Supplemental Online Content

Siggs OM, Qassim A, Han X, et al. Association of high polygenic risk with visual field worsening despite treatment in early primary open-angle glaucoma. *JAMA Ophthalmol*. Published online November 10, 2022. doi:10.1001/jamaophthalmol.2022.4688

eAppendix
eReferences

This supplemental material has been provided by the authors to give readers additional information about their work.

eAppendix.

Glaucoma progression dataset

We sampled 1,103 genotyped participants from the PROGRESSA (Progression Risk Of Glaucoma: RElevant SNPs with Significant Association) study. Participants were recruited and monitored at multiple public and private ophthalmology practices across Australia between 2012 and 2020. Of those with a self-reported ancestry, >95% of PROGRESSA participants were European. Individuals with an optic nerve head appearance suspicious or probable for glaucoma,[1] open drainage angles on gonioscopy, visual field mean deviation >-6 dB in both eyes, and no secondary cause of elevated intraocular pressure (IOP) were enrolled for longitudinal follow-up. Visual field testing was performed 6-monthly using a Humphrey Visual Field SITA Standard 24–2 protocol, or sooner if clinically indicated. ≥4 reliable fields defined by false positive and fixation loss rates <33% were required for longitudinal analysis (1,563 eyes from 808 individuals met inclusion criteria). Visual field worsening was defined by modified Hodapp-Parish-Anderson criteria; i.e., two consecutive reliable field tests showing a new visual field defect, or extension into a new region, defined by 3 contiguous test locations showing pattern standard deviation (PSD) <5%, one of which was at <1%, in the a visual field region (nasal, paracentral, Bjerrum's, or temporal areas). If the glaucoma hemifield test was “Outside Normal Limits” or the global PSD was <5%, then test locations being <5% on PSD was considered sufficient. Grading was performed using the aforementioned criteria via a custom script implemented in R, and validated against clinician grading. Participants with known pathogenic or likely pathogenic *MYOC* variants were not included in the PRS comparison analysis. We utilized all visual field data available for analysis including those predating PROGRESSA enrollment, and both eyes were included where sufficient reliable visual fields were available. Structural progression was measured as the rate of pRNFL thinning per annum in the fastest-thinning superior or inferior quadrant for each eye, as measured by OCT on the same device (Cirrus HD-OCT [Carl Zeiss], or SPECTRALIS [Heidelberg Engineering]). Scans with artifacts or segmentation errors were excluded, as were Cirrus scans with a quality score <6. Increasing rates of pRNFL change, which reflect non-pathological interscan thickness variations, were set to 0. A minimum of 2 years and 4 scans were required to generate a reliable rate of pRNFL change (1,777 eyes from 896 individuals met inclusion criteria). Treatment intensity was measured by the maximal number of topical glaucoma drops recorded throughout follow-up, with each selective laser trabeculoplasty (SLT) or trabeculectomy procedure counted as the equivalent of one or three topical medications, respectively. All treatment decisions were made at the discretion of the treating ophthalmologist, without knowledge of PRS. For population controls we used 17,642 genotyped individuals from the population-based QSkin cohort (93% of whom were of self-reported white European ancestry): a prospective cohort aged 40–69 years, randomly sampled from Queensland, Australia.[2] This study was approved by the Southern Adelaide Clinical Human Research Ethics Committee, in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants, and study participants received no compensation or incentives to participate.

Deriving the glaucoma polygenic risk score

The glaucoma polygenic risk score (PRS) was obtained using summary statistics of genome-wide association studies (GWAS) of glaucoma (case and control) and its endophenotypes.[3] Endophenotypes included intraocular pressure (IOP), vertical cup-to-disc ratio (VCDR) and disc-diameter adjusted VCDR GWAS obtained from the UK Biobank and International Glaucoma Genetics Consortium cohorts. These correlated traits were combined using a multivariate analysis of the GWAS data which improved the overall discovery power.[3] All cohorts used in deriving the PRS were independent of our study cohort. The derived PRS is measured for each individual as a numerical score representing the weighted number of single nucleotide variants (SNPs) the individual carries that is associated with glaucoma and its endophenotypes. The PRS was further optimized by using a reference group of advanced glaucoma cases enrolled in a glaucoma registry, adjusting the P-value threshold of the SNPs to $P \leq 0.001$ with linkage disequilibrium clumping at $r^2 = 0.1$, totalling to 2,673 uncorrelated SNPs.

Validation of the visual field worsening using the Collaborative Initial Glaucoma Treatment Study [CIGTS] criteria

The CIGTS visual field score was calculated for each visual field using an scripted implementation of the criteria (in-house script implemented in R). Details and validation of the CIGTS criteria are described elsewhere, but summarized here briefly for reference.[4] Each visual field location is scored based on the intensity of the defect using the age-matched total deviation probability plot, if the location belongs to a cluster of ≥ 3 defective locations. Progression was defined as a 3 point or more increase in the CIGTS score in subsequent fields, sustained over 3 follow-up fields. Using a similar approach to our reported primary analysis, we fitted a mixed-effect multivariable Cox proportional hazard model adjusting for age, sex, and baseline field mean deviation with progression per CIGTS criteria as our outcome. The top 5% PRS group were 1.77 times greater risk of visual field worsening 95% confidence interval 1.1–2.9, P =.03) compared to the bottom 95%.

eReferences

1. Bayer A, Harasymowycz P, Henderer JD, Steinmann WG, Spaeth GL. Validity of a new disk grading scale for estimating glaucomatous damage: correlation with visual field damage. *Am J Ophthalmol*. 2002;133(6):758-763.
2. Olsen CM, Green AC, Neale RE, et al. Cohort profile: the QSkin Sun and Health Study. *Int J Epidemiol*. 2012;41(4):929-929i.
3. Craig, J. E., Han, X., Qassim, A., Hassall, M., Cooke Bailey, J. N., Kinzy, T. G., ... & MacGregor, S. (2020). Multitrait analysis of glaucoma identifies new risk loci and enables polygenic prediction of disease susceptibility and progression. *Nature genetics*, *52*(2), 160-166.
4. Gillespie, B. W., Musch, D. C., Guire, K. E., Mills, R. P., Lichter, P. R., Janz, N. K., & Wren, P. A. (2003). The collaborative initial glaucoma treatment study: baseline visual field and test–retest variability. *Investigative ophthalmology & visual science*, *44*(6), 2613-2620.