nature portfolio

Peer Review File

Multi-ancestry genome-wide association analyses of refractive error augment genetic discovery and polygenic prediction

Corresponding Author: Professor Jian Yang

A version of this paper was originally rejected for publication by Nature Genetics, however that decision was reconsidered after appeal by the authors.

Version 0:

Decision Letter:

5th December 2023

Dear Jian,

Your Article "Cross-ancestry genome-wide association study augments genetic insights and clinical potentials for refractive error" has been seen by three referees. You will see from their comments below that, while they find your work of potential interest, they have raised substantial criticisms that must be addressed. In light of these comments, we cannot accept the manuscript for publication at this time, but we would be interested in considering a suitably revised version that addresses the referees' concerns.

We hope you will find the referees' comments useful as you decide how to proceed. If you wish to submit a substantially revised manuscript, please bear in mind that we will be reluctant to approach the referees again in the absence of major revisions.

To guide the scope of the revisions, the editors discuss the referee reports in detail within the team, including with the chief editor, with a view to identifying key priorities that should be addressed in revision, and sometimes overruling referee requests that are deemed beyond the scope of the current study. In this case, we ask that you address all technical queries related to the association and polygenic risk score analyses, extending the analyses to include additional benchmarking where appropriate, adjusting interpretations where needed, and revising the presentation for clarity throughout. We hope you will find this prioritized set of referee points to be useful when revising your study. Please do not hesitate to get in touch if you would like to discuss these issues further.

If you choose to revise your manuscript taking into account all reviewer and editor comments, please highlight all changes in the manuscript text file. At this stage, we will need you to upload a copy of the manuscript in MS Word .docx or similar editable format.

We are committed to providing a fair and constructive peer-review process. Do not hesitate to contact us if there are specific requests from the reviewers that you believe are technically impossible or unlikely to yield a meaningful outcome.

If revising your manuscript:

*1) Include a “Response to referees” document detailing, point-by-point, how you addressed each referee comment. If no action was taken to address a point, you must provide a compelling argument. This response will be sent back to the referees along with the revised manuscript.

*2) If you have not done so already, please begin to revise your manuscript so that it conforms to our Article format instructions, available here.
Refer also to any guidelines provided in this letter.

*3) Include a revised version of any required Reporting Summary: https://www.nature.com/documents/nr-reporting-summary.pdf
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If you wish to submit a suitably revised manuscript, we hope to receive it within 3-6 months. If you cannot send it within this time, please let us know. We will be happy to consider your revision so long as nothing similar has been accepted for publication at Nature Genetics or published elsewhere. Should your manuscript be substantially delayed without notifying us in advance and your article is eventually published, the received date would be that of the revised, not the original, version.

Please do not hesitate to contact me if you have any questions or would like to discuss the required revisions further.

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Thank you for the opportunity to review your work.

Sincerely,
Kyle

Kyle Vogan, PhD
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Nature Genetics
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Referee expertise:

Referee #1: Genetics, ocular disorders

Referee #2: Genetics, myopia

Referee #3: Genetics, complex traits

Reviewers' Comments:

Reviewer #1:
Remarks to the Author:

The Authors present the largest study to date evaluating the genetic architecture of refractive error (and its closely associated endophenotypes such as myopia and age of spectacle wear) in 600,211 participants of European ancestry and 59,784 participants of Asian ancestry. It was a tremendous pleasure reading this manuscript, which performed:

a) standard genome-wide association analysis followed by meta-analysis and comparison of ancestry specific signals.
b) fine-mapping to identify potential causal variants.
c) polygenic risk score analysis.
d) secondary analysis evaluating the relationship between polygenic risk scores and disease onset, severity and progression.

I have some significant technical and interpretational questions for the authors to clarify:

1. Results, main text, lines 148-149: Could the authors explain in detail how did 6.8% of the SNPs explain >68% of SNP-based heritablity in EAS? This is a large claim, and clear analytical evidence would help the reader.

2. Results, main text, lines 145-146: It is not fair to analyse for "similarity and difference" between European and Asian ancestry given that the European ancestry participants have a >10-fold increase in sample size compared to participants of Asian ancestry. It could be challenging to draw firm conclusions from 60K participants of Asian ancestry when the European ancestry dataset comprises 600K.

3. Results, main text, line 202: How would SNP rs634990 in GJD2, a common, non-coding SNP, be causal? A 'causal' statement these days might neccesitate biological evidence obtained by experimental perturbation. Same goes for rs429358 in APOE, which is a common, non-synonymous variant predicted to be benign. To term such a variant as ‘causal’ compared to others in linkage disequilibrium with it may necessitate additional experimentation.

4. Results, main text, line 221: The 10-fold cross validation method is poorly explained in the manuscript text, as well as in Supplementary Figure 2. Could the Authors please clarify in detail which samples are involved in which stage of the analysis (training vs test)?

5. Figure 6, main manuscript: Could the Authors clarify from which cohorts are the results drawn from? Are they from UK Biobank only? Or are other collections such as 23andMe, ALSPAC, etc. included? For panel 6D, what do the colours mean?

Reviewer #2:
Remarks to the Author:

This exciting manuscript described a high quality GWAS meta-analysis of refractive error that identified 80 novel loci by combing GWAS summary statistics from EUR-ancestry (n=600k) and EAS ancestry (n=60k) samples. Systematic fine mapping integrating functional annotation information was carried out for 534 independent GWAS loci, which identified variants with a high posterior inclusion probability (PIP >= 0.9) at many sites. The work also claimed to have built on many other recently reported findings.

Main points

1. Popcorn analysis. The current work applied a popcorn analysis to evaluate the degree of shared genetic architecture between EUR and EAS samples, which is a topic of keen interest in refractive error genetics research. The new analysis extended an earlier popcorn analysis reported by Tedja et al. (ref #9). The Tedja et al. analysis evaluated a GWAS for refractive error in EUR and a GWAS for refractive error in EAS. Here, it appeared that an MTAG meta-analysis of GWASs in EUR for refractive error was evaluated against a GWAS for myopia case-control status in EAS. In colloquial language, the new popcorn analysis was a ‘GWAS for apples’ in EUR vs. a ‘GWAS for pears’ in EAS. Please can the authors provide theoretical or simulation-based evidence that a popcorn analysis will provide valid results when different traits are measured in the two ancestry groups?

2a) PRS accuracy. The current work deviated from most previous assessments of refractive error PRS accuracy by reporting performance during cross-validation. In general, performance during cross-validation yields inflated estimates of accuracy. Hence, please could the authors only report PRS performance in independent samples. For instance, the performance in cross validation was an incremental R2=21.6%, whereas in the EPIC-Norfolk and ALSPAC samples accuracy was R2=17.5% and R2=16.6%, respectively. The data in Figure 5 and Figure 6 also should be obtained in an independent sample, to allow comparison with previous work (refs #7, 8, 9, 11).

2b) PRS accuracy in EAS. There is keen interest in improving the transportability of a PRS for refractive error, especially for use in children of EAS ancestry [1]. Scant information was presented about the accuracy of the new PRS in EAS samples compared with existing PRSs [1,2]. Did the new PRS offer any improvement?

2c) Including GWAS summary statistics from the 23andMe Inc. company can restrict full details of a PRS being made openly available to the scientific community. If the new PRS reported in the current work is only available for use by the authors and not by others, please explain why the knowledge of the performance of the new PRS will be of scientific interest.

3. A proportion of the claimed novel discoveries were of questionable novelty. The authors should provide evidence that their discoveries exceed similar findings reported in recent studies:

3a) Improved PRS accuracy (lines 219-277). Clark et al. [2] reported a PRS that attained an incremental R2 of approximately 21% in cross-validation, which is similar to the 21.6% reported in the current study. In independent samples, the Clark et al. PRS appeared to perform as well as or better than the current PRS. The Clark et al. PRS is publicly available, hence the authors should perform side-by-side comparisons to confirm that their new PRS is more accurate. (To be consistent with standard practice in refractive error genetics research, the evaluation of the PRS should be done in a large, independent sample of participants).

3b) Accuracy of the PRS improved as individuals progressed from infancy to early adulthood (lines 279-285). The current results in the ALSPAC cohort were extremely similar to results for the ALSPAC cohort and Generation R cohort reported by

Clark et al. [2]. The gold standard method for assessing refractive error in children is cycloplegic autorefraction; this was used in the Generation R study but not in the ALSPAC study. Thus, the Clark et al. study is more convincing than the current work in this regard.

3c) Non-linearity of genetic effect sizes for SNPs associated with refractive error (lines 318-337). There was already a large body of evidence reporting non-linear effects of SNPs associated with refractive error [3-6]. Furthermore, the justification for testing different models of non-linearity was not clear. Likewise, testing the non-linearity of a PRS may have been reliant on the assumption that all risk variants for refractive error exhibit the same pattern of non-linearity, which prior research suggests is not the case [3,4]. In summary, in was not clear what value this section added and what novelty it provided.

3d) Pleiotropy between outdoor activity and educational attainment (lines 309-315). The Mendelian randomization analysis of outdoor activity as a causal risk factor for myopia was problematic, since time outdoors was assessed in adults, which is after the age at which myopia develops. For this Mendelian randomization analysis to be scientifically plausible, the authors need to demonstrate that SNPs associated with outdoor activity in adults are similarly associated with the time children spend outdoors. In fact, this topic was the focus a very recent multivariable Mendelian randomization study [7]. The newly published study did confirm that genetic predisposition to outdoor activity tracked from childhood to adulthood. The published study also documented the pleiotropy between outdoor activity and educational attainment in relation to myopia development. Hence, this section of the manuscript did not appear to add new knowledge.

4. The EAS GWAS analyses were performed for the traits ‘myopia case-control status’ (ToMMo cohort) and ‘high myopia case-control status’ (ref #14). Accordingly, how valid was a METAL fixed-effect EUR + EAS meta-analysis, in view of the different traits that were studied in the EUR and EAS groups? The units of measurement for the traits would be different – hence, was the meta-analysis based on Z-scores? Was the assessment of cross-ancestry effect size heterogeneity (line 150-165) valid if different traits were measured in the EUR and EAS samples?

Minor points

1. Line 42. Myopia does not affect over half of the global population. The authors are referring to a prediction for the year 2050 (ref #3).

2. Line 43. Strictly speaking, ‘blindness’ is a clinical sign, not a symptom (or not a symptom of visual impairment caused by myopia).

3. Line 46. The value n=848 is likely to mislead most readers. Instead, it would be more informative to report the total number of independent loci now known to be associated with refractive error and myopia (n=534).

4. Line 50. Combining polygenic prediction and self-reported RE onset is a bizarre concept that would never been done clinically. Once the age of onset of myopia is known, high myopia can be predicted confidently without the need for a PRS (ref #40). Please avoid such a contrived analysis.

5. Line 51-53. This ‘conclusion’ was already well-established prior to the current study (refs #11, 46-53).

6. Line 57. This should be “cornea and lens”, not “cornea and/or lens”. The cornea or lens alone will never match the eye axial length.

7. Line 74. Quoting the heritability from twin studies appears to have been done for dramatic effect. As the authors point out later, the ‘SNP heritability’ is more relevant than the broad sense heritability. Also, please quote a more up to date value of exiting PRS performance [2].

8. Lines 111-124. GREML-based assessment of refractive error has been reported many times before, including studies cited by the authors and other studies. However, the new report of a GREML-LDMS estimate of heritability is novel. This section of the manuscript could be shortened considerably to just focus on the new result. I could not find out from the supplementary methods section which sample(s) were used for the GREML heritability calculations; please specify in the text.

9. Line 132. Did the current study identify more or fewer genetic loci associated with refractive error in EUR subjects than the Hysi et al., 2020 study (ref #8)? (Hysi et al, reported 449 loci, whereas the current study reports only 431 loci). If the Hysi et al. study identified more loci, would be it be more powerful to use the (publicly available) Hysi et al. summary stats for the meta-analysis with the EAS GWAS summary statistics?

10. Line 192. The 848 SNPs with CARMA PIP>0.9 were distributed across 583 loci. At how many of the 582 quasi-independent loci was no SNP with PIP>0.9 identified (how often was fine-mapping unsuccessful)?

11. Figure 1. Given that the current study followed a well-established study design for a modern GWAS meta-analysis, this figure could be omitted or moved to the supplement.

12. Figure 2. In my view, Figure 2 added no additional value compared to a statement that genetic predisposition to refractive error was polygenic.

13. Figure 3. Increasing the font size would improve this figure.

14. Figure 5. I strongly recommend removing the graph bar for 'UKB + 23andMe + Funct + covs'. Age and sex have strong associations with refractive error in UKB, which likely relate to changes in the level of education in the UK over the past few decades [8]. The point of a PRS is to allow prediction. Knowledge about changes in education decades in the future will not be known ahead of time, hence using them for 'prediction' is not justified.

15. Figure 6. Please explain which samples were evaluated.

16. Supplementary Tables 3, 5, 6. Please define units of beta coefficient.

17. Supplementary Table 4. Please add column headings.

References

1. Lanca, C., Kassam, I., Patasova, K., Foo, L.L., Li, J., Ang, M., Hoang, Q.V., Teo, Y.Y., Hysi, P.G. and Saw, S.M. (2021) New Polygenic Risk Score to Predict High Myopia in Singapore Chinese Children. Transl Vis Sci Technol 10, 26.

2. Clark, R., Lee, S.S.-Y., Du, R., Wang, Y., Kneepkens, S.C.M., Charng, J., Huang, Y., Hunter, M.L., Jiang, C., Tideman, J.W.L., Melles, R.B., et al. (2023) A new polygenic score for refractive error improves detection of children at risk of high myopia but not the prediction of those at risk of myopic macular degeneration. EBioMedicine 91, 104551.

3. Pozarickij, A., Williams, C., Hysi, P.G., Guggenheim, J.A. and U. K. Biobank Eye and Vision Consortium. (2019) Quantile regression analysis reveals widespread evidence for gene-environment or gene-gene interactions in myopia development. Communications Biology 2, 167.

4. Clark, R., Pozarickij, A., Hysi, P.G., Ohno-Matsui, K., Williams, C., Guggenheim, J.A. and UK Biobank Eye and Vision Consortium. (2022) Education interacts with genetic variants near GJD2, RBFOX1, LAMA2, KCNQ5 and LRRC4C to confer susceptibility to myopia. PLoS Genet. 18, e1010478.

5. Pozarickij, A., Enthoven, C.A., Ghorbani Mojarrad, N., Plotnikov, D., Tedja, M.S., Haarman, A.E.G., Tideman, J.W.L., Polling, J.R., Northstone, K., Williams, C., Klaver, C.C.W., et al. (2020) Evidence That Emmetropization Buffers Against Both Genetic and Environmental Risk Factors for Myopia. Invest Ophthalmol Vis Sci 61, 41.

6. Pozarickij, A., Williams, C., Guggenheim, J.A. and U.K. Biobank Eye and Vision Consortium. (2020) Non-additive (dominance) effects of genetic variants associated with refractive error and myopia. Molecular Genetics and Genomics 295, 843-853.

7. Clark, R., Kneepkens, S.C.M., Plotnikov, D., Shah, R.L., Huang, Y., Tideman, J.W.L., Klaver, C.C.W., Atan, D., Williams, C., Guggenheim, J.A., for the, U.K.B.E., et al. (2023) Time Spent Outdoors Partly Accounts for the Effect of Education on Myopia. Invest Ophthalmol Vis Sci 64, 38.

8. Williams, K.M., Bertelsen, G., Cumberland, P., Wolfram, C., Verhoeven, V.J.M., Anastasopoulos, E., Buitendijk, G.H.S., Cougnard-Grégoire, A., Creuzot-Garcher, C., Erke, M.G., Hogg, R., et al. (2015) Increasing Prevalence of Myopia in Europe and the Impact of Education. Ophthalmology 122, 1489–1497.

Reviewer #3:
Remarks to the Author:

Thank you for the opportunity to review the manuscript by Cheng et al., which presents a large-scale cross-ancestry genetic analysis on refractive errors (RE). The study's identification of novel loci and its use of statistical fine-mapping methods to prioritize causal variants are commendable. The manuscript also highlights the potential clinical application of genetic risk prediction modeling in RE. While the manuscript presents these findings, it faces critical issues that need addressing.

Some key aspects:

Sample comparison:
The authors have included European (EUR) and East Asian (EAS) populations. However, the increase in sample size compared to previous studies (Hysi et al., 2020; Tedja et al., 2018) is modest. Notably, the EUR population sample overlaps significantly with those used in Hysi et al., 2020. The addition of new samples from EAS populations, though valuable, is limited in scale, which impacts the robustness of novel signal identification.

Specifically, for the EUR populations, in the current study, the authors included UKB and 23andMe, both of which were already included in Hysi et al 2020, with nearly the same samples in the analysis (e.g. the nearly same ~100k individuals with mean spherical equivalent from UKB; the identical 23andME samples; imputed MSE based on age of onset of

spectacle wear (Cheng et al) versus myopia status estimated based on age of first spectacle wear (Hysi et al 2020)). Additionally, Hysi et al. 2020 included an extra 34,998 individuals (measured MSE) from the GERA Study and 34,079 participants from the CREAM.
In the context of EAS populations, the current study included samples from the Tohoku Medical Megabank Organization (ToMMo), encompassing 14,786 myopes, and from WeGene, totaling 5,952 samples, though lacking specific details about the number of myopic individuals.

Despite this inclusion, the overall increment in sample size remains modest. This limitation is reflected in the identification of only one novel genetic signal (as per Line 139), out of 18 total signals, with the remaining 17 previously reported in EUR populations.

MTAG analysis considerations:
In the MTAG analysis, the authors should reconsider the use of "perfect_gencov" due to the varied definitions of MSE and different traits involved (eg. measured MSE, inferred MSE, and myopia status). The "perfect_gencov" flag could potentially lead to many spurious genomic signals.

Clarity on novel loci:
The methodology for defining novel loci lacks clarity. The authors employ a clumping+threshold approach (P 5e-8, r2 0.01, window 250 kb) but do not adequately describe how to define novel loci from those identified in prior studies. A conditional analysis on previous GWAS summary statistics could provide a clearer distinction.

Phenotypic data and prediction accuracy:
The authors used polynomial models to predict MSE using age, sex, year of birth, and age of onset of spectacle wear. However, the prediction accuracy is limited in the sense for phenotypic data (r2=0.3, Line 50 in supplement). Although the authors show a relatively high genetic correlation of “inferred MSE”, the GWAS on very low accuracy “inferred MSE” is likely to include many non-MSE or unrelated factors.

Contextualizing genetic findings:
The authors should offer some biological interpretations for the newly identified loci.

Contextualizing polygenic risk score:
The application of polygenic risk scores in myopia research is well-documented in previous publications. Contextualizing the findings of this study (Lines 218-300) against existing literature (referenced PMIDs: PMID: 37055258; PMID: 31670792; PMID: 35358591) is crucial for understanding its novelty and applicability.

Contextualizing lifestyle modifications:
The novelty and importance of the findings of lifestyle modifications (Lines 52-53, 301) should reference well-established clinical trial findings (referenced PMIDs: PMID: 26372583; PMID: 29371008; PMID: 35779695; PMID: 28951126).

Other comments:
Clarification is required on the term “self-reported RE onset” mentioned in Line 50.

The manuscript lacks specific information on the number of myopia cases within the WeGene cohort's self-reported myopia status (Lines 101 and 165, supplement).

An explanation on interpreting the negative selection on RE variants is needed (Line 123).

I suggest comparing the authors' simulation analysis of nonlinear causal relationships with established nonlinear MR methods for robustness, e.g. fractional polynomial method (PMID: 28317167) or doubly-ranked stratification method (PMID: 37390109).

A power calculation explanation for the non-association of the significant loci rs12193446 with RE in EAS populations would be beneficial (Lines 345-348).

The clarity of the statistical fine-mapping illustration in Figure 1 could be improved by directly including the two statistical methods.

The foundational sample description should ideally be included in the online methods section for clarity and accessibility (Line 521).

Version 1:

Decision Letter:

14th May 2024

Dear Jian,

Your revised Article entitled "Cross-ancestry genome-wide association study augments genetic insights and clinical potentials for refractive error" has been seen by the original referees, whose comments are included below. In the light of their ongoing concerns, we have decided that we cannot offer to publish your manuscript in Nature Genetics.

In particular, while the referees find aspects of the study improved, they raise overlapping concerns about the level of advance your findings represent over earlier work and the strength of the novel conclusions that can be drawn at this stage. We are persuaded that these reservations are sufficiently important as to preclude publication of this study in Nature Genetics.

I am sorry we cannot be more positive on this occasion, but we hope you will find our referees' comments helpful when preparing your paper for submission elsewhere.

Sincerely,
Kyle

Kyle Vogan, PhD
Senior Editor
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Referee expertise:

Referee #1: Genetics, ocular disorders

Referee #2: Genetics, myopia

Referee #3: Genetics, complex traits

Reviewers' Comments:

Reviewer #1:
Remarks to the Author:

The Authors have been very responsive to the first round of review. Specifically in their revised manuscript, they have indicated that the predictor described in the main text explained (up to) 22.2% of the total trait variance in refractive error (line 51, Abstract).

Context was provided in the main text (line 77; 3.4% to 19%). To increase helpfulness to the readers, the authors should consider presenting analyses / data for the following pieces (because it is extremely relevant given the sample size and cross-ancestry nature of this study):

1. Estimated twin heritability was 75-88 percent, line 76, main text
2. How much of this could reasonably be attributed to SNPs, given diminishing returns based on common variant GWAS methods (this paper uses GWAS data)?
3. On which end of the spectrum did the description of the (up to) 22.2% of the total trait variance of RE lie?

Reviewer #3 brought up a very profound point on #Sample Comparison# which I missed in the previous round of review. This section closes with the following comment from Reviewer #3:

"Despite this inclusion, the overall increment in sample size remains modest. This limitation is reflected in the identification of only one novel genetic signal (as per Line 139), out of 18 total signals, with the remaining 17 previously reported in EUR populations."

The authors included FinnGen (3,534 myopia cases and 361,237 controls) and GERA (34,998 individuals with measured MSE) in this revised manuscript. Granted that the vast majority of the FinnGen collection were controls, could the authors explain how did the addition of 3,534 myopia cases and 34,998 individuals with MSE result in the identification of "68 independent genetic associations that have not been reported previously", which is a very major increase from one as pointed out by Reviewer #3?

Thank you.

Reviewer #2:
Remarks to the Author:

The revised draft of the manuscript is much improved. Many of my original comments have been addressed in full. Please find below points where additional clarification or justification would be helpful.

Main points

1. The meta-analysis now includes a myopia case-control GWAS reported by FinnGen (3,534 cases, 361,237 controls). Myopia is heavily under-reported in the FinnGen dataset (myopia prevalence ~1%), which is not surprising for an EHR-based study. Please provide evidence that the MTAG analysis was robust to the severe case and control misclassification errors in the FinnGen GWAS.

2. The cross-ancestry meta-analysis was performed using a fixed effects model. Why was the fixed effects model justified for the cross-ancestry meta-analysis? (MTAG was used in preference to a fixed effects model for the EUR samples meta-analysis).

3. The section on PRS (lines 296–312) currently reports the accuracy in predicting myopia. This section and Table 1 would benefit from also reporting the accuracy in predicting high myopia (which has been a main focus of previous work [1,2]).

4. There was a convincing improvement in the accuracy of predicting high myopia when combining AOSW + PRS, compared to AOSW alone (Figure 6F). However, it would be prudent to discuss two caveats. First, AOSW in UK Biobank was self-reported in 40 to 70-year-olds and therefore may be subject to recall bias. Recall bias would lead to AOSW having lower accuracy in UK Biobank compared to its predictive accuracy in a sample in which AOSW was assessed without error. Second, ref #48 was cited to support the argument that AOSW + family history has equal predictive accuracy as AOSW alone. However, ref #48 was a study from Singapore, which unlike the UK, has seen a massive surge in myopia in the past generation. In the UK, it is possible that AOSW + family history is a better predictor than AOSW alone.

5. The manuscript reports the improved accuracy of the PRS with children's age as if this was a novel finding (lines 326-332; 431-434). However, this result was already reported (Figure 1 of [3]; Figure 4 of [4]; Figure 4a of [2]).

6. The manuscript reports that genetic effects on refractive error are age dependent as if this was a novel finding (line 331). However, this result was already reported (Figure 1 of [5]; Figure 3 of [3]).

7. The MR results reported in lines 349-360 (and 436-446) largely recapitulated the results of a recent study [6]. The novel aspects of the authors' current work should be highlighted, as the key findings were simply confirmatory. As an example, the manuscript states, "We identified EA and OA as two major risk factors for RE" (line 363). Yet this was already very well-established [7].

8. The manuscript reports non-linear PRS x E interactions as if they were novel (lines 53-55; 363-382). However, this result was already reported (Figure 3 of [8]; Figure 2B of [9]).

9. The manuscript reports a Non-linear MR analysis (lines 381-382). There is a genuine concern that the use of non-linear MR analysis methods in samples such as UK Biobank is unreliable (when using either the standard NLMR method or the double-ranking method) [10,11]. The authors may wish to omit the NLMR results.

10. In reply to my suggestion that the SNP-based heritability section could be shortened, in their Response Letter, the authors say, "to the best of our knowledge, no report of GREML analysis using individual-level data for estimating the SNP-based heritability for refractive error exists." Here are such reports, including analyses in UK Biobank: Table 2 of [12]; Figure 1 of [13].

Minor/technical points

1. Abstract line 49. The term "eye development" is generally used to describe ocular development during pregnancy rather than post-natal effects. Please can I check that this was the meaning intended by the authors?

2. Supplementary Tables. The results tables beginning on page 19 and page 37 appear to have information missing, such as a missing data column for 'chromosome' or column headers that are incomplete.

References

1. Kassam, I., Foo, L.-L., Lanca, C., Xu, L., Hoang, Q.V., Cheng, C.-Y., Hysi, P. and Saw, S.-M. (2022) The potential of current polygenic risk scores to predict high myopia and myopic macular degeneration in multi-ethnic Singapore adults. Ophthalmology 129, 890-902.
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3. Tideman, J.W., Fan, Q., Polling, J.R., Guo, X., Yazar, S., Khawaja, A., Hohn, R., Lu, Y., Jaddoe, V.W., Yamashiro, K., Yoshikawa, M., et al. (2016) When do myopia genes have their effect? Comparison of genetic risks between children and adults. Genet Epidemiol 40, 756-766.
4. Ghorbani Mojarrad, N., Williams, C. and Guggenheim, J.A. (2018) A genetic risk score and number of myopic parents independently predict myopia. Ophthalmic Physiol Opt 38, 492–502.

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Reviewer #3:
Remarks to the Author:

My major concerns remain unsolved.

Concern 1: sample comparison
The authors claim to have increased the sample size of European (EUR) ancestry data from 600K to approximately 1M. However, this increase (400K) primarily comprises control samples (361,237 controls) from publicly available GWAS data from FinnGen, specifically including "binary myopia status of 3,534 cases and 361,237 controls from the FinnGen cohort." In my initial comments, I differentiated the samples of European and Asian populations and compared the sample sizes in the current study with those from previous studies (Hysi et al., 2020; Tedja et al., 2018). “Specifically, for the EUR populations, in the current study, the authors included UKB and 23andMe, both of which were already included in Hysi et al. 2020, with nearly the same samples in the analysis (e.g. the nearly same ~100k individuals with mean spherical equivalent from UKB; the identical 23andMe samples; imputed MSE based on age of onset of spectacle wear (Cheng et al.) versus myopia status estimated based on age of first spectacle wear (Hysi et al. 2020)). Additionally, Hysi et al. 2020 included an extra 34,998 individuals (measured MSE) from the GERA Study and 34,079 participants from the CREAM.”

In the revised manuscript, the authors included the same 34,998 individuals (measured MSE) from the GERA Study previously included in Hysi et al. 2020. The new samples of binary myopia status from the FinnGen cohort represent an additional sample size of 14K, calculated as 4*(3534 * 361237)/(3534 + 361237). Compared with the inclusion of 34,079 participants from the CREAM in Hysi et al. 2020, the current study even has a smaller sample size of the European population.

Overall, for EUR populations, the current study incorporates UKB, 23andMe, and the GERA Study, all previously included in Hysi et al. 2020, with nearly identical samples. The modest increase in sample size from FinnGen (effective sample size 14K) is minimal compared to Hysi et al. 2020, which also included additional participants from CREAM (34K).

The new samples from EAS populations are limited in scale and have not robustly identified novel signals (as further detailed in MTAG method considerations below).

Concern 2: MTAG analysis considerations
The authors have conducted new analyses using the MTAG default setting to address potential inflation from the "perfect_gencov" flag, which assumes perfect genetic correlation between multiple input traits. They have noted a higher LDSC intercept for MTAG-‘perfect_gencov’ compared to MTAG-default (1.08 versus 0.99) and have opted for a conservative approach. However, for some crucial results, the authors continue to utilize results from MTAG-‘perfect_gencov’, which is not suitable for analyzing multiple diverse traits, e.g., the GJD2 locus; rs258669 within CACNA2D1; examples in Figure 2.

The authors presented simulated genetic correlations for continuous and binary traits to demonstrate shared genetic architecture. However, they failed to present empirical genetic correlations for the included continuous and binary myopia-related traits.

Concern 3: clarity on novel loci:
The authors reported 427 genetic loci using MTAG-default, similar to the 435 loci reported by Hysi et al. 2020, which was

expected given that most cohorts included in the two studies are nearly identical, with similar power in identifying loci. In the EAS population, the authors reported 13 loci, all previously identified in EUR populations.

Overall, the incremental increase in novel loci from the modest sample size increase is minimal.

In the conditional analysis, the authors utilized 435 lead SNPs from Hysi et al. 2020 and reported 42 conditionally significant loci, most of which are just above the 5e-8 significance level. A more appropriate approach would involve conditioning on all genome-wide significant loci (not just independent SNPs) from Hysi et al. 2020.

Concern 4: questionable novelty of polygenic risk score:
As also pointed out by Reviewer 2 in comments 2 and 3, the slight increase in PRS accuracy and claims of novel discoveries were of questionable novelty.

Concern 5: questionable novelty of lifestyle modifications:
Although the authors have shown a potential nonlinear association between educational attainment and outdoor activities with the risk of refractive error (RE), given the available evidence from similar Mendelian randomization studies and well-established clinical trial findings (referenced PMIDs: PMID: 26372583; PMID: 29371008; PMID: 35779695; PMID: 28951126), the novelty of the main finding in the section “Educational attainment and outdoor activities are two main environmental risk factors for RE” was of questionable novelty.

Overall, the authors have used nearly overlapping European samples with Hysi et al. 2020 (Concern 1), used inflated MTAG setting for heterogeneity myopia related traits (MTAG-‘perfect_gencov’, Concern 2), identified very few novel loci (Concern 3), achieved a modest increase in PRS (Concern 4), and reported lifestyle risk factors that are well-established from previous clinical trials (Concern 5). These factors defer the novelty of this current study, representing very incremental work in the field of myopia genetics.

**Although we cannot publish your paper, it may be appropriate for another journal in the Nature Portfolio. If you wish to explore other journals and transfer your manuscript, please use our manuscript transfer portal. You will not have to re-supply manuscript metadata and files, unless you wish to make modifications. For more information, please see our manuscript transfer FAQ page.

Version 2:

Decision Letter:

IMPORTANT: Please note the reference number: NG-A63948R1-Z Yang. This number must be quoted whenever you communicate with us regarding this paper.

30th May 2025

Dear Jian,

Thank you for asking us to reconsider our decision on your manuscript "Cross-ancestry genome-wide association study augments genetic insights and clinical potentials for refractive error". I have discussed your proposed resubmission with my editorial colleagues, and we invite you to revise your manuscript along the lines that you propose for further consideration and peer review.

When preparing a revision, please ensure that it fully complies with our editorial requirements for format and style; details can be found in the Guide to Authors on our website (http://www.nature.com/ng/).

Please be sure that your manuscript is accompanied by a separate point-by-point response detailing the changes you have made and your response to the points raised. At this stage, we will need you to upload:

1) A copy of the manuscript in MS Word .docx format.

2) The Editorial Policy Checklist:
https://www.nature.com/documents/nr-editorial-policy-checklist.pdf

3) The Reporting Summary:
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(Here you can read about the role of the Reporting Summary in reproducible science:
https://www.nature.com/news/announcement-towards-greater-reproducibility-for-life-sciences-research-in-nature-1.22062)

Please use the link below to be taken directly to the site and view and revise your manuscript:

Link Redacted

With best wishes,
Kyle

Kyle Vogan, PhD
Senior Editor
Nature Genetics
https://orcid.org/0000-0001-9565-9665

Version 3:

Decision Letter:

3rd July 2025

Dear Jian,

Your revised Article "Cross-ancestry genome-wide association study augments genetic insights and clinical potentials for refractive error" has been seen by the original referees. You will see from their comments below that, while they find the work improved, they have raised a few ongoing concerns. We remain interested in the possibility of publishing your study in Nature Genetics, but we would like to consider your response to these ongoing concerns in the form of a further revision before we make a final decision on publication.

To guide the scope of the revisions, the editors discuss the referee reports in detail within the team, including with the chief editor, with a view to identifying key priorities that should be addressed in revision, and sometimes overruling referee requests that are deemed beyond the scope of the current study. In this case, while we are persuaded that the level of advance over previous work is sufficient to justify ongoing consideration, we ask that you fully address all remaining technical points. We hope that you will find this prioritized set of referee points to be useful when revising your study. Please do not hesitate to get in touch if you would like to discuss these issues further.

We therefore invite you to revise your manuscript taking into account all reviewer and editor comments. Please highlight all changes in the manuscript text file. At this stage, we will need you to upload a copy of the manuscript in MS Word .docx or similar editable format.

We are committed to providing a fair and constructive peer-review process. Do not hesitate to contact us if there are specific requests from the reviewers that you believe are technically impossible or unlikely to yield a meaningful outcome.

When revising your manuscript:

*1) Include a “Response to referees” document detailing, point-by-point, how you addressed each referee comment. If no action was taken to address a point, you must provide a compelling argument. This response will be sent back to the referees along with the revised manuscript.

*2) If you have not done so already, please begin to revise your manuscript so that it conforms to our Article format instructions, available
here.
Refer also to any guidelines provided in this letter.

*3) Include a revised version of any required Reporting Summary: https://www.nature.com/documents/nr-reporting-summary.pdf
It will be available to referees (and, potentially, statisticians) to aid in their evaluation if the manuscript goes back for peer review.
A revised checklist is essential for re-review of the paper.

Please be aware of our guidelines on digital image standards.

EXTENDED DATA FIGURES

When re-submitting your manuscript, please ensure that any supplementary figures and tables that are crucial to the manuscript’s conclusions are converted into Extended Data figures and tables to increase visibility of these data. Extended Data figures and tables are online-only (present in the online PDF and full-text HTML versions of the paper), peer-reviewed

display items that provide essential background to the article but are not included in the main article due to space constraints. A maximum of ten Extended Data display items (figures and tables) is permitted.

Please use the link below to submit your revised manuscript and related files:

Link Redacted

Note: This URL links to your confidential home page and associated information about manuscripts you may have submitted, or that you are reviewing for us. If you wish to forward this email to co-authors, please delete the link to your homepage.

We hope to receive your revised manuscript within 8-12 weeks. If you cannot send it within this time, please let us know.

Please do not hesitate to contact me if you have any questions or would like to discuss these revisions further.

Nature Genetics is committed to improving transparency in authorship. As part of our efforts in this direction, we are now requesting that all authors identified as 'corresponding author' on published papers create and link their Open Researcher and Contributor Identifier (ORCID) with their account on the Manuscript Tracking System (MTS), prior to acceptance. ORCID helps the scientific community achieve unambiguous attribution of all scholarly contributions. You can create and link your ORCID from the home page of the MTS by clicking on 'Modify my Springer Nature account'. For more information, please visit www.springernature.com/orcid.

We look forward to seeing the revised manuscript and thank you for the opportunity to review your work.

Sincerely,
Kyle

Kyle Vogan, PhD
Senior Editor
Nature Genetics
https://orcid.org/0000-0001-9565-9665

Referee expertise:

Referee #1: Genetics, ocular disorders

Referee #2: Genetics, myopia

Referee #3: Genetics, complex traits

Reviewers' Comments:

Reviewer #1 (Remarks to the Author):

The Authors have likely tried their best to respond to the previous round of peer review.

As a due diligence measure, could the Authors reconcile the N=932 RE-associated variants with Figure 1, whereby summing up all the EUR, EAS, and AFR variants did not yield N=932. However, N=932 was consistent in Supplementary Table 9. Key numbers in the abstract and main display items should have consistency, and if there are alternate explanations, these should be clarified.

Reviewer #2 (Remarks to the Author):

Revision 2 of the manuscript is much improved. The inclusion of new GWAS samples from the MVP, AllofUs and 23mofang cohorts has resulted in the discovery of 241 novel, quasi-independent variants associated with refractive error, as well as 6 EAS-specific signals and 1 AFR-specific signal. The polygenic score (PRS) derived by the authors has improved prediction accuracy in EUR (R2 = 21.4%), EAS (R2 = 9.7%) and AFR (R2 = 4.2%) compared to previously published polygenic scores for refractive error.

In the later sections of the manuscript that I previously criticized for claims of novelty when describing findings that had already been published, the authors do now cite the earlier studies.

Main points

1. Please include a data availability statement.

2. (a) The analyses reported in Figure 7a,b used genetic variants associated specific behaviors in adults as instrumental variables for childhood behaviours (since childhood is the age when myopia typically develops). Therefore, these analyses relied on the assumption that the genetic component of these behaviors is conserved throughout the life course from childhood to adulthood. This assumption is questionable, because parents may play a major role in determining children's behavior. In the Discussion, the authors acknowledge the need to confirm that instrumental variables are associated with childhood behaviors yet they themselves failed to do this, e.g. television viewing behavior. (b) Genetic nature could explain a proportion of the association between an individual's genotype and their phenotype for traits related to behavioral risk factors (Kong et al. 2018; Guggenheim et al. 2022). The authors should at least acknowledge that their Mendelian randomization analyses could be biased if genetic nurture is important for these behaviors.

Minor points

1. When reporting GWAS findings in the supplementary tables, please indicate which allele is the effect allele, e.g. label the alleles EA=Effect Allele and NEA=Non-Effect Allele.

2. Line 177. When reporting the popcorn analysis results, clarify if the values are the "genetic effect correlation" or the "genetic impact correlation" that are produced by popcorn.

3. Line 194. Ancestry-specific GWAS signals (lines 169-172) appeared to be assumed if a variant was strongly associated ($P < 5e-08$) in one ancestry but not associated in the other ancestries ($P > 0.05$). A test for heterogeneity was reported (Supplementary Table 8) but it was not clear if this was the statistical test was used to confirm that ancestry-specific signals were not simply due to chance. Please clarify the criteria used to classify a variant as ancestry-specific.

4. Line 482. Cite a reference to support the statement, "near work becomes substantially detrimental only when exceeding certain durations".

5. Line 670. Please provide the link for accessing the publicly available MVP summary statistics (I could not find summary statistics for myopia using the generic weblink provided in the manuscript).

6. Line 694. Typo "diptor".

7. Line 762. With regards to estimating how much of the SNP-based heritability in EAS and AFR was explained by the top EUR variants: (a) Explain how boundaries were defined with respect to "the lead SNPs along with their upstream and downstream boundaries". (b) Was this SBayesRC analysis restricted to hapmap3 variants or were all variants included? (c) What was the size of the reference panel for this SBayesRC analysis.

References

Guggenheim JA, R Clark, T Zayats, C Williams & UK Biobank Eye and Vision Consortium (2022): Assessing the contribution of genetic nurture to refractive error. Eur. J. Hum. Genet. 30: 1226-1232.

Kong A, G Thorleifsson, ML Frigge, BJ Vilhjalmsson, AI Young, TE Thorgeirsson, et al. (2018): The nature of nurture: Effects of parental genotypes. Science 359: 424-428.

Reviewer #3 (Remarks to the Author):

The manuscript presents a large-scale cross-ancestry GWAS of refractive error (RE), reports additional genome-wide associations, proposes improved polygenic risk scores (PRS) and explores gene–environment interactions.

While the dataset is larger and more diverse than earlier efforts, the biological and translational advances derived from this added scale are, in most places, confirmatory or modest extensions of established knowledge. Many of the major concerns raised in prior rounds remain unresolved.

Sample-size (previous Concern 1): The EUR meta-analysis still relies heavily on UKB (direct + AOSW) and 23andMe—the core of Hysi et al. 2020.

The expansion of "1M to 1.5M" is almost entirely from published MVP data (DOI: 10.1126/science.adj1182, with 65,574 cases and 333,242 controls) and All of Us (only 5,339 cases, and additional 91,024 controls). Therefore, the ~860 K "new" EUR participants (MVP, AllofUs) contain <70 K additional cases and thus generate a far smaller effective sample-size gain than the headline number suggests.

In EAS and AFR, although sample sizes increase, the absolute numbers remain modest, maintaining a substantial imbalance relative to EUR and limiting power to detect ancestry-specific biology.

Novel loci (previous Concern 3): The author reported 708 quasi-independent variants, and 168 previously unknown quasi-independent associations after conditional analysis of the Hysi et al. 2020 study.

However, from table S5, there are 25 SNPs with P value > 5e-8 (e.g. rs4234239 p=1.033e-01; rs41276453 = 7.106e-04). Without filtering the marginal P value < 5e-8, these 25 loci cannot be considered significant. Moreover, of the remaining 143 SNPs (marginal P < 5e-8), most SNPs (71% SNPs, n=102) have P value > 1e-9 or in an overlap position (±250 kb) with Hysi et al. 2020 study, suggesting limited power to detect novel SNPs.

PRS improvement remains modest (previous Concern 4): In Europeans, the SBayesRC PRS explains 21.4 % (vs. 19.0 % in Clark et al. 2023; +2.4%). In EAS the cross-ancestry model doubles R^2 (4.5 -> 9.7 %), but absolute accuracy remains low and clinically marginal.

The authors reported that the combined AOSW + PRS AUC of 0.92 for high myopia, yet the incremental gain over AOSW alone (0.88 -> 0.92) indicates the clinical translation is not yet compelling.

As pointed out in the reviewer 2's comments 7, 8, and 9, the Gene–environment and MR analyses largely confirm prior work (previous Concern 5), and most reported interactions and causal inferences replicate earlier findings (e.g., education, outdoor activity), offering little beyond confirmation of established relationships.

Overall, the manuscript offers the largest RE dataset so far. However, the resulting findings are primarily incremental.

Version 4:

Decision Letter:

Our ref: NG-A63948R3

16th December 2025

Dear Jian,

Thank you for submitting your revised manuscript "Cross-ancestry genome-wide association study augments genetic insights and clinical potentials for refractive error" (NG-A63948R3). Based on your responses and clarifications, we will be happy in principle to publish your study in Nature Genetics as an Article pending final revisions to comply with our editorial and formatting guidelines.

We are now performing detailed checks on your paper, and we will send you a checklist detailing our editorial and formatting requirements soon. Please do not upload the final materials or make any revisions until you receive this additional information from us.

Thank you again for your interest in Nature Genetics. Please do not hesitate to contact me if you have any questions.

Sincerely,
Kyle

Kyle Vogan, PhD
Senior Editor
Nature Genetics
https://orcid.org/0000-0001-9565-9665

Responses to the Reviewers

We thank the reviewers for their constructive comments, which have greatly helped improve our manuscript. We have addressed all the reviewers' comments point-by-point below (in blue) in this document and have made corresponding changes in the manuscript files. Here is a summary of the main changes:

1) We have increased the sample size of the European (EUR) ancestry data from 600K to approximately 1M by incorporating summary statistics from the FinnGen and GERA cohorts. This effort has led to improved power in discovering genetic associations in both the EUR ancestry and cross-ancestry analyses.
2) We have added a GWAS meta-analysis using MTAG with the default setting, on top of the original MTAG analysis with the 'perfect-gencov' setting. We found that the default setting showed a slightly lower level of inflation in test statistics, compared to the "perfect-gencov" setting. Hence, we opted for a conservative approach, using summary statistics from MTAG-default for the subsequent analyses.
3) We have performed a series of gene prioritization analyses as well as a functional enrichment analysis to better interpret the GWAS findings and prioritized 20 genes involved in the biological processes related to eye development.
4) We have benchmarked our refractive error polygenic risk score (PRS) against the best performing PRS from the published studies. Our PRS consistently outperformed the published PRS by 17.3-59.0% across multiple validation datasets. In addition, we constructed a cross-ancestry PRS, which improved the prediction accuracy in East Asian (EAS) ancestry by 6.2% compared to our EUR PRS and by 61.3% compared to the published PRS.
5) We have made every effort to address all the technical queries made by the reviewers and have meticulously revised the manuscript to improve its readability.

Reviewers' Comments:

Reviewer #1:
Remarks to the Author:

The Authors present the largest study to date evaluating the genetic architecture of refractive error (and its closely associated endophenotypes such as myopia and age of spectacle wear) in 600,211 participants of European ancestry and 59,784 participants of Asian ancestry. It was a tremendous pleasure reading this manuscript, which performed:

a) standard genome-wide association analysis followed by meta-analysis and comparison of ancestry specific signals.
b) fine-mapping to identify potential causal variants.
c) polygenic risk score analysis.
d) secondary analysis evaluating the relationship between polygenic risk scores and disease onset, severity and progression.

Re: We thank the reviewer for the summary of our study and the positive remarks.

I have some significant technical and interpretational questions for the authors to clarify:

1. Results, main text, lines 148-149: Could the authors explain in detail how did 6.8% of the SNPs explain >68% of SNP-based heritablity in EAS? This is a large claim, and clear analytical evidence would help the reader.

Re: The fundamental concept of this analysis is to estimate variance explained by all SNPs in loci, which are identified as trait-associated from the EUR data, in the EAS data. Deriving the estimate involves two steps, which we have explicated in the **Methods** section (lines 715-721). First, we annotated the trait-associated loci, i.e., the lead SNPs along with the upstream and downstream boundaries, from the EUR GWAS analysis. Second, we performed an SBayesRC analysis using the EAS GWAS summary statistics and an LD reference from UKB-EAS to estimate the proportion of h^2_{SNP} explained by annotated SNPs when all the SNPs were fitted jointly.

Due to the increased sample size of the EUR dataset, we have updated the statement in the revised manuscript. It now reads: “We estimated that 60.5% of the SNP-based heritability in EAS could be explained by the SNPs in the GWAS loci identified in EUR, which only accounted for 6.7% of the genetic variants” (lines 169 - 171).

2. Results, main text, lines 145-146: It is not fair to analyse for "similarity and difference" between European and Asian ancestry given that the European ancestry participants have a >10-fold increase in sample size compared to participants of Asian ancestry. It could be challenging to draw firm conclusions from 60K participants of Asian ancestry when the European ancestry dataset comprises 600K.

Re: We agree with the reviewer and have revised the sentence to read as follows: “We next estimated the genetic correlation between EUR and EAS using Popcorn, a method that accounts for the difference in sample size between datasets.” (lines 167 - 169)

When discussing ancestry-specific associations, we paid attention not only to the p-values, which are dependent on sample size, but also to the allele frequencies of the variants in each ancestry (lines 173 – 177).

3. Results, main text, line 202: How would SNP rs634990 in GJD2, a common, non-coding SNP, be causal? A 'causal' statement these days might neccesitate biological evidence obtained by experimental perturbation. Same goes for rs429358 in APOE, which is a common, non-synonymous variant predicted to be benign. To term such a variant as ‘causal’ compared to others in linkage disequilibrium with it may necessitate additional experimentation.

Re: We agree and have amended it to ‘fine-mapped lead variant’ (lines 234-235).

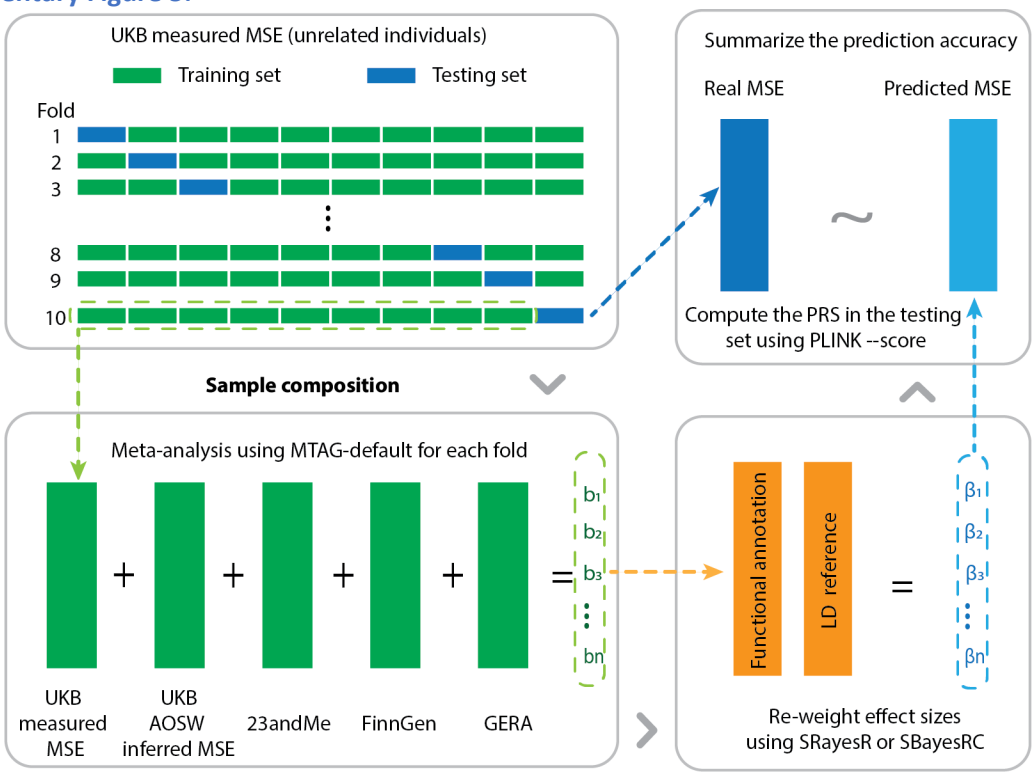
In addition, we have included the statement, “However, the causal roles of these fine-mapped lead variants warrant further experimental evidence” to avoid any potential misinterpretation (lines 239-240).

4. Results, main text, line 221: The 10-fold cross validation method is poorly explained in the manuscript text, as well as in Supplementary Figure 2. Could the Authors please clarify in detail which samples are involved in which stage of the analysis (training vs test)?

Re: We have included a description of the 10-fold cross-validation analysis in the **Methods** section (lines 781 – 792; also see below) and a schematic diagram of the analysis in **Supplementary Figure 5** (also see below) and cited both of them in the main text (line 256).

"Cross-validation of the PRS
We assessed the performance of the PRS using a 10-fold cross-validation analysis. First, we randomly divided the unrelated UKB individuals (genetic relatedness < 0.05) with directly measured mean spherical equivalent (MSE) into ten groups. Each group was used iteratively as a testing set, while the remaining groups were used as a part of the training set. Second, for each cross-validation fold, we conducted a meta-analysis using the GWAS summary statistics of UKB directly measured MSE (excluding the testing set), UKB AOSW inferred MSE (excluding the testing set), 23andMe, FinnGen, and GERA. Third, the meta-analyzed GWAS summary data, along with functional annotations and LD reference, were used to compute the joint effect sizes of genetic variants using SBayesR (without functional annotations) or SBayesRC (with functional annotations). Fourth, the estimated joint effects of the genetic variants were then used as weights to construct a PRS for each individual in the testing set. We evaluated the prediction performance by correlating the observed MSE with the PRS, adjusting for sex, age, and 10 genotype principal components (PCs)."

Supplementary Figure 5:



5. Figure 6, main manuscript: Could the Authors clarify from which cohorts are the results drawn from? Are they from UK Biobank only? Or are other collections such as 23andMe, ALSPAC, etc. included? For panel 6D, what do the colours mean?

Re: We have clarified in the revised manuscript that for **Figure 6**, a subset of the UKB individuals was

used as the testing set. The SNP weights for generating PRS were computed using summary statistics from a training set of the UKB directly measured MSE (excluding individuals in the testing set), UKB AOSW inferred MSE (excluding individuals in the testing set), 23andMe, FinnGen, and GERA (lines 534-537).

The 23andMe dataset was used as part of the training set. The ALSPAC dataset was not included in the analysis presented in Figure 6 but was subsequently used to demonstrate the performance of the PRS in completely independent samples (**Table 1, Supplementary Figure 6**).

For panel 6D, color code does not have any specific meaning. We have modified it to avoid confusion.

Reviewer #2:
Remarks to the Author:

This exciting manuscript described a high quality GWAS meta-analysis of refractive error that identified 80 novel loci by combing GWAS summary statistics from EUR-ancestry (n=600k) and EAS ancestry (n=60k) samples. Systematic fine mapping integrating functional annotation information was carried out for 534 independent GWAS loci, which identified variants with a high posterior inclusion probability (PIP >= 0.9) at many sites. The work also claimed to have built on many other recently reported findings.

Re: We thank the reviewer for the summary of our study and the positive comments.

Main points

1. Popcorn analysis. The current work applied a popcorn analysis to evaluate the degree of shared genetic architecture between EUR and EAS samples, which is a topic of keen interest in refractive error genetics research. The new analysis extended an earlier popcorn analysis reported by Tedja et al. (ref #9). The Tedja et al. analysis evaluated a GWAS for refractive error in EUR and a GWAS for refractive error in EAS. Here, it appeared that an MTAG meta-analysis of GWASs in EUR for refractive error was evaluated against a GWAS for myopia case-control status in EAS. In colloquial language, the new popcorn analysis was a 'GWAS for apples' in EUR vs. a 'GWAS for pears' in EAS. Please can the authors provide theoretical or simulation-based evidence that a popcorn analysis will provide valid results when different traits are measured in the two ancestry groups?

Re: We thank the reviewer for raising this point.

The core question underlying this comment is whether there is a shared genetic basis between refractive error (RE), myopia, and high myopia. Under a threshold-liability model, where RE is considered as a continuous disease liability and myopia or high myopia is a disease status ascertained based on the disease liability, it is expected that the three traits have a shared genetic basis. We have validated this theory by simulation. When we simulated a quantitative trait and dichotomized it to a binary case-control trait given a prevalence of 0.3 (to mimic myopia) or 0.05 (to mimic high myopia), the estimated genetic correlation between RE and myopia, or between RE and high myopia, had a mean of approximately 1 across simulation replicates, even if cases were over-sampled to achieve a case-control ratio of 1:1 (**Supplementary Figure 2**). This is also supported by empirical evidence that the estimated genetic correlation between RE and myopia in the UKB was close to unity [genetic correlation = 1.0002, standard error (s.e.) = 0.0056], consistent with the previous theoretical findings from the LDSC method (PMID: 26414676, "There is no distinction between observed and liability scale genetic correlation for

case/control traits, so we can define and estimate genetic correlation between a case/control trait and a quantitative trait and genetic correlation between pairs of case/control traits without the need to specify a scale"). These results support a shared genetic basis between RE and myopia even if cases are oversampled.

A more straightforward analysis to address the reviewer's concern is to perform a Popcorn analysis using EUR myopia case-control data (generated by MTAG using 23andMe as the target GWAS) and EAS myopia case-control data. This would be a 'pears' to 'pears' comparison. The estimate of the cross-ancestry genetic correlation remained unchanged [0.800 (s.e. 0.043) vs. 0.808 (s.e. 0.042)].

We thank the referee again for raising this important point. We have incorporated the simulation study above into the revised manuscript (**Supplementary Figure 2**) to demonstrate a shared genetic basis between refractive error and myopia (or high myopia). We have also reported the Popcorn estimate of cross-ancestry genetic correlation from the analysis only with case-control data to demonstrate that the estimated cross-ancestry genetic correlation is not affected by the inclusion of both refractive error and myopia case-control phenotypes (lines 168-169).

2a) PRS accuracy. The current work deviated from most previous assessments of refractive error PRS accuracy by reporting performance during cross-validation. In general, performance during cross-validation yields inflated estimates of accuracy. Hence, please could the authors only report PRS performance in independent samples. For instance, the performance in cross validation was an incremental R2=21.6%, whereas in the EPIC-Norfolk and ALSPAC samples accuracy was R2=17.5% and R2=16.6%, respectively. The data in Figure 5 and Figure 6 also should be obtained in an independent sample, to allow comparison with previous work (refs #7, 8, 9, 11).

Re: We agree with the reviewer that indeed, cross-validation could lead to an inflated estimate of prediction accuracy in the presence of confounding (typically population stratification), and/or relatedness between individuals in the training and testing sets. However, this does not necessarily imply that the prediction accuracy quantified in cross-validation is invariably inflated. On the other hand, the prediction accuracy quantified in an out-of-sample setting is not always unbiased but could be deflated due to differences in LD, MAF, and/or genetic effects between populations or subpopulations. Hence, in the absence of confounding, the prediction accuracy quantified in the cross-validation using unrelated individuals provides an upper limit of the performance of a PRS, and its performance decays as the genetic distance between the training population and testing population increases (PMID: 37198491). We hope to provide a more balanced view by reporting from both cross-validation and out-of-sample prediction in multiple testing datasets.

We have applied a stringent analysis protocol to minimize the confounding effect and avoid sample relatedness between training and testing sets. Specifically, we have estimated the LDSC intercept and attenuation ratio in the training data, both of which indicated a minimal level of population stratification effect. Prior work (Wood et al. 2014 Nature Genetics) has shown that the effect of population stratification can be largely corrected by fitting genotype PCs in the PRS analysis even in the presence of population stratification in the training set. Therefore, in our cross-validation analysis, 10 PCs were included in the PRS analysis to further avoid the potential effect of population stratification. Moreover, all the analyses were performed on unrelated individuals of the UKB, meaning that all the individuals in the testing set were, in the conventional definition, not related to any individuals in the training set (genetic relatedness < 0.05). Additionally, the cross-validation analysis was also applied in the Clark et al.

2023, *eBioMedicine*. Hence, the cross-validation results reported in this study provided a direct comparison to those presented in Clark et al.

We have incorporated some of the discussions above into the revised manuscript (lines 286-288,296-297). In response to the reviewer's suggestion, we have also presented the results for the EPIC-Norfolk and ALSPAC cohorts in the manuscript (**Supplementary Figure 6**).

2b) PRS accuracy in EAS. There is keen interest in improving the transportability of a PRS for refractive error, especially for use in children of EAS ancestry [1]. Scant information was presented about the accuracy of the new PRS in EAS samples compared with existing PRSs [1,2]. Did the new PRS offer any improvement?

Re: We thank the reviewer for this suggestion. Unfortunately, refractive error or myopia GWAS data from children of EAS ancestry are not available to us. However, when tested in UKB adult individuals of EAS ancestry, our EUR PRS and cross-ancestry PRS demonstrated 59.0% and 61.3% higher accuracy, respectively, compared to that from Clark et al. (2023, *eBioMedicine*).

We have included these results in the revised manuscript (lines 415-421).

2c) Including GWAS summary statistics from the 23andMe Inc. company can restrict full details of a PRS being made openly available to the scientific community. If the new PRS reported in the current work is only available for use by the authors and not by others, please explain why the knowledge of the performance of the new PRS will be of scientific interest.

Re: We thank the reviewer for raising this issue.

First, we will release the PRS without the 23andMe data, which is 10% more accuracy than that from Clark et al. (2023, *eBioMedicine*), to satisfy the demands of both the scientific community and clinical researchers.

Second, we will release the full summary statistics of the meta-analysis excluding the 23andMe data. The summary statistics from 23andMe can be accessed by rational application (https://research.23andme.com/dataset-access/), which has been extensively utilized by the research community, including the genetic studies for refractive error (refs 8 and 11). We will release easy-to-use scripts for meta-analyzing our released data and the 23andMe data, and for generating the PRS given the meta-analysis summary data.

Third, as more datasets become available in the future, it will pave the way for more genetic association discoveries in all ancestries. Our effort at the current stage is to explore the clinical potentials based on the available datasets. The insights of PRS in predicting RE stratification, onset, progression, severity, and interaction with environmental factors could be informative for future studies equipped with more powerful sample sizes and advanced methodologies.

3. A proportion of the claimed novel discoveries were of questionable novelty. The authors should provide evidence that their discoveries exceed similar findings reported in recent studies:

3a) Improved PRS accuracy (lines 219-277). Clark et al. [2] reported a PRS that attained an incremental R2 of approximately 21% in cross-validation, which is similar to the 21.6% reported in the current study.

In independent samples, the Clark et al. PRS appeared to perform as well as or better than the current PRS. The Clark et al. PRS is publicly available, hence the authors should perform side-by-side comparisons to confirm that their new PRS is more accurate. (To be consistent with standard practice in refractive error genetics research, the evaluation of the PRS should be done in a large, independent sample of participants).

Re: We thank the reviewer for the comment. However, we could not find a cross-validation prediction R^2 of 21% in *Clark et al. 2023, eBioMedicine*. We noticed that they reported a cross-validation prediction R^2 of 19.0% in UKB individuals of European ancestry, which was lower than that of our updated PRS with R^2 of 22.2%. In accordance with the reviewer's request, we have provided a side-by-side comparison of prediction accuracy between our PRS and the Clark et al. PRS in multiple testing sets, as shown in the table below (also in **Supplementary Note 4**). Across the testing sets, our PRS improved the prediction accuracy by 17.3-59.0% compared with the Clark et al. PRS.

Testing dataset	**Sample size**	**Our PRS** (improvement compared with Clark et al.,)	**The Clark et al. PRS**
UKB-AFR	4,302	0.037 (37.0%)	0.027
UKB-SAS	4,947	0.120 (20.0%)	0.100
UKB-EAS	839	0.097 (59.0%)	0.061
ALSPAC-mother	1,512	0.163 (17.3%)	0.139

3b) Accuracy of the PRS improved as individuals progressed from infancy to early adulthood (lines 279-285). The current results in the ALSPAC cohort were extremely similar to results for the ALSPAC cohort and Generation R cohort reported by Clark et al. [2]. The gold standard method for assessing refractive error in children is cycloplegic autorefraction; this was used in the Generation R study but not in the ALSPAC study. Thus, the Clark et al. study is more convincing than the current work in this regard.

Re: We have updated the PRS analysis using our updated GWAS meta-analysis data in the ALSPAC cohort, with individuals stratified into different age groups. Our PRS showed a 3.6%-14.0% increased prediction accuracy compared to the Clark et al. PRS across these age groups (see the table below). In addition, 1) the upward trend of the PRS accuracy from infancy to early adulthood and 2) the association between PRS and refractive error progression are both novel findings that have not been previously reported and discussed (lines 326-332, 430-434). We agree that cycloplegic autorefraction provides a more accurate measure of refractive error in children. Unfortunately, despite multiple attempts, we were unable to gain access to the Generation R data.

Testing dataset	**Sample size**	**Our PRS** (improvement compared with Clark et al.)	**The Clark et al. PRS**
ALSPAC 12-month	889	0.0173 (3.6%)	0.0167
ALSPAC 7-year	6,175	0.0583 (11.7%)	0.0522
ALSPAC 10-year	5,892	0.0931 (14.0%)	0.0817
ALSPAC 15-year	4,054	0.1120 (10.2%)	0.1016

ALSPAC progression between 7-year and 15-year	3,588	0.0424 (6.0%)	0.0400

3c) Non-linearity of genetic effect sizes for SNPs associated with refractive error (lines 318-337). There was already a large body of evidence reporting non-linear effects of SNPs associated with refractive error [3-6]. Furthermore, the justification for testing different models of non-linearity was not clear. Likewise, testing the non-linearity of a PRS may have been reliant on the assumption that all risk variants for refractive error exhibit the same pattern of non-linearity, which prior research suggests is not the case [3,4]. In summary, in was not clear what value this section added and what novelty it provided.

Re: We appreciate the reviewer's comment, which has helped to improve the clarity of our manuscript (lines 367-368, 381-382, 453-460).

The non-linearity explored in this work pertains to the non-linear effect of an exposure (e.g., outdoor activities) on RE, rather than non-linear genetic effects of SNPs (known as genotype-by-exposure/environment interaction, or GxE) on RE. Our observation was that the effect of an exposure, such as educational attainment (EA) and outdoor activities (OA), on RE varies depending on the level of RE. We employed the PRS as a surrogate for RE in the interaction model, RE ~ exposure + RE-PRS + RE-PRS * exposure, to assess the interaction term, i.e., whether the influence of exposure on RE is dependent on genetic risk of RE. In fact, a non-linear relationship between exposure and RE, if present, can be detected directly via a quartile regression between exposure and RE without the use of RE-PRS. The advantage of using RE-PRS to assess the relationship between an exposure and RE is that the observed association is less likely to be attributed to potential confounding factors.

The reviewer is correct that in a scenario where the exposure has a non-linear causal effect on the outcome trait, theoretically, all genetic variants associated with the "exposure" will appear to have a non-linear effect on the outcome. However, in practice, these mediated genetic effects are often orders of magnitude smaller than the direct genetic effects and thus remain almost undetectable at single-variant level. This explains why GxE are not prevalent for most complex traits as suggested in previous studies (Wang et al., 2019, Science Advances; Marderstein et al., 2021, AJHG). Therefore, our results are not inconsistent with those reported in Pozarickij et al. (2019) and Clark et al. (2022).

The value of this section is twofold. Firstly, we are the first to report the PRS x exposure interaction for RE, suggesting that EA and OA have a larger effect on RE for individuals with a higher genetic risk for myopia. Through simulation, we proposed that an asymmetric non-linear causal relationship could explain the PRS x exposure interaction. In this revision, we have also incorporated a non-linear MR analysis (**Supplementary Figure 7**), which indicated uneven localized average causal effects across different strata for both EA and OA, further validating our hypothesis. Secondly, our findings of PRS x exposure interaction and non-linear causal relationship can provide clinical insights. For individuals with a higher genetic risk for myopia, lifestyle interventions, such as increased outdoor activities, could be more effective in preventing myopia progression. Furthermore, the non-linear MR analysis indicated that even a small amount of daily outdoor activity could have a substantial protective effect against myopia (lines 453-460).

3d) Pleiotropy between outdoor activity and educational attainment (lines 309-315). The Mendelian randomization analysis of outdoor activity as a causal risk factor for myopia was problematic, since time

outdoors was assessed in adults, which is after the age at which myopia develops. For this Mendelian randomization analysis to be scientifically plausible, the authors need to demonstrate that SNPs associated with outdoor activity in adults are similarly associated with the time children spend outdoors. In fact, this topic was the focus a very recent multivariable Mendelian randomization study [7]. The newly published study did confirm that genetic predisposition to outdoor activity tracked from childhood to adulthood. The published study also documented the pleiotropy between outdoor activity and educational attainment in relation to myopia development. Hence, this section of the manuscript did not appear to add new knowledge.

Re: We thank the reviewer for this comment. In the revised manuscript, we have raised the caveat that the Mendelian randomization analysis for OA relies on the assumption that SNP effects on OA in adults are similar to those on OA in childhood and acknowledged that the Clark et al. study confirmed that genetic predisposition to outdoor activity is largely consistent from childhood to adulthood (lines 437-439). We have also acknowledged that the causal effects of EA and OA on RE have been established in the Clark et al. study and have accordingly condensed the related text where appropriate (line 436).

In the revised text, we emphasize the analysis where we used MR to extensively assess the putative causal effects of other exposures such as time spent watching television, income, and fruit intake. We demonstrated that most of the significant causal effects of these exposures on RE became non-significant when conditioning on the effect of EA. This finding has important implications in RE epidemiological studies. As mentioned in the Discussion section (lines 441-448), epidemiological studies based on a univariate model could yield inconsistent conclusions about the associations between an exposure and RE – for instance, television watching could be found to have either a positive, negative, or no effect on RE. Our finding provides a suggestion for further epidemiological design to consider the confounding effect of EA on RE.

4. The EAS GWAS analyses were performed for the traits 'myopia case-control status' (ToMMo cohort) and 'high myopia case-control status' (ref #14). Accordingly, how valid was a METAL fixed-effect EUR + EAS meta-analysis, in view of the different traits that were studied in the EUR and EAS groups? The units of measurement for the traits would be different – hence, was the meta-analysis based on Z-scores? Was the assessment of cross-ancestry effect size heterogeneity (line 150-165) valid if different traits were measured in the EUR and EAS samples?

Re: We have demonstrated in our response to comment #1 from this reviewer a shared genetic basis between refractive error, myopia, and high myopia. Hence, a meta-analysis of the three traits is valid and increases the power of detection. Nevertheless, even if the genetic overlaps between the traits are imperfect, a meta-analysis is still valid and can improve the power of detection for shared genetic loci, despite reduced power for loci that are heterogeneous between traits. This concept extends to the meta-analysis of data from different ancestry groups, between which genetic heterogeneity is expected.

As clarified in the Methods section (lines 701-703), the meta-analysis was performed based on z-scores. The heterogeneity test of cross-ancestry effect size was also based on z-scores and effective sample sizes. Hence, the difference in measurement for the traits has been accounted for.

Minor points

1. Line 42. Myopia does not affect over half of the global population. The authors are referring to a prediction for the year 2050 (ref #3).

Re: We have clarified in the revised manuscript (line 43) that the statement refers to refractive error, not merely myopia. A meta-analysis showed that the global estimated pool prevalence of myopia, hyperopia, and astigmatism in adults was 26.5% (95% CI: 23.4–29.6), 30.9% (95% CI: 26.2–35.6), and 40.4% (95% CI: 34.3–46.6), respectively (PMID: 29564404).

2. Line 43. Strictly speaking, 'blindness' is a clinical sign, not a symptom (or not a symptom of visual impairment caused by myopia).

Re: We thank the reviewer for pointing out this and have replaced 'symptom' with 'consequences' (line 44).

3. Line 46. The value n=848 is likely to mislead most readers. Instead, it would be more informative to report the total number of independent loci now known to be associated with refractive error and myopia (n=534).

Re: We have reported the total number of independent loci in the abstract (lines 46-47). We believe that it is also useful to show the number of fine-mapped variants with high confidence (line 48).

4. Line 50. Combining polygenic prediction and self-reported RE onset is a bizarre concept that would never been done clinically. Once the age of onset of myopia is known, high myopia can be predicted confidently without the need for a PRS (ref #40). Please avoid such a contrived analysis.

Re: We acknowledge that the clinical utility of the PRS might be limited once the age of onset is known. We have revised the relevant text in the abstract to read (lines 50-52): "This predictor explained 22.2% of the variation in RE, was capable of stratifying the onset, progression, and severity of myopia, and achieved an AUC of 0.813 for predicting high myopia".

However, we maintain that it is valuable for the research community to understand that the PRS can enhance the prediction of high myopia even when the age of onset is already known. While age of onset alone and RE-PRS alone yielded AUCs of 0.883 and 0.813, respectively, combining RE-PRS and age of onset improved the AUC to 0.921 for high myopia prediction (lines 341-345). In addition to the age of onset of myopia, RE-PRS can provide added value for predicting high myopia, leading to an identification of 51.1% extra patients in the top 1% of population of high risk (8.2% extra patients in the top 10% of population of high risk). Compared to the age of onset of myopia, RE-PRS alone can provide comparable prediction accuracy for high myopia at the birth of an individual, providing substantial opportunities for early intervention and prevention.

To clarify this point, we have included a corresponding discussion in the revised manuscript (lines 424-429): "It has been shown in a previous study that the age of myopia onset alone is a significant predictor of high myopia, with no additional predictive value from parental myopia status48. Our study demonstrates that an accurate RE-PRS, rather than parental myopia status, can provide an additional benefit for predicting high myopia. Compared to the age of onset of myopia, RE-PRS alone can provide comparable prediction accuracy for high myopia at the birth of an individual, providing substantial opportunities for early intervention and prevention."

5. Line 51-53. This 'conclusion' was already well-established prior to the current study (refs #11, 46-53).

Re: Reference #11 discusses the integration of EA GWAS data into a PRS for RE prediction, and references #46-53 show the associations and causal relationships between environmental risk factors and RE. The text in lines 51-53 (now lines 53-55) emphasizes non-linear effects of environmental risk factors on RE, a finding that has not been established in any of the existing studies.

6. Line 57. This should be “cornea and lens”, not “cornea and/or lens”. The cornea or lens alone will never match the eye axial length.

Re: We thank the reviewer for pointing this out. We have corrected it in the revised manuscript (line 59).

7. Line 74. Quoting the heritability from twin studies appears to have been done for dramatic effect. As the authors point out later, the ‘SNP heritability’ is more relevant than the broad sense heritability. Also, please quote a more up to date value of exiting PRS performance [2].

Re: We have modified it in Line 77.

8. Lines 111-124. GREML-based assessment of refractive error has been reported many times before, including studies cited by the authors and other studies. However, the new report of a GREML-LDMS estimate of heritability is novel. This section of the manuscript could be shortened considerably to just focus on the new result. I could not find out from the supplementary methods section which sample(s) were used for the GREML heritability calculations; please specify in the text.

Re: The estimate of SNP-based heritability for RE has been reported previously by Tedja et al. (2018 Nat Genet), estimated from summary statistics using the LDSC method. However, to the best of our knowledge, no report of GREML analysis using individual-level data for estimating the SNP-based heritability for refractive error exists. Nevertheless, we have condensed the description of the LDSC and GREML estimates and highlighted the estimate from GREML-LDMS in the revised manuscript (lines 126-136).

We have also clarified in the revised manuscript that we used individual-level genotypes and directly measured MSE data from the unrelated UKB-EUR individuals for the GREML and GREML-LDMS analyses (N=95,519, lines 115-116, **Method** lines 670-671,682-683, **Supplementary Table 1**).

9. Line 132. Did the current study identify more or fewer genetic loci associated with refractive error in EUR subjects than the Hysi et al., 2020 study (ref #8)? (Hysi et al, reported 449 loci, whereas the current study reports only 431 loci). If the Hysi et al. study identified more loci, would be it be more powerful to use the (publicly available) Hysi et al. summary stats for the meta-analysis with the EAS GWAS summary statistics?

Re: In the revised manuscript, with the addition of GWAS data from two more cohorts, we identified 648 quasi-independent signals in 509 non-overlapping loci for the EUR ancestry using MTAG with the “perfect_gencov” setting, which assumes different measures of the same trait. As suggested by Reviewer #3, we identified 77 novel variants by conditioning on the top variants identified by the Hysi et al., 2020 study. Our current dataset is more powerful than that of the Hysi et al., 2020 study, exhibiting an even smaller attenuation ratio (0.043 in our study vs. 0.097 in Hysi et al.), as we employed the MTAG method, which outperforms the conventional fixed-effect meta-analysis method in accounting for potential confounding effects due to population stratification (**Supplementary Note 3**).

As suggested by Reviewer #3, in the revised manuscript, we have also conducted a meta-analysis with MTAG with the default setting, which accommodates imperfect genetic correlation among datasets. This approach reduced the attenuation ratio to less than 0, indicating a minimal level of inflation due to population stratification, if there is any.

10. Line 192. The 848 SNPs with CARMA PIP>0.9 were distributed across 583 loci. At how many of the 582 quasi-independent loci was no SNP with PIP>0.9 identified (how often was fine-mapping unsuccessful)?

Re: In the revised version, using MTAG with the default setting, we have identified 540 quasi-independent signals in 427 non-overlapping loci for the EUR ancestry. Out of the 427 loci, 119 had no SNP with a PIP greater than 0.9. However, it doesn't necessarily mean fine-mapping was unsuccessful in these loci. Of 119 loci, 117 had at least one 0.99-credible set, which captures a causal variant with a posterior probability of 0.99.

11. Figure 1. Given that the current study followed a well-established study design for a modern GWAS meta-analysis, this figure could be omitted or moved to the supplement.

Re: We agree and have moved **Figure 1** to **Supplementary Figure 1**.

12. Figure 2. In my view, Figure 2 added no additional value compared to a statement that genetic predisposition to refractive error was polygenic.

Re: We had intended to use **Figure 2** (now **Figure 1**) to show 1) the distribution of EUR and EAS signals across the genome under the current sample size, 2) the observation that most EAS signals are overlapped with the EUR signals, and 3) the number and the distribution of the novel GWAS signals. We have also improved the figure by adding explicit annotations about the number of significant variants for each chromosome.

13. Figure 3. Increasing the font size would improve this figure.

Re: We have improved the figure with a larger font size.

14. Figure 5. I strongly recommend removing the graph bar for 'UKB + 23andMe + Funct + covs'. Age and sex have strong associations with refractive error in UKB, which likely relate to changes in the level of education in the UK over the past few decades [8]. The point of a PRS is to allow prediction. Knowledge about changes in education decades in the future will not be known ahead of time, hence using them for 'prediction' is not justified.

Re: We have removed the graph bar for 'UKB + 23andMe + Funct + covs' from **Figure 5**.

15. Figure 6. Please explain which samples were evaluated.

Re: We have clarified it in the figure legend about the training and testing sets, which now reads (lines 534-537): "A subset of the UKB individuals was used as the testing set. The SNP weights for generating RE-PRS were computed using summary statistics from a training set of the UKB directly measured MSE (excluding individuals in the testing set), UKB AOSW inferred MSE (excluding individuals in the testing set), 23andMe, FinnGen, and GERA".

Additionally, as suggested by reviewer #1, we have updated **Supplementary Figure 5** to illustrate the 10-fold cross-validation. All unrelated UKB-EUR individuals with directly measured MSE had an out-of-sample PRS under the 10-fold cross-validation framework. The final sample size of the testing set was 67,654, less than the overall sample size of 95,519 for the UKB measured MSE because we removed individuals with estimated genetic relatedness greater than 0.05 with individuals of UKB AOSW inferred MSE to avoid potential sample overlap between training and testing sets.

16. Supplementary Tables 3, 5, 6. Please define units of beta coefficient.

Re: Done. The beta coefficient is in standard deviation units.

17. Supplementary Table 4. Please add column headings.

Re: Done.

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8. Williams, K.M., Bertelsen, G., Cumberland, P., Wolfram, C., Verhoeven, V.J.M., Anastasopoulos, E., Buitendijk, G.H.S., Cougnard-Grégoire, A., Creuzot-Garcher, C., Erke, M.G., Hogg, R., et al. (2015) Increasing Prevalence of Myopia in Europe and the Impact of Education. Ophthalmology 122, 1489–1497.

Reviewer #3:
Remarks to the Author:

Thank you for the opportunity to review the manuscript by Cheng et al., which presents a large-scale cross-ancestry genetic analysis on refractive errors (RE). The study's identification of novel loci and its use of statistical fine-mapping methods to prioritize causal variants are commendable. The manuscript also highlights the potential clinical application of genetic risk prediction modeling in RE. While the manuscript presents these findings, it faces critical issues that need addressing.

Re: We thank the reviewer for the summary of our study.

Some key aspects:

Sample comparison:
The authors have included European (EUR) and East Asian (EAS) populations. However, the increase in sample size compared to previous studies (Hysi et al., 2020; Tedja et al., 2018) is modest. Notably, the EUR population sample overlaps significantly with those used in Hysi et al., 2020. The addition of new samples from EAS populations, though valuable, is limited in scale, which impacts the robustness of novel signal identification.
Specifically, for the EUR populations, in the current study, the authors included UKB and 23andMe, both of which were already included in Hysi et al 2020, with nearly the same samples in the analysis (e.g. the nearly same ~100k individuals with mean spherical equivalent from UKB; the identical 23andME samples; imputed MSE based on age of onset of spectacle wear (Cheng et al) versus myopia status estimated based on age of first spectacle wear (Hysi et al 2020)). Additionally, Hysi et al. 2020 included an extra 34,998 individuals (measured MSE) from the GERA Study and 34,079 participants from the CREAM.
In the context of EAS populations, the current study included samples from the Tohoku Medical Megabank Organization (ToMMo), encompassing 14,786 myopes, and from WeGene, totaling 5,952 samples, though lacking specific details about the number of myopic individuals.

Despite this inclusion, the overall increment in sample size remains modest. This limitation is reflected in the identification of only one novel genetic signal (as per Line 139), out of 18 total signals, with the remaining 17 previously reported in EUR populations.

Re: We thank the reviewer for raising this concern. During the revision process, we have managed to include two additional large datasets of European ancestry: FinnGen (3,534 myopia cases and 361,237 controls) and GERA (34,998 individuals with measured MSE), which has increased the overall sample size to 999,980 for EUR and 59,784 for EAS, compared to 542,934 for EUR and no sample for EAS in the Hysi et al 2020 study. With the increased sample size, we now have 68 independent genetic associations that have not been reported previously. Together with the high-confidence putative causal variants identified through fine-mapping analysis and putative causal genes pinpointed by gene prioritization

analysis, these findings provide important leads for future research aimed at uncovering previously unknown genes and biological pathways that play a pivotal role in the onset and progression of refractive error.

Regarding the EAS data, we have included detailed information of the ToMMo, WeGene, and the Meguro et al. datasets in **Methods** and **Supplementary Note 1**. Although the sample size of the EAS data was much smaller than that of the EUR data, it has been approximately 5 times larger than that of the published largest EAS GWAS for RE and related traits (Tedja. et al, 2018). The inclusion of the EAS data not only led to the discovery of RE associated loci, especially those specific to the EAS ancestry, but also improved the portability of the PRS. During the revision process, we have trained the PRS model with both the EUR and EAS data using a newly developed model, SBayesRC-multi. The cross-ancestry PRS model improved the prediction accuracy by 61.3% in the EAS ancestry compared to the best performing PRS from the published studies (lines 306-312, lines 420-421).

MTAG analysis considerations:
In the MTAG analysis, the authors should reconsider the use of "perfect_gencov" due to the varied definitions of MSE and different traits involved (eg. measured MSE, inferred MSE, and myopia status). The "perfect_gencov" flag could potentially lead to many spurious genomic signals.

Re: The 'perfect_gencov' flag in MTAG assumes a genetic correlation of 1 among the traits/datasets involved in the meta-analysis but allows each trait has a different level of heritability. The assumption is valid based on our simulation that all RE traits theoretically share a genetic architecture with a genetic correlation of 1 (**Supplementary Figure 2**). This assumption is less stringent when compared to the assumption of the fixed-effect meta-analysis method implemented in previous studies (e.g., Hysi et al. 2020 Nat Genet).

In response to the reviewer's concern, we have rerun the meta-analysis using two additional methods, the conventional fixed-effect meta-analysis method and MTAG with the default setting that allows imperfect genetic correlation among datasets. We then estimated the corresponding genomic inflation factor, LDSC intercept, and attenuation ratio using the HapMap3 SNPs, as shown in the table below. Compared to the fixed-effect meta-analysis, the results from the two MTAG analyses yielded relatively low levels of inflation. We chose the summary statistics from MTAG-default, which showed no evidence of inflation due to potential confounding effects, for the post-GWAS analyses. Nevertheless, we have also reported the genome-wide significant loci from both MTAG-default and MTAG-'perfect_gencov'.

We have incorporated the discussion above in **Supplementary Note 3** and have updated the main text accordingly (lines 140-165).

	Fixed-effect model	**MTAG-perfect_gencov**	**MTAG-default**
Lambda GC	2.1502	1.9465	1.7767
Mean χ^2 value	3.172	2.9652	2.7055
LDSC intercept	1.2304 (0.0178)	1.0816 (0.0166)	0.9877 (0.0156)
Attenuation ratio	0.1061 (0.0082)	0.0415 (0.0085)	< 0

Clarity on novel loci:
The methodology for defining novel loci lacks clarity. The authors employ a clumping+threshold approach (P 5e-8, r2 0.01, window 250 kb) but do not adequately describe how to define novel loci from

those identified in prior studies. A conditional analysis on previous GWAS summary statistics could provide a clearer distinction.

Re: We thank the reviewer for this suggestion.

We first used GCTA-COJO to conduct a conditional GWAS analysis, conditioning on the lead SNPs of the 435 independent loci from Hysi et al. (2020 *Nature Genetics*). We then performed a clumping analysis of the conditional GWAS results, with a *P*-value threshold of 5e-8, an LD r-squared threshold of 0.01, and a window size of 250 kb. The clumped variants from the conditional analysis can be regarded as novel association signals. We finally defined stringent independent novel variants for those clumped variants that did not overlap with the regions reported by Hysi et al. (**Supplementary Table 5**). We have clarified this in the revised manuscript (lines 155-159, Method lines 706-714).

Phenotypic data and prediction accuracy:
The authors used polynomial models to predict MSE using age, sex, year of birth, and age of onset of spectacle wear. However, the prediction accuracy is limited in the sense for phenotypic data (r2=0.3, Line 50 in supplement). Although the authors show a relatively high genetic correlation of "inferred MSE", the GWAS on very low accuracy "inferred MSE" is likely to include many non-MSE or unrelated factors.

Re: The accuracy of the predictor is limited by the proportion of variance in MSE explained by the factors, i.e., age, sex, year of birth (YOB), and age of onset of spectacle wear (AOSW), used in the prediction model. The question arises as to whether these prediction errors will introduce genetic associations irrelevant to MSE but linked to other unrelated factors in our GWAS results. We believe such instances would be extremely rare considering the observations below. First, we noted a strong genetic correlation between the predicted MSE and the observed MSE. Therefore, genetic loci specific to the predicted MSE but not the observed MSE are uncommon. Second, genetic loci specific to the predicted MSE are unlikely to be corroborated by the other datasets involved in the meta-analysis and, as such, are very unlikely to be identified as genome-wide significant loci in the meta-analysis. We have tested this empirically. Comparing GWAS summary statistics between the directly measured (N=107,247) and predicted MSE (N=301,121), we found that none of the genetic loci specific to the predicted MSE remained genome-wide significant in the EUR GWAS meta-analysis.

Contextualizing genetic findings:
The authors should offer some biological interpretations for the newly identified loci.

Re: We appreciate the reviewer's suggestion.

To facilitate the biological interpretation of the GWAS loci, including the newly discovered loci, we have performed a series of analyses to prioritize genes responsible for the genetic associations. These include: 1) a gene-based association test using MAGMA and mBAT-combo; 2) a similarity-based gene prioritization analysis that integrates the gene-based test statistics and gene connectivity information (e.g., pathways, protein-protein interactions, and gene co-expression networks) to prioritize genes that have similar biological functions using PoPS; and 3) locus-based prioritization analyses that integrate GWAS summary statistics with molecular quantitative trait locus (xQTL) data using COLOC, SMR, and FUSION-TWAS. We have also conducted an enrichment analysis to test the enrichment of the prioritized genes in biological pathways and processes. The revised manuscript now includes the updated results and visualization of this analysis (lines 205-220; **Figure 3**).

Contextualizing polygenic risk score:
The application of polygenic risk scores in myopia research is well-documented in previous publications. Contextualizing the findings of this study (Lines 218-300) against existing literature (referenced PMIDs: PMID: 37055258; PMID: 31670792; PMID: 35358591) is crucial for understanding its novelty and applicability.

Re: This comment is related to comment 3 from reviewer #2. We have performed a head-to-head comparison of our PRS with those recently developed in the literature, including those mentioned in the reviewer's comment above. These results have also been presented in **Supplementary Note 4**. Briefly, among all the existing PRSs tested in our testing sets (including those in the cross-validation), the one from Clark et al. 2023 demonstrated the best performance. Compared with the Clark et al. PRS, our PRS trained with the EUR data was 17.3% more accurate in the EUR testing set, and 59.0% more accurate in the EAS testing set. Moreover, our PRS trained with the cross-ancestry meta-analysis data was 61.3% more accurate than the Clark et al. PRS in the EAS testing set. We have included these results in the revised manuscript (lines 286-312, lines 415-421).

Contextualizing lifestyle modifications:
The novelty and importance of the findings of lifestyle modifications (Lines 52-53, 301) should reference well-established clinical trial findings (referenced PMIDs: PMID: 26372583; PMID: 29371008; PMID: 35779695; PMID: 28951126).

Re: We appreciate the reviewer's recommendation of the references. These have been duly referenced and discussed in the revised manuscript (Lines 436-460).

Other comments:
Clarification is required on the term "self-reported RE onset" mentioned in Line 50.

Re: The term "Self-reported RE onset" was intended to represent "age of onset of spectacle wear (AOSW)" as obtained from UKB data-field 2217, which is the touchscreen question, "What age did you first start to wear glasses or contact lenses?". In the revised version (line 52), we reported only the prediction performance for high myopia of RE-PRS instead.

The manuscript lacks specific information on the number of myopia cases within the WeGene cohort's self-reported myopia status (Lines 101 and 165, supplement).

Re: We have included the relevant information in **Supplementary Note 1** (lines 185-210).

An explanation on interpreting the negative selection on RE variants is needed (Line 123).

Re: We have commented on the interpretation of the negative selection on RE variants in the revised manuscript (lines 126-136).

I suggest comparing the authors' simulation analysis of nonlinear causal relationships with established nonlinear MR methods for robustness, e.g. fractional polynomial method (PMID: 28317167) or doubly-ranked stratification method (PMID: 37390109).

Re: We appreciate the reviewer's suggestion. We have carried out a nonlinear MR using the doubly-ranked stratification method and incorporated the results in the Results section (Lines 381-382, **Supplementary Figure 7**). These findings corroborate our conclusion of the non-linear causal effects of education attainment and outdoor activities on RE.

A power calculation explanation for the non-association of the significant loci rs12193446 with RE in EAS populations would be beneficial (Lines 345-348).

Re: The SNP rs12193446 is monomorphic in EAS according to both the 1000 Genomes Project (1KGP) phase 3 and the genome Aggregation Database (gnomAD) datasets. Hence, it is not a power issue. We have clarified it in the revised manuscript (lines 175-177).

The clarity of the statistical fine-mapping illustration in Figure 1 could be improved by directly including the two statistical methods.

Re: We have revised **Figure 1** (now **Supplementary Figure 1**) to incorporate the two statistical methods for clearer understanding.

The foundational sample description should ideally be included in the online methods section for clarity and accessibility (Line 521).

Re: We have added the sample description in the Online Methods section (lines 569-662).

Response to reviewers

We thank the reviewers for their constructive comments and valuable suggestions, which have greatly helped us to improve our manuscript. We have addressed all the reviewers' comments point-by-point below (in blue) in this document and made the corresponding changes in the manuscript files (highlighted in yellow). Below is a summary of the main changes.

1) Increased sample sizes and inclusion of African ancestry data: We have further increased the sample size for both European (EUR) and East Asian (EAS) ancestries, and we have included data from African (AFR) ancestry. Specifically,

- For EUR ancestry, we further increased the sample size from 1M to 1.5M by incorporating summary statistics from the Million Veteran Program (MVP) and the All of Us Research Program (AllofUs).
- For EAS ancestry, the sample size has increased from 60K to 121K by including data from the 23mofang cohort.
- We have performed GWAS meta-analysis for the AFR ancestry using the MVP and AllofUs cohorts, comprising 21,224 cases and 123,513 controls.

These expansions have increased the number of novel quasi-independent associations to 241 in the cross-ancestry meta-analysis and led to the discovery of 6 EAS-specific signals and 1 AFR-specific signal.

2) Meta-analysis methodology update: We adopted the sample size weighted fixed-effect method for both ancestry-stratified and cross-ancestry GWAS meta-analyses (previously, MTAG was used for ancestry-stratified analysis). This decision was made because MTAG can bias test statistics due to errors in estimating the variance-covariance matrices of SNP effects and SNP effects estimation errors, as confirmed by our simulations (**Supplementary Note 4** and **Supplementary Figure 7**). Such biases become more prominent with a large number of cohorts, especially when some have relatively small sample sizes (Turley et al., Nat Genet, 2018). To avoid potential biases and ensure consistency across analyses, we chose the commonly used sample size-weighted meta-analysis method, which our simulation and real data analyses showed to be more robust with higher statistical power. Previously, MTAG was chosen for its better performance in the cross-validation PRS analysis in the EUR ancestry (see our response to comment #3 from reviewer #1 for more details); however, this advantage diminished as more cohorts were added. Notably, in the sample size-weighted meta-analysis, we adjusted the test statistics using the estimated LD Score regression intercept in cohorts with an attenuation ratio greater than 0.1 (Lee et al., Nat Genet, 2018) to account for potential inflation in GWAS statistics. For completeness, we will also release MTAG meta-analysis summary statistics for the ancestry-stratified analyses.

3) The novelty of our polygenic risk score (PRS) lies primarily in the improved cross-ancestry prediction. By increasing the discovery samples from under-represented ancestries, we have significantly enhanced the polygenic risk prediction in non-European populations. Compared with the existing best predictor, we have improved EAS prediction accuracy (R^2)

from 4.5% to 9.7% (a 116% improvement) and AFR prediction accuracy from 2.7% to 4.2% (a 55.6% improvement).

4) To explore the molecular mechanisms underlying RE, statistical fine-mapping pinpointed 16 high-confidence putative causal variants. Gene prioritization analyses highlighted 23 genes involved in eye development.

Reviewer #1:
The Authors have been very responsive to the first round of review. Specifically in their revised manuscript, they have indicated that the predictor described in the main text explained (up to) 22.2% of the total trait variance in refractive error (line 51, Abstract).

Context was provided in the main text (line 77; 3.4% to 19%). To increase helpfulness to the readers, the authors should consider presenting analyses / data for the following pieces (because it is extremely relevant given the sample size and cross-ancestry nature of this study):

1. Estimated twin heritability was 75-88 percent, line 76, main text

Re: We have clarified in the revised manuscript that this refers to the pedigree-based heritability (h^2_{ped}), estimated from 345 monozygotic and 267 dizygotic twin pairs using an ACE model (PMID: 17065484). This estimate is broadly consistent with our h^2_{ped} estimate (0.699, s.e. = 0.024) from the related individuals in the UK Biobank (**Supplementary Table 1**).

The revised text now reads (lines 83-85): "Heritability estimated from twin studies is reported to be between 75 and 88%[6,10], while the proportion of variance explained by common genetics variants is estimated to be 38.7%[11]".

2. How much of this could reasonably be attributed to SNPs, given diminishing returns based on common variant GWAS methods (this paper uses GWAS data)?

Re: We interpret "this" as referring to the estimate of h^2_{ped}. The GREML-LDMS approach is used to estimate the proportion of variance attributed to all SNPs (denoted by SNP-based heritability or h^2_{SNP}), which considers all the small effects captured by the SNPs. The estimated h^2_{SNP} using all imputed common variants was 0.395 (minor allele frequency, MAF≥0.01), and when considering all the imputed common and rare variants, it increased to 0.460 (minor allele count, MAC≥3). We have revised the manuscript to clarify this.

The revised text now reads (lines 146-149): "The results from GREML-LDMS analysis showed that while common variants (MAF≥0.01) accounted for the majority of the overall genetic variance (0.395 / 0.460), rare variants (MAF<0.01) contributed to more genetic variance than expected under an evolutionary neutral model".

3. On which end of the spectrum did the description of the (up to) 22.2% of the total trait variance of RE lie?

Re: The value of 22.2% represented the proportion of variance in the RE phenotype (as measured by mean spherical equivalent, MSE) explained by the PRS derived from all common variants included in our analysis. We have clarified this in the revised manuscript. The revised text now reads (lines 271-274): "Incorporating functional annotations by SBayesRC improved the prediction accuracy (i.e., variance in MSE explained by the PRS) further to 21.4%, accounting for 54.2% of h^2_{SNP} estimated using the same set of common variants in the testing set". This places our PRS towards the higher end of the spectrum in terms of prediction accuracy achievable with current methodologies and available data.

In the cross-validation analysis presented in the previous version of the manuscript, the training set comprised UKB individuals with directly measured MSE (excluding the testing set), UKB individuals with AOSW inferred MSE, 23andMe, FinnGen, and GERA cohorts. The testing set comprised the left-out UKB individuals with directly measured MSE. In the revised manuscript, we added two more cohorts, MVP and AllofUs, to the training set. Despite including these additional cohorts, the variance explained by the SBayesRC PRS in the testing set decreased slightly from 22.2% to 21.4%. We believe there are two primary reasons for this reduction:

1) Decrease in SNP-based heritability. The SNP-based heritability in the training set decreased from 25.1% to 21.4% (Table R1), likely due to increased phenotype heterogeneity introduced by the MVP and AllofUs cohorts. Consequently, the ratio of the variance explained by the SBayesRC PRS in the testing set to the SNP-based heritability in the training set increased after adding these cohorts.

2) Change in GWAS meta-analysis method. We shifted from using MTAG to the sample size-weighted meta-analysis method in the ancestry-stratified meta-analyses. As noted at the beginning of this document, MTAG can bias test statistics due to errors in estimating variance-covariance matrices of SNP effects and estimation errors (Turley et al., Nat Genet, 2018). Our simulations indicated a deflation in MTAG test statistics owing to a downward bias from using the LDSC estimate of genetic correlation to approximate true effect size correlation between datasets, leading to inflated standard errors of SNP effect estimates. This is evidenced by the observation that the variance explained by the PRS constructed based on genome-wide significant variants (after clumping) identified by MTAG decreased from 10.9% to 10.6% with the addition of the two cohorts. In contrast, for the sample size-weighted meta-analysis, the variance explained by the PRS constructed based on genome-wide significant variants increased from 10.7% to 11.2% (see Table R1 below). Therefore, for completeness, we will provide MTAG meta-analysis summary statistics for the ancestry-stratified analyses in the Data Availability section.

Table R1 Cross-validation analysis in different versions of the manuscripts

	First Version	1st Revision	Current Version
Training Set	UKB+23andMe	UKB+23andMe+GERA+ FinnGen	UKB+23andMe+GERA+ FinnGen+MVP+AllofUs
Sample size	579,074	978,843	1,474,022
PRS (SBayesRC)	16.9%(fixed_effect) 21.6% (MTAG)	21.2%(fixed_effect) 22.2% (MTAG)	21.4% (fixed_effect) 22.3% (MTAG)
PRS (P+T)	10.2%(fixed_effect) 10.2% (MTAG)	10.7% (fixed_effect) 10.9% (MTAG)	11.2% (fixed_effect) 10.6% (MTAG)
SBayesRC h^2_{SNP}	35.8%	25.1%	21.4%

PRS (P+T): PRS using the pruning and thresholding method. SBayesRC h^2_{SNP}: estimate of variance explained by all common SNPs using SBayesRC.

The observation that the variance explained by the SBayesRC PRS in the testing set (21.4%) closely matched the SBayesRC estimate of heritability (21.4%) in the training set suggests that the prediction accuracy is approaching the theoretical upper limit. To further improve the prediction accuracy, cohorts with precise phenotypic measurement are required. We have revised the manuscript (lines 453-456): “Last but not least, further efforts to improve the prediction accuracy for RE should not only focus on increasing the discovery sample size but also incorporate datasets with precise phenotypic measurements to diminish misclassification errors”.

Reviewer #3 brought up a very profound point on #Sample Comparison# which I missed in the previous round of review. This section closes with the following comment from Reviewer #3:

"Despite this inclusion, the overall increment in sample size remains modest. This limitation is reflected in the identification of only one novel genetic signal (as per Line 139), out of 18 total signals, with the remaining 17 previously reported in EUR populations."

The authors included FinnGen (3,534 myopia cases and 361,237 controls) and GERA (34,998 individuals with measured MSE) in this revised manuscript. Granted that the vast majority of the FinnGen collection were controls, could the authors explain how did the addition of 3,534 myopia cases and 34,998 individuals with MSE result in the identification of "68 independent genetic associations that have not been reported previously", which is a very major increase from one as pointed out by Reviewer #3?

Re: For the EAS ancestry, during the second revision, we made significant progress by adding 47,305 self-reported myopes and 8,209 non-myopes from 23mofang, Inc., increasing the overall sample size from 60K to 121K. **This increased the total number of genome-wide significant quasi-independent associations from 13 to 44 in EAS, with 6 EAS-specific novel associations**. Additionally, we included data from the AFR ancestry with a sample size of 145K (21,224 myopia cases and 123,513 controls), leading to the discovery of an AFR-specific novel association. Furthermore, we increased the sample size

for the EUR ancestry to 1.5M. **The cross-ancestry meta-analysis identified quasi-independent 932 RE-associated variants, including 241 previously unknown associations.**

We have revised the manuscript accordingly to update the changes in sample sizes and the corresponding major results.
Update text (lines 50-54): "We conducted ancestry-stratified and cross-ancestry meta-analyses of genome-wide association studies for RE in individuals of European (N=1,495,159), East Asian (N=121,172), and African (N=144,737) ancestries. The cross-ancestry meta-analysis identified 932 RE-associated variants, including 241 previously unknown associations, 6 EAS-specific associations, and 1 AFR-specific association."
Update text (lines 89-93): "we conducted multi-ancestry GWAS meta-analyses for RE, integrating data from 10 cohorts that included 1,495,159 individuals of EUR ancestry, 121,172 individuals of East Asian (EAS) ancestry, and 144,737 individuals of African (AFR) ancestry, which allowed us to investigate both ancestry-specific and shared genetic effects associated with RE."
Update text (lines 388-392): "The expanded sample size compared to the largest previous study[8] was due to a larger sample size for the imputed MSE from AOSW (301K vs. 172K) in EUR, as well as the inclusion of additional data from 860K individuals of EUR ancestry (FinnGen, MVP, and AllofUs), 121K individuals of EAS ancestry, and 145K individuals of AFR ancestry."

Reviewer #2:
The revised draft of the manuscript is much improved. Many of my original comments have been addressed in full. Please find below points where additional clarification or justification would be helpful.

We thank the reviewer for the positive remark that the manuscript was much improved.

Main points

1. The meta-analysis now includes a myopia case-control GWAS reported by FinnGen (3,534 cases, 361,237 controls). Myopia is heavily under-reported in the FinnGen dataset (myopia prevalence ~1%), which is not surprising for an EHR-based study. Please provide evidence that the MTAG analysis was robust to the severe case and control misclassification errors in the FinnGen GWAS.

Re: As stated at the beginning of this document and in the responses to comments from reviewer #1, we have adopted the sample size weighted fixed-effect method for both ancestry-stratified and cross-ancestry GWAS meta-analyses. This decision was made because MTAG can bias test statistics due to errors in estimating the variance-covariance matrices of SNP effects and SNP effects estimation errors, as confirmed by our simulations. Such biases become more prominent with a large number of cohorts, especially when some have relatively small sample sizes (Turley et al., Nat Genet, 2018).

To avoid potential biases and ensure consistency across analyses, we chose the commonly used sample size-weighted meta-analysis method, which our simulation and real data analyses showed to be more robust with higher statistical power (**Supplementary Notes 4** and **5**). Previously, MTAG was chosen for its better performance in the cross-validation PRS analysis in the EUR ancestry (see our response to comment #3 from reviewer #1 for more details); however, this advantage diminished as more cohorts were added. Notably, in the sample size-weighted meta-analysis, we adjusted the test statistics using the estimated LD Score regression intercept in cohorts with an attenuation ratio greater than 0.1 (Lee et al., Nat Genet, 2018) to account for potential inflation in GWAS statistics. In addition, we accessed the robustness of both meta-analysis approaches in dealing with data with underreporting issue by both real data analysis and simulation. For completeness, we will also release MTAG meta-analysis summary statistics for the ancestry-stratified analyses.

In our EUR meta-analysis with real data, the increase in the LDSC intercept from 1.171 to 1.175 (standard error of 0.018 for both estimates) due to the inclusion of FinnGen data was minimal, representing only 14.3% of the increase in the mean chi-squared value from 2.509 to 2.537. This suggests that the majority of the increase in the test statistics resulting from the inclusion of the FinnGen data is attributable to polygenic signals.

Consistently, our simulation analysis demonstrated that integrating a dataset with under-reporting into the meta-analysis did not inflate test-statistics for null variants for the sample size-weighted approach, although a slight deflation was observed for the MTAG approach **(Supplementary Note 4** and **Supplementary Figure 7a)**. However, the effect on statistical power for causal variants depended on the degree of misclassification errors, as detailed in **Supplementary Note 4** and **Supplementary Figure 7b**. In instances of severe misclassification errors, the meta-analysis statistical power was compromised. Therefore, it is essential to thoroughly evaluate whether the inclusion of a dataset with under-reporting genuinely enhances the power of the analysis to detect true genetic associations.

We have incorporated these analyses in the revised manuscript (lines 392-396): “Despite potential misclassification errors that could arise from under-reporting in electronic health records, such as those in the FinnGen cohort, the inclusion of this dataset indeed enhanced the statistical power of our meta-analysis, as demonstrated by an increase in the mean χ^2 from 2.51 to 2.54 with a minimal increase in the estimate of LDSC intercept (**Supplementary Note 4** and **Supplementary Figure 7**)”.

2. The cross-ancestry meta-analysis was performed using a fixed effects model. Why was the fixed effects model justified for the cross-ancestry meta-analysis? (MTAG was used in preference to a fixed effects model for the EUR samples meta-analysis).

Re: In the previous version of the manuscript, we did not use MTAG for the cross-ancestry meta-analysis because MTAG relies on LD score regression to estimate the variance-covariance matrices for SNP effects. This approach is not applicable to data from different

ancestries due to substantial differences in LD patterns and allele frequencies. Applying MTAG across ancestries without accounting for these differences can lead to inflated test statistics.

As mentioned in our response to the previous comment, in the current version of the manuscript, we have employed the sample size-weighted fixed effects model for both the ancestry-stratified and cross-ancestry meta-analyses. This meta-analysis method does not rely on assumptions regarding homogeneity of allele frequencies and LD patterns between datasets, making it suitable for cross-ancestry analyses. Indeed, the fixed effects model is the commonly used approach in cross-ancestry meta-analyses (PMID: 36224396; PMID: 37945903).

3. The section on PRS (lines 296–312) currently reports the accuracy in predicting myopia. This section and Table 1 would benefit from also reporting the accuracy in predicting high myopia (which has been a main focus of previous work [1,2]).

Re: We have included the accuracy of predicting high myopia in Table 1 (also see the table below).

Cohort	Phenotype	Ancestry	Validation sample size	R^2 for RE	AUC for myopia (s.e.)	AUC for high myopia (s.e.)
UKB	Measured MSE	EUR	10-fold cross-validation	0.214	0.741(0.006)	0.806 (0.011)
		AFR	4,302	0.040	0.612(0.009)	0.655(0.026)
		SAS	4,947	0.111	0.672(0.008)	0.722(0.017)
		EAS	839	0.088	0.654 (0.019)	0.711(0.027)
EPIC-Norfolk	Measured MSE	EUR	7,069	0.180	0.722 (0.007)	0.809(0.016)
ALSPAC	Measured MSE	EUR	1,493	0.166	0.728 (0.015)	0.808(0.032)

4. There was a convincing improvement in the accuracy of predicting high myopia when combining AOSW + PRS, compared to AOSW alone (Figure 6F). However, it would be prudent to discuss two caveats. First, AOSW in UK Biobank was self-reported in 40 to 70-year-olds and therefore may be subject to recall bias. Recall bias would lead to AOSW having lower accuracy in UK Biobank compared to its predictive accuracy in a sample in which AOSW was assessed without error. Second, ref #48 was cited to support the argument that AOSW + family history has equal predictive accuracy as AOSW alone. However, ref #48 was a study from Singapore, which unlike the UK, has seen a massive surge in myopia in the past generation. In the UK, it is possible that AOSW + family history is a better predictor than AOSW alone.

Re: We thank the reviewer for the suggestion and have included these caveats in the revised manuscript. The relevant texts read:
"However, this finding should be interpreted with caution because there has been a dramatic increase in myopia prevalence in the past generation in Singapore[67,68]" (lines 441-443). "In addition, AOSW in the UKB was self-reported between the ages of 40 and 70, which may be subject to recall bias, potentially affecting the predictive power for AOSW" (lines 446-448).

5. The manuscript reports the improved accuracy of the PRS with children's age as if this was a novel finding (lines 326-332; 431-434). However, this result was already reported (Figure 1 of [3]; Figure 4 of [4]; Figure 4a of [2]).

Re: We have revised the manuscript to recognize the previous findings and clarify that our discovery of the association between PRS and RE progression (difference in MSE of the same individual at the ages of 7 and 15) is novel. The revised texts read as follows "We also observed that the prediction accuracy of the PRS increased with age, in line with the observations in previous studies[14,50,51]" (lines 325-326) and "Furthermore, we found a strong association (R^2=4.0%, P value= 4.0×10^{-35}) between RE progression, as measured by the differences in MSE for the same individual between ages 7 and 15 years, and RE-PRS." (lines 328-330).

6. The manuscript reports that genetic effects on refractive error are age dependent as if this was a novel finding (line 331). However, this result was already reported (Figure 1 of [5]; Figure 3 of [3]).

Re: We have revised the manuscript accordingly to recognize the previous findings. The relevant text now reads as follows "An intriguing avenue for further understanding the genetic architecture of RE involves investigating the temporal genetic regulation during childhood and adolescence with large-scale longitudinal datasets, as indicated in previous studies[50,69]. Our analyses showed consistent evidence that the prediction accuracy of RE-PRS increased from 12 months to 18 years of age. Additionally, RE-PRS was found to be associated with changes in MSE over time" (lines 448-453).

7. The MR results reported in lines 349-360 (and 436-446) largely recapitulated the results of a recent study [6]. The novel aspects of the authors' current work should be highlighted, as the key findings were simply confirmatory. As an example, the manuscript states, "We identified EA and OA as two major risk factors for RE" (line 363). Yet this was already very well-established [7].

Re: We have removed the statement "From the analysis above, we identified EA and OA as two major causal risk factors for RE" and revised a preceding statement "In the MR analysis for each risk factor separately, the results showed that most of these factors have a significant causal effect on RE (FDR < 0.05), with the largest effects from educational

attainment (EA) and outdoor activities (OA) (**Figure 7a**), in line with previous findings[54,56]" (lines 352-355).

8. The manuscript reports non-linear PRS x E interactions as if they were novel (lines 53-55; 363-382). However, this result was already reported (Figure 3 of [8]; Figure 2B of [9]).

Re: The PRS-by-EA and SNP-by-EA interactions have been reported previously. We have revised the manuscript accordingly to acknowledge the previous findings. The relevant texts now read: "Second, individuals with a higher genetic risk for myopia tended to be more susceptible to environmental risk factors, a reflection of PRS x E interaction as indicated in previous work[58,59]." (lines 367-369).

Our finding of PRS-by-OA interaction is novel. Furthermore, we have proposed a non-linear causal effect of OA on RE as a potential reason for the PRS-by-OA interaction, which could have implications in clinical practices. The relevant texts now read: "Specifically, a moderate amount of daily outdoor activity is effective, with marginal additional benefits beyond this level. A previous randomized controlled trial also showed no dose-response effect between an additional 40 minutes and 80 minutes of outdoor time in reducing the risk of myopia[73]. Meanwhile, near work becomes substantially detrimental only when exceeding certain durations, which might explain the disproportionately high prevalence of myopia in East and Southeast Asia due to overloaded schoolwork." (lines 478-484).

9. The manuscript reports a Non-linear MR analysis (lines 381-382). There is a genuine concern that the use of non-linear MR analysis methods in samples such as UK Biobank is unreliable (when using either the standard NLMR method or the double-ranking method) [10,11]. The authors may wish to omit the NLMR results.

Re: We have removed the analysis from the revised manuscript as per the reviewer's suggestion.

10. In reply to my suggestion that the SNP-based heritability section could be shortened, in their Response Letter, the authors say, "to the best of our knowledge, no report of GREML analysis using individual-level data for estimating the SNP-based heritability for refractive error exists." Here are such reports, including analyses in UK Biobank: Table 2 of [12]; Figure 1 of [13].

Re: We apologize for this oversight. We have now revised the text as follows: "The estimate of h^2_{SNP} from GREML[27] was 0.370, with a standard error (s.e.) of 0.005, consistent with a previous report[11]" (lines 139-140).

The GREML-LDMS analysis reported in the subsequent text, which considered rare variants and differences in LD score among genetic variants, is novel.

Minor/technical points

1. Abstract line 49. The term “eye development” is generally used to describe ocular development during pregnancy rather than post-natal effects. Please can I check that this was the meaning intended by the authors?

Re: According to Gene Ontology (GO), “eye development” is defined as “the process whose specific outcome is the progression of the eye over time, from its formation to the mature structure” (source: https://amigo.geneontology.org/amigo/term/GO:0001654). According to this definition, this term covers both prenatal and postnatal ocular development.

2. Supplementary Tables. The results tables beginning on page 19 and page 37 appear to have information missing, such as a missing data column for ‘chromosome’ or column headers that are incomplete.

Re: We apologized for this issue and will try to avoid this problem in the next submission.

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Reviewer #3:

My major concerns remain unsolved.

Concern 1: sample comparison
The authors claim to have increased the sample size of European (EUR) ancestry data from 600K to approximately 1M. However, this increase (400K) primarily comprises control samples (361,237 controls) from publicly available GWAS data from FinnGen, specifically including "binary myopia status of 3,534 cases and 361,237 controls from the FinnGen cohort." In my initial comments, I differentiated the samples of European and Asian populations and compared the sample sizes in the current study with those from previous studies (Hysi et al., 2020; Tedja et al., 2018). “Specifically, for the EUR populations, in the current study, the authors included UKB and 23andMe, both of which were already included in Hysi et al. 2020, with nearly the same samples in the analysis (e.g. the nearly same ~100k individuals with mean spherical equivalent from UKB; the identical 23andMe samples; imputed MSE based on age of onset of spectacle wear (Cheng et al.) versus myopia status estimated based on age of first spectacle wear (Hysi et al. 2020)). Additionally, Hysi et al. 2020 included an extra 34,998 individuals (measured MSE) from the GERA Study and 34,079 participants from the CREAM.”

In the revised manuscript, the authors included the same 34,998 individuals (measured MSE) from the GERA Study previously included in Hysi et al. 2020. The new samples of binary myopia status from the FinnGen cohort represent an additional sample size of 14K,

calculated as 4*(3534 * 361237)/(3534 + 361237). Compared with the inclusion of 34,079 participants from the CREAM in Hysi et al. 2020, the current study even has a smaller sample size of the European population.

Overall, for EUR populations, the current study incorporates UKB, 23andMe, and the GERA Study, all previously included in Hysi et al. 2020, with nearly identical samples. The modest increase in sample size from FinnGen (effective sample size 14K) is minimal compared to Hysi et al. 2020, which also included additional participants from CREAM (34K).

The new samples from EAS populations are limited in scale and have not robustly identified novel signals (as further detailed in MTAG method considerations below).

Re: During the second revision, we have significantly expanded our datasets. Our study now differs from the Hysi et al. 2020 study in the following aspects: 1) a much larger sample size for the imputed MSE from age of onset of spectacle wear (n = ~301K) compared to that in Hysi et al. (n = ~172K); 2) 74,447 myopia cases and 785,503 controls of EUR ancestry from the FinnGen, MVP, and AllofUs cohorts altogether; 3) 121,172 individuals of EAS ancestry from the ToMMo, WeGene, 23mofang, MVP, and Japanese high myopia cohorts; 4) 21,224 cases and 123,513 controls of AFR ancestry from the MVP and AllofUs cohorts. The overall sample sizes have reached 1.5M for EUR, 121K for EAS, 145K for AFR, representing the largest GWAS for RE to date.

The significance of this study lies not only in its scale but also in the diverse ancestral representation. We have identified several ancestry-specific signals and develop more accurate cross-ancestry PRS, enhancing our understanding of the genetic architecture of RE.

We have revised manuscript as follows: “The expanded sample size compared to the largest previous study[8] was due to a larger sample size for the imputed MSE from AOSW (301K vs. 172K) in EUR, as well as the inclusion of additional data from 860K individuals of EUR ancestry (FinnGen, MVP, and AllofUs), 121K individuals of EAS ancestry, and 145K individuals of AFR ancestry.” (lines 388-392).

Concern 2: MTAG analysis considerations

The authors have conducted new analyses using the MTAG default setting to address potential inflation from the "perfect_gencov" flag, which assumes perfect genetic correlation between multiple input traits. They have noted a higher LDSC intercept for MTAG-‘perfect_gencov’ compared to MTAG-default (1.08 versus 0.99) and have opted for a conservative approach. However, for some crucial results, the authors continue to utilize results from MTAG-‘perfect_gencov’, which is not suitable for analyzing multiple diverse traits, e.g., the GJD2 locus; rs258669 within CACNA2D1; examples in Figure 2.

The authors presented simulated genetic correlations for continuous and binary traits to

demonstrate shared genetic architecture. However, they failed to present empirical genetic correlations for the included continuous and binary myopia-related traits.

Re: First, as stated at the beginning of this document and in the responses to comments from reviewer #1, we have adopted the sample size weighted method for both ancestry-stratified and cross-ancestry GWAS meta-analyses. This decision was made because MTAG can bias test statistics due to errors in estimating the variance-covariance matrices of SNP effects and SNP effects estimation errors, as confirmed by our simulations. Such biases become more prominent with a large number of cohorts, especially when some have relatively small sample sizes (Turley et al., Nat Genet, 2018). To avoid potential biases and ensure consistency across analyses, we chose the commonly used sample size-weighted meta-analysis method, which our simulation and real data analyses showed to be more robust with higher statistical power (**Supplementary Notes 4** and **5**). Previously, MTAG was chosen for its better performance in the cross-validation PRS analysis in the EUR ancestry (see our response to comment #3 from reviewer #1 for more details); however, this advantage diminished as more cohorts were added. Notably, in the sample size-weighted meta-analysis, we adjusted the test statistics using the estimated LD Score regression intercept in cohorts with an attenuation ratio greater than 0.1 (Lee et al., Nat Genet, 2018) to account for potential inflation in GWAS statistics. For completeness, we will also release the MTAG-default meta-analysis summary statistics for the ancestry-stratified analyses.

Regardless of the change in the meta-analysis method in the ancestry-stratified meta-analyses, we have presented the empirical genetic correlations for the continuous and binary RE traits in **Supplementary Note 1**. Pairwise genetic correlation estimates among the 23andMe, UKB directly measured MSE, and UKB AOSW-inferred MSE data were all close to 0.93, as estimated using the bivariate LDSC method (Supplementary Note lines 89–91). Specifically, the genetic correlation between UKB directly measured MSE (continuous) and 23andMe (binary) data was 0.93 (s.e. = 0.02).

As for the two loci, *GJD2* and *CACNA2D1*, mentioned by the reviewer, in the revised manuscript, the signal in the *GJD2* locus is the most significant for both the EAS (p= 5.76e-62) and AFR (p= 4.81e-15) ancestries (**Supplementary Figure 3**), and the association in the *CACNA2D1* locus remains a significant EAS-specific signal (p=2.02e-8) . All the p-values reported here are based on sample size weighted meta-analysis. Note that in Figure 2, we have replaced *CACNA2D1* with another EAS specific association, *PDE4B*, because the association of *PDE4B* has been supported by a prior knockout experiment, and we have relocated *CACNA2D1* to **Supplementary Figure 4**.

Concern 3: clarity on novel loci:
The authors reported 427 genetic loci using MTAG-default, similar to the 435 loci reported by Hysi et al. 2020, which was expected given that most cohorts included in the two studies are nearly identical, with similar power in identifying loci. In the EAS population, the authors reported 13 loci, all previously identified in EUR populations.

Overall, the incremental increase in novel loci from the modest sample size increase is minimal.

In the conditional analysis, the authors utilized 435 lead SNPs from Hysi et al. 2020 and reported 42 conditionally significant loci, most of which are just above the 5e-8 significance level. A more appropriate approach would involve conditioning on all genome-wide significant loci (not just independent SNPs) from Hysi et al. 2020.

Re: With the expanded sample sizes, the statistical power of our study has been further improved. In the EUR-stratified GWAS meta-analysis, we have now identified 708 quasi-independent variants within 539 non-overlapping genetic loci. Conditioning on all the jointly significant associations identified from the Hysi et al. 2020, 168 quasi-independent associations remained genome-wide significant, identified from PLINK clumping analysis of the conditional GWAS summary statistics.

It should be noted that because genome-wide significant SNPs are often in strong LD, fitting all of them in the conditional analysis model can lead to collinearity issue. In this revision, we selected all the jointly significant SNPs from the loci reported by the Hysi et al. using GCTA-COJO-slct, including multiple independent signals within a single locus, and fitted them as covariates in the conditional analysis model using GCTA-COJO-cond.

In addition, the increased sample sizes in EAS and AFR ancestries has added six EAS-specific associations and one AFR-specific association.

We have revised the manuscript to include the comparison with the Hysi et al. study. Updated text (lines 166-168): “Conditioning on all the jointly significant associations identified from the largest published study[8], we identified 168 previously unknown quasi-independent associations”.

Concern 4: questionable novelty of polygenic risk score:
As also pointed out by Reviewer 2 in comments 2 and 3, the slight increase in PRS accuracy and claims of novel discoveries were of questionable novelty.

Re: To summarize our response to the previous comments 2 and 3 from reviewer #2: In European ancestry, our study improved the proportion of variance in RE explained by PRS from the previously reported 19.0% by Clark et al. to 21.4% and improved prediction by 11.0%-48.1% across multiple testing cohorts. More importantly, our cross-ancestry PRS using both EUR and EAS GWAS summary statistics improved over the existing EUR PRS by 115.6% in EAS (4.5% using previously published RE-PRS vs. 9.7% using our cross-ancestry RE-PRS), a region with the highest myopia around the globe. The improvement stems from both the increased sample size and the advancement of PRS method, SBayesRC, which incorporates functional annotations and considers a mixture distribution of SNP effects.

Concern 5: questionable novelty of lifestyle modifications:
Although the authors have shown a potential nonlinear association between educational attainment and outdoor activities with the risk of refractive error (RE), given the available evidence from similar Mendelian randomization studies and well-established clinical trial findings (referenced PMIDs: PMID: 26372583; PMID: 29371008; PMID: 35779695; PMID: 28951126), the novelty of the main finding in the section "Educational attainment and outdoor activities are two main environmental risk factors for RE" was of questionable novelty.

Re: In the manuscript, we have moderated our conclusion to state, "Our MR analysis corroborated previous finding that EA and OA are causal risk factors for RE" (lines 458-459). Previous understanding of these two major risk factors is based on the association effect sizes from epidemiological studies or clinical trials, whereas we derived the conclusion based on the conditional MR analysis, where the effects of other causal factors diminished when considering the effect of education attainment. As emphasized in the discussion section, the significance of our study lies in: 1) the need to be aware of potential confounding effects induced by these two major risk factors, as they could clarify some controversial findings from observational studies (lines 461-469); and 2) a potential non-linear causal relationship, which can guide the development of more efficient and tailored prevention strategies (lines 477-484).

Overall, the authors have used nearly overlapping European samples with Hysi et al. 2020 (Concern 1), used inflated MTAG setting for heterogeneity myopia related traits (MTAG-'perfect_gencov', Concern 2), identified very few novel loci (Concern 3), achieved a modest increase in PRS (Concern 4), and reported lifestyle risk factors that are well-established from previous clinical trials (Concern 5). These factors defer the novelty of this current study, representing very incremental work in the field of myopia genetics.

Re: Here is a summary of our response to these concerns.
1) Study power: The sample size of our EUR data was 952K larger than the EUR data used in Hysi et al., and together with the 121K EAS and 145K AFR samples, this study represents the largest GWAS for RE to date.
2) Our analysis identified 539 and 575 associated regions in the EUR ancestry and cross-ancestry meta-analyses, respectively, representing 20.0% and 28.1% increases in the number of GWAS loci compared with the largest previous meta-analysis (i.e., the Hysi et al. study).
3) Our cross-ancestry RE-PRS improved the prediction R^2 over the best-performing existing PRS by 115.6% in EAS and 55.6% in AFR. Our EUR RE-PRS improved prediction R^2 by 11.0%-48.1% across multiple testing cohorts.
4) While EA and OA are well established risk factors for RE, it is novel to observe that certain risk effects on RE diminished after accounting for EA using conditional analysis. Furthermore, our study is the first to show a PRS-by-OA interaction effect on RE, and to

demonstrate that this interaction likely arises from non-linear causal effects of these two major risk factors on RE.

Collectively, this study has provided comprehensive insights into the cross-ancestry genetic architecture of refractive errors and demonstrate the clinical relevance of our findings. We believe that our work will contribute to mitigating the high prevalence and societal burden of refractive errors, particularly high myopia.

Responses to Reviewers' Comments

Reviewer #1

The Authors have likely tried their best to respond to the previous round of peer review.

Re: We thank the reviewer for the positive remark.

As a due diligence measure, could the Authors reconcile the N=932 RE-associated variants with Figure 1, whereby summing up all the EUR, EAS, and AFR variants did not yield N=932. However, N=932 was consistent in Supplementary Table 9. Key numbers in the abstract and main display items should have consistency, and if there are alternate explanations, these should be clarified.

Re: We thank the reviewer for pointing this out. The N=932 represents the total number of unique, quasi-independent variants identified from cross-ancestry GWAS meta-analysis, which is larger than the sum of the variants from the ancestry-stratified analyses, because of the increased power of the cross-ancestry meta-analysis. We have clarified this in the revised manuscript.

Update text (lines 205-208): "We identified 932 quasi-independent variants within 575 non-overlapping genomic regions, which is larger than the sum of the numbers from the ancestry-stratified analyses, because of the increased power of the cross-ancestry meta-analysis".

Reviewer #2

Revision 2 of the manuscript is much improved. The inclusion of new GWAS samples from the MVP, AllofUs and 23mofang cohorts has resulted in the discovery of 241 novel, quasi-independent variants associated with refractive error, as well as 6 EAS-specific signals and 1 AFR-specific signal. The polygenic score (PRS) derived by the authors has improved prediction accuracy in EUR (R2 = 21.4%), EAS (R2 = 9.7%) and AFR (R2 = 4.2%) compared to previously published polygenic scores for refractive error.

In the later sections of the manuscript that I previously criticized for claims of novelty when describing findings that had already been published, the authors do now cite the earlier studies.

Re: We thank the reviewer for the positive remark and the summary of our revision.

Main points

1. Please include a data availability statement.

Re: We thank the reviewer for the reminder and have now provided a data availability statement in the revised manuscript.

Updated text (lines 886-909): "

Data availability

The ancestry-stratified and cross-ancestry GWAS summary datasets and polygenic risk scores from this study are available for research use at https://yanglab.westlake.edu.cn/re.html, excluding the 23andMe participants due to the 23andMe data transfer agreement. For bona fide researchers who meet the criteria for accessing the GWAS summary statistics from 23andMe (https://research.23andme.com/dataset-access/), we provide easy-to-use scripts for meta-analyzing our released summary statistics and the 23andMe summary statistics, and for generating the PRS given the meta-analyzed summary statistics at https://yanglab.westlake.edu.cn/re.html. Individual-level data from the UK Biobank are publicly available to bona fide researchers (https://www.ukbiobank.ac.uk); GWAS summary statistics from the FinnGen cohort can be downloaded through the link https://storage.googleapis.com/finngen-public-data-r9/summary_stats/finngen_R9_H7_MYOPIA.gz; GWAS summary statistics from the MVP cohort can be downloaded at https://ftp.ncbi.nlm.nih.gov/dbgap/studies/phs002453/analyses/GIA/phs002453.MVP_R4.1000G_AGR.GIA.PheCodes_SenseOrgans_batch1.analysis-PI.MULTI.tar; GWAS summary statistics from the All of Us Research Program are accessible to researchers via the All of Us Researcher Workbench at https://www.researchallofus.org; Individual-level data from the ALSPAC cohort can be accessed upon request (https://www.bristol.ac.uk/alspac/researchers/access/); Individual-level data from the

EPIC-Norfolk cohort can be accessed upon request (https://www.epic-norfolk.org.uk/for-researchers/data-sharing/data-requests/); GWAS summary statistics from the Meguro et al. study, the WeGene cohort, the GERA cohort, the 23mofang cohort, and the ToMMo cohort can be accessed upon request."

2. (a) The analyses reported in Figure 7a,b used genetic variants associated specific behaviors in adults as instrumental variables for childhood behaviours (since childhood is the age when myopia typically develops). Therefore, these analyses relied on the assumption that the genetic component of these behaviors is conserved throughout the life course from childhood to adulthood. This assumption is questionable, because parents may play a major role in determining children's behavior. In the Discussion, the authors acknowledge the need to confirm that instrumental variables are associated with childhood behaviors yet they themselves failed to do this, e.g. television viewing behavior. (b) Genetic nature could explain a proportion of the association between an individual's genotype and their phenotype for traits related to behavioral risk factors (Kong et al. 2018; Guggenheim et al. 2022). The authors should at least acknowledge that their Mendelian randomization analyses could be biased if genetic nurture is important for these behaviors.

Re: We thank the reviewer for reminding us of this limitation. Well-powered GWAS data for childhood behaviours are not yet available, which would be required to select a sufficient number of genetic instruments for the MR analysis. We, therefore, used genetic instruments from adult behaviour GWAS as an approximation under the assumption that the genetic effects are largely shared between adult and childhood behaviours. However, if this assumption does not hold, the MR results can be biased. We have added a caveat in the revised manuscript, along with a caveat regarding the potential confounding from genetic nurture. Note that as per a comment from Reviewer #3, we have decided to move the entire MR section to Section 5 of the **Supplementary Note**.

Updated text (lines 357-363 in the supplementary): "Notably, the MR analysis was performed under the assumption that genetic predisposition to outdoor activities remains consistent from childhood to adulthood, an assumption largely supported by prior work[44]. However, if this assumption does not hold, these MR results could be biased. Furthermore, these results do not account for potential confounding from genetic nurture, which should be considered when interpreting these associations[45,46]. Future studies with a sufficiently

powered childhood-behaviour GWAS are warranted to select appropriate genetic instruments and validate our findings."

Minor points

1. When reporting GWAS findings in the supplementary tables, please indicate which allele is the effect allele, e.g. label the alleles EA=Effect Allele and NEA=Non-Effect Allele.

Re: Done.

2. Line 177. When reporting the popcorn analysis results, clarify if the values are the "genetic effect correlation" or the "genetic impact correlation" that are produced by popcorn.

Re: We have clarified in the manuscript that we are referring to the genetic impact correlation.

Updated text (lines 181-183): "We next estimated the genetic correlation (r_g) for RE among the three ancestries using Popcorn[35] (specifically, the *'genetic impact correlation'* output), a method that accounts for the difference in sample size between datasets."

3. Line 194. Ancestry-specific GWAS signals (lines 169-172) appeared to be assumed if a variant was strongly associated (P < 5e-08) in one ancestry but not associated in the other ancestries (P > 0.05). A test for heterogeneity was reported (Supplementary Table 8) but it was not clear if this was the statistical test was used to confirm that ancestry-specific signals were not simply due to chance. Please clarify the criteria used to classify a variant as ancestry-specific.

Re: In our previous version of the manuscript, an ancestry-specific signal was defined as a variant that was genome-wide significant ($P < 5.0 \times 10^{-8}$) in one ancestry but not in the others, and showed significant heterogeneity in effect size across ancestries (Cochran's Q test FDR < 0.05). In the revised manuscript, we have added the condition that the variant showed no evidence of an overlapping signal (LD $r^2 < 0.1$) in the other ancestries. This more stringent criterion revised the number of EAS-specific signals from six to four, but all other results remained unchanged.

Updated figure: Extended Figure 3.

Updated text (lines 695-698): "We defined an ancestry-specific signal as a variant that reached genome-wide significance ($P < 5.0 \times 10^{-8}$) in one ancestry but not in the others, showed significant heterogeneity in effect size across ancestries (Cochran's Q test FDR < 0.05), and had no evidence of an overlapping signal (LD $r^2 < 0.1$) in the other ancestries".

4. Line 482. Cite a reference to support the statement, "near work becomes substantially detrimental only when exceeding certain durations".

Re: Done. We have cited the Ha et al., 2025, *JAMA Netw Open* (PMID:39982728) for this statement (**lines 389-390** in Section 5 of the **Supplementary Note)**.

5. Line 670. Please provide the link for accessing the publicly available MVP summary statistics (I could not find summary statistics for myopia using the generic weblink provided in the manuscript).

Re: Done.

Updated text (lines 899-902): "GWAS summary statistics from the MVP cohort can be downloaded at https://ftp.ncbi.nlm.nih.gov/dbgap/studies/phs002453/analyses/GIA/phs002453.MVP_R4.1000G_AGR.GIA.PheCodes_SenseOrgans_batch1.analysis-PI.MULTI.tar".

6. Line 694. Typo "diptor".

Re: Fixed.

7. Line 762. With regards to estimating how much of the SNP-based heritability in EAS and AFR was explained by the top EUR variants: (a) Explain how boundaries were defined with respect to "the lead SNPs along with their upstream and downstream boundaries". (b) Was this SBayesRC analysis restricted to hapmap3 variants or were all variants included? (c) What was the size of the reference panel for this SBayesRC analysis.

Re: We thank the reviewer for these questions and have provided the requested details in the Methods section. (a) The boundaries for lead SNPs were defined as an LD r^2 of 0.1

upstream and downstream, as specified in the Supplementary Table 3. (b) This SBayesRC analysis included all variants. (c) For EUR, we used a random sample of 20,000 unrelated UKB individuals of European ancestry as the LD reference. For AFR, we used 7,015 unrelated UKB individuals of AFR ancestry. For EAS, we used 2,256 unrelated UKB individuals of EAS ancestry. All the LD references can be downloaded at https://github.com/zhilizheng/SBayesRC.

Updated text (lines 708-712): "First, we annotated the trait-associated loci, i.e., the lead SNPs along with their upstream and downstream boundaries based on an LD $r^2 < 0.1$ from the EUR GWAS analysis. Second, we performed an SBayesRC analysis using the EAS/AFR GWAS summary statistics and an LD reference from UKB-EAS (N = 2,256) or UKB-AFR (N = 7,015) to estimate the proportion of h^2_{SNP} explained by the annotated SNPs when all SNPs were fitted jointly".

References

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Kong A, G Thorleifsson, ML Frigge, BJ Vilhjalmsson, AI Young, TE Thorgeirsson, et al. (2018): The nature of nurture: Effects of parental genotypes. Science 359: 424-428.

Reviewer #3

The manuscript presents a large-scale cross-ancestry GWAS of refractive error (RE), reports additional genome-wide associations, proposes improved polygenic risk scores (PRS) and explores gene–environment interactions.

While the dataset is larger and more diverse than earlier efforts, the biological and translational advances derived from this added scale are, in most places, confirmatory or modest extensions of established knowledge. Many of the major concerns raised in prior rounds remain unresolved.

Re: We thank the reviewer for the careful reading of our manuscript again and for raising the additional comments.

Sample-size (previous Concern 1): The EUR meta-analysis still relies heavily on UKB (direct + AOSW) and 23andMe—the core of Hysi et al. 2020.

The expansion of "1M to 1.5M" is almost entirely from published MVP data (DOI: 10.1126/science.adj1182, with 65,574 cases and 333,242 controls) and All of Us (only 5,339 cases, and additional 91,024 controls). Therefore, the ~860 K "new" EUR participants (MVP, AllofUs) contain <70 K additional cases and thus generate a far smaller effective sample-size gain than the headline number suggests.

Re: While this study represents the largest cross-ancestry RE GWAS, we agree with the reviewer that the increase in sample size in EUR compared to the Hysi et al. 2020 study is primarily due to the inclusion of the FinnGen, MVP and All of Us data, as well as a larger sample size for the AOSW-derived RE phenotype in the UKB. We have included this as limitations in the revised manuscript.

Updated text (lines 424-434): "This study is not without limitations. First, while our study presents the largest cross-ancestry RE GWAS to date, the gain in statistical power in the EUR ancestry compared to the largest previous study[8] is primarily due to the inclusion of the FinnGen, MVP, and AllofUs datasets, as well as a larger sample for the AOSW-derived RE phenotype in the UKB. This may partly explain the modest improvement of our study over the Clark et al. study[14] in PRS (R^2 of 21.4% vs. 19.0%). Second, while the overall sample size of the meta-analysis surpassed 1.7M, for some of the included case-control cohorts, the number of myopia cases was limited, meaning that the effective sample size could be substantially smaller than the observed overall sample size. More effort is warranted in the future to further expand the sample sizes to unveil the full genetic landscape of RE in different ancestries and improve the performance of PRS, particularly in underrepresented ancestries".

In EAS and AFR, although sample sizes increase, the absolute numbers remain modest, maintaining a substantial imbalance relative to EUR and limiting power to detect ancestry-specific biology.

Re: While we had made every effort to assemble data for the largest cross-ancestry GWAS meta-analysis for RE to date, we agree with the reviewer that the genetic associations discovered in this study, particularly those in EAS and AFR ancestries, still represent only a small fraction of the complete genetic architecture. Further efforts are certainly warranted to expand the sample sizes of RE GWAS, especially for underrepresented populations. As noted in the response to a comment above, we have incorporated this point into the limitations section of our revised manuscript.

Updated text (lines 429-434): "Second, while the overall sample size of the meta-analysis surpassed 1.7M, for some of the included case-control cohorts, the number of myopia cases was limited, meaning that the effective sample size could be substantially smaller than the observed overall sample size. More effort is warranted in the future to further expand the sample sizes to unveil the full genetic landscape of RE in different ancestries and improve the performance of PRS, particularly in underrepresented ancestries".

Novel loci (previous Concern 3): The author reported 708 quasi-independent variants, and 168 previously unknown quasi-independent associations after conditional analysis of the Hysi et al. 2020 study.

However, from table S5, there are 25 SNPs with P value > 5e-8 (e.g. rs4234239 p=1.033e-01; rs41276453 = 7.106e-04). Without filtering the marginal P value < 5e-8, these 25 loci cannot be considered significant. Moreover, of the remaining 143 SNPs (marginal P < 5e-8), most SNPs (71% SNPs, n=102) have P value > 1e-9 or in an overlap position (±250 kb) with Hysi et al. 2020 study, suggesting limited power to detect novel SNPs.

Re: We want to clarify that a variant can be non-significant in the marginal analysis but become significant in a joint analysis because of a masking effect (i.e., the product of the effects of two variants and the LD correlation between their effect alleles being negative) or statistical sampling (i.e., a variant with a borderline significance in the marginal analysis becomes significant in the conditional analysis, or vice versa). Nevertheless, we thank the reviewer for pointing this out and have added text to clarify that 25 of the conditionally significant variants did not have genome-wide significant marginal p-values.

Updated text (lines 166-169): "Conditioning on all the jointly significant associations identified from the largest published study, we identified 168 previously unknown quasi-

independent associations, of which 143 remained significant after filtering out those with marginal P-values $\geq 5x10^{-8}$ (**Supplementary Table 4**)".

We have also integrated the reviewer's comments about the locations and significance levels of the conditionally significant variants into the revised manuscript.

Updated text (lines 169-174): "Of these 143 conditionally significant variants, 71% were in an overlapping position (±250 kb) with those identified in previous work[8] or had a P-value > $1x10^{-9}$. This suggests a clustering of trait-associated variants in specific genomic regions, a known pattern for polygenic traits like height[34], and indicates that much larger sample sizes are needed to discover the genetic variants explaining the remaining SNP-heritability."

PRS improvement remains modest (previous Concern 4): In Europeans, the SBayesRC PRS explains 21.4 % (vs. 19.0 % in Clark et al. 2023; +2.4%). In EAS the cross-ancestry model doubles R^2 (4.5 -> 9.7 %), but absolute accuracy remains low and clinically marginal.

Re: As noted in the responses above, while we have made our best effort to assemble GWAS datasets from three main ancestries, all of the largest sample sizes to date, for the cross-ancestry analysis, we agree with the reviewer that the improvement in PRS in EUR was modest. Although we have substantially improved the performance of the PRS in other ancestries (e.g., from 4.5% to 9.7% in EAS as noted by the reviewer), future efforts are needed to further improve PRS performance, particularly in under-represented populations. As noted above, we have incorporated this into the limitations section of the revised manuscript.

Updated text (lines 424-438): "This study is not without limitations. First, while our study presents the largest cross-ancestry RE GWAS to date, the gain in statistical power in the EUR ancestry compared to the largest previous study[8] is primarily due to the inclusion of the FinnGen, MVP, and All of Us datasets, as well as a larger sample for the AOSW-derived RE phenotype in the UKB. This may partly explain the modest improvement of our study over the Clark et al. study[14] (R^2 of 21.4% vs. 19.0%). Second, while the overall sample size of the meta-analysis surpassed 1.7M, for some of the included case-control cohorts, the number of myopia cases was limited, meaning that the effective sample size could be substantially smaller than the observed overall sample size. More effort is warranted in the future to further expand the sample sizes to unveil the full genetic landscape of RE in

different ancestries and improve the performance of PRS, particularly in underrepresented ancestries".

The authors reported that the combined AOSW + PRS AUC of 0.92 for high myopia, yet the incremental gain over AOSW alone (0.88 -> 0.92) indicates the clinical translation is not yet compelling.

Re: We have revised the text to moderate the statement.

Updated text (lines 400-402): "While it may not be compelling in clinical translation, the RE-PRS in conjunction with AOSW achieves an AUC of 0.92 for high myopia prediction in EUR."

As pointed out in the reviewer 2's comments 7, 8, and 9, the Gene–environment and MR analyses largely confirm prior work (previous Concern 5), and most reported interactions and causal inferences replicate earlier findings (e.g., education, outdoor activity), offering little beyond confirmation of established relationships.

Re: While our MR and gene-environment interaction analyses provided some novel insights, we agree with the reviewer that some of the results are confirmatory. We have therefore moved these analyses to the **Supplementary Note** (Section 5) and have retained a very brief mention them in the main text.

Updated supplementary item: Section 5 of the **Supplementary Note.**

Updated text (lines 419-422): "Last but not least, we investigated the effects of environmental risk factors, including educational attainment (EA) and outdoor activities (OA), on RE and found potentially non-linear effects of EA and OA and RE (Section 5 of the **Supplementary Note** and **Extended Figure 6**)".

Overall, the manuscript offers the largest RE dataset so far. However, the resulting findings are primarily incremental.

Re: We thank the reviewer for acknowledging that this study offers the largest RE GWAS dataset so far. We hope that the genetic findings, and possibly more importantly, the summary statistics generated from this study, can serve as a stepping stone toward fully

revealing the genetic architecture of RE and have an impact on future prevention and intervention strategies.