nature portfolio

Peer Review File

Uncovering Genetic Loci and Biological Pathways Associated with Age-Related Cataracts through GWAS Meta-Analysis

REVIEWER COMMENTS

Reviewer #1 (Remarks to the Author):

Diaz-Torres and co-authors conducted a meta-analysis using data from 127,985 cases and 837,371 controls of European ancestry and identified genetic loci and biological pathways associated with age-related cataracts. Compared to the cited Choquet 2021 cataract study of ~585,000 samples, the authors added GWAS summary statistics from ~380,000 more samples. They also performed Mendelian Randomization and PRS analyses. However, there are several points that require clarification.

1. The authors stated that they used subjects of European ancestry. There are non-European subjects in the GERA cohort. Did the authors only use European subjects in the GERA cohort? Similarly, did the authors exclusively use European participants in the UK Biobank cohort? If the authors did not re-analyze the GERA and the UKB cohorts, where and how were the GWAS summary statistics obtained?
2. The authors' analysis of the FinnGen cohort or acquisition of the GWAS summary statistics is unclear. The cited FinnGen study (Ref. 16) does not appear to contain cataract information.
3. For the Mass General Brigham Biobank (Lines 120-126), the methodology for the cataract analysis and disease definition/classification is unclear.
4. Were cataracts defined similarly across all cohorts used? Please provide a summary of this information in a table.
5. In the Methods section, the authors only explained that Regenie was used in the CLSA study. The methodology for analyzing other cohorts is unclear. Was Regenie used in other cohorts, or was a different approach employed?
6. The authors used an imputation quality score of 0.8 to filter variants. Was the 0.8 score used in each cohort analysis originally? Or, did the authors obtain GWAS summary statistics from each cohort, possibly using different initial cutoffs, and then further filtered using the imputation quality score of 0.8?
7. Did the authors assess heterogeneity in their meta-analysis results?
8. Please explain why the Raine Study was mentioned in the method and results sections but excluded from Figure 1.
9. It is puzzling that the authors only mentioned the causal relationship between type 1 diabetes and cataracts and did not address the relationship between type 2 diabetes and cataracts until line 354. The authors should consider mentioning the previously published relationship between type 2 diabetes and cataracts early in the manuscript to prevent confusion.
10. Lines 165-157: What is the difference between gene-based and gene-set analyses?
11. Line 160: What is the origin of the 18,488 gene-sets, and how were they defined?
12. Line 50, are all 126 genes novel?
13. Line 227: Please clarify the procedure for "excluded through a principal component analysis," including the number of principal components and any cutoffs used.
14. BHAS participants are of European ancestry. What was the rationale for using the TOPMed reference panel, which includes many non-European ancestry subjects? Were all cohorts imputed similarly or differently? How many variants were genotyped in BHAS, and how many remained after QC?

15. The authors identified 126 genes associated with cataracts. It is unclear whether these 126 genes were derived from single-variant analysis or gene-based analysis. If from gene-based analysis, which type? Was the MAGMA gene-based analysis similar or different from recent gene-based analyses (e.g., Burden and SKAT), or does MAGMA have apparent advantages over recently developed gene-based methods?
16. Line 235: Please consider using "PLINK 2.0" (the official name on the PLINK website) instead of "Plink v 2."
17. Line 277: Are all 20 genes involved in "drug-gene interactions among 20 genes" novel, or have some of these genes been reported previously without investigating the drug-gene relationship?
18. Lines 299 - 304: In the PRS section, why was only BHAS mentioned, while the Raine Study Gen2 was not? However, in the following paragraph (Lines 305-310), both the Raine Study Gen2 and the BHAS cohorts were used.
19. Regarding PRS, could the authors provide prediction accuracy metrics in addition to the association p-values, such as AUC?
20. For the PRS analysis, please also construct PRS using previously published loci and compare with your new PRS results. Do the new genes/results yield better PRS outcomes or produce similar findings?
21. In the Manhattan plot, the newly discovered genes show relatively much lower statistical significance than the previously reported ones. Can the authors comment on the biological significance differences between the newly discovered genes and the previously reported ones? Can we assume previously reported ones carry much more biological significance because they show much stronger statistical significance and possibly larger effect sizes as well?
22. Please provide a section for web resources detailing the software tools used and GWAS results accessed.
23. Are the the authors' meta-analysis summary statistics available to the public?

Reviewer #2 (Remarks to the Author):

Diaz-Torres et al present a large GWAS meta-analysis on cataracts and find several novel loci. They performed gene enrichment analysis identifying 126 genes and mention 4 that are involved in drug-interactions. The performed Mendelian Randomization and polygenic risk score analysis to explore the relationship between type 2 diabetes and UVR exposure and cataracts.

I think the paper would benefit from a main table showing the novel variants found. It would also be good to discuss the set of variants, for instance add information on how many coding variants were found. It is a bit odd that a GWAS does not discuss any individual variants. Were there no novel variants of interest, for instance loss of function or missense variants?

Supplementary Table 1 should have more information, like the coding change of each variant, the minor allele frequency, the mapped/closest gene, the odds ratio and then results from individual cohorts. It is not clear what units the BETA is in.

It is not clear why the UK Biobank and GERA cohort are grouped together before the meta-analysis. Did the authors use the previously published meta-analysis to use in their meta-analysis instead of summary statistics from the individual cohorts?

Comparison between the cohorts is missing, and GWAS results from individual cohorts should be in the Supplement. Reporting for example correlation between effect sizes in different cohorts would be helpful, and perhaps add figures comparing effect sizes for different cohorts for the 85 variants.

Reporting on ethnicity is lacking for most of the cohorts (except CLSA). Did the authors only use individuals of European ancestry, and if not how did they adjust for ethnicity?

There is no information about the genotypic data used in the meta-analysis, whether the cohorts had whole genome sequence data and imputation, or just chip typed data. How many variants were tested?

In a previous GWAS cataract study, sex specific analysis found some additional loci. Did the authors perform such analysis?

For the Mendelian randomization (MR) analysis, it is not clear what variants were used as instrumental variables. Was it the 85 independent variants? Further, I think the presentation of the MR results could be more informative with effect plots instead of just the OR for different MR analysis. For example, by plotting the effect on cataract against effect on T2D, for the risk increasing allele for all the variants associating with cataracts, and add the regression line from a weighted linear regression. Then do the same thing, except for variants that have been reported to associate with T2D. An example of this kind of presentation can be found in “Rare SLC13A1 variants associate with intervertebral disc disorder highlighting role of sulfate in disc pathology” by Bjornsdottir et al.

The authors discuss that lipid metabolism has been associated with cataracts. Can the authors explore the causality of lipids measurement through MR analysis?

In Figure 2 it is difficult to read the labels.

In the drug-gene interaction discussion, it is not clear to the reader what these interactions mean, and the speculations of the authors in the discussion feel a bit far-fetched.

The authors emphasize on how their findings have important implications for the treatment of cataracts. I am not a cataract specialist, but I think that cataracts today are only treated with surgery. It would be good if the authors would mention that in the introduction and give a bit of background on the current research on non-surgical treatment of cataracts. Also, when the authors talk about treatment, it is not always clear if they mean to prevent or to cure cataracts.

Why did the authors not explore rare variant associations?
The only genes mentioned in the paper are known genes, and then 4 genes with drug-interactions. Are those 4 already known or novel? That should be mentioned.

Regarding the concluding sentence “The results of this study have important implications for both the diagnosis and treatment of cataracts and open up exciting research avenues for future research.”
How do these findings have important implications for diagnosis of cataracts? The PRS does associate with cataracts in an independent cohort, but the predicted power seems to be really low, and is not really explored in the paper except for the reported r2=0.002. It would be interesting to see the OR for cataracts for different deciles of the PRS.
And how do they have important implication treatment of cataracts? I think the authors need to tone this down or show/explain further how exactly these results can have important implications.

I do not understand the sentence in lines 87-89.

Thank you for taking the time to review our manuscript. We appreciate your insightful comments and suggestions. We have carefully considered each of your points and believe that our responses will address any concerns you may have had. In the following pages, we provide a point-to-point answer to each of your comments and explain how we have revised the manuscript accordingly.

Reviewer 1:

1. The authors stated that they used subjects of European ancestry. There are non-European subjects in the GERA cohort. Did the authors only use European subjects in the GERA cohort? Similarly, did the authors exclusively use European participants in the UK Biobank cohort? If the authors did not re-analyze the GERA and the UKB cohorts, where and how were the GWAS summary statistics obtained?

Thank you to the reviewer for the comment. As the reviewer is pointing out in the comment, GERA and UK Biobank have data of multiple ancestries. We obtained the cross-ancestry GWAS summary statistics based on the publication of Choquet et al. 2021 (A large multiethnic GWAS meta-analysis of cataracts identifies new risk loci and sex-specific effects). In order to make this clear, we have included this phrase in the last paragraph of the introduction: "**In this study, we present the largest GWAS meta-analysis of cataracts using data from 125,065 cases and 820,244 controls of multiple ancestry.**"

2. The authors' analysis of the FinnGen cohort or acquisition of the GWAS summary statistics is unclear. The cited FinnGen study (Ref. 16) does not appear to contain cataract information.

Thanks to the reviewer for the comment. As the reviewer is pointing out, the cited study (Ref. 16) is describing FinnGen but not the cataract GWAS summary statistics. Therefore, we decided to include more information to clarify the release version of FinnGen for the summary statistics and where to find additional information. We included the following statement: "T**he summary statistics used in this study included 50,961 cases and 287,330 controls and are labeled as 'senile cataracts' as part of Finngen release eight (https://r8.finngen.fi/pheno/H7_CATARACTSENILE).**"

3. For the Mass General Brigham Biobank (Lines 120-126), the methodology for the cataract analysis and disease definition/classification is unclear.

Thanks to the reviewer for the comment, we have included further information for clarification "The Mass General Brigham Biobank (formerly Partners HealthCare Biobank) is a long-term medical research repository allocated within the Partners HealthCare System in Boston, Massachusetts [15]. The Biobank is dedicated to collecting and storing biospecimens, such as blood and tissue samples, from over 130,000 participants and genomic data from over 65,000

participants; of which 47% are over 60 years of age. The Partners HealthCare Biobank operates with the highest ethical standards and follows the Declaration of Helsinki to ensure the protection of the rights and welfare of study participants.
We performed a GWAS in 2,920 cases and 17,127 controls using PLINK 1.90beta. The phenotype was defined based on ICD-9/ICD-10 codes in the electronic health records of the participants."

And further details in the supplementary table 6 "**Quality control was conducted on the single consolidated dataset. We excluded duplicated or related individuals and those with an overall missingness > 0.05, unresolved gender mismatches, or heterozygosity rates greater than three s.d. from the mean. Relatedness among samples was determined using 279,933 LD-independent SNPs, and pairwise identity by descent estimation was conducted using PLINK". For any related samples (pi-hat between 0.1875 and 0.9), one case with a higher genotyping call rate was selected and kept, while the others were excluded. For those paired samples with pi-hat > 0.9, both cases were excluded. Only individuals of self-reported European ancestry were considered for analysis. Amongst these, ancestry outliers were identified using principal component analysis (smartpca) and excluded. SNP-based quality control included excluding markers on non-autosomal chromosomes, those with minor allele frequency < 0.01, missing genotype data > 0.05, showing deviation from Hardy-Weinberg equilibrium in the healthy controls (p < 0.001), or with differential missingness in cases vs. controls. The resulting dataset was processed, and genotypes were imputed using minimac3 and the Haplotype Reference Consortium's (HRC1.1 2016) reference panel on the Michigan Imputation Server**".

4. Were cataracts defined similarly across all cohorts used? Please provide a summary of this information in a table.

Thank you to the reviewer for the suggestion. To provide additional details on phenotype definition, genotyping, QC, and imputation procedures, we have included Supplementary Table 6. This table offers comprehensive information for each study and cohort included in the meta-analysis or PRS analysis.

Cataracts were clinically diagnosed, and the definition remains consistent across previous meta-analyses, Finngen, and the Mass General Brigham Biobank. However, during the paper review process, it came to our attention that CLSA definition of cataracts was heterogeneous which could include different aetiologies and cataract subtypes due to the self-reported nature of the phenotype. Subsequent analysis revealed that the inclusion of CLSA was also diminishing the number of associations in the GWAS and distorting the observed effects.

Further scrutiny of the phenotype's consistency unveiled that the SNP heritability was inconsistent with the other studies included in the meta-analysis (h2 = 0.02, se = 0.02 [LDSC]). Consequently, we made the decision to remove CLSA from the study. This action was taken to address the reviewer's suggestion to ensure utilizing cleaner and consistent phenotypes across the studies, ultimately resulting in reduced noise in both GWAS and post-GWAS analyses.

5. In the Methods section, the authors only explained that Regenie was used in the CLSA study. The methodology for analyzing other cohorts is unclear. Was Regenie used in other cohorts, or was a different approach employed?

Thanks to the reviewer for the comment. Regenie was used for CLSA, which has been excluded from the analysis. In the case of The Mass General Brigham Biobank, PLINK was used, and this information is provided in the methods section: "**We conducted a GWAS with PLINK 1.90beta on 2,920 cases and 17,127 controls.**" Further details about the software utilized and the procedures employed are now available in Supplementary Table 6, as described in the methods section of the manuscript "**Detailed information regarding phenotype definition, genotyping, QC and imputation procedures on all the cohorts are detailed in Supplementary Table 6.**"

6. The authors used an imputation quality score of 0.8 to filter variants. Was the 0.8 score used in each cohort analysis originally? Or, did the authors obtain GWAS summary statistics from each cohort, possibly using different initial cutoffs, and then further filtered using the imputation quality score of 0.8?

Imputation QC procedures are now included in Supplementary Table 6. However, there are differences between the thresholds used for the different GWA analyses used in the meta-analysis (e.g. Choquet et al. use an r2 threshold over 0.6). In order to assess if our results remain consistent, we have included heterogeneity estimates (Supplementary Table 1) and effect plots (Figure 2) comparing our study with the previously published meta-analysis. We have also included a genetic correlation analysis of the cohorts included in the meta-analysis "**Prior to the meta-analysis, we assessed the genetic correlation between studies using LD score regression, which showed a consistent correlation between Choquet et al. 2021 meta-analysis and FinnGen (rg=0.94, se=0.04, p=1.03e-92). LDSC was not able to be performed in The Mass General Brigham Biobank study, likely due to the restricted sample size. After the meta-analysis, we correlated the effect estimates between the meta-analysis and previous cataract GWAS, using independent and genome-wide significant loci, to ensure consistency in the magnitude and direction of the effect. Results showed a high correlation of the effect between our meta-analysis, FinnGen (r=0.98, p<2.2e-16), Choquet et al. 2021 (r=0.99, p<2.2e-16), and a lower correlation with MGBB (r=0.52, p<1.7e-05) with a wider 95% CIs due to the smaller sample size in that study (Figure 3).**"

7. Did the authors assess heterogeneity in their meta-analysis results?

Heterogeneity estimates have been incorporated into Supplementary Table 1. Notably, within the vast majority of loci, there is a notable absence of heterogeneity, exhibiting heterogeneity p-values over 0.05. Given the limited occurrence of heterogeneous loci, we consider that an inverse variance-weighted approach is a more suitable fit for the data compared to alternative methods, such as random efficient meta-analysis, which exhibit greater robustness in the presence of heterogeneity but compromise statistical power.

8. Please explain why the Raine Study was mentioned in the method and results sections but excluded from Figure 1.

Thank you for your comments. To provide additional clarity on the utilization of the Raine cohort, we have included the relevant information in Figure 1. Initially, the intention was to present a figure exclusively for displaying the GWAS meta-analysis, excluding any post-meta-analysis analyses. However, we acknowledge that this intention was not clearly conveyed. Consequently, we have included more information in the plot.

9. It is puzzling that the authors only mentioned the causal relationship between type 1 diabetes and cataracts and did not address the relationship between type 2 diabetes and cataracts until line 354. The authors should consider mentioning the previously published relationship between type 2 diabetes and cataracts early in the manuscript to prevent confusion.

Thanks to the reviewer for the suggestion, to make clear why we are interested in the association between type 1 diabetes and cataracts we included the next information at the end of the introductory section of the study "**In addition, we investigated the potential causal association between type 1 diabetes and cataracts using a Mendelian randomization (MR) framework to gain further insights into the relationship between diabetes and cataracts. Previous research has already established a causal association between type 2 diabetes and cataracts [9]. Furthermore, we estimated polygenic risk scores (PRS) to assess the association between cataract risk and UVR-related lesions in both young and older adults.**"

10. Lines 165-157: What is the difference between gene-based and gene-set analyses?

We have now explained this in the "Functional annotation and eQTL" section of the manuscript for clarity "**Gene-based analysis was used to prioritize associations between genes and cataracts. Gene-set tests, also known as pathway analysis or enrichment analysis, were employed to assess groups of genes that are functionally related and collaborate in biological pathways that are associated with cataracts aetiology process.**"

11. Line 160: What is the origin of the 18,488 gene-sets, and how were they defined?

Gene sets were obtained from Msigdb v7.0 for "Curated gene sets" and "GO terms" as part of the magma analysis and are incorporated in FUMA V.108 pipeline. This has been also added to the manuscript "Functional annotation and eQTL" section " **Gene sets were obtained from Msigdb v7.0 for "Curated gene sets" and "GO terms" as part of the magma analysis and are incorporated in FUMA V.108 pipeline**"

12. Line 50, are all 126 genes novel?

Thanks for the question. No, the 126 genes (now 134 after excluding CLSA) are the ones that were prioritized by MAGMA. We are highlighting the ones that are "novel" or have not been reported before in the results where we state "**We identified 101 independent genome-wide significant loci associated with cataracts (Supplementary Table 1). Of these, 44 loci had been previously reported, and 57 were unreported; as per Figure 2 and Supplementary Table 1**." We also include the definition of known and novel loci in the manuscript for clarity "We conducted an inverse variance weighted fixed-effect meta-analysis of 125,065 cases and 820,244 controls using METAL [15]. All variants were aligned to the positive strand on build GRC37 hg19 and those with a minor allele frequency (MAF) < 1%. Linkage disequilibrium (LD) clumping was used to identify the independent genome-wide significant loci within a 1Mb window. Clumping of the results was conducted in PLINK 1.9 with a P-value cut-off of 5e-8 and r-squared < 0.001 using the 1000 Human Genome Project reference panel. **Employing the same configuration for the clumping analysis, we estimated the independent loci for the previous, larger meta-analysis, resulting in 44 independent loci. Loci that were shared between the previous and this study's meta-analysis were considered known, while those unique to this study were deemed as novel.**"

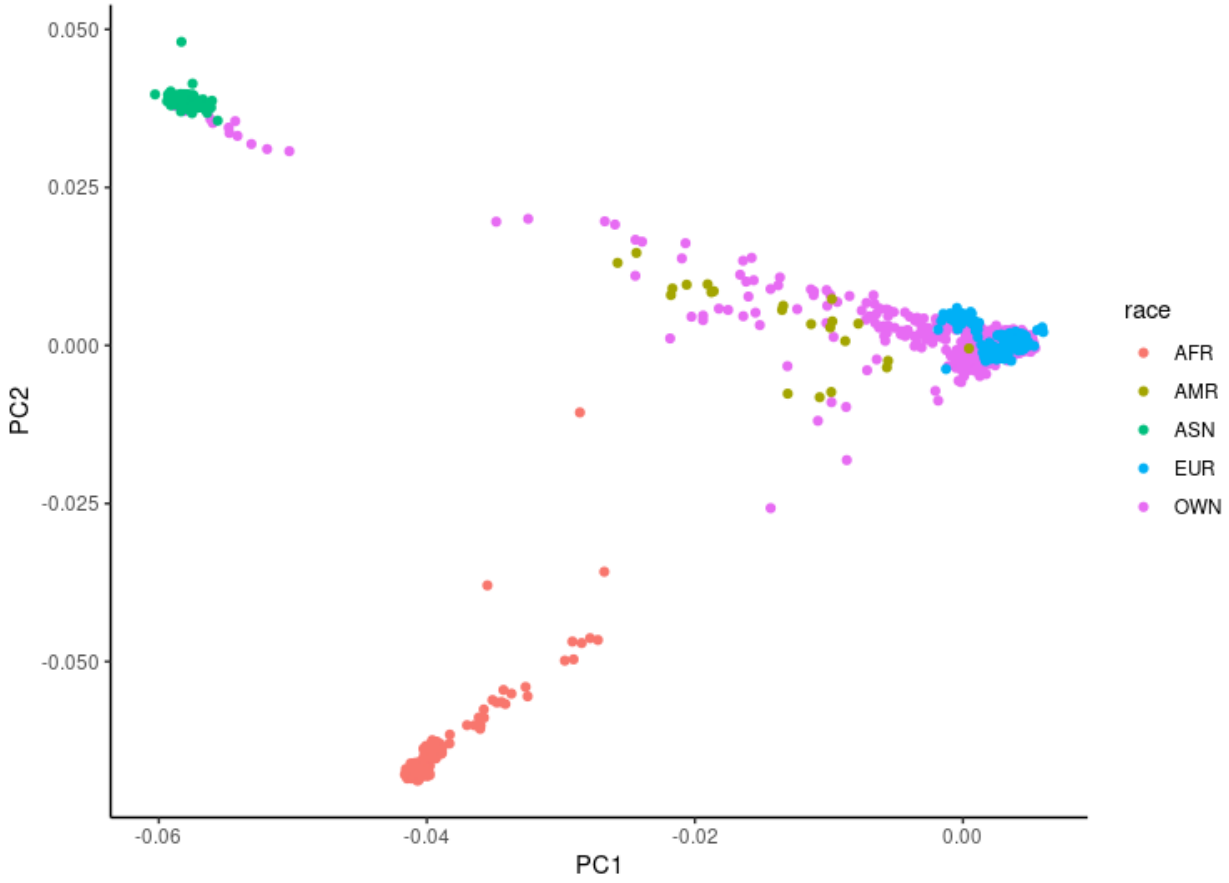
13. Line 227: Please clarify the procedure for "excluded through a principal component analysis," including the number of principal components and any cutoffs used.

Thanks to the reviewer for the comment. To clarify we included the following information in Supplementary Table 6 of the paper "**To exclude other ancestries, we used principal component analysis for participants of known European ancestry, using 1000 Genomes. All the samples beyond six standard deviations from the European centroid were excluded"**.

14. BHAS participants are of European ancestry. What was the rationale for using the TOPMed reference panel, which includes many non-European ancestry subjects? Were all cohorts imputed similarly or differently? How many variants were genotyped in BHAS, and how many remained after QC?

BHAS participants are primarily of European ancestry, however, the dataset encompasses individuals with Asian ancestry, as shown in the plot below (OWN=BHAS). Including individuals from various ancestry populations in the reference panel can enhance the imputation accuracy for a broader range of genetic variants. This approach is particularly useful for capturing variants that may be more common in non-European populations but remain relevant to individuals of European ancestry. Moreover, the reference panel contains over 300 million genetic variants, offering a much broader genome coverage compared to other reference panels.

Raine was imputed using TOPMed, GERA and the Mass General Brigham Biobank were imputed using HRC, and Finngen was imputed using the population-specific Sequencing Initiative Suomi (SISu) v.3 imputation reference panel. Additional details on imputation and QC can be found in Supplementary Table 6. Regarding the BHAS imputation, 654,027 variants were genotyped, and 497,840 remained after QC.



15. The authors identified 126 genes associated with cataracts. It is unclear whether these 126 genes were derived from single-variant analysis or gene-based analysis. If from gene-based analysis, which type? Was the MAGMA gene-based analysis similar or different from recent gene-

based analyses (e.g., Burden and SKAT), or does MAGMA have apparent advantages over recently developed gene-based methods?

Thanks to the reviewer for the comment. The 126 genes (now 134 after excluding CLSA) were identified using MAGMA, as stated in the manuscript: "**A gene enrichment analysis using MAGMA on 19,491 genes identified 134 genes associated with cataracts, after a Bonferroni correction for multiple testing (p < 2.56e-6) (Supplementary Table 2**)"

Regarding the comparison between MAGMA and other gene-based analysis methods like Burden and SKAT, the performance and advantages of these methods can vary depending on the dataset, the underlying genetic architecture of the trait, and the specific research question being addressed. MAGMA is a gene-based analysis method commonly used in the context of common variants, while Burden and SKAT are particularly effective in identifying associations driven by multiple rare variants within the same gene.

16. Line 235: Please consider using "PLINK 2.0" (the official name on the PLINK website) instead of "Plink v 2."

Thanks to the suggestion, we are now including Plink as PLINK 2.0

17. Line 277: Are all 20 genes involved in "drug-gene interactions among 20 genes" novel, or have some of these genes been reported previously without investigating the drug-gene relationship?

Thanks to the reviewer for the question. Most of the genes have been reported (12/20) before for cataracts, but the drug-gene interaction was unexplored. Out of the 20 genes, four had drug-gene interactions, and we have clarified in the manuscript which of these are novel associations. "Drug-gene interactions among 20 genes associated with cataracts identified four genes (GNL3, JAG1, METTL21A, and CREB1) that interact with eight drugs. **Three of these genes (CREB1, METTL21A and GNL3) have not previously been associated at a genome-wide significant level with cataracts.** GNL3 exhibited interactions with epirubicin, cyclophosphamide, and fluorouracil. Meanwhile, JAG1 was found to interact with hydrocortisone. Interactions between METTL21A and citalopram were also observed. Similarly, CREB1 displayed interactions with citalopram, lithium, nicotine, and alcohol".

18. Lines 299 - 304: In the PRS section, why was only BHAS mentioned, while the Raine Study Gen2 was not? However, in the following paragraph (Lines 305-310), both the Raine Study Gen2 and the BHAS cohorts were used.

In the first paragraph, we tested the PRS derived from the GWAS meta-analysis in BHAS as a way to validate the predictive ability of the loci identified through the meta-analysis for determining

the risk of cataracts. The Raine cohort is composed of people who are under 30 years of age and thus do not have age-related cataracts. We made this clearer in the manuscript "**The Raine cohort consists of individuals under 30 years of age, which means they do not have age-related cataracts. Therefore, only estimates for UVR-related phenotypes (as explained in the section below) were assessed in this cohort.**"

In the subsequent paragraph, we are testing the PRS of cataracts, as opposed to presence of cataract itself, against CUVAF as a way to investigate associations between UV early exposure and the risk of cataracts in younger adults through Raine and later in life using BHAS. We included all cohorts in Figure 1 to make this clearer.

19. Regarding PRS, could the authors provide prediction accuracy metrics in addition to the association p-values, such as AUC?

Thank you to the reviewer for the suggestion. AUC estimates have now been incorporated alongside the variance explained and the p-value. This is included now in the results of the manuscript "**We used a pruning and threshold approach for the PRS analysis, where the genome-wide loci were selected to evaluate the phenotypic variance explained by the genetic risk of cataracts. We found that the genetic risk of cataracts was associated with cataracts in BHAS, an independent cohort, confirming that these loci are likely associated with cataracts (AUC = 0.54, R-squared = 0.001, p = 0.01).**"

20. For the PRS analysis, please also construct PRS using previously published loci and compare with your new PRS results. Do the new genes/results yield better PRS outcomes or produce similar findings?

As suggested by the reviewer, we have now included a comparison between the PRS derived from the published and new loci. "The PRS analysis showed an association between the genome-wide loci and the development of cataracts ($p<0.05$), confirming that these loci are likely associated with cataracts disease (AUC = 0.54, *R-squared = 0.001, p=0.01)***. The PRS demonstrated similar predictive efficacy when contrasted with the PRS derived from the previous meta-analysis (AUC = 0.53, *R-squared = 0.001, p=0.02*). DeLong's test was performed to compare the ROC curves of the two models. The test yielded a p-value over 0.05 (p = 0.918), suggesting no statistically significant difference in AUC between the two models.**"

21. In the Manhattan plot, the newly discovered genes show relatively much lower statistical significance than the previously reported ones. Can the authors comment on the biological significance differences between the newly discovered genes and the previously reported ones? Can we assume previously reported ones carry much more biological significance because they show much stronger statistical significance and possibly larger effect sizes as well?

Thanks to the reviewer for the question. It is important to clarify that statistical significance alone does not directly reflect the biological significance of a gene or a variant. The biological significance of a gene depends on several factors, and mere statistical significance does not automatically imply that a gene is more biologically relevant. Biological significance can be assessed by factors such as functional relevance, which we are evaluating through our gene-based analysis, gene pathway analysis and the integration of tissue-specific expression through eQTL data. Additionally, some genes may be more relevant to specific tissues or cell types, making their overall biological significance context-dependent. Furthermore, genes involved in disease pathways and those with potential as therapeutic targets hold significant biological importance, even if their statistical significance might be lower. While larger effect sizes might indicate more immediate practical importance, smaller effects can still be biologically relevant.

22. Please provide a section for web resources detailing the software tools used and GWAS results accessed.

Thanks to the reviewer's suggestion, we have included a section in the manuscript that highlights all the data accessibility and the software used in our analysis.

23. Are the the authors' meta-analysis summary statistics available to the public?

Yes, summary statistics are now available in Zenodo.

Reviewer 2 :

1- I think the paper would benefit from a main table showing the novel variants found. It would also be good to discuss the set of variants, for instance add information on how many coding variants were found. It is a bit odd that a GWAS does not discuss any individual variants. Were there no novel variants of interest, for instance loss of function or missense variants.

Thanks to the reviewer for this comment. Supplementary Table 1 and Figure 2 now highlight all the novel variants. We also highlight genes that were prioritized through QTL analysis using MAGMA and SMR in Supplementary Table 2. Additionally, we have included functional information in Supplementary Table 1, which highlights genes that were prioritized through MAGMA, annotation, and exonic regions.

2- Supplementary Table 1 should have more information, like the coding change of each variant, the minor allele frequency, the mapped/closest gene, the odds ratio and then results from individual cohorts. It is not clear what units the BETA is in.

Thanks to the reviewer's suggestion. We have added further information to Supplementary Table 1. This table now highlights the effect allele, nearest gene, unit of effect, heterogeneity estimates for each variant, and novel loci.

3- It is not clear why the UK Biobank and GERA cohort are grouped together before the meta-analysis. Did the authors use the previously published meta-analysis to use in their meta-analysis instead of summary statistics from the individual cohorts?

Thanks to the reviewer for the comment. As part of our meta-analysis we included the previous large meta-analysis of Choquet et al. 2021 that includes UK and GERA. We have made this clearer in the method section "**The current meta-analysis includes the previous larger cataract meta-analysis that encompassed the Genetic Epidemiology Research in Adult Health and Aging (GERA) and the UK Biobank (UKB)[4], using their publicly available summary statistics. The GERA cohort contains clinical and genomic data of over 110,000 participants; 33,145 patients who have undergone cataract surgery and 64,777 controls were included in this study. The UKB is a large prospective study following the health of approximately 500,000 participants, including 34,699 cataract cases defined as "participants with a self-reported cataract operation (f20004 code 1435) or/and a hospital record including a diagnosis code (ICD-10: H25 or H26)" [4] , and 452,622 controls were included in the meta-analysis. Cataract was clinically diagnosed in GERA, The Mass General Brigham Biobank, and FinnGen. Detailed information regarding phenotype definition, genotyping, QC and imputation procedures on all the cohorts are detailed in Supplementary Table 6.**"

4- Comparison between the cohorts is missing, and GWAS results from individual cohorts should be in the Supplement. Reporting for example correlation between effect sizes in different cohorts would be helpful, and perhaps add figures comparing effect sizes for different cohorts for the 85 variants.

Thanks to the reviewer's suggestion, we have now included a correlation of the effect between the cohorts used in the meta-analysis. Please refer to Figure 3. We have also included the following text in the manuscript detailing the correlation between the studies prior to our meta-analysis "**Prior to the meta-analysis, we assessed the genetic correlation between studies using LD score regression, which showed a consistent correlation between Choquet et al. 2021 meta-analysis and FinnGen (rg=0.94, se=0.04, p=1.03e-92). LDSC was not able to be performed in The Mass General Brigham Biobank study, likely due to the restricted sample size. After the meta-analysis, we correlated the effect estimates between the meta-analysis and previous cataract GWAS, using independent and genome-wide significant loci, to ensure consistency in the magnitude and direction of the effect. Results showed a high correlation of the effect between our meta-analysis, FinnGen (r=0.98, p<2.2e-16), Choquet et al. 2021 (r=0.99, p<2.2e-**

16), and a lower correlation with MGBB (r=0.52, p<1.7e-05) due to the smaller sample size in that study (Figure 3)."

5- Reporting on ethnicity is lacking for most of the cohorts (except CLSA). Did the authors only use individuals of European ancestry, and if not how did they adjust for ethnicity?

Quality controls for population stratification for each cohort are detailed in Supplementary Table 6. As we made clear now in the manuscript "cohorts" section "**Detailed information regarding phenotype definition, genotyping, QC and imputation procedures on all the cohorts are detailed in Supplementary Table 6**."

6- There is no information about the genotypic data used in the meta-analysis, whether the cohorts had whole genome sequence data and imputation, or just chip typed data. How many variants were tested?

Details on the genotypic data are detailed in Supplementary Table 6.

7- In a previous GWAS cataract study, sex specific analysis found some additional loci. Did the authors perform such analysis?

Thank you for the suggestion. We recognize that previous GWAS meta-analyses have conducted sex-specific analyses. However, for this meta-analysis, we lacked access to individual-level data for the included cohorts, with the exception of the Mass General Brigham Biobank. Given the restricted sample size of the Mass General Brigham Biobank, it is unlikely that we will find any novel sex-specific loci.

8- For the Mendelian randomization (MR) analysis, it is not clear what variants were used as instrumental variables. Was it the 85 independent variants? Further, I think the presentation of the MR results could be more informative with effect plots instead of just the OR for different MR analysis. For example, by plotting the effect on cataract against effect on T2D, for the risk increasing allele for all the variants associating with cataracts, and add the regression line from a weighted linear regression. Then do the same thing, except for variants that have been reported to associate with T2D. An example of this kind of presentation can be found in "Rare SLC13A1 variants associate with intervertebral disc disorder highlighting role of sulfate in disc pathology" by Bjornsdottir et al.

Thanks to the reviewer for the suggestion. It was unclear to us if the reviewer was referring to T1D OR T2D for this analysis, so we decided to include an answer for the two scenarios.

For T2D, despite the good insight that the proposed analysis could bring to the association between diabetes and cataracts, this has been the focus of previous studies that have explored the genetic overlap between the conditions and evaluated the effects of common and rare variants. A good example of this can be found in (https://www.medrxiv.org/content/10.1101/2021.08.05.21261684v1.full) and (https://pubmed.ncbi.nlm.nih.gov/34999863/).

In the case where the reviewer is referring to T1D, we appreciate the reviewer's suggestion and agree that further comparisons between T2D and T1D will help clarify if the association with cataracts is mediated through the effects of variants previously linked to T2D. To better elucidate the independence of the effect of T1D on cataracts, we conducted a multivariate MR analysis and adjusted for the effect of T2D. The results are now included in the manuscript. "**The association between Type 1 diabetes and cataracts remained significant in the MVMR analysis after adjusting for Type 2 diabetes (OR 1.008, 95% CI 1.002 - 1.01, p=0.01).**"

9- The authors discuss that lipid metabolism has been associated with cataracts. Can the authors explore the causality of lipids measurement through MR analysis?

Thanks to the reviewer for the suggestion. We have included further analysis in the manuscript to clarify the relationship between lipid metabolites and cataracts, which is now described in the method section, presented in the results, and discussed in the manuscript.

Methods "**Cataracts and lipid metabolites**

Considering the association between lipid metabolites and cataracts highlighted by the gene-set analysis, we further assessed a potential causal relationship through a Mendelian randomization framework. We utilized summary statistics data from 249 metabolic markers, including amino acids and metabolites related to glycolysis and fatty acids. This data was generated through metabolic profiling conducted by Nightingale Health's NMR metabolomics platform, which analyzed over 118,000 participants from the UK Biobank. We employed Generalized Summary-data-based Mendelian Randomization (GSMR) v1.91 to investigate the potential causal relationship between cataracts and the mentioned phenotypes. This method only requires GWAS summary statistics to estimate MR effect sizes and considers correlated SNP instruments by modeling LD from a pre-specified reference panel. Additionally, it uses the HEIDI-outlier statistical test to look for heterogeneous SNP outliers. We applied specific parameters to select independent instrumental variants (--clump-r2 0.001, --gwas-thresh 5e-8, and --clump kb 1000 --heidi-thresh 0.01). To control for Type-1 Error, we applied the Bonferroni correction to our association p-values. We used the following corrected threshold: p = 0.05 / (249 metabolites) = 2e-04. To avoid sample overlap with the UKB participants, we excluded GERA from the meta-analysis."

Results "**<u>Cataracts and lipid metabolites</u>**

Out of the 249 metabolites tested through GSMR, 28 showed an association with cataracts, including total fatty acids, Omega 6, the ratio of phospholipids to total lipids, cholesterol in small HDL, among others; for a complete list of nominal associations, see Supplementary Table 7. However, only the association with β-Hydroxybutyric acid was maintained after multiple testing correction. β-Hydroxybutyric acid was highlighted as a protective factor for cataracts in our results (OR=0.41 95% CI 0.29 - 0.56, p= 7.24e-08)."

Discussion "**We further assessed blood lipid metabolites and their association with cataracts. Notably, we observed a nominal causal association between the ratio of phospholipids to total lipids and the risk of cataracts. This finding aligns with prior research that has emphasized the phospholipid-to-cholesterol ratio as a factor contributing to cataract etiology. However, among all the tested associations, only β-Hydroxybutyric acid demonstrated sustained statistical significance following correction for multiple testing, and there is no established link between β-Hydroxybutyric acid, a ketone body produced during fasting or ketosis, and the development or prevention of cataracts. Consequently, further research is necessary to elucidate the specific role of lipid metabolites in the etiology of cataracts.**"

10 -In Figure 2 it is difficult to read the labels.

Thank you for the suggestion. We have now included the novel loci in Supplementary Table 1 for added clarity.

11- In the drug-gene interaction discussion, it is not clear to the reader what these interactions mean, and the speculations of the authors in the discussion feel a bit far-fetched.

We thank the reviewer for the comment. We have included an introductory phrase at the beginning of the paragraph and toned down the discussion "**Drug-gene interactions involve genes that encode proteins potentially influenced by drug-like molecules, predicted based on sequence and structural similarity to existing drug targets. These interactions can aid in the identification of potential risk factors for cataract development and in uncovering the mechanisms of interaction with drugs. Our results suggest that specific interactions between genes and drugs highlight mechanisms that may play a role in the development of cataracts. In particular, the gene *CREB1* exhibits interactions with lithium, nicotine, and alcohol, indicating their potential relevance to cataract formation. Consistently, previous studies have linked lithium [42], nicotine [43], and alcohol consumption [44] with an increased risk of developing cataracts. Citalopram, an antidepressant known as a selective serotonin reuptake inhibitor [45], has been associated with an increased risk of cataract surgery [46]. The interaction between *METTL21A* and *CREB1* with citalopram could be highlighting a mechanism that is triggered by citalopram and leads to an increased risk of cataracts.**

Meanwhile, the interaction of *JAG1* with hydrocortisone underlines a potential drug that could be used for the treatment of cataracts. Hydrocortisone is a corticosteroid drug that has been evaluated for its potential as an anti-cataract steroidal drug [47], which is consistent with the findings of this study. The mechanism of interaction with other drugs such as epirubicin, cyclophosphamide, and fluorouracil is not well understood, so further research is needed to determine if this interaction could lead to potential treatments. This information sheds light on the complex genetic basis of cataracts and provides new insights into the underlying mechanisms behind gene-drug interaction."

The authors emphasize on how their findings have important implications for the treatment of cataracts. I am not a cataract specialist, but I think that cataracts today are only treated with surgery. It would be good if the authors would mention that in the introduction and give a bit of background on the current research on non-surgical treatment of cataracts. Also, when the authors talk about treatment, it is not always clear if they mean to prevent or to cure cataracts.

Thanks for the suggestion. Cataracts are primarily treated through surgery; however, there are individuals who are ineligible for surgery due to specific conditions, such as advanced diabetes, corneal disease, or macular degeneration, requiring alternative treatments. Currently, drugs targeting the negative regulation of lipid biosynthesis have shown promise as potential cataract treatments. However, in the context of our study, most of the associations between drug targets and cataracts likely involve drugs that could contribute to cataract development. To make this clearer, we have included the following information in the introduction: "**The identification of novel genetic variants associated with cataracts could have significant medical implications for the prevention of cataracts and visual impairment, understanding the disease's etiology, and identifying modifiable lifestyle factors that contribute to the development of the disease. In addition, drug-gene interactions can further elucidate the relationship between genes encoding proteins influenced by specific drug targets, shedding light on potential contributors to cataract development and deepening our understanding of how drugs interact with these genes. Moreover, a deeper understanding of the underlying genetic architecture could provide a way to screen individuals at high risk of developing cataracts, using methods such as polygenic risk scores (PRS). This insight could also shed light on the relationship between genetic risk for cataracts and potential contributing factors that may increase the likelihood of developing cataracts later in life.**"

12 - Why did the authors not explore rare variant associations?

Thank you for the question. There are several reasons for our decision to exclude rare variants from our analysis:

By definition, rare variants are less common in the population, with a frequency of less than 1%. A significant portion of the analyses in this paper relies on imputation. Given the low allele frequency of rare variants, their imputation quality is often low, making them difficult to verify. We acknowledge that having access to other data, such as WES or WGS data, would be helpful to further elucidate the etiology of cataracts. Unfortunately, we did not have access to such data to explore the rare variant association.

13 - The only genes mentioned in the paper are known genes, and then 4 genes with drug-interactions. Are those 4 already known or novel? That should be mentioned.

Thanks to the reviewer for the question. We are emphasizing these genes as they appear to have greater biological significance for cataract etiology. These genes were consistent across multiple levels of analysis, including gene-based analysis, gene-set analysis, expression through eQTL analysis, and drug-gene interaction studies. Regarding the aspect of whether these genes were known or unknown before this study, three out of four were previously unknown. We are including the following statement in the manuscript for clarity "Drug-gene interactions among 20 genes associated with cataracts identified four genes (GNL3, JAG1, METTL21A, and CREB1) that interact with eight drugs. **Three of these genes (CREB1, METTL21A and GNL3) have not previously been associated at a genome-wide significant level with cataracts.** GNL3 exhibited interactions with epirubicin, cyclophosphamide, and fluorouracil. Meanwhile, JAG1 was found to interact with hydrocortisone. Interactions between METTL21A and citalopram were also observed. Similarly, CREB1 displayed interactions with citalopram, lithium, nicotine, and alcohol".

14 - Regarding the concluding sentence “The results of this study have important implications for both the diagnosis and treatment of cataracts and open up exciting research avenues for future research.” How do these findings have important implications for diagnosis of cataracts? The PRS does associate with cataracts in an independent cohort, but the predicted power seems to be really low, and is not really explored in the paper except for the reported r2=0.002. It would be interesting to see the OR for cataracts for different deciles of the PRS.

Thanks to the reviewer for the suggestion. We have now added some additional tests and information to the PRS analysis. “**To further test the association between these genome-wide independent loci and cataracts, we conducted a PRS analysis using data from 442 cataract cases and 4698 controls in the BHAS (Figure 5). We used a pruning and threshold approach for the PRS analysis, where the genome-wide loci were selected to evaluate the phenotypic variance explained by the genetic risk of cataracts. We found that the genetic risk of cataracts was associated with cataracts in BHAS, an independent cohort, confirming that these loci are likely associated with cataracts (AUC = 0.54, R-squared = 0.001, p = 0.01). Participants in the highest PRS decile were more likely to have cataracts (odds ratio = 1.12, 95% CI =**

0.81–1.56) compared to the lower decile (odds ratio = 0.85, 95% CI = 0.60–1.20). The PRS demonstrated similar predictive efficacy when contrasted with the PRS derived from the previous meta-analysis (AUC = 0.53, *R-squared = 0.001, p=0.02*). DeLong's test was performed to compare the ROC curves of the two models. The test yielded a p-value over 0.05 (p = 0.918), suggesting no statistically significant difference in AUC between the two models."

And how do they have important implication treatment of cataracts? I think the authors need to tone this down or show/explain further how exactly these results can have important implications.

Thanks to the reviewer for the comment we have now toned this down and included a closing paragraph that describes the importance of the study "**This study contributes to our understanding of the genetic basis of cataracts and has yielded several noteworthy findings. Our analysis identified 101 loci and 134 genes associated with cataracts, doubling the number of known loci. Notably, we have identified a potential overlap between negative regulation of lipid biosynthesis and the development of cataracts, as well as drug-gene interactions that may expand the range of therapeutic options available for the treatment of cataracts. Furthermore, our findings provided evidence of a putative causal relationship between genetic predisposition to type 1 diabetes and an increased risk of cataracts. Finally, we highlight a potential association between exposure to UVR and risk to develop age-related cataracts; however, further research is required to establish the reliability of this as a marker of cataract risk. The results of this study contribute to the understanding of biological mechanisms involved in the development of cataracts and open up potential new avenues for the treatment of cataracts**."

15 - I do not understand the sentence in lines 87-89.

Thanks to the reviewer for the comment to make this clearer. We have changed the paragraph from " Moreover, understanding the underlying genetic architecture could provide a way to develop novel methods to screen individuals at high risk of developing cataracts through methods such as polygenic risk scores (PRS), and contribute to elucidating the relationship between cataracts and ultraviolet radiation (UVR) as a potential contributing factor that could elevate the risk of developing cataracts later in life."

To " **Moreover, understanding the underlying genetic architecture could provide a way to screen individuals at high risk of developing cataracts, using methods such as polygenic risk scores (PRS). This understanding could also shed light on the relationship between genetic risk for cataracts and potential contributing factors that may increase the likelihood of developing cataracts later in life**."

REVIEWER COMMENTS

Reviewer #1 (Remarks to the Author):

The authors have addressed my remarks in a satisfactory fashion.

Reviewer #2 (Remarks to the Author):

First, here are comments to the previous comment-answers as numbered in the Response to Referees Letter file:

2. I still can not find the minor allele frequency of the variants per dataset, and effects and p-values for different datasets.

4. Again, the GWAS results per dataset is only presented in the figure, not in a supplementary table.
I find it strange that LDSR could not have performed for the MGBB data and the sample size should not be the problem (~20k).
It does not really make sense to report correlation between the meta effects and individual cohort effects, since the meta is derived from the other cohorts. I would like to see the pairwise correlation plots between each of the individual datasets. And In the figures when computing the correlation, it is better to always use the allele that has an increasing effect. Otherwise the two clusters (where minor allele has increasing vs decreasing effect) will always create some correlation. I am assuming they used the effect allele as minor allele, but that is not clear. When computing the correlation, it should be weighted by the frequency of the variants.

6. I think some of the information in supplementary table 6 needs to be in methods as a text.

8. In my previous comment I misspelled T1D as T2D. I apologize for that. The authors have not answered my comment so I repeat it here after correcting from T1D to T2D:
For the Mendelian randomization (MR) analysis, it is not clear what variants were used as instrumental variables. Was it the 85 independent variants? Further, I think the presentation of the MR results could be more informative with effect plots instead of just the OR for different MR analysis. For example, by plotting the effect on cataract against effect on T1D, for the risk increasing allele for all the variants associating with cataracts, and add the regression line from a weighted linear regression. Then do the same thing, except for variants that have been reported to associate with T1D. An example of this kind of presentation can be found in “Rare SLC13A1 variants associate with intervertebral disc disorder highlighting role of sulfate in disc pathology” by Bjornsdottir et al.

Other comments:

Figure 1 would benefit from more info like sample size (N cases and N controls per cohort)

Did the authors account for the inflation in the test statistics that they observed using LDSR?

In the PRS results section, they say that participants in the highest PRS are more likely to have cataracts compared to the rest of the cohort but the 95% CI of 0.81-1.56 shows that the difference is not significant, which is alarming. I wouldn't say that the risk is higher unless it is significant.

In line 374, P=0.05/2=0.025, and from the supplementary table the P value is 0.029 which is larger than 0.025, so it is not significant after multiple testing as is stated.

Thank you for taking the time to review the changes to our manuscript. We appreciate your comments and suggestions. We have carefully considered each of your points and believe that our responses will address any concerns you may have had.

Reviewer 2:

2. I still can not find the minor allele frequency of the variants per dataset, and effects and p-values for different datasets.

Thank you for your comment. We have now included the minor allele frequency for the independent variants from the meta-analysis in Supplementary Table 1. We have not shown MAF per dataset due to the absence of study-specific MAF across all studies (please see details below). Additionally, we have provided the effect sizes and p-values for each dataset in Supplementary Table 8. Full summary statistics for the previous meta-analysis and FinnGen are publicly available as part of their respective publications. The Mass General Brigham Biobank cataracts data can be accessed through application, and more information is available on their portal: https://www.massgeneralbrigham.org/en/research-and-innovation/participate-in-research/biobank/for-researchers
We would also like to clarify that we do not have MAF for all the datasets, specifically for GERA and MGBB. In such cases, the MAF was added based on a reference panel of Europeans from UKBB. This was not clearly specified in the manuscript, so we have added the following information: "All variants were aligned to the positive strand on build GRC37 hg19, and those with a minor allele frequency (MAF) < 1% were removed. **In cases where the MAF was not available for the study (i.e., GERA and MGBB), it was derived from a reference panel based on five thousand healthy individuals from UKBB**".

4. (a) Again, the GWAS results per dataset is only presented in the figure, not in a supplementary table.
(b) I find it strange that LDSR could not have performed for the MGBB data and the sample size should not be the problem (~20k).
(c) It does not really make sense to report correlation between the meta effects and individual cohort effects, since the meta is derived from the other cohorts. (d) I would like to see the pairwise correlation plots between each of the individual datasets. (e) And In the figures when computing the correlation, it is better to always use the allele that has an increasing effect. Otherwise the two clusters (where minor allele has increasing vs decreasing effect) will always create some correlation. I am assuming they used the effect allele as minor allele, but that is not clear. When computing the correlation, it should be weighted by the frequency of the variants.

Thank you for the comments and suggestions.

(a) As mentioned in the response to the previous comment (2.), GWAS results per dataset are now included for the genome-wide significant independent variants, and full summary GWAS results are publicly available or accessible upon request.

(b) The reviewer notes that the sample size is around 20k (n=20,047), which is a total of cases (n=2,920) and controls (n=17,127). To determine if the MGBB GWAS has sufficient power for this analysis, we examined the heritability estimate for MGBB using LDSC and found a small heritability with a large standard error 0.0211 (0.0489); i.e z is <1. Following the advice in the LDscore paper, we hence have reported the rg as NA. Which states that when heritability estimates are too noisy you should not report genetic correlations. Specifically, the paper says "Remove all traits with heritability z-score below 4. Genetic correlation estimates for traits with heritability z-score below 4 are generally too noisy to report." (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4797329/)

(c) We reported the correlation between the meta-analysis effects and the effects on other cohorts as a means to confirm the consistency of the estimates. However, as the reviewer points out, it is important to include the correlation between the effects of the individual datasets. We have now included these as supplementary material (Supplementary Figures 3 to 5).

(d) Plots are now included; please refer to Supplementary Figures 3 to 5.

(e) We appreciate the reviewer's observation. Including both risk-decreasing and increasing alleles can indeed increase the correlation estimates. We opted to weight the correlation based on the inverse of squared standard error of the effect estimates (inverse variance weighted), which is equivalent to the reviewers suggestion of weighting by the allele frequency. As the reviewer suggested, we used the risk-increasing allele in the study with the larger sample size, and aligned the other studies to match the same allele. These adjustments are now included in the results section of the manuscript, and the results are described as follows.“**Prior to the meta-analysis, we assessed the genetic correlation between studies using LD score regression, which showed a consistent correlation between Choquet et al. 2021 meta-analysis and FinnGen (rg=0.94, se=0.04, p=1.03e-92). The univariate LDSC results for The Mass General Brigham Biobank study were too noisy to allow us to reliably estimate a genetic correlation. We then proceeded to correlate the effect estimates of the genome-wide significant independent loci, weighting variants by the square of the standard error of their effect estimates (also known as inverse variance weighted). Risk-increasing alleles in the larger study were selected, and the other study was aligned to match the same allele. The weighted correlation between the previous meta-analysis, and FinnGen was high (r = 0.84), in comparison with the other two studies. The correlation between MGBB and the previous meta-analysis, and between MGBB and FinnGen, was 0.1 and 0.19, respectively, as shown in Supplementary Figures 3 to 5. This is consistent with the results of LDSC, where the previous meta-analysis and FinnGen**

maintained a high correlation throughout the genome. Given the smaller sample size of MGBB (approximately 3k cases) in comparison with the previous meta-analysis (approximately 68k cases) and FinnGen (approximately 51k cases), larger confidence intervals and lower statistical power were expected. Nevertheless, the correlation still maintains a consistent positive correlation of effects between studies. We further tried a 'leave-one-out' approach, where we excluded MGBB from the meta-analysis. The results did not affect the number of loci associated with cataracts, leading us to conclude that including MGBB in the meta-analysis does not significantly affect the results. However, given the consistency in the direction of the effect correlations, and a slight improvement in the effect estimates we decided to include it in the meta-analysis."

6. I think some of the information in supplementary table 6 needs to be in methods as a text.

Thank you to the reviewer for the suggestion. We agree that the information in Supplementary Table 6 is important. However, including it in the main body of the study would cause us to exceed the word limit stipulated by the journal, thereby reducing our ability to describe and discuss our results in depth.

8. In my previous comment I misspelled T1D as T2D. I apologize for that. The authors have not answered my comment so I repeat it here after correcting from T1D to T2D:
a) For the Mendelian randomization (MR) analysis, it is not clear what variants were used as instrumental variables. Was it the 85 independent variants? b) Further, I think the presentation of the MR results could be more informative with effect plots instead of just the OR for different MR analysis. For example, by plotting the effect on cataract against effect on T1D, for the risk increasing allele for all the variants associating with cataracts, and add the regression line from a weighted linear regression. Then do the same thing, except for variants that have been reported to associate with T1D. An example of this kind of presentation can be found in "Rare SLC13A1 variants associate with intervertebral disc disorder highlighting role of sulfate in disc pathology" by Bjornsdottir et al.

a) In the MR method section, we specify the variables selected through the clumping process: "We selected independent instrumental variables based on clumping using PLINK 1.9, with the following parameters: --clump-r2 0.001, --clump-p1 5e-8, --clump-kb 1000". This means that we included only variants that are under an r-squared of 0.001 in a 1000 kb window and are genome-wide significant for the exposure (T1D). However, upon reviewing the clumping thresholds used for this analysis, we realized that the clumping used for defining the independent loci in the GWAS was less stringent (--clump-r2 0.01, --clump-p1 5e-8, --clump-kb 1000). This has been rectified in the methods section of the manuscript." **Linkage disequilibrium (LD) clumping was used to identify the independent genome-wide significant loci within a 1Mb window. Clumping of**

the results was conducted in PLINK 1.9 with a P-value cut-off of 5e-8 and r-squared < 0.01 using the 1000 Human Genome Project reference panel. ”

The clumping used for the MR method resulted in 43 independent loci. Two SNPs (rs2596560 and rs9468541) were identified as pleiotropic outliers and eight as palindromic The remaining 33 SNPs were used in the MR analysis. This is now clearer in the results section of the manuscript “**Analysis with MR-PRESSO identified two potential pleiotropic outliers (rs2596560 and rs9468541) and eight palindromic SNPs with intermediate allele frequencies. Once the outliers and palindromic SNPs were excluded, MR results based on IVW showed that genetic predisposition to diabetes is likely to have a causal association with higher cataract risk** ”

b) Based on the reviewer's suggestion, we have now included additional information in Figure 4. We added Figure 4B, which shows the effect of variants associated with Cataracts (Outcome) and Type 1 Diabetes (Exposure), including the inverse variance weighted and MR-Egger fit results. Furthermore, the reviewer suggested doing the same for variants reported to associate with T1D, but we assume the reviewer meant T2D. To address this part of the suggestion, we have added Supplementary Figure 6 and included the following statement in the MR results section. “**We further tested the association between type 1 diabetes and cataracts by excluding SNPs associated with type 2 diabetes ($p < 0.05$). The association remained significant (OR = 1.009, 95% CI 1.002 - 1.015, p = 4.1e-3), as shown in Supplementary Figure 6. These results were consistent with the MVMR analysis, which adjusted for type 2 diabetes-related variants as a possible confounder in the association between type 1 diabetes and cataracts (OR = 1.008, 95% CI 1.002 - 1.014, p = 0.01).**”

Other comments:

Figure 1 would benefit from more info like sample size (N cases and N controls per cohort)

Thanks to the reviewer we have now included the sample size in Figure 1.

Did the authors account for the inflation in the test statistics that they observed using LDSR?

Thank you to the reviewer for the question. As the reviewer suggests, a common approach is to adjust the GWAS test statistics for genomic inflation lambda (by deflating the chi-square for each SNP through division by the value of lambda). However, given that the LDSC intercept is close to 1 there is no evidence of any inflation due to biases such as that caused by population stratification. Then the inflation of test statistics is likely a result of true polygenicity. Hence, the test statistics do not require adjustment for inflation.

In the PRS results section, they say that participants in the highest PRS are more likely to have cataracts compared to the rest of the cohort but the 95% CI of 0.81-1.56 shows that the difference is not significant, which is alarming. I wouldn't say that the risk is higher unless it is significant.

Thanks to the reviewer for the comment. We agree and modified the manuscript accordingly ". **Participants in the highest PRS decile seem more likely to have cataracts (odds ratio = 1.12, 95% CI = 0.81–1.56) compared to the rest of the cohort, however, results were not statistically significant.**"

In line 374, P=0.05/2=0.025, and from the supplementary table the P value is 0.029 which is larger than 0.025, so it is not significant after multiple testing as is stated.

Thank you to the reviewer for the comment. However, we have already clearly stated in the manuscript that the results were nominally significant. "**The PRS for cataracts was nominally associated with pterygium (p = 0.029) area in young adults (the Raine Study Gen2) after multiple testing correction (p = 0.05/2 traits = 0.025). In older adults (BHAS) there was a nominal association between the PRS of cataracts with CUVAF (p<0.05) (Supplementary Table 5)**."

REVIEWERS' COMMENTS

Reviewer #1 (Remarks to the Author):

1. The minor allele frequency (MAF) of the variants per dataset is still missing.

2. The GWAS results per dataset are not included in the supplementary table provided. The table should also include MAF.

3. Some of the information, such as the phenotype definition for each dataset in Supplementary Table 6, should be included in the Methods section as text.

4. When describing the results in Supplementary Table 5, please use the actual p-values, e.g., $P = 0.047$ instead of $P < 0.05$. Also, include the two non-significant p-values, i.e., $P = 0.189$ and $P = 0.201$.

Thank you for taking the time to review the changes to our manuscript. We appreciate your comments and suggestions. We have carefully considered each of your points and believe that our responses will address any concerns you may have had.

Reviewer 1

1. The minor allele frequency (MAF) of the variants per dataset is still missing.

Thank you for the comment. As we mentioned before, we are not including MAF per dataset due to the absence of study-specific MAF across all studies, specifically for GERA and MGBB. In such cases, the MAF was derived from a reference panel of Europeans from UKBB. This is already specified in the manuscript. "**All variants were aligned to the positive strand on build GRC37 hg19, and those with a minor allele frequency (MAF) < 1% were removed. In cases where the MAF was not available for the study (i.e., GERA and MGBB), it was derived from a reference panel based on five thousand healthy individuals from UKBB**"

2. The GWAS results per dataset are not included in the supplementary table provided. The table should also include MAF.

Thank you for the suggestion. GWAS results for the meta-analysis are publicly available. Including GWAS summary statistics per study in a supplementary table would require presenting effect estimates for millions of variants, resulting in a massive dataset that is already publicly available for the studies included in the meta-analysis (e.g., GERA and FinnGen datasets).

3. Some of the information, such as the phenotype definition for each dataset in Supplementary Table 6, should be included in the Methods section as text.

Thank you for the suggestion. We have provided further detailed information about the phenotype definition in the Methods section of the manuscript. "The current meta-analysis also includes the previous larger cataract multi-ancestry meta-analysis that encompassed GERA and UKB[4], using their publicly available summary statistics. The GERA cohort contains clinical and genomic data of over 110,000 participants; 33,145 patients who have undergone cataract surgery and 64,777 controls were included in this study. The UKB is a large prospective study following the health of approximately 500,000 participants, including 34,699 cataract cases defined as "participants with a self-reported cataract operation (f20004 code 1435) or/and a hospital record including a diagnosis code (ICD-10: H25 or H26)" [4], and 452,622 controls were included in the meta-analysis. **Cataracts were clinically diagnosed based on ICD-9/ICD-10 criteria in GERA, the Mass General Brigham Biobank, and FinnGen**. Detailed information regarding phenotype definition, genotyping, QC, and imputation procedures for all cohorts is provided in Supplementary Table 6."

4. When describing the results in Supplementary Table 5, please use the actual p-values, e.g., P = 0.047 instead of P < 0.05. Also, include the two non-significant p-values, i.e., P = 0.189 and P = 0.201.

Thank you to the reviewer for the suggestion. We have now included this information in the manuscript: “We investigated the association between the PRS for cataracts and measures of ocular UVR exposure in the Raine Study Gen2 and BHAS cohorts. **The PRS for cataracts was nominally associated with pterygium area in young adults (the Raine Study Gen2; p =0.029) after multiple testing corrections (p = 0.05/2 traits = 0.025), but not in older adults (BHAS; p = 0.189). Furthermore, the PRS for cataracts was not associated with CUVAF in young adults (p = 0.201) and was nominally associated in older adults (p = 0.047) (Supplementary Table 5).**”