors state that PRS was estimated in each ancestry group separately, and then merged and normalised to identify ancestry level differences. It is unclear how the data were normalised. Given the PRS are mostly based on European individuals, with different numbers of East and South Asian individuals included, we expect PRS distributions to be different. To express the trans-ancestry PRS on the same scale across ancestries, several methods have been proposed for ancestry adjustment e.g. https://doi.org/10.1186/s13073-022-01074-2. Could the authors provide methodological details on how this was done for this analysis?

The aim of this analysis was to compare the contribution of polygenic risk to disease between the three ancestry groups. To achieve this, we calculated the PRS for each cohort participant using the same set of PRS-specific genetic variants. We then applied Z-score standardization across all participants, to enable relationships to disease risk, and differences between ancestral groups, to presented on a per SD scale. This approach facilitates comparisons of effect size and ancestry-based risk scores between the PRS evaluated. We have updated the **Methods** section (page 20) accordingly.

In contrast, using methods for ancestry adjustment such as residualizing PCs (*https://doi.org/10.1186/s13073-022-01074-2*) would remove the difference due to the ethnicity, and thus would not enable us to address our specific scientific question**.**

2) Methylation analysis

a. Previous studies have shown that methylation risk scores can predict a trait independently of a PRS of the same trait, suggesting the majority of methylation differences may be related to environmental effects and not genetic effects (Shah et al PMID 26119815). Methylome-wide association studies (MWAS) have been shown to identify different genes to GWAS of the same trait (e.g. the strongest predictor of smoking status based on methylation data is in the AHRR gene which has not been identified in GWAS of smoking). This would suggest methylation may

capture consequences of exposure while GWAS captures casual pathways that determine the exposure. In Figure 4d the authors tested PRS for traits such as smoking and alcohol consumption. This likely captures behaviours that drive intake and there the associations with methylation PCs may not be as strong as actual exposure. Could the authors add the association of actual alcohol intake and smoking status with the different PCs?

We agree with the reviewer's observations that methylation markers capture important environmental exposures, and highlight our work in relation to aetiology and prediction of diabetes using data from the HELIOS study, which strongly supports this view (DOI: 10.1101/2025.02.14.25322264).

The association of the methylation PCs with alcohol consumption and smoking status were provided in **Supplementary Table 9**. Taking into account corrections for multiple testing, alcohol and smoking habit are not associated with PC1 of DNA methylation, as assessed by direct measurement or PRS. We note that smoking and alcohol consumption rates are both low in our Asian populations, which we speculate limits their contribution to molecular variation in the population. We have revised the manuscript to better highlight these results (pages 7-8).

b. Given the traits in Fig 4 have different units, have these been standardised for comparison of the b_estimate? Please could this be clarified in the legend and methods.

We confirm that all quantitative traits have been standardized using a z-scored transformation to ensure comparability of the β-estimates across traits with different units. This has been clarified in the legend and **Methods** section (page 22).

c. Given rice and chapati intake seem to be strongly associated with PC1 of the methylation data, could the authors add rice intake to the food consumption figure 2d to understand how the intake is different in the 3 groups.

In our original **Figure 4b**, 'rice' referred to fermented rice/lentil items, such as idli and thosai. The labels have now been corrected to reflect this detail, in revised **Figure 4b**. Thosai (but not idli) is amongst the top 20 foods significantly different across ethnicities (significantly higher consumption by Indians, $p<2.2e-308$) and already included in **Figure 2b**.

<u>Minor comments</u>

1. Fig 2h has interesting results but does not seem to be discussed at all. There are interesting ethnic differences here which may point towards different effective lifestyle interventions for different groups. For example, physical activity, despite being lowest in the Chinese group, has a significant negative association with DBP, which is not seen in the Indian and Malay groups. Similarly higher DASH scores are associated with lower DBP in Indians but not in the other two groups, suggesting diet as a more effective intervention for reducing blood pressure in Indians. Could the authors please add a discussion around these results.

Thank you for highlighting this issue. We agree that the data is suggestive of a differential effect of health behaviours on cardiovascular risk between the ethnic groups, which may have clinical relevance in relation to management of modifiable risk factors in key population subgroups. We

have added estimates of heterogeneity of effect to enable the reader to better interpret these differences (**Fig 2h**) and have also incorporated it into the **Discussion** section (pages 12-13).

2. There are examples of differential associations with DBP and SBP. Do DASH scores have similar associations with SBP and DBP?

We found higher DASH scores to be associated lower levels of both SBP and DBP. Effect sizes [β (SE)] for SBP per 1SD increase in DASH score were: -0.01 (0.03) in Chinese, -0.04 (0.03) in Indians, and -0.02 (0.01) in Malays. There is little evidence for heterogeneity of effect between the ethnic groups in the associations of DASH with DBP (I^2=11.1%, P=0.308) or SBP (I^2=0%, P=0.421). Heterogeneity of effect is more striking for DASH diet and lipid traits.

3. The Chinese groups is reported to have lower self-reported physical activity and lower DASH scores than Indians, yet the prevalence of disease is said to be higher. This is not discussed at all. Can the authors elaborate on this finding.

We believe the reviewer is commenting on the observation that: i. Physical activity and DASH scores are lowest in Chinese, but highest amongst Indians, and that ii. Since increased physical activity and DASH diet score are considered favourable for health, it is intriguing that the Indian population has higher rates of cardiovascular and metabolic disease compared to Chinese. We agree that these are important findings. They provide key evidence for our limited understanding of risk factors and health outcomes in Asian populations, and show that the dietary scores developed in North American and European settings may not be reliable or valid in Asians populations. This provides the primary motivation for the present study, in which we create a resource focussed on the health outcomes of Asian people, using state-of-the-art approaches.

For example, we highlight our use of metabolite profiling to better understand dietary exposures in Asian populations, using the HELIOS cohort. We link these dietary metabolite exposures robustly to cardiometabolic outcomes in Asians, providing an objective framework for Precision Nutrition in Asian populations (https://doi.org/10.1101/2023.12.04.23299350). We have expanded on this motivation in the **Discussion** section of the revised manuscript (pages 12-13).

4. Page 19–line 626. eQTLGen data were used for the SMR and coloc analysis. eQTLGen data is generated from primarily European cohorts. As both SMR and coloc assume the LD structure to be similar in the two datasets being compared, using eQTL data from European individuals with HELIOS data may lead to inaccurate estimation of the posterior probability (in the case of SMR, the estimation of the HEIDI p-value). This limitation should be discussed.

We agree with the reviewer's remark. To address the concern around the European-centric nature of eQTLGen data, we replicated our leading associations using the eQTL data available for N=1,228 HELIOS study participants. Although the sample size is smaller than eQTLGen, the majority of our top associations replicated (**Supplementary Tables 14 and 15**). We have expanded on these points in our revised **Discussion** section (page 14).

5. Figure 3 – The authors report much larger number of ancestry-unique SNPs and INDELs in Chinese individuals compared to the Malay and Indian cohorts. Is this simply a reflection of sample size? Can the authors provide an interpretation in the Discussion?

We agree that this is likely due to the larger proportion of Chinese individuals in the HELIOS cohort. We have elaborated upon this in the **Discussion** section (page 13).

6. Page 9, line 274-276. The authors stated that the clinical characteristics of the cohort are "broadly representative of the population from which they were recruited". However, comparison statistics from the population were not provided. Please provide the population statistics, and perform statistical tests to confirm the similarity between the cohort and the population. In addition, could the authors also discuss the potential "healthy volunteer bias", which is a problem present in many large Biobanks (e.g. UK Biobank). The assumption is that if there is not significant difference in disease prevalence in HELIOS compared to the general population, there shouldn't be a healthy volunteer bias. Along the same lines, are disease rates similar to those living in China, Malaysia, India?

We thank the reviewer for the comments. We now present a comparison of the characteristics of HELIOS study participants to national statistics for the general population of Singapore (**Supplementary Table 1**). In keeping with our recruitment strategy which aimed to oversample the Indian and Malay ethnic groups, the proportion of people reporting Chinese ethnicity was lower than for the general population. There was some evidence for responder bias, with a higher proportion of women, lower cigarette smoking rates, and greater total years in education amongst study participants, compared to national averages. Nevertheless, the prevalence rates for diabetes, hypertension, hypercholesterolemia and obesity among HELIOS participants were similar to those reported in national statistics, indicating a minor healthy cohort effect overall. We have added these observations to the revised manuscript (page 5).

Supplementary Table 1. Comparison of HELIOS Study with Singapore National Statistics.

	HELIOS			Singapore National Statistics		
Median Age (years)	53.7			40.8[1*]		
Sex (Male: Female**)**	1:1.5			1:1.04[1]		
Ethnicity (Chinese: Malay: Indian**)**	68:14:18			74:13:9[1]		
Education (total year of education**)**	13.8 ± 3.4			11.1[1]		
	Chinese	Malay	Indian	Chinese	Malay	Indian
Lifestyle						
Current smoking, %	6.0	17.8	10.8	8.6[2]	21.1[2]	8.9[2]
Regular alcohol consumption, %	1.6	1.9	0.2	2.4[2]	-	-
Overall health						
Diabetes (Self-reported or fasting glucose ≥ 7.0 mmol/L), %	7.1	16.0	20.1	8.2[2]	14.4[2]	14.2[2]
Hypertension (Self-reported or SBP/DBP ≥ 140/90 mmHg), %	28.4	31.8	32.8	36.1[2]	37.5[2]	29.5[2]
Hypercholesterolemia (Self-reported or LDL≥ 4.100 mmol/L), %	41.8	43.3	42.6	39.6[2]	39.2[2]	37.5[2]
Obesity (BMI ≥ 27.5 Kg/m^2), %	14.3	49.3	41.0	16.1[2]	38.7[2]	31.8[2]

[1]Data were taken from the Population Trend 2018, Singapore Department of Statistics https://www.singstat.gov.sg/-/media/files/publications/population/population2018.pdf.
[2]Data were taken from the National Population Health Survey 2020 (Household Interview and Health Examination) for Singapore residents aged 18 to 74 years, Ministry of Health (https://www.moh.gov.sg/others/resources-and-statistics/nphs-2020).
*The national data were presented in median only for all age groups include children.

We have also provided a comparison of the prevalence for major chronic disease amongst the Chinese, Malay and Indian participants of HELIOS with the populations of China, Malaysia and India (overall, urban and rural) based on statistics from NCD Risk Factor Collaboration (NCD-RisC) and the ICMR-INDIAB-17 study (**Supplementary Table 2**). The health status, and health disparities, of ethnic groups in the HELIOS study, mirror those seen in China and Malaysia and Urban India. These findings have been added to the results section of the revised manuscript (page 5).

Supplementary Table 2. A comparison of disease prevalence between HELIOS and the populations of China, Malaysia and India (Overall, Urban and Rural).

		HELIOS study			NCD Risk Factor Collaboration[1]			INDIAB study[2]	
		Chinese	Malay	Indian	China	Malaysia	India	Urban	Rural
Diabetes[3] (%)	Men	10.0	21.7	26.7	11.7	23.8	20.3	19.2	10.4
	Women	7.4	16.4	21.5	8.8	24.9	22.0		
Hypertension[4] (%)	Men	36.2	38.1	36.1	30.2	40.5	31.6	40.7	33.0
	Women	23.3	28.4	29.7	24.1	41.0	30.5		
HDL cholesterol (mmol/L)	Men	1.4	1.2	1.2	1.2	1.2	0.9	1.1	1.1
	Women	1.7	1.5	1.4	1.3	1.4	1.0		
Total cholesterol (mmol/L)	Men	5.1	5.3	4.9	4.6	5.0	4.2	4.5	4.3
	Women	5.4	5.3	5.1	4.7	5.3	4.2		
BMI ≥25 Kg/m^2 (%)	Men	40.0	70.8	60.6	41.0	51.5	26.1	39.6	23.1
	Women	24.9	68.5	65.4	31.4	57.5	30.8		

[1]Statistics were drawn from NCD Risk Factor Collaboration statistics at year of 2020 for diabetes and obesity, 2019 for hypertension and 2018 for cholesterol https://www.ncdrisc.org/index.html.
[2]Anjana, Ranjit Mohan, et al. "Metabolic non-communicable disease health report of India: the ICMR-INDIAB national cross-sectional study (ICMR-INDIAB-17)." The Lancet Diabetes & Endocrinology 11.7 (2023): 474-489.
[3]Fasting plasma glucose ≥ 7.0 mmol/L, HbA1c ≥6.5% or taking medication for diabetes.
[4]SBP/DBP ≥140/90 mmHg

7. Extended Table 1 and Extended Figure 1 – the authors mentioned that Indian and Malay participants had a higher prevalence for some conditions. What was the statistical test used to confirm the statistical significance of these differences? It would also be great to provide the percentages of disease prevalence in a table.

Thank you for the suggestion. We added the percentages of disease prevalence in **Extended Table 1.** Comparisons between the ethnic groups were done using generalized linear models for categorical measurements and linear regression for continuous variables, with adjustment for age and sex. This has been added to the footnote to **Extended Table 1** and **Methods** section (page 18).

8. Page 6 Line 186. 'Indians and Malays had lower levels of lipid metabolites'. Could the authors discuss if this is related to medication use? Is there greater statin use amongst these two groups?

Statin use was slightly higher in Indian and Malay participants compared to Chinese (21.1% and 17.3% vs 15.7% respectively; $P=3.5x10^{-5}$). However, Indians and Malays had lower levels of lipid metabolites, even after exclusion of individuals on statins, indicating that medication use is

unlikely to explain the biological observation. This has been added to the **Results** section (page 8).

9. In extended figure 1 – the authors presented the prevalence of diagnosed and undiagnosed cases in each group. However, the same clinical thresholds (e.g. blood pressure of 140/90 and BMI of >=30) were used in all three ethnic groups, despite evidence showing that ethnicity-specific cut-offs are needed to achieve the same level of clinical surveillance (e.g. 10.1016/S2213-8587(21)00088-7). Could the authors clarify which guidelines they are using to determine the thresholds, and highlight the ethnicity-specific cut-offs as a limitation of the paper?

We appreciate the comment and recognise that thresholds for clinical diagnosis or intervention may be ethnicity-specific for body mass index (BMI) (DOI: 10.1016/S2213-8587(21)00088-7) and blood pressure (DOI: 10.1186/s12916-024-03259-5). We have added the references used for determining disease prevalences, together with disease prevalences using both global and ethnicity-specific cutoffs in **Extended Table 1** and updated **Extended Figure 1** based on ethnic specific thresholds, where these exist.

10. Page 4, line 91 – what are the "other ethnicities"?

Ethnicity of participants was assessed based on their National Registration Identity Card (NRIC). The term 'other ethnicities' refers to people who did not identify as being of i. Chinese or other East Asian, ii. Indian or other South Asian, or iii. Malay or other Southeast Asian ethnic group. The most common backgrounds for people of 'other ethnicity' were European, Eurasian and Arabic. We included this information in the footnote to **Extended Table 1**.

11. Please standardise the colour scheme used to represent the three different ethnic groups. For example, the colour scheme was not the same between Extended Figure 3c and Figure 4a.

Thank you for highlighting this. We have now standardised the colour coding scheme for Extended Figure 3c, as suggested.

12. Figure 3d, the legend shows Indian as purple, but there are no points of purple colour on the plot.

Thank you for pointing this out. This has been corrected.

13. Though individual level data is not available, could the authors add a data availability statement for any summary statistics (e.g. metabolite GWAS). Will these be made publicly available through online catalogues?

We thank the reviewer for the question. Individual level data are accessible on a collaborative basis through application to the HELIOS study Data Access Committee. The summary statistics generated in the present study will also be made publicly available through GWAS catalogue and our GitHub repository upon publication. We have updated the **Data availability** statement to the manuscript (pages 24-25) to provide the specific details.

Reviewer #2 (Remarks to the Author):

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

Reviewer #3 (Remarks to the Author):

Wang and colleagues describe a unique biobank from Singapore, a country with excellent healthcare system and three major ancestry groups (Chinese, Indian and Malay). Given the scarcity of biobank-grade data on Asian populations other than Chinese and Japanese, this is a welcome contribution. The high quality of data collected on the participants and its linkage to medical records make up for the small cohort size. The paper is very light on discoveries other than comparative analyses between the three groups. It seems that the much anticipated discoveries are reserved for an accompanying submission that this reviewer has no access to. Additionally, there are several concerns that should be addressed:

Major:

1. The RNA-seq data are completely missing, which is not acceptable given that this is one modality of omics that was specifically mentioned by the authors. Needless to say, the transcriptomics data will provide a wealth of information for this comparative analysis. Please refer to PMID: 39383868 as an example of what readers should expect from a study of this nature.

We thank the reviewer for the comment. The eQTL data generated in the HELIOS study participants were used in the initial manuscript, to help identify biological pathways underlying divergent metabolite concentrations between our Asian ethnic groups.

In response to the reviewer's comments we have now added a systematic evaluation of the differences in gene expression between the ethnic groups, that both mirrors and complements the comparison of methylation and metabolite levels between the three main ethnic groups. Results show that 2,972 of the 12,434 genes successfully assayed are differentially expressed between the three ethnic groups ($P<1.34x10^{-6}$). Pathways analysis suggests that the stratified genes identified are enriched in inflammatory processes, as well as thermogenesis and COVID-19 susceptibility. We also observe enrichment for neurological disease associated gene sets such as Amyotrophic lateral sclerosis, Alzheimer's, Huntington and Parkinson's. Genetic association analysis of the gene expression data identified cis-eQTLs for 1,177 of these stratified genes ($P<7.3x10^{-10}$). Compared to the largest publicly available blood based eQTL database, eQTLgen, we identify novel cis-eQTLs for 70 genes (40 protein coding), including *TXNIP,* which encodes a key regulator of glucose uptake. These new data have been added to the revised manuscript (page 9).

We have also updated the **Data availability** statement of the manuscript (pages 24-25) to clarify access to both the individual level data and summary statistics (including the eQTLs) generated in the present study.

2. Similarly, the authors should provide data on the genetic determinants of the CpG methylation pattern differences between the three groups.

The current manuscript includes analyses to show how methylation markers differ between ethnic groups, and how these differences relate to behavioural (eg diet), physiological (eg adiposity) and genomic factors (eg ancestry-based PCs). We agree that carrying out a complete analysis of the genetic determinants of the CpG methylation differences is an important question. MethQTL analyses are a major piece of work, and ongoing within our group, and through collaboration with multiple partners. We believe that our methQTL work is best presented as a dedicated companion paper.

3. Since this is a precision medicine cohort, one would expect the authors to provide a more medically relevant context for their findings. For example, the content of figure 6 is mentioned almost as a note in passing when highlighting the potential of this biobank to inform a deeper understanding of the pathogenesis of chronic diseases without providing details. More such examples should be provided.

The primary purpose of our manuscript is to describe the motivation, design and ambition of the HELIOS study, and to illustrate the potential for this resource to provide new, precision medicine insights relevant to the health of Asian populations.

We fully agree that this resource will provide the basis for deeper understanding of the pathogenesis of chronic disease in Asia. There are already a number of companion manuscripts that are reporting deep evaluation of the dataset to provide insights into i. *FDX1* as a novel determinant of cholesterol metabolism and cardiovascular in Asians (DOI: 10.1101/2024.05.14.24307316); ii. understanding the impact of diet on metabolic variation and chronic disease in the population (Precision nutrition, DOI: 10.1101/2023.12.04.23299350), iii. the relationships of visceral adiposity with metabolic health between the three Asian groups (DOI:10.1016/S2213-8587(24)00195-5) and iv. deep exploration of biological pathways linking DNA methylation to diabetes in Asia (DOI: 10.1101/2025.02.14.25322264). These companion papers are published, or available on MedRxiv. The current primary paper is intended as the gateway to these highly focussed companion efforts, whose depth and scope goes well beyond what we can include in this current flagship resource paper.

4. In line with point #3 above, it is surprising that the authors did not comment at all about the contribution, or lack thereof, of rare variants to the differences between the three groups in their disposition to a wide range of phenotypes. They simply listed the types of variants identified without any clinical context. This is a major deficiency that must be addressed.

In the current report, we have used our WGS data to support our quantitative trait analyses (expression, methylation and metabolite), both in single variant and PRS analyses. As noted in the manuscript, sample size is anticipated to increase ~100,000 by end 2025 and will be the basis for a systematic analysis of rare variants in relation to health outcomes, as a companion paper.

5. The authors assert that the linkage to medical records provide numerous opportunities but the only benefit shown was the demonstration of reproducibility of measurements. For example, could

the "undiagnosed" diseases identified by the investigators through the biobanking process have been predicted from the linked medical records?

We would like to clarify that to assess the reproducibility of our study methods we carried out a structured reassessment of 398 participants, as a recall study.

In relation to the EHR data linked through TRUST, our analyses go substantially beyond the summary set out by the reviewer. In particular, we carry out three primary analyses:

- We show that linked data can be retrieved for 95% of participants, including 712 biochemistry, 84 medication and 14 medical records per participant. This demonstrates the depth and breadth of the linked EHR data, with the potential for EHR linkage to bring a wide range of additional phenotype data into the cohort study.
- We make a comparison of the research phenotype and corresponding EHR phenotypes, to assess the validity of data linked through the EHR. We show moderate to high concordance between the research and EHR traits providing strong evidence for the validity of the EHR datasets.
- Thirdly we use diabetes as an exemplar 'use case' to demonstrate the potential utility of the EHR linkage for ascertaining and exploring longitudinal health outcomes. We successfully show identification of people with new onset diabetes, (~1% incidence per year, see **Extended Figure 5**), and demonstrate risk associations with age, adiposity, glucose and metabolic traits. This analysis acts a as 'positive control' by providing proof for the validity and utility of the linked EHR data for identification of longitudinal health trajectories and their risk factors.

We note that while EHR linkage is routine and well established in US and Europe, there is less experience or implementation of EHR linkage in Asia. Indeed, the present report describes the first approved use of the TRUST platform for EHR linkage in Singapore. In that context, we believe that our analyses well considered, by providing initial evidence for the depth, breadth, validity and utility of linked EHR datasets in Singapore, and the opportunity to accelerate Precision Medicine research for Asian populations. The results set the scenes for the deeper use of the EHR data to advance understanding of chronic disease in future research.

Minor:

1. Please provide more explanation of Figure S4.

We have updated the figure legend of **Extended Figure 4**, to further explain the findings, as suggested.

2. There is an error in the color code of Figure 3B.

Thank you for pointing this out. This has now been corrected.

Reviewer #4 (Remarks to the Author):

The manuscript describes the data collection and first analyses of a novel cohort of 10k individuals of Asian descent, separated into Chinese, Malay and Indian background. Samples were collected by random sampling as much as possible, to represent these general populations, and extensive phenotyping was performed at baseline, enriched with various medical/lifestyle data-sources, and the population is being followed-up. Extensive genomic measurements have been performed, including WGS on all individuals, and RNAseq, methylation, metabolomics, microbiomics on subsets. Initial results are performed to demonstrate the generalizability of the cohort, confirming a number of expected but interesting associations, and zooming in on the differences between the three ancestry groups to highlight the value of this dataset for clinical and fundamental research. The work has been performed in accordance with current state-of-the-art practices and is well written. I agree with the authors that this data-source is a valuable contribution to the scientific field in general, and the alleviate the missing data on Asian population in specific. I have some – mostly clarifying – questions.

1. Recruitment of these samples was done via random sampling as much as possible. The characteristics of the cohort are displayed in table 1. To understand the generalizability of the cohort to the wider Singapore (or Asian) population, it would be helpful to include a paragraph on how these characteristics compare to these generalized populations. For example, what was the participation rate, and do the authors envision any selection biases (e.g., healthier or higher educated participants compared to general population, as is common in population studies) that potential users of this resource should be aware of?

We thank the reviewer for their comments, and for noting the valuable nature of the resource that we have created. As noted in our responses to Reviewer #1, we now provide a comparison between the characteristics of HELIOS study participants, and the national statistics for the general population of Singapore (**Supplementary Table 1**). There was some evidence for responder bias, with a higher proportion of women, lower cigarette smoking rates, and greater total years in education amongst study participants, compared to national averages. Nevertheless, the prevalence rates for diabetes, hypertension, hypercholesterolemia and obesity among HELIOS participants were similar to those reported in national statistics, indicating a minor healthy cohort effect overall. We have added these observations to the revised manuscript (Page 5).

2. Similarly, the authors compare and comment on differences between the three subgroups. It would be useful to either comment on the expected generalizability of these findings to their respective populations, and/or subset the three subgroups to similar phenotypic characteristics and repeat the omics-phenotype associations to allow for more direct comparisons. For example, the authors note that the rate of T2D is higher in the Indian/Malay population, and also note that there are CpGs differential in these populations, and finally that these overlap to some extent. Is the CpG difference caused by the different ancestries, or by the different rate of cases?

As noted in response to the reviewer's first comment, the characteristics of the study participants are similar those of the general population. As such, we believe that the differences observed in

molecular phenotypes between the ethnic groups mirrors differences that exist in the wider population.

We have carried out a number of analyses to identify the risk factors and disease traits factors that are driving the observed differences in methylation between the ethnic groups. We show that the differences in methylation reflect both behavioural exposures such as dietary factors, as well as genetic background (as assessed by ancestry related genetic PCs). We also use genetic instruments to assess whether the observed relationship between exposures and methylation levels is likely to be causal. For example, we identify that BMI, body fat and central obesity are strongly associated with the methylation markers that are divergent between the ethnic groups, and then use PRS to show that the effect of adiposity on these methylation markers is likely to be causal. We have updated the text to better describe the differences in methylation between ethnic groups, and how these relate to behavioural and genetic exposures (pages 7-8).

Minor comments

3. It is not explicitly stated if the consent/data access allows for international sharing of this resource, and if so which level of data can be shared (e.g., summary statistics only, vs access to processed omics data and phenotypes). Some more details may be useful.

We thank the reviewer for the question. Individual level data are accessible through application to the HELIOS study Data Access Committee. The summary statistics generated in the present study will also be made publicly available through GWAS catalogue and our GitHub repository upon publication. We have updated the **Data availability** statement to the manuscript to describe this (pages 24-25).

4. I believe at least some analyses were stratified on sex. It may be nice to illustrate more explicitly if/when some sex-specific results were observed.

The current manuscript is focused on comparisons between the three main Asian ethnic subgroups. We adjusted for sex in our regression models that explore differences in traits and disease between the ethnic groups, but did not carry out any sex stratified analysis. We have clarified where we adjust for sex as a covariate in the revised manuscript.

5. Of the coding variants, 88,995 are protein altering, 82,831 of those are indels - is that correct? In other databases, such as gnomAD, the number of missense single nucleotide variants is much higher than the number of frameshift indels. Perhaps I misunderstood the comparison made here, but then please clarify.

We thank the reviewer for pointing this out. There was a mistake in our annotations which has now been fixed. The number of missense variants (non-synonymous variants, N = 1,373,981) is significantly higher than indels (N = 108,744). We have revised the results, table and figure accordingly to reflect the changes.

6. P5, line 24 - without the methods it's a bit hard to place this in context, please add a line stating how many PRS were tested, and perhaps where they came from.

Thank you. We now provide details of the PRS tested in **Supplementary Table 4**, and in the **Methods** section of the revised manuscript (page 20).

7. P10, line 1, seems to be a typo around "are linked".

Thank you for pointing this out. This has been corrected.

8. The figures 5 and 6 have a partially transparent background, causing them to be displayed incorrectly against dark backgrounds.

Thank you for highlighting this. We have corrected the figures accordingly in the revised manuscript.

Thank you again for your consideration. We look forward to hearing from you.

Yours Sincerely,

John Chambers
Professor of Cardiovascular Epidemiology
Lee Kong Chian School of Medicine
Nanyang Technological University

Reviewer #1

Thank you the authors for addressing my comments. I have additional comments on the PRS analysis.

a. The authors make statements such as "Mean values for PRS vary between populations (Figure 3c). The strongest separation was observed for depression, with higher PRS amongst Indians compared to Chinese and Malays (P=2.8x10-162)."

"Based on the difference in PRS between the populations, and the observed risk ratios for T2D, we estimate that the small increase in PRS observed in Indian and Malay populations accounts for only ~5% of their three-fold excess risk of T2D compared to Chinese participants (Indians: 5.7 [95%CI: -1.3 to 12.6]%; Malay: 3.1 [95%CI: -5.4 to 11.6]%, Supplementary Methods).

The difference in PRS means across populations is confounded by differences in LD and allele frequency across different populations. It is not indicative of a difference in genetic risk burden between populations. Therefore, analyses looking at whether a difference in mean can explain the observed differences in prevalence are not appropriate.

We thank the reviewer for their perspective.

We agree that the relationship of PRS with disease risk is influenced by effect size, allele frequency and LD between the causal and measured variant. We also agree that this may impact the transferability of ancestry specific PRS to populations from which they were not derived. We have therefore removed reference to comparisons of PRS across ethnic groups, where these PRS are based on *single ancestry* data (e.g. PRS derived solely from European or East Asian populations).

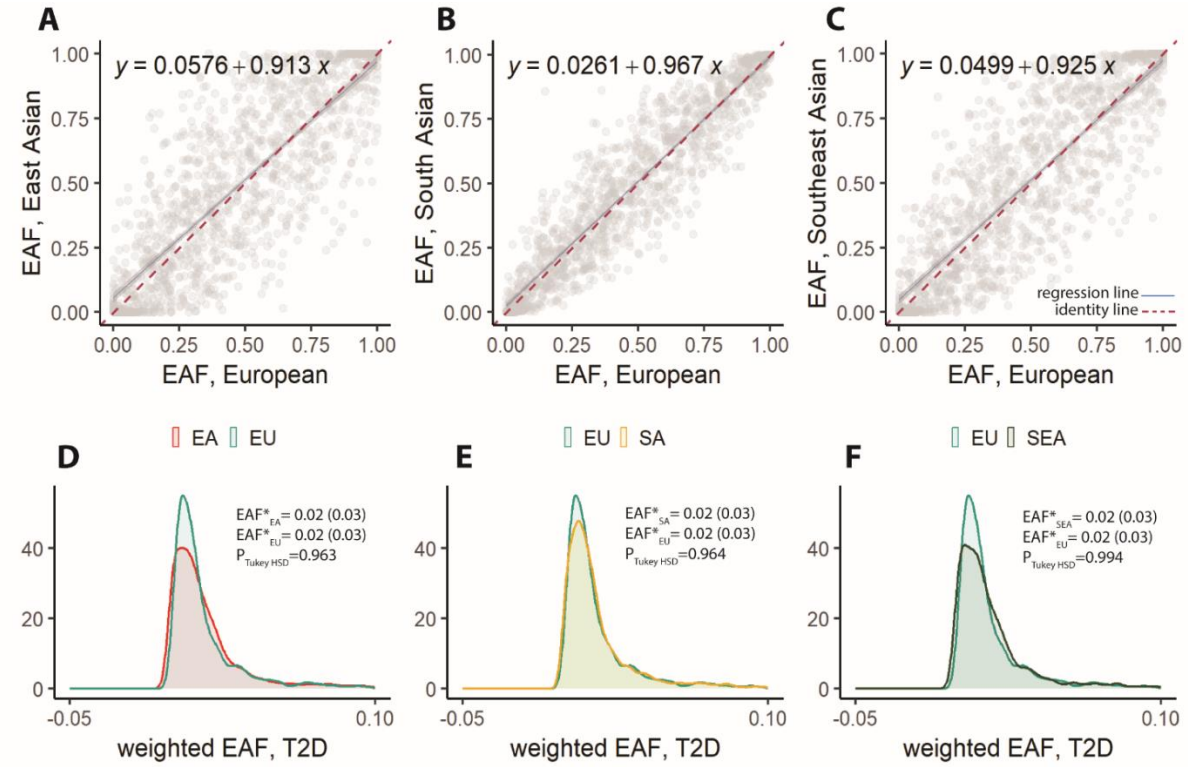
In contrast, *trans-ethnic* PRS are now based on robust estimates for each of the necessary components, especially when these incorporate large sample size, as is the case for the current iteration of T2D GWAS. Our recent collaborative studies show that trans-ethnic PRS for T2D have highest discrimination and prediction compared to ancestry specific PRS, and are also generalisable across the out-of-Africa populations, including Europeans (EUR), South Asians (SAS), East Asians (EAS), and Americans (AMR). In support of this statement, we provide the key results from our current manuscript. In particular, we highlight the 6th paragraph of the **Results** section, and **Supplementary Figure 3**. We also highlight that these analyses are based on GWA datasets from 360,000 cases and 1.8 million controls, and include multiple global ancestral groups.

Our evaluation of the T2D PRS is further strengthened by study design. We investigated three distinct Asian populations in the same environmental setting, using best practice, standardised clinical and molecular characterisation. We show a 3-fold differences in T2D prevalence between the Asian ethnic groups, that is consistent with disease burden in other urban Asian populations. We also use whole genome sequencing to give precise estimates of effect allele frequency, avoiding the errors which can arise based on sparse genotyping and imputation from ancestrally inaccurate reference panels.

To provide additional evidence in support of our view that the genetic variants currently identified by GWAS do not explain differences in risk of diabetes *between* populations we highlight that in the most recent T2D GWAS [DOI: 10.1038/s41586-024-07019-6], >90% of genetic effects are shared between populations with no evidence for heterogeneity of effect between EUR, SAS and EAS. We also show (**Revision Figure 1** and new **Supplementary Figure 3**) that distributions for: i. Effect Allele Frequency, and ii. Effect Allele Frequency weighted by effect size (beta), are overlapping in our populations, providing direct evidence that there is no systematic shift in genetic susceptibility between the populations of the magnitude needed to explain a 3-fold difference in disease risk between populations.

Given the strengths of our approach, and the additional lines of evidence above, we suggest retaining the data and arguments set out, but adding the qualification that although the analysis is based on the best available trans-ethnic PRS, there may be residual limitations arising from differences in LD structure. We hope this meets with the reviewer's support.

Revision Figure 1. Distribution of EAF (Panels **A** to **C**) and EAF weighted by effect size (Panels **D** to **F**) in East Asian, Southeast Asian, South Asian and European populations, for the 1270 genetic variants reported to be associated with T2D [DOI: 10.1038/s41586-024-07019-6]. EAF is based on WGS data for our HELIOS Study participants.



b. On the analysis using ancestry-specific PRS, the authors state "results show that the performance of these ancestry specific PRS as assessed by both odds ratio and R2 values is similar to the trans-ancestry model based PGS Scores". However, some of the ancestry-specific PRS only have a small number of variants (Suppl Table 4. 10 for EAS CAD PRS and 275 for SAS T2D PRS) compared to >1 million variants in the Euro-centric PRS for these 2 traits. It is well-known that P+T underperforms compared to more recent Bayesian method. To make R2 more comparable, the same method should be applied to the summary

statistics from different GWAS, which are publicly available. Bayesian methods like SBayesR, SBayesRC, LDpred2 do not require training/validation sets and therefore can simply be applied to GWAS summary statistics to obtain independent SNP sets and weights that can then be used in the target population.

We thank the reviewer for the suggestion. We agree that there are numerous emerging methods for estimation of PRS, that are improving assessment of genetic risks in the population. The questions posed by the reviewer are fair and valid, but are a major piece of work, that would hold sufficient work to represent a full manuscript on their own. The present communication is focussed on describing the existence, strengths and opportunities of our Precision Medicine cohort, and to set the foundations for future efforts using the resource. An analysis of PRS will be a separate, dedicated report.

c. For the Eurocentric PRS, it would help for to put the R2 values in context. For example, the R2 for depression ranges from 0.005 - 0.3%, compared to T2D which range from 5-7%. Is this a reflection of a lower genetic contribution (i.e. SNP-based heritability) in general to depression compared to T2D or a lower generalisability of the European PRS for depression? Could the authors add the R2 reported in published literature for these PRS in Europeans as this would provide a better understanding of the difference in R2 across traits.

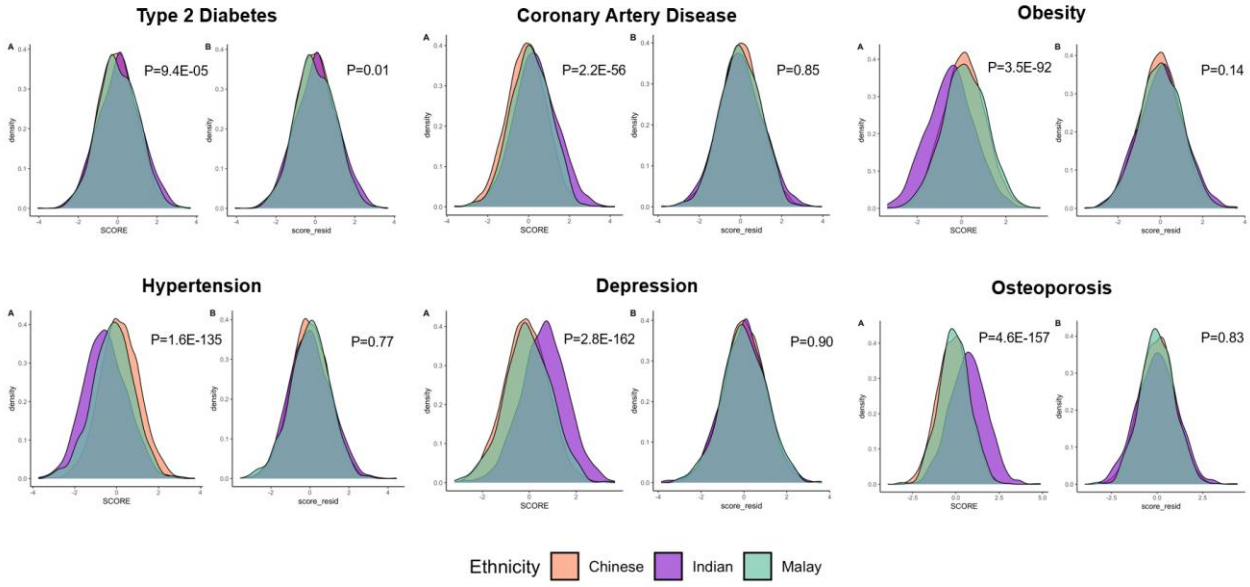
Thank you. We have added this information to the **Supplementary Table 5**, where it is available in the respective publication.

d. The response to my comment on PRS recalibration is as follows: "The aim of this analysis was to compare the contribution of polygenic risk to disease between the three ancestry groups. To achieve this, we calculated the PRS for each cohort participant using the same set of PRS-specific genetic variants. We then applied Z-score standardization across all participants, to enable relationships to disease risk, and differences between ancestral groups, to presented on a per SD scale. This approach facilitates comparisons of effect size and ancestry based risk scores between the PRS evaluated. We have updated the Methods section (page 20) accordingly. In contrast, using methods for ancestry adjustment such as residualizing PCs (https://doi.org/10.1186/s13073-022-01074-2) would remove the difference due to the ethnicity, and thus would not enable us to address our specific scientific question."

As highlighted above, the difference in PRS distribution across the 3 ancestry groups is reflective of differences in LD and allele frequency in these populations compared to a European population. It does not indicate whether the genetic risk burden is higher in one group compared to another. This is why post-hoc methods for standardising the PRS for genetic ancestry onto a standard scale have been proposed so that OR can be directly compared. In addition, the post-hoc PC adjustment in the referenced paper is done within each ancestry group because genetic ancestry is continuous (see https://www.nature.com/articles/s41586-023-06079-4). The referenced paper uses the method by Wang et al. https://pubmed.ncbi.nlm.nih.gov/32762905/ who use this framework to evaluate a CAD PRS for South Asians.

The analysis we have carried out aims to test whether differences in *trans-ancestry* PRS **between** the ethnic groups contribute to their documented 3-4 fold variation in risk of T2D. Respectfully, we believe that using genetic PCs as covariates in such a model is circular, as the effect of correcting for genetic PCs is to regress out genetic ancestry (i.e. it removes the very outcome measure being tested). To illustrate this, we show below the impact of adjusting for genetic PCs on all PRS evaluated, whether these are based on single or trans-ancestry approaches (**Revision Figure 2**). As we expect, in each case, there is no difference in PRS between the ancestral groups *after adjusting for PCs of genetic ancestry*.

Revision Figure 2. Distribution of PRS values amongst Chinese, Indian and Malay individuals before (Panela A) and after (Panel B) adjusting for the top 5 PCs of genetic ancestry. Anova - P values are for comparison of respective PRS values between the ethnic groups.



We note a separate issue around the application of PRS across ethnicity that may be perceived as a cause for concern; specifically that the odds ratios for disease association **within** a group may be confounded by genetic structure if they are estimated in an admixed or structured population. To counter this possibility, we already assessed disease risk relationships in the three Asian ancestral groups separately, which should remove confounding by population structure. Nevertheless, to address this potential concern, we now also show that additional correction for genetic PCs has no material effect on relationships to T2D (**Revision Table 1**). This provides further evidence of the validity of our approach.

e. The entry for the South Asian PGS catalog lists 282 variants, whereas Suppl Table 4 lists 275. What is the reason for this discrepancy?

Many thanks. This is because there were 7 variants which are not available in our QC-ed genomic dataset. This is clarified in the revised manuscript.

Revision Table 1. Odds ratio for disease per 1SD change in respective PRS, before and after adjustment for PCs of genetic ancestry.

	OR (95% CI) ***Without PCs***	**OR (95% CI)** ***With PCs***
Chinese		
Type 2 diabetes	2.17 [1.91-2.47]	2.14 [1.89-2.43]
Coronary artery disease	1.25 [1.12-1.41]	1.24 [1.12-1.40]
Obesity	1.41 [1.25 -1.58]	1.39 [1.24-1.55]
Hypertension	1.71 [1.57-1.85]	1.68 [1.55-1.82]
Depression	1.15 [1.03-1.29]	1.15 [1.03 -1.30]
Osteoporosis	1.26 [1.13 -1.41]	1.25 [1.12-1.39]
Indian		
Type 2 diabetes	2.17 [1.91-2.47]	2.14 [1.89-2.43]
Coronary artery disease	1.25 [1.12-1.41]	1.24 [1.12-1.40]
Obesity	1.41 [1.25 -1.58]	1.39 [1.24-1.55]
Hypertension	1.71 [1.57-1.85]	1.68 [1.55-1.82]
Depression	1.15 [1.03-1.29]	1.15 [1.03 -1.30]
Osteoporosis	1.26 [1.13 -1.41]	1.25 [1.12-1.39]
Malay		
Type 2 diabetes	2.17 [1.91-2.47]	2.14 [1.89-2.43]
Coronary artery disease	1.25 [1.12-1.41]	1.24 [1.12-1.40]
Obesity	1.41 [1.25 -1.58]	1.39 [1.24-1.55]
Hypertension	1.71 [1.57-1.85]	1.68 [1.55-1.82]
Depression	1.15 [1.03-1.29]	1.15 [1.03 -1.30]
Osteoporosis	1.26 [1.13 -1.41]	1.25 [1.12-1.39]

Reviewer #2

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

Reviewer #3

I thank the authors for their thoughtful response. The inclusion of transcriptomic data is a welcome change. While I understand the authors' desire to delay a comprehensive rare variant analysis for a subsequent publication, it is still important to highlight at least a few examples based on what they already have.

We are grateful to the reviewer for their comments.

As requested, we have added an exemplar rare variant analysis (the 5th paragraph of the **Results** section, **Supplementary Table 4** and **Supplementary Figure 2**). This focusses on three genes that cause Familial Hypercholesterolaemia (ApoB, PCSK9 and LDLR). We evaluate the carrier frequencies of rare pathogenic variants across the three ethnic groups, and show clinical correlation. We demonstrate differences in the frequency pathogenic mutations between populations, as well as the presence of clinically important allelic heterogeneity. We observe the highest burden of ClinVar supported pathogenic variants amongst Chinese individuals (0.82%) compared to Malay (0.31%) and Indians (0.13%). This difference was reduced by inclusion of predicted pathogenic variants, suggesting under-reporting of Malay-specific variants in curated databases. We thank the reviewer for the suggestion, which has strengthened the report.

Reviewer #4

My questions have been sufficiently answered, I appreciate the extra work that was performed.