prevalent POAG/glaucoma by evaluating metabolomic pro les measured in either serum[6–8], plasma[9–11], aqueous humor[12–15], tear[16], and optic nerve[17] samples. However, these studies are limited by relatively small sample sizes (the largest study included 211 cases and 295 controls[7]), focus on treated POAG cases[9–11,13–18] which may result in bias due to treatment, and use of convenience controls with other eye conditions[13–15,19,20]. Additionally, the use of prevalent cases may be problematic for the discovery of changes related to early disease, as consequences of advanced disease or treatment are likely to impact circulating metabolite pro les in glaucoma. Our study included 599 incident cases and 599 matched controls in a nested case-control study of pre-diagnostic circulating plasma metabolites from ~10 years before POAG diagnosis, and to con rm the ndings, we evaluated the metabolomic data in prevalent glaucoma cases from the UK Biobank.

Here, we show that higher levels of diglycerides and triglycerides are adversely associated with incident POAG in three health professional cohorts with stronger associations for POAG with paracentral visual eld (VF) loss. We con rmed the adverse associations for glycerides in a cross-sectional analysis performed in the UK Biobank.

S d a –N e

and the inverse associations with cholesteryl esters and organic acids and derivatives (which includes amino acids) were robust (FDR <

compared to peripheral VF loss[21–23]. Therefore, we separately evaluated the associations between metabolite classes and POAG de ned by VF loss patterns (paracentral (Fig. 3a) versus peripheral VF loss (Fig. 3b)). Of the 599 cases, VF loss patterns derived from Humphrey visual eld test were available in 509 cases. As shown in Fig. 3

BMI > 25 kg/m^2 and those diagnosed closer in time to blood draw (within a decade).

UK B ba

To assess whether the associations observed in NHS/NHSII/HPFS might also be observed in the UK Biobank, we conducted metabolomic analyses of the outcome of glaucoma, de ned based on self-reported glaucoma, use of glaucoma medications and ICD codes (2238 glaucoma cases and 44723 non-cases). In general, glaucoma cases were older, had more diabetes, and higher systolic blood pressure than controls (Table 2).

In multivariable-adjusted analyses of individual metabolites (Fig. 4 Supplementary Data 2), we observed that 6 TG metabolites were nominally associated with higher glaucoma risk ($p < 0.05$). Tyrosine (NEF < 0.05), glucose (NEF < 0.05), glutamine (NEF<0.2), and one TG metabolite (NEF < 0.2) were also signi cantly associated with higher glaucoma risk. Speci c organic acids and derivatives, such as acetate, 3-hydroxybutyrate, citrate, pyruvate, and lactate were inversely associated with glaucoma (NEF < 0.05). Notably, data on glucose, acetate, 3-hydroxybutyrate, citrate, pyruvate, and lactate were not available in NHS/NHSII/HPFS; however, there were null associations between tyrosine, valine, glutamine, and phenylalanine with POAG (Supplementary Data 1).

Figure 5 shows results from evaluating metabolite classes in glaucoma in the UK Biobank. Amino acids and TGs were positively associated while ketone bodies were inversely associated with glaucoma (FDR < 0.05). Glycolysis-related metabolites were inversely associated with glaucoma at FDR < 0.2.

Pre-clinical plasma metabolite pro ling indicates that higher levels of DGs and TGs are adversely associated with incident POAG in 3 health professional cohorts with stronger associations for POAG with paracentral VF loss. The adverse associations for glycerides were con rmed in a cross-sectional analysis performed in the UK Biobank. While this study was the rst to evaluate the relation between pre-diagnostic plasma metabolites, the replication of our ndings in a prevalent glaucoma dataset supports a role for altered lipid regulation in glaucoma.

A systematic review[5] identi ed 13 studies to date on the metabolomics of open-angle glaucoma. Of these, three evaluated serum[6,7,26], and three evaluated plasma[9–11] while others evaluated aqueous humor[12–15], tear[27], and optic nerve[17] samples. These studies have collectively assessed ~140 different metabolites. Compared to existing studies (where the largest study included 211 cases and 295 controls[7]), our study was unique in that the sample size in NHS/NHSII/HPFS was the largest to date (599 cases and 599 controls), did not use a convenience control sample (e.g., those with cataract or other non-glaucoma eye conditions) and importantly, evaluated pre-diagnostic plasma collected a mean of 10.3 years before POAG diagnosis which is unaffected by

glaucoma with hyperlipidemia as well as hypertriglyceridemia (OR = 1.42; 95%CI 1.04, 1.93; based on pooling of 4 studies). Our study con-

glaucoma outcome de nition described above. In addition, UK Bio-

future analyses given the hypothesis-generating aspect of this study. All statistical tests were two-sided.

Re a

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

[illegible]

Data from the UK Biobank cannot be shared per our Material Transfer Agreement. UK Biobank data requests can be made directly to the UK Biobank via https://www.ukbiobank.ac.uk/enable-your-research/apply-for-access. Data from the health professional cohorts (NHS,

35. S , M. K. a. Pa d c m c a c a a d a d ca a m a m ma -a a c ma. Transl. Vis. Sci. Technol. **10**, 13–13 (2021).
36. A c , L., D , S., Pa , A. & Ma da , A. C m a : a d . d a . Front. Physiol. **3**, 486 (2012).
37. H , K. a. C c da a d m a a c ca . A m ' d a . Nat. Commun. **11**, 5698 (2020).
38. L a , D. A. & D a a a a , V., A m ' D. a N ma , I. S m a d a am a d - a d a am A m ' d. a m c a d dc m m dc m a - m . J. Alzheimer's Dis. **80**, 311–319 (2021).
39. W d, P. L. a. Ta d d m c d a b- m dc m a m (MCI) a d a - A - m ' d. a (LOAD). BBA Clin. **5**, 25–28 (2016).
40. M , P. J. a. P a ma d fi m a a o a d ab a d 2 d ab . PLoS One **8**, 74341 (2013).
41. M a, E. M. & Ha c , G. M. M c d a : m c d a c . J. Bioenerg. Biomembr. **48**, 99–112 (2016).
42. L , P. a. T m c a m a d c d m . Life Sci. **247**, 117443 (2020).
43. L , S. a. A a ma m ab mc a a d m a ao d , ac ca , a d a m . Investig. Ophthalmol. Vis. Sci. **59**, 1025–1032 (2018).
44. H , L. T. Y. a. R a a d c ao d a a c ma, a m d a a a d fib c ac . Biochim. Biophys. Acta Mol. Basis Dis. **1866**, 165560 (2020).
45. H , M. a. A a d c ao d a a a c a a d a c ma b . Investig. Ophthalmol. Vis. Sci. **59**, 693–701 (2018).
46. M b c , S. M. & B a ac a a, S. K. A a d c m a c ma . Investig. Ophthalmol. Vis. Sci. **61**, 60 (2020).
47. Ed a d , G., A b d , K., G a, Y., L , R. K. & B a ac a a, S. K. P fi c a d a c ma ma a m . Biochimie **101**, 232–247 (2014).
48. A b d , K., G a, Y., L , R. K. & B a ac a a, S. K. C m a a fi c a d a c ma ma ab - c a m . Investig. Ophthalmol. Vis. Sci. **54**, 3037–3044 (2013).
49. A a , A. J. a. H ma ab c a m fi a d c amd fi a d a a c m m a m. Investig. Ophthalmol. Vis. Sci. **55**, 3413–3422 (2014).
50. A a , A. J., M ba, G. C., G a, Y., L , R. K. & B a ac a a, S. K. S d a d c amd ma a m . Mol. Vis. **19**, 1966 (2013).
51. C a a , M. Z., Va oa, A.-K., P a , M. C., E -A oa , M. & B a ac a a, S. K. O c d m c a m a d c d a a a c ma. Investig. Ophthalmol. Vis. Sci. **60**, 1789–1798 (2019).
52. Kam , K., Fa , M. & O'B , C. M c d a d c a : c a c ma. Mitochondrion **35**, 44–53 (2017).
53. L , H., M o ca, K. & P c , V. M c d a ma a a d ma -a a c ma. J. Glaucoma **29**, 295–303 (2020).
54. K , G. Y., Va B , N. J., T c , I. A. & C , J. G. M c d a d c a d a c ma. J. Glaucoma **18**, 93–100 (2009).
55. C m , V., R a a, F., T c , I. A. & C , J. G. O da a d m c d a d c a c ma. Curr. Opin. Pharm. **13**, 12–15 (2013).
56. I , A., Ba , A. & Sacca, S. C. T da a c ma. Mutat. Res. **612**, 105–114 (2006).
57. B d'A , C., P a, B., C amba a, F. & D , F. O da- a d a da m a c c a c ma: a - ma c a d m a-a a . PLoS One **11**, 0166915 (2016).
58. W am , P. A. a. V am B3 m d a m c d a - ab a d a c ma a d mc . ScienceTcGroups}ScienceTcGroups

77. K a aa, A. P. a. G . m - d a a . . d . 68 . , o a . oa d . , a c , a h . . a d m h . . h d c , . h , ma . h . -a , a c ma. Nat. Genet. **50**, 778–782 (2018).
78. K m, J. a. I a c , a h . . , a c ma, a d d a ca , c . . m h , . : a . -d , ac , . . d . m UK B. ba . Ophthalmology **128**, 866–876 (2021).
79. K m, J. a. S a , . . , a , . . . a c , a h . . , a c ma, a d . c , a c . . c . m a h a a m . . UK B. ba . Investig. Ophthalmol. Vis. Sci. **63**, 31 (2022).
80. Ha , X. a. A . . o a , ... , h a h . a , a c ma a d a - , a d mac , a d . a , . . : a a a . . . , U , d K. d m B. ba a d Ca a d a , . . , d . a . d . . a , . BMC Med **19**, 104 (2021).
81. Pa . , N.P. a. M ab , c h d c . . . , o d . c . . a a d . a , . . m . . Circulation **137**, 841–853 (2018)
82. O'S , a , J. F. a. D m , a d . a , c ao d . a ma . , a a d h d c . d ab . . J. Clin. Invest **127**, 4394–4402 (2017).