nature portfolio

Peer Review File

Large-scale GWAS of strabismus identifies risk loci and provides support for a link with maternal smoking

Corresponding Author: Mr Weixiong He

This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 0:

Reviewer comments:

Reviewer #2

(Remarks to the Author)
This manuscript presents a large-scale meta-analysis of strabismus using GWAS results from 7 European ancestry groups and 3 definitions of strabismus subtypes. They further use Mendelian randomization to evaluate the presence of genetic support for a causal relationship between maternal smoking during pregnancy and strabismus in offspring, evaluating birth weight, which may also be mediated by maternal smoking during pregnancy. Overall, this is a very interesting study and is, for the most part, well-presented. I have a few questions and suggestions for the authors, below:

In the Abstract, lines 46-47 state: "...large-scale meta-analyses using 11 strabismus GWAS of European ancestry from 7 cohorts..."
However, the Introduction, lines 86-87, states: "Here, we conducted a European ancestry meta-analysis GWAS of strabismus analyses combining multiple summary statistics from 7 sources." The methods state "We included 7 sources with 11 sets of GWAS summary statistics..." which I personally find to be the most clear way of stating this information. Please clarify and present this language consistently.

In the Introduction, Lines 68-70 state: "The prevalence of ET and XT varies across ancestries. For example, among European ancestry, ET has 2.5% prevalence, while XT has a prevalence of
1%." But then no additional information is given about the actual variation in prevalence of ET and XT across ancestral groups. It would be helpful to have an idea of these estimates, despite that the paper focused on individuals of European descent. It might also be helpful to present, if available/known, the variation in rates of maternal smoking across ancestries.

In the Introduction, Line 84 states: "furthermore" - should this be "further"?

In the Methods section, Statistical Analyses (line 519), GWAS meta-analyses (line 520) - please explain whether variants that were not present in all datasets were included in the analyses. If variants that were not present in all datasets were included, was there a threshold number of studies in which each variant was present in order to be analyzed?

Given the differences in sample sizes of the meta-analyzed datasets, are the authors concerned about a particular dataset overly influencing the overall result? Were any analyses done to evaluate this?

In the Methods section, lines 576-578 state: "The MR approach tests for evidence of a causal effect of an exposure on an outcome and, conditional on some additional assumptions, allows an estimation of the magnitude of the causal effect" - it would be helpful to expand on those additional assumptions and how they apply to this particular analysis.

Table 2, where the "original paper" is mentioned, it would be helpful to include a reference for each variant of interest.

Please ensure consistent font type and size throughout the tables. The tables in general are not formatted well.

The information in Column 3 of the Supplementary Table was cut off.

The discussion should discuss the focus on European ancestry data as a limitation.

Reviewer #3

(Remarks to the Author)
This is a relatively straightforward meta-GWAS of strabismus and it's main subtypes. Benefits are large case sample sizes, novel loci, follow-up of some clinical epi hypothesis with MR. Weakness are lack of assessment of variants with aspects of strabismus such as amblyopia.

Line 108, please provide Cis or SE for h2 in main results (SE are provided in sup table 2) so that can see if they overlap or not.

No need to use the word 'gene' after using the approved gene symbol.

Table 1 is difficult to read in the main text. I would suggest to start it on a new page and switch orientation to landscape so that it is easier to follow. Also to reduce font size.

It would be useful to have a table (? Supplement) that shows the results for any snp associated with any of the 3 outcomes for all 3 outcomes so that we can see how different the association results are between outcomes. There is no indication of the direction of effect across the different cohorts, nor what the sample size is, since presumably not all cohorts imputed the same set of variants.

Supp Table 7 only has SNP id, but no chromosome or position information which makes it difficult to interpret. Please define the use of bC, bC_se, bC_pval. Presumably these are conditional results, but conditional on which other SNPs? This table is referred to on line 237, but it implies it is genetic correlation, which appears to be instead in Table S6.

The MR of maternal smoking requires better explanation. Can you provide details about the GWAS of maternal smoking. When I look at the methods and reference list, it appears the GWAS was of smoking, not maternal smoking. If this is the case, then this needs to be much more clearly described. On line 256 the authors refer to Saunders et al., 2022, but I do not find this in the reference list. Can there be a parallel analysis of paternal smoking to compare? If not, why not? Should instead they refer to parental smoking, rather than maternal smoking?

Can bi-directional MR be performed for maternal smoking, and birth weight.

Can multivariate MR be performed for (maternal) smoking and birth weight?

On line 9 of the supplementary methods, a GWAS from 2022 (ref 72) is cited, but there are no separate references for the supplement, and ref #72 in the main text is a methods paper published in 2018.

Fig 1 the manhattan plots look very odd. Generally the higher number chrs are shorter than the lower number chromosomes. But some (e.g. chr 17, 10) appear to be longer than chr 1. Is it due to ALT haplotypes? If so, this needs to be clarified and/or fixed.

Fig 2. There is no legend, just title. Please state where the LD that was used for the r2 presentation derive from.

Why was there no analysis of the X chrom?

There is inconsistent details about imputation panels used for the different cohorts. No imputation is described for FinnGen or Lifelines nor the previously published USA/Australia/UK GWAS ?
I could find no Qq plots, either overall of by cohort/outcome.

The top chr 15 snp is closer to CHRNB4 rather than CHRNA3.

There is no discussion of GWAS summary statistic availability.

The abstract could benefit from a case count for the main analysis (20,464).

Table 2 has column with Log(OR) with brackets, that are not defined. Is it possible to convert log(OR) to regular OR to help understanding. The top left cell of the table indicates that the 'OR and p in original paper', so where are the results from the replication provided? It would be useful to have a direct comparison between previous effect size (and allele frequency) and that from the replication data.

Version 1:

Reviewer comments:

Reviewer #2

(Remarks to the Author)

The authors have sufficiently addressed my concerns.

(Remarks on code availability)

Reviewer #3

(Remarks to the Author)
The authors have been mostly responsive to my previous comments.

The presentation of the qq plots (Fig1) now rises the question about genomic control lambda since the first traits appear to have inflated type 1 error due to early departure by -log10p around 1. Were double genomic control applied in the gwas and meta analysis? What is the genomic control lambda. If inflated, what do the lambdas look like separately by study and by MAF strata.

(Remarks on code availability)

Version 2:

Reviewer comments:

Reviewer #2

(Remarks to the Author)
My reviews and those of Reviewer #3 have been addressed.

(Remarks on code availability)

Thank you for reviewing our manuscript. We appreciate your insightful comments and suggestions. We have carefully considered each of your suggestions and believe our revisions have comprehensively addressed your concerns. In the following pages, we provide a detailed response to each point raised, along with explanations of the corresponding changes made to the manuscript. Additionally, we have reformatted all tables to enhance their clarity and presentation.

REVIEWER COMMENTS

Reviewer #1 (Remarks to the Author):

This manuscript presents a large-scale meta-analysis of strabismus using GWAS results from 7 European ancestry groups and 3 definitions of strabismus subtypes. They further use Mendelian randomization to evaluate the presence of genetic support for a causal relationship between maternal smoking during pregnancy and strabismus in offspring, evaluating birth weight, which may also be mediated by maternal smoking during pregnancy. Overall, this is a very interesting study and is, for the most part, well-presented. I have a few questions and suggestions for the authors, below:

In the Abstract, lines 46-47 state: "...large-scale meta-analyses using 11 strabismus GWAS of European ancestry from 7 cohorts..."
However, the Introduction, lines 86-87, states: "Here, we conducted a European ancestry meta-analysis GWAS of strabismus analyses combining multiple summary statistics from 7 sources." The methods state "We included 7 sources with 11 sets of GWAS summary statistics..." which I personally find to be the most clear way of stating this information. Please clarify and present this language consistently.
Response: We agree that our initial descriptions varied and could lead to confusion. We have now uniformly described the composition of our meta-analysis as "11 strabismus GWAS of European ancestry from 7 sources" across the Abstract, Introduction, and Methods sections for clarity.

See lines 44-46: Here, we conducted large-scale meta-analyses using 11 strabismus GWAS of European ancestry from 7 sources to discover new genetic variants for strabismus and its sub-types.

Lines 82-84: Here, we conducted a European ancestry meta-analysis GWAS of strabismus analyses combining 11 summary statistics from 7 sources.

In the Introduction, Lines 68-70 state: "The prevalence of ET and XT varies across ancestries. For example, among European ancestry, ET has 2.5% prevalence, while XT has a prevalence of
1%." But then no additional information is given about the actual variation in prevalence of ET and XT across ancestral groups. It would be helpful to have an idea of these estimates, despite that the paper focused on individuals of European descent. It might also be helpful to present, if available/known, the variation in rates of maternal smoking across ancestries.
Response: Thanks for your suggestion. We've added lines 64-67: The prevalence of ET and XT varies across populations. For example, among European regions, ET has a

prevalence of 2.17%, whereas XT has a prevalence of approximately 1.53%. By contrast, theAfrican region shows a prevalence of 0.13% for ET and 0.14% for XT (Hashemi et al. 2019)

We have expanded our discussion to include prevalence rates of ET and XT across different ancestries and added commentary on the variation in maternal smoking rates across ancestries. See lines 268-275: Third, for our MR sensitivity analysis based on multiple SNP IVs, our estimates were derived assuming that the offspring genotype for each SNP was indicative of maternal smoking risk. Irrespective of the precise magnitude of the risk, our MR analyses provide an independent line of evidence for a link between maternal smoking and strabismus. In comparison to previous observational studies (Y. Yang et al. 2019; Cotter et al. 2011), MR studies are subject to different sources of confounding bias. Therefore, combining past evidence from observational studies (Y. Yang et al. 2019) and the new evidence from our MR study, there is consistent evidence of a causal relationship between maternal smoking and strabismus.

In the Introduction, Line 84 states: "furthermore" - should this be "further"?
Response: Upon your suggestion, we replaced "furthermore" with "further". See lines 81-82: Obtaining a larger sample size increases the statistical power of GWAS and is expected to lead to the discovery of further strabismus risk variants.

In the Methods section, Statistical Analyses (line 519), GWAS meta-analyses (line 520) - please explain whether variants that were not present in all datasets were included in the analyses. If variants that were not present in all datasets were included, was there a threshold number of studies in which each variant was present in order to be analyzed?
Response: We thank the reviewer for the question concerning the inclusion criteria for variants in our GWAS meta-analyses. Due to the different QC and imputation strategies in each input GWAS summary statistics, some SNPs may be missed in some input datasets and we didn't set a threshold number of studies. We've added a degree of freedom and heterogeneity tests columns in Table 1. The significant loci reported in Table 1 were presented in at least two input datasets and showed P>0.05 in heterogeneity tests. We also added a statement in the Method section, see lines 523-525: Given the variation in QC and imputation strategies across the input GWAS summary statistics, variants that were not present in all datasets were included without establishing a specific threshold for the number of studies.

Given the differences in sample sizes of the meta-analyzed datasets, are the authors concerned about a particular dataset overly influencing the overall result? Were any analyses done to evaluate this?
Response: We thank the reviewer for raising this point. We clarified our approach by stating that "variants that were not present in all datasets were included without establishing a specific threshold for the number of studies" (lines 524-525). This decision was based on maximizing the inclusivity of relevant genetic data. As listed in Table 1, we utilized the I^2 statistic to quantify the extent of heterogeneity among the studies. We conducted subgroup analyses to explore whether specific characteristics of the datasets (e.g., population ancestry, genotyping platform) contributed to the observed heterogeneity. All p-values from the I^2 test are larger than 0.05, suggesting our results are broadly homogeneous.

In the Methods section, lines 576-578 state: "The MR approach tests for evidence of a causal effect of an exposure on an outcome and, conditional on some additional assumptions, allows an estimation of the magnitude of the causal effect" - it would be helpful to expand on those additional assumptions and how they apply to this particular analysis.
Response: The main assumptions for MR are (1) the genetic marker is associated with the exposure, (2) the genetic marker is independent of the outcome given the exposure and all confounders of the exposure-outcome association, and (3) the genetic marker is independent of factors that confound the exposure-outcome relationship (VanderWeele et al. 2014). A fourth assumption is that the exposure-outcome effect is linear. These assumptions are just the basic assumption for MR. In the interests of space, we did not describe these in detail; the main point we wanted to make was that MR can be used to a) perform a hypothesis test for there being a causal effect and b) estimate the exposure-outcome effect size. Our main interest in the paper was in a).

Upon revision, we find this sentence superfluous and have removed it.

Table 2, where the "original paper" is mentioned, it would be helpful to include a reference for each variant of interest.
Response: Each variant of interest now includes a citation, facilitating easier reference to the source studies. See Table 2.

Please ensure consistent font type and size throughout the tables. The tables in general are not formatted well.
Response: Thank you for flagging this issue. All tables have now been reformatted for consistency in font type and size.

The information in Column 3 of the Supplementary Table was cut off.
Response: Thank you for bringing this to our attention. We have thoroughly reviewed all supplementary tables associated with our study, but we did not find any indication of the information in Column 3 being cut off. That could result from the error when converting the table into PDF in the Manuscript Tracking System. We've re-submitted these tables and that should be fixed.

The discussion should discuss the focus on European ancestry data as a limitation.
Response: A paragraph discussing the limitation of focusing on European ancestry data has been added to the discussion, acknowledging the potential impact on the generalizability of our findings.

Lines 275-282: Fourth, our study focused on individuals of European descent. Due to the lack of samples, we were unable to seek to replicate our GWAS lead loci in non-European cohorts. Although maternal smoking rates differ across countries (Hashemi et al. 2019; Lange et al. 2018), maternal smoking is associated with strabismus in conventional observational studies across various ancestry groups (Zhang et al. 2021; Y. Yang et al. 2019; Cotter et al. 2011). Our MR findings in Europeans provide support for a causal link between maternal smoking and strabismus. Future genetic studies should be conducted in a wider range of ancestries to expand the scope of gene mapping and MR studies.

Reviewer #2 (Remarks to the Author):

This is a relatively straightforward meta-GWAS of strabismus and its main subtypes. Benefits are large case sample sizes, novel loci, follow-up of some clinical epi hypothesis with MR. Weakness are lack of assessment of variants with aspects of strabismus such as amblyopia.

Line 108, please provide Cis or SE for h2 in main results (SE are provided in sup table 2) so that can see if they overlap or not.
Response: Thank you for the suggestion, we've added the 95% CI to the rg values listed in the result section.

No need to use the word 'gene' after using the approved gene symbol.
Response: We've removed "gene" after gene symbols in the main text.

Table 1 is difficult to read in the main text. I would suggest to start it on a new page and switch orientation to landscape so that it is easier to follow. Also to reduce font size.
Response: Thank you. We've changed it to landscape.

It would be useful to have a table (? Supplement) that shows the results for any snp associated with any of the 3 outcomes for all 3 outcomes so that we can see how different the association results are between outcomes. There is no indication of the direction of effect across the different cohorts, nor what the sample size is, since presumably not all cohorts imputed the same set of variants.
Response: Thank you for your suggestion. We have incorporated your feedback and now include Table S3 in the supplementary materials. Table S3 provides the SNP beta coefficients and corresponding p-values for each analysis conducted. Additionally, we have included information on the direction of effect across different cohorts for each GWAS lead SNP, as per your request.

We have included detailed information on the sample sizes for each cohort and analysis in Table S1 of the supplementary materials.

Supp Table 7 only has SNP id, but no chromosome or position information which makes it difficult to interpret. Please define the use of bC, bC_se, bC_pval. Presumably these are conditional results, but conditional on which other SNPs? This table is referred to on line 237, but it implies it is genetic correlation, which appears to be instead in Table S6.
Response: We've added chromosomes and positions to Table S8 (was Table S7). Table S8 was referred to in lines 135-137: We also applied the above procedures for the results of the GWAS for ET and for XT. All four XT lead SNPs and two out of four ET lead SNPs (rs228636 and rs8070929) retained genome-wide significance in the conditional analysis (Table S8 (was Table S7)). We also swapped the bC, bC_se, and bC_pval to mtCOJO effect size, mtCOJO standard error, and mtCOJO p-value to make it clearer.

The MR of maternal smoking requires better explanation. Can you provide details about the GWAS of maternal smoking. When I look at the methods and reference list, it appears the GWAS was of smoking, not maternal smoking. If this is the case, then this needs to be much more clearly described. On line 256 the authors refer to Saunders et al., 2022, but I do not find this in the reference list. Can there be a parallel analysis of paternal smoking to

compare? If not, why not? Should instead they refer to parental smoking, rather than maternal smoking?
Response: The variant used to proxy maternal smoking is from Yang, Q., Millard, L. A. C. & Davey Smith, G. Proxy gene-by-environment Mendelian randomization study confirms a causal effect of maternal smoking on offspring birthweight, but little evidence of long-term influences on offspring health. Int. J. Epidemiol. 49, 1207–1218 (2020). In this paper, they said "Each additional smoking-increasing allele of participants' (G1) rs16969968 was associated with a 1.02 [95% confidence interval (CI): 1.01, 1.03; P-value = 5 × 10–3] higher odds of their mothers' (G0) smoking in pregnancy". We've added "The detailed description of this instrument has been described by Yang et al. (2020)" (Lines 592-593) to notify readers.

Saunder's paper was shown in the reference list from the supplementary file, but by mistake was not included in the main text, now it has been added to the main text. Using paternal smoking in pregnancy as a negative control could further validate the proxy MR findings and help to separate intrauterine effects from other effects of parental smoking on offspring outcomes. Unfortunately, such paternal smoking data were not available.

Can bi-directional MR be performed for maternal smoking, and birth weight.
Response: Thank you for your inquiry. Exposure needs to happen before outcome therefore we cannot test whether MR can be used to test the effect offspring birth weight on maternal smoking during pregnancy. Potentailly, we can test whether offspring birth weight affects maternal smoking after giving birth but this will require individual-level mother-offspring pair data to test it. We do not have access to the necessary data. As such, we are unable to perform the requested analysis at this time.

Can multivariate MR be performed for (maternal) smoking and birth weight?
Response: Same to the last question. Unfortunately, due to the unavailability of GWAS data for maternal smoking, we are unable to conduct multivariate MR at this time.

On line 9 of the supplementary methods, a GWAS from 2022 (ref 72) is cited, but there are no separate references for the supplement, and ref #72 in the main text is a methods paper published in 2018.
Response: We are sorry for the inconsistency between the main text and supplementary methods. We've corrected the reference list in supplementary methods.

Fig 1 the manhattan plots look very odd. Generally the higher number chrs are shorter than the lower number chromosomes. But some (e.g. chr 17, 10) appear to be longer than chr 1. Is it due to ALT haplotypes? If so, this needs to be clarified and/or fixed.

Response: Thanks for your suggestion. We have updated the Manhattan plot. See Figure 1

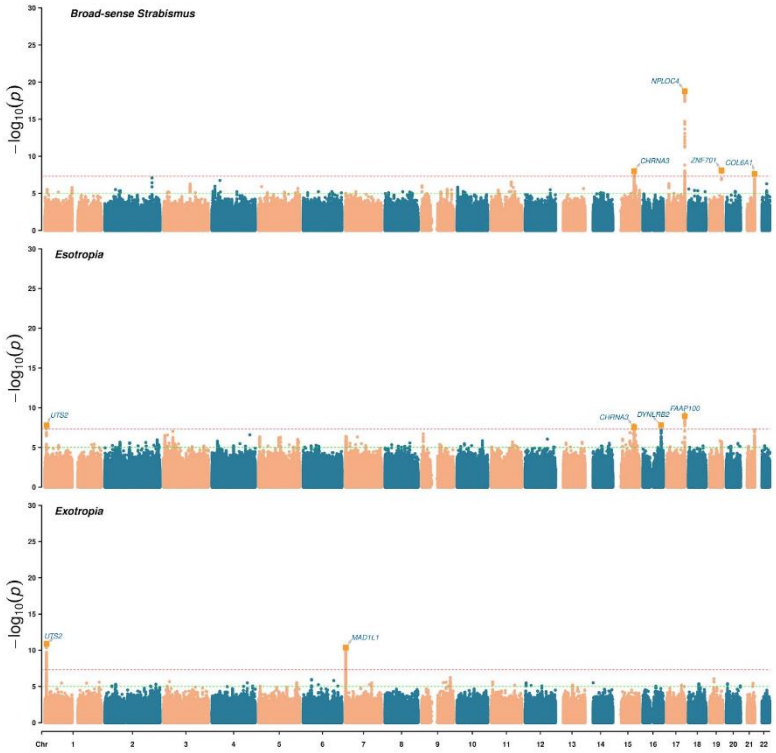


Fig 2. There is no legend, just title. Please state where the LD that was used for the r2 presentation derive from.
Response: We thank the reviewer for this comment. We have added the figure legend for Figure 2. And listed the genome build and LD information.

Why was there no analysis of the X chrom?
Response: Our study was designed to maximize the comparability of our findings with the broader body of GWAS literature, which predominantly focuses on autosomal variants. In this study, we only focus on autosomes because X-chromosome was excluded in some GWAS sources and we need to keep the input GWAS consistent. However, we acknowledge the importance of the X chromosome and excluding it might overlook potentially significant associations. Future studies, possibly with larger sample sizes and more advanced methodologies specifically tailored for sex chromosome analysis, could provide valuable insights into the role of the X chromosome. We added an statement regarding to this, see line 525-527: Besides, due to X-chromosome was excluded in some GWAS sources, we limited our analysis to autosomes to keep the input GWAS consistent.

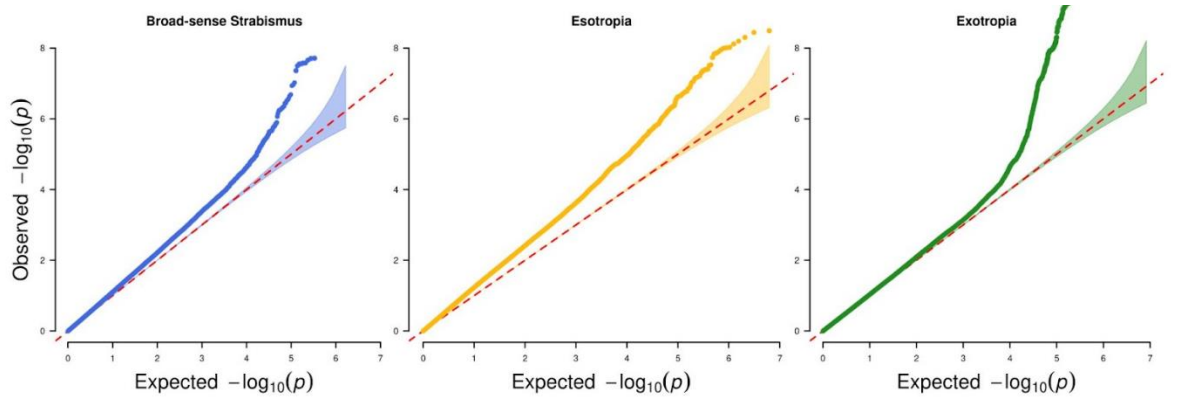
There is inconsistent details about imputation panels used for the different cohorts. No imputation is described for FinnGen or Lifelines nor the previously published USA/Australia/UK GWAS ?
Response: Thanks for your suggestion. We cited the described documents for FinnGen, and Lifelines and published GWAS. We noticed it could sometimes make it hard to read. Thus we briefly added the imputation sentences in our method section. For FinnGen, line 379-385: The variants were genotyped using ThermoFisher Axiom custom array v2 that contains 723,376 probesets for 664,510 markers. In addition to the core GWAS markers (about 500,000) it contains about 116,000 coding variants enriched in Finland

(https://www.finngen.fi/en/researchers/genotyping). Genotype imputation was conducted by using the population-specific SISu v4.2 imputation reference panel (contains 8,554 whole-genome sequencing (WGS) data of Finnish individuals). The detailed QC and imputation procedures have been described at https://finngen.gitbook.io/documentation/.

For Lifelines, line 439-446: SNP data obtained from the array were imputed using human reference genomes including the Genome of The Netherlands (GoNL) release 5 (Genome of the Netherlands Consortium ...) and the 1000 Genomes phase1 v3 reference panels (1000 Genomes Project Consortium et al...), using Minimac (version 2012.10.3 9) (Howie et al. 2012). Prior to imputation, SHAPEIT2 was employed for genotype pre-phasing (Delaneau et al. 2013), and the Genotype Harmonizer was used to align the genotypes with the reference panels to address strand issues (Deelen et al. 2014). Cleaned pedigree files and in- and output files for imputation algorithms were created in PLINK (Purcell et al. 2007). The imputation analysis was conducted using Beagle (version 3.1.0 8) (Browning and Browning 2009).

For published esotropia GWAS, line 476-481: The sample collection and GWAS analysis procedures have been described in detail in the paper (Shaaban et al. 2018). Briefly, 337,204 SNPs were genotyped using OmniExpress arrays and passed QC, these SNPs were imputed against 1000 Genomes phase1 v3 European reference panels (1000 Genomes Project Consortium et al...) using IMPUTE2 program (version 2) (Howie et al. 2009).

I could find no Qq plots, either overall or by cohort/outcome.
Response: Thanks for your suggestion. We've added QQ-plots for strabismus, esotropia and exotropia analyses along with the Manhattan plot (Figure 1).



The top chr 15 snp is closer to CHRNB4 rather than CHRNA3.
Response: We thank the reviewer for indicating this issue. We annotated our GWAS significant SNPs on OpenTarget Genetics, based on the source from the website (https://genetics.opentargets.org/Variant/15_78664464_T_C/associations) the distance from the variant to the transcription start site (TSS) of *CHRNA3* is 43,169, of *CHRNB4* is 63,290. But we agree this could be misleading. We've replaced *CHRNA3* with *CHRNB4* to make it consistent with our locus zoom plot. Both *CHRNA3* and *CHRNA4* have been associated with smoking behaviours. Thus, this will not affect our conclusion in the manuscript.

There is no discussion of GWAS summary statistic availability.

Response: We've added the data availability section. See lines 599-604: UK Biobank data are available through the UK Biobank Access Management System at https://www.ukbiobank.ac.uk/. FinnGen Data are available via https://www.finngen.fi/en. The data that support the findings of this study are available on request from the

corresponding author WH. The data are not publicly available due to the individual-level data is from multiple resources that could compromise different research participant privacy/consent.

The abstract could benefit from a case count for the main analysis (20,464).
Response: Thanks for the comment, we have the case count for each analysis in the abstract. See lines 44-51: Here, we conducted large-scale meta-analyses using 11 strabismus GWAS of European ancestry from 7 cohorts to discover new genetic variants for strabismus and its sub-types. We identified 4 genome-wide significant independent loci (*NPLOC4-TSPAN10-PDE6G-FAAP100*, *COL6A1*, *ZNF701* and *CHRNA4*) in the meta-analysis of broadly defined strabismus (20,464 cases and 954,921 controls). We also identified 5 (*UTS2*, *CHRNA4*, *DYNLRB2*, *NPLOC4-TSPAN10-PDE6G-FAAP100* and *MAD1L1*) independent loci by narrowing down the strabismus definition to esotropia (5,963 cases and 588,794 controls) and exotropia (3,998 cases and 583,468 controls).

Table 2 has column with Log(OR) with brackets, that are not defined. Is it possible to convert log(OR) to regular OR to help understanding. The top left cell of the table indicates that the 'OR and p in original paper', so where are the results from the replication provided? It would be useful to have a direct comparison between previous effect size (and allele frequency) and that from the replication data.
Response: Thanks for your advice. We've updated Table 2 to make a more direct comparison.

Reference

VanderWeele, Tyler J., Eric J. Tchetgen Tchetgen, Marilyn Cornelis, and Peter Kraft. 2014. "Methodological Challenges in Mendelian Randomization." *Epidemiology* 25 (3): 427–35.

Response to reviewer

The presentation of the qq plots (Fig1) now rises the question about genomic control lambda since the first traits appear to have inflated type 1 error due to early departure by -log10p around 1. Were double genomic control applied in the gwas and meta analysis? What is the genomic control lambda. If inflated, what do the lambdas look like separately by study and by MAF strata.

Response: Thank you for your comments regarding the QQ plots. We have calculated the genomic control lambda values as follows. We have further analysed the lambda values by MAF. We have calculated the lambda values for both input GWAS and meta-analysis. The specific lambda values are detailed in the tables below and included in Table S1 and Table S3 in the supplementary materials. Reassuringly each of the input sets shows no strong inflation in genomic control lambda (<1.1 for all datasets). However, we did observe some inflation in the lambda values for the meta-analysed datasets. It has been shown (https://pubmed.ncbi.nlm.nih.gov/21407268/) that the genomic control lambda is expected to increase when sample sizes increase via meta-analysis. The exact genomic control value obtained varies by meta-analysed phenotype and is largest for esotropia.

To further assess the inflation, we investigated the linkage disequilibrium score regression (LDSC) attenuation ratio. The LDSC attenuation ratio was 0.87, indicating partial inflation. This suggests that the esotropia trait may exhibit a higher degree of polygenicity compared to the other strabismus traits.

We did not apply double genomic control in our GWAS and meta-analysis. Literature suggests that double genomic control may be ineffective in correcting for population stratification in meta-analyses (https://pubmed.ncbi.nlm.nih.gov/23269928/).

Overall, while we acknowledge the observed inflation in the meta-analysis results, the lambda values remain within acceptable limits, by considering the increased sample sizes and inherent variability across the input genetic association studies.

Lambda MAF_Strata GWAS		
Meta-analysis	**Lambda**	**MAF**
Strabismus	1.12	all
Strabismus	1.16	[0.1 - 0.5]
Strabismus	1.08	[0.01 - 0.05)

Strabismus	1.15	[0.05 - 0.1)
Esotropia	1.35	all
Esotropia	1.43	[0.1 - 0.5]
Esotropia	1.22	[0.01 - 0.05)
Esotropia	1.42	[0.05 - 0.1)
Exotropia	1.03	all
Exotropia	1.05	[0.1 - 0.5]
Exotropia	1.02	[0.01 - 0.05)
Exotropia	1.02	[0.05 - 0.1)

GWAS	**Lambda**
Strabismus	
GERA	1.02
UKB self-reported strabismus	1.05
Fin STRABBOTH	1.04
AGDS	1.01
The 2018 Shaaban study non-accommodative esotropia discovery set	1.03
The 2018 Shaaban study accommodative esotropia discovery set	1.02
The 2018 Shaaban study non-accommodative esotropia replication set	0.99
The 2018 Shaaban study accommodative esotropia replication set	1.01
Lifelines CytoSNP	1.02
Lifelines GSA	1.00
Estonian Biobank Broad-sense strabismus	1.05

Esotropia	
GERA esotropia	1.03
FinnGen Convergent concomitant strabismus	1.03
Estonian Biobank esotropia	1.02
The 2018 Shaaban study non-accommodative esotropia discovery set	1.03
The 2018 Shaaban study accommodative esotropia discovery set	1.02
The 2018 Shaaban study non-accommodative esotropia replication set	0.99
The 2018 Shaaban study accommodative esotropia replication set	1.01
Exotropia	
GERA exotropia	1.01
FinnGen Divergent concomitant strabismus	1.05
Estonian Biobank exotropia	1.01