

Supplementary Methods

Mendelian Randomization of smoking and strabismus

To investigate the causal effect between smoking and strabismus using a person's own genes as exposure to strabismus, we performed MR analyses using the same procedure with the intergenerational MR. For smoking exposure data, we applied independent genome-wide significantly associated SNPs for smoking initiation from the GWAS published in 2022 (Saunders et al. 2022). The summary statistics from our GWAS for esotropia were used as the 'outcome' data. The genetically inferred causal association was estimated by the inverse variance-weight (IVW) estimator and exponentiated to reflect the OR for strabismus per SD change in smoking phenotype via package TwoSampleMR (Hemani et al. 2018). To check the robustness of our IVW estimates, we also conducted MR analyses using various alternative MR methods, weighted median, MR-Egger regression and weighted mode. We also applied MR-PRESSO (Pleiotropy Residual Sum and Outlier) (Verbanck et al. 2018) to identify and adjust for outliers that may represent SNPs with horizontally pleiotropic effects on both smoking and strabismus. The Rucker framework was applied by the "mr_rucker" function incorporated into the TwoSampleMR package to determine whether heterogeneity influences the IVW or MR-Egger regression models (Bowden et al. 2017).

Supplementary Results

Genetic association between smoking traits and strabismus

The gene-smoking interaction has been well-studied and this interaction can be passed from parents to offspring (Saleheen et al. 2017; Joehanes et al. 2016). Given a subset of our strabismus risk loci were associated with smoking, we also investigated the causal link between smoking and strabismus using individuals' own gene rather than a proxy instrument variable from their mothers. Using the genome-wide significant SNPs from large-scale smoking GWAS (Saunders et al. 2022) as the instrument variables, we performed two-sample MR and evaluated the causal link between three smoking phenotypes smoking initiation and

three strabismus phenotypes (Table S10). The causal association was estimated by the inverse variance-weight (IVW) estimator. The MR results of alternative MR inverse-variance weighted models suggested nominal evidence for an association (OR for the IVW estimate was 1.046, 95% CI 1.008–1.084; $P_{IVW} = 0.017$), although after Bonferroni correction for the 3 phenotypes tested, the P-value exceeded 0.05. For the nominally significant smoking initiation result, there was no evidence for horizontal pleiotropy (MR Egger intercept = -1.37×10^{-5} , SE = 0.00048, P = 0.98) and after excluding outliers through MR-PRESSO outlier-corrected tests, the estimate was essentially unchanged (Table S10). We also compared the difference of models with or without heterogeneity by using MR-Egger over IVW using the Rucker framework (Bowden et al. 2017). The difference was small and non-significant (difference = 0.001; P = 0.97).

Supplementary References

- Bowden, Jack, Fabiola Del Greco M, Cosetta Minelli, George Davey Smith, Nuala Sheehan, and John Thompson. 2017. “A Framework for the Investigation of Pleiotropy in Two-Sample Summary Data Mendelian Randomization.” *Statistics in Medicine* 36 (11): 1783–1802.
- Hemani, Gibran, Jie Zheng, Benjamin Elsworth, Kaitlin H. Wade, Valeriia Haberland, Denis Baird, Charles Laurin, et al. 2018. “The MR-Base Platform Supports Systematic Causal Inference across the Human Phenome.” *eLife* 7 (May). <https://doi.org/10.7554/eLife.34408>.
- Joehanes, Roby, Allan C. Just, Riccardo E. Marioni, Luke C. Pilling, Lindsay M. Reynolds, Pooja R. Mandaviya, Weihua Guan, et al. 2016. “Epigenetic Signatures of Cigarette Smoking.” *Circulation. Cardiovascular Genetics* 9 (5): 436–47.
- Saleheen, Danish, Wei Zhao, Robin Young, Christopher P. Nelson, Weangkee Ho, Jane F. Ferguson, Asif Rasheed, et al. 2017. “Loss of Cardioprotective Effects at the ADAMTS7 Locus as a Result of Gene-Smoking Interactions.” *Circulation* 135 (24): 2336–53.
- Saunders, Gretchen R. B., Xingyan Wang, Fang Chen, Seon-Kyeong Jang, Mengzhen Liu, Chen Wang, Shuang Gao, et al. 2022. “Genetic Diversity Fuels Gene Discovery for Tobacco and Alcohol Use.” *Nature* 612 (7941): 720–24.
- Verbanck, Marie, Chia-Yen Chen, Benjamin Neale, and Ron Do. 2018. “Detection of Widespread Horizontal Pleiotropy in Causal Relationships Inferred from Mendelian Randomization between Complex Traits and Diseases.” *Nature Genetics* 50 (5): 693–98.