nature portfolio

Peer Review File

Exome-wide genetic risk score (ExGRS) to predict high myopia across multi-ancestry populations

Corresponding Author: Professor Jianzhong Su

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
This paper develops a method for combining both common and rare variants into polygenic risk score calculations, using high myopia as the phenotype of interest. The method uses a relatively standard approach for the common variants, but then considers rare variants per gene, with greater weighting to variants that have likely impacts on the protein. The study uses exome sequencing data and calculates effect sizes for common variants and genes with rare variants in a training set of data from Han Chinese, before testing the resulting scores in two smaller groups of Han Chinese and the UK Biobank.
Comments for the authors to address:
1. Please clarify the numbers of individuals at each stage and ensure the methods match the details given in the results and discussion. It is confusing as to how many people were in the training set, variably presented as 6300 cases and 6300 controls (figure 4), or 12000 (discussion) or 12600 i (results). Similarly, Figure 4 does not reflect the 8682 individuals from the UKB, referring only to 1200 UKB samples used in the final testing stage.

2. The methods makes reference to an overall study size for MAGIC of 21,227 and 130,494 for UKB. Please explain how the subsets of samples from each of these cohorts was selected.

3. Please provide the definition of high myopia used in the study.

4. My interpretation of the methods is that the validation and testing of the exGRS in the two smaller Han Chinese cohorts used all the available variants that met inclusion and filtering criteria, but when the score was tested in UK Biobank (European) samples, only variants that overlapped between the two datasets were included. The authors then conclude, (lines 196 to 203) that rare variants explain less heritability than common variants in Europeans, in contrast to their findings in Han Chinese. I would contend that to make this comparison, the score should be re-derived in a European discovery set and include rare variants unique to those data. An alternative conclusion for the data presented is that the Han Chinese exGRS is not very useful in Europeans.

5. When annotating rare variants, how were variants with discordant annotations between the three tools (CADD, SIFT, Polyphen) handled?

6. Please carefully review the English grammar, particularly in the introduction.

7. Can the authors please elaborate/clarify what they mean by “No more clinical interventions or examinations are required…” at line 62 in the introduction. I think they are trying to indicate that we can assess genetic risk for almost any complex trait from a single blood sample, but it reads as if the blood test eliminates the need for clinical care of those diseases.

Reviewer #3

(Remarks to the Author)
1. The current study focused solely on the genetic components of high myopia, without considering the important roles of environmental and lifestyle factors. How would the authors address the accuracy and reliability of the results?

2. Two distinct ancestral populations (Han Chinese and European) were studied. Would it be the potential introduction of population stratification? which can lead to spurious genetic associations if not properly accounted for.

3. Although 12,000 individuals from the MAGIC cohort and 8,682 from the UK Biobank are relatively large sample sizes, still may be insufficient to fully capture the genetic complexity of high myopia, especially for rare variant analysis. Would there be any sample size bias in the study?

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)

The authors have largely addressed my questions and made appropriate updates to the manuscript, however several minor things remai:

1. Introduction Lines 62-63: Thank you for clarifying this confusion, however, I somewhat disagree. The genetic test may able to predict risk, but an individual will still require tests or examinations for diagnosis. The genetic test may indicate that these should be more frequent than for someone at lower risk, but predicting risk does not eliminate the need for additional tests.

2. Line 66: Please define the abbreviation AUROC at first use and if it is not used again, please spell out in full.

3. Results line 85: The number of variants should probably be 2.2x10^6 for 2.2 million and 2.6x10^6 for 2.6 million (not 2.2x10^-6 which is 0.000022)

Reviewer #2

(Remarks to the Author)

Point-to-point response to the reviewers (COMMSMED-24-0347-T)

Reviewer #1

Comments for the Author

This paper develops a method for combining both common and rare variants into polygenic risk score calculations, using high myopia as the phenotype of interest. The method uses a relatively standard approach for the common variants, but then considers rare variants per gene, with greater weighting to variants that have likely impacts on the protein. The study uses exome sequencing data and calculates effect sizes for common variants and genes with rare variants in a training set of data from Han Chinese, before testing the resulting scores in two smaller groups of Han Chinese and the UK Biobank.

Reply - We greatly appreciate reviewer #1's recognition to the quality and importance of our work.

Comments for the authors to address:

1. Please clarify the numbers of individuals at each stage and ensure the methods match the details given in the results and discussion. It is confusing as to how many people were in the training set, variably presented as 6300 cases and 6300 controls (figure 4), or 12000 (discussion) or 12600 (results). Similarly, Figure 4 does not reflect the 8682 individuals from the UKB, referring only to 1200 UKB samples used in the final testing stage.

Reply - Thank you for this observation. We acknowledge the inconsistencies in the reported numbers and have made the following adjustments: In the Methods section, we have now clarified that the training set consisted of 6300 cases and 6300 controls, making a total of 12,600 individuals. The previous mention of 12,000 individuals was incorrect, and we have corrected it accordingly. In the Results and Discussion sections, the numbers have been consistently revised to reflect the correct total of 12,600 individuals in the training dataset, 5,400 individuals in the validation dataset and 1,219 individuals in the testing dataset. We also revised the figures to correctly indicate the number of samples used. Regarding the UKB dataset, we have revised manuscript to correctly show the total number of 8,682 individuals used in the final testing stage. This ensures that the figure now accurately reflects the different stages of the analysis. These changes have been carefully implemented to align all reported figures across the manuscript and improve overall clarity. We appreciate the reviewer's attention to these details, which has strengthened the consistency of our work.

2. The methods makes reference to an overall study size for MAGIC of 21,227 and 130,494 for UKB. Please explain how the subsets of samples from each of these cohorts was selected.

Reply - Thank you for your thoughtful advice. We have added the description of the sample selection in the Methods section of the manuscript. "The analysis presented here is based on 21,227 unrelated human samples collected from epidemiological studies of myopia. After removing samples showing

poor sequencing quality or ambiguous sex status, population outliers identified by principal component analysis (PCA), we random selected approximately 70% (12,600) of participants as training samples, whereas the remaining 30% (5,400) were assigned as validation samples.

UKB measured refractive error of 130,494 participants by non-cycloplegic autorefraction using a TomeyRC-5000 AutoRefractor Keratometer. We excluded unreliable refractometry results and calculated the spherical equivalent (SE) as spherical refractive error plus half the cylindrical error. In addition, samples identified as outliers in heterozygosity and missing rates, participants with sex discrepancy, and individuals of non-Caucasian ancestry were removed in our study according to the sample QC provided by UKB. We estimated relatedness in each cohort by PLINK and only kept one of any pair of individuals with relatedness ($\hat{\pi}$) > 0.2. Finally, we identified 2,096 myopia cases (participants with SER of single eye ≤ -6.0D) and 6,586 controls (participants with SER of single eye > -0.25D)."

3. Please provide the definition of high myopia used in the study.

Reply - Thank you for your thoughtful advice. In the study, high myopia is defined as a spherical equivalent refraction (SER, sphere + [cylinder/2]) of single eye -6.00 diopters(D) or less.

4. My interpretation of the methods is that the validation and testing of the exGRS in the two smaller Han Chinese cohorts used all the available variants that met inclusion and filtering criteria, but when the score was tested in UK Biobank (European) samples, only variants that overlapped between the two datasets were included. The authors then conclude, (lines 196 to 203) that rare variants explain less heritability than common variants in Europeans, in contrast to their findings in Han Chinese. I would contend that to make this comparison, the score should be re-derived in a European discovery set and include rare variants unique to those data. An alternative conclusion for the data presented is that the Han Chinese exGRS is not very useful in Europeans.

Reply - Thank you for your insightful comment. We agree that the comparison between the Han Chinese and European populations is complex due to differences in genetic architecture, including the distribution and effect sizes of rare variants. We have made the following clarifications and adjustments.

1. Actually, based on all 2.2×10^{-6} variants in UKB, only 0.05 of the phenotypic variance is accounted for rare variants but 0.17 for common variants, which suggested rare variants explain less genetic heritability than common variants in the UKB European populations (Figure1).

2. We have revised the results that the rvGRS provides limited improvement over cvGRS in the prediction of HM risk in lines 196 to 203.

3. We acknowledge that the Han Chinese ExGRS does not fully capture the genetic risk for high myopia in European populations. We have revised the Discussion section to emphasize this limitation, noting

that rare variants contributing to heritability in Han Chinese may not be directly transferable to European populations.

4. We appreciate the suggestion regarding an alternative conclusion. In line with this, we have revised our conclusions to reflect that while the Han Chinese ExGRS is robust within the Han Chinese population, its utility in European cohorts is limited. We now emphasize the need for population-specific genetic risk scores to optimize prediction accuracy across diverse ancestries.

We believe these revisions address the concerns raised and provide a more balanced interpretation of our findings.

5. When annotating rare variants, how were variants with discordant annotations between the three tools (CADD, SIFT, Polyphen) handled?

Reply - Thank you for your insightful comment. We understand that discordant annotations between different tools (CADD, SIFT, PolyPhen) can introduce variability in the classification of rare variants. In our study, when all three tools produced conflicting results, we adopted a conservative strategy by excluding such variants from downstream analysis. This ensured that only variants with consistent or high-confidence deleterious predictions were retained. We have updated the Methods section to include this explanation, ensuring that the process for handling discordant annotations is transparent and reproducible. We hope this addresses the reviewer's concern.

6. Please carefully review the English grammar, particularly in the introduction.

Reply - Thank you for your thoughtful advice. We have thoroughly reviewed the introduction for grammatical issues and made several improvements to enhance clarity and readability. Specifically, we focused on: Correcting sentence structures to avoid run-on sentences. Ensuring subject-verb agreement and consistent verb tenses throughout the introduction. Clarifying ambiguous phrases to improve overall readability. We believe these revisions address the concerns raised and improve the overall quality of the manuscript. We appreciate the reviewer's attention to these details, which has helped us strengthen the paper.

7. Can the authors please elaborate/clarify what they mean by "No more clinical interventions or examinations are required…" at line 62 in the introduction. I think they are trying to indicate that we can assess genetic risk for almost any complex trait from a single blood sample, but it reads as if the blood test eliminates the need for clinical care of those diseases.

Reply - Thank you for pointing out the potential ambiguity in our wording. We acknowledge that the original phrasing may have inadvertently suggested that clinical care or further interventions would no longer be necessary after genetic risk assessment, which was not our intention. To clarify, our intended meaning was that genetic risk scores, derived from a blood or saliva sample, provide a non-invasive and

efficient way to assess predisposition to complex traits or diseases without requiring additional invasive tests for the purpose of risk prediction alone. The revised text now reads:“ Blood or saliva samples can be used to predict a wide range of conditions, eliminating the need for any additional invasive examinations or tests.”

Reviewer #2

Comments for the Author

1. The current study focused solely on the genetic components of high myopia, without considering the important roles of environmental and lifestyle factors. How would the authors address the accuracy and reliability of the results?

Reply - Thank you for highlighting this important aspect. We agree that environmental and lifestyle factors, such as near-work activities, outdoor exposure, and educational attainment, play significant roles in the development and progression of high myopia. While our study primarily focused on the genetic architecture of high myopia, we acknowledge that incorporating environmental and lifestyle data could provide a more comprehensive model for risk prediction. To address this limitation, we have included a discussion in the revised manuscript acknowledging the impact of non-genetic factors and emphasizing that our genetic risk score (ExGRS) is intended to be one component of a broader, multifactorial approach to myopia risk assessment. Additionally, we have highlighted the need for future studies to integrate both genetic and environmental data to improve prediction accuracy. We believe our findings still provide valuable insights into the genetic predisposition to high myopia, but we recognize the need for caution when interpreting the results in isolation from other contributing factors.

2. Two distinct ancestral populations (Han Chinese and European) were studied. Would it be the potential introduction of population stratification? which can lead to spurious genetic associations if not properly accounted for.

Reply - We appreciate the reviewer's concern regarding population stratification. To mitigate the risk of spurious associations due to population structure, we firstly calculated and adjusted for the first 20 principal components in both the MAGIC and UK Biobank cohorts to account for population stratification within each cohort. To minimized the risk of cross-ancestry biases affecting the results, we used variants with concordant direction-of-effect between MAGIC and UKB to improve the trans-ethnic performance of the ExGRS. We have carefully interpreted the results when comparing findings between the Han Chinese and European cohorts, recognizing that differences in linkage disequilibrium patterns, allele frequencies, and genetic architecture may contribute to variations in the performance of the genetic risk scores across populations. Finally, we have included a discussion in the revised manuscript to highlight this potential limitation and suggest further studies using larger, more diverse multi-ancestry datasets to refine cross-population genetic predictions.

3. Although 12,000 individuals from the MAGIC cohort and 8,682 from the UK Biobank are relatively

large sample sizes, still may be insufficient to fully capture the genetic complexity of high myopia, especially for rare variant analysis. Would there be any sample size bias in the study?

Reply - Thank you for your insightful comment. While our study includes a considerable sample size, we acknowledge that the detection of rare variants associated with high myopia remains challenging, and larger sample sizes are required to achieve robust statistical power in rare variant analyses. To mitigate potential sample size bias, we used aggregated burden tests (e.g., SKAT-O) that increase power by combining information across multiple rare variants within genes. We also validated the findings in distinct cohorts (5,400 individuals in the validation dataset and 1,219 individuals in the testing dataset from MAGIC cohort and UKB cohort), which increases the robustness of our results and helps to minimize potential biases arising from limited sample sizes. We emphasize the need for larger, well-powered studies in the future to further validate these associations. We believe our study provides important initial insights into the genetic basis of high myopia, but we fully agree that larger cohorts will be crucial for uncovering additional rare variants with significant effects.

Point-to-point response to the reviewers (COMMSMED-24-0347A)

Reviewer #1

Comments for the Author

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Reply - We greatly appreciate reviewer #1's recognition to the quality and importance of our work.

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Reply - Thank you for pointing out the potential ambiguity in our wording. The revised text now reads: "Blood or saliva samples can be used to predict a wide range of conditions, providing the complementary need for any additional examinations or tests for diagnosis."

2. Line 66: Please define the abbreviation AUROC at first use and if it is not used again, please spell out in full.

Reply - Thank you for your thoughtful advice. The revised text now reads: "The best area under the receiver operating characteristic curve (AUROC) for HM is 0.783 and 0.672 in European and East Asian populations, respectively."

3. Results line 85: The number of variants should probably be 2.2x10^6 for 2.2 million and 2.6x10^6 for 2.6 million (not 2.2x10^-6 which is 0.000022)

Reply - Thank you for your thoughtful advice. We have changed "2.2x10^-6 variants" to "2.6 and 2.2 million variants" in our revised manuscript.