Pyruvate and Related Energetic Metabolites Modulate Resilience Against High Genetic Risk for Glaucoma

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Running Head: Pyruvate as a Modulator of Resilience Against High Genetic Risk for Glaucoma

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Abbreviations and Acronyms:

AUC, area under the curve; BMI, body mass index; CI, confidence interval; HDL, high-density lipoprotein; ICD, International Classification of Diseases; IOP, intraocular pressure; mRNFL, macular retinal nerve fiber layer; MET, metabolic equivalent of task; MRS, metabolic risk score; MTAG, Multi-Trait Analysis of Genome-wide association study data; NEF, number of effective; NMR, nuclear magnetic resonance; OAG, open-angle glaucoma; OR, odds ratio; POAG, primary open-angle glaucoma; PRS, polygenic risk score; RGC, retinal ganglion cell; ROC, receiver operating characteristic; SD, standard deviation; SNP, single nucleotide polymorphism; TCA, Tricarboxylic acid; ATP, Adenosine triphosphate; VCDR, vertical cup-disc ratio; VLDL, very low-density lipoprotein.

Abstract

A glaucoma polygenic risk score (PRS) can effectively identify disease risk, but some individuals with high PRS do not develop glaucoma. Factors contributing to this resilience remain unclear. Using 4,658 glaucoma cases and 113,040 controls in a cross-sectional study of the UK Biobank, we investigated whether plasma metabolites enhanced glaucoma prediction and if a metabolomic signature of resilience in high-genetic-risk individuals existed. Logistic regression models incorporating 168 NMR-based metabolites into PRS-based glaucoma assessments were developed, with multiple comparison corrections applied. While metabolites weakly predicted glaucoma (Area Under the Curve=0.579), they offered marginal prediction improvement in PRS-only-based models (P=0.004). We identified a metabolomic signature associated with resilience in the top glaucoma PRS decile, with elevated glycolysis-related metabolites—lactate (P=8.8E-12), pyruvate (P=1.9E-10), and citrate (P=0.02)—linked to reduced glaucoma prevalence. These metabolites combined significantly modified the PRS-glaucoma relationship ($P_{interaction}$=0.011). Higher total resilience metabolite levels within the highest PRS quartile corresponded to lower glaucoma prevalence (Odds $Ratio_{highest\ vs.\ lowest\ total\ resilience\ metabolite\ quartile}$=0.71, 95% Confidence Interval=0.64–0.80). As pyruvate is a foundational metabolite linking glycolysis to tricarboxylic acid cycle metabolism and ATP generation, we pursued experimental validation for this putative resilience biomarker in a human-relevant Mus musculus glaucoma model. Dietary pyruvate mitigated elevated intraocular pressure (P=0.002) and optic nerve damage (P<0.0003) in *Lmx1b*^{V265D} mice. These findings highlight the protective role of pyruvate-related metabolism against glaucoma and suggest potential avenues for therapeutic intervention.

Introduction

Glaucoma is a polygenic, progressive neurodegenerative disease and a leading cause of irreversible blindness.[1] The disease is typically asymptomatic until advanced visual field loss occurs, and around 50 to 70% of people affected remain undiagnosed.[2,3] Early detection and intervention are essential to stop disease progression and prevent visual impairment in glaucoma-affected individuals, as there is no cure available; however, population-based glaucoma screening is not cost-effective from a public health perspective.[4–6]

Glaucoma is well-suited for developing and applying a polygenic risk score (PRS) to facilitate disease identification and risk stratification as it is a condition with several endophenotypes, such as elevated intraocular pressure (IOP) and thinning of the retinal nerve fiber layer (RNFL), and it is highly heritable.[7–9] Glaucoma risk is influenced by both genetic and metabolic factors, with emerging evidence suggesting that gene-environment interactions may play a greater role in conferring disease risk than independent exposures alone.[10–15]

Glaucoma was five times more likely in the UK Biobank (UKBB) among participants with the highest genetic risk decile versus the lowest decile (7.4% vs 1.3%).[9] While the relatively low glaucoma prevalence in the highest decile group could be explained by disease under-ascertainment or a PRS that incompletely reflects the glaucoma genetic architecture, it is possible that resilience biomarkers could explain this result, in addition to protective genetic or epigenetic factors.

Recent advancements in metabolomics have opened up avenues to explore metabolites as potential biomarkers for glaucoma.[16] Metabolites are intermediate and end products of cellular processes critical for driving cellular growth and tissue homeostasis.[17] These small molecules provide a holistic measure of physiological status, reflecting both genetic predispositions and

environmental influences. Previous studies have indicated a potential role for plasma metabolites to stratify glaucoma risk; however, these studies were limited by small sample sizes,[18–22] with restricted coverage of metabolites. In addition, the potential benefits of the integration of metabolomics with genetics to identify individuals at the highest risk of glaucoma remain unexplored.

This study aims to evaluate plasma metabolites as risk factors for glaucoma. First, we explored whether incorporating plasma metabolite data improved the predictive accuracy of a PRS for glaucoma risk based on available data in the UKBB, where genetic and metabolomic data were available for 117,698 participants. Additionally, we evaluated interactions between glaucoma PRS and metabolomics for further stratifying individuals with high genetic risk but without glaucoma. We undertook an agnostic approach to identify a metabolite signature associated with resilience to high glaucoma genetic risk. Finally, we experimentally validated a top resilience metabolite by assessing its ability to rescue the ocular phenotype in a human-relevant, genetic mouse model of glaucoma.

Results

UK Biobank Study Characteristics

A total of 117,698 participants (4,658 glaucoma cases and 113,040 non-cases) were included in this study. UKBB participants were predominately of European ancestry (85.8%) but were also of admixed American (0.22%), Asian (2.77%), and African (1.73%) ancestry. The design of the human studies is depicted graphically in **Figures 1 and 2**. There were significant differences seen in baseline demographic and clinical characteristics between the glaucoma cases and the non-cases presented in **Table 1**. Notably, characteristics associated with glaucoma included male gender, older age, genetic African ancestry (Privé et al[23] describes the method for

determining genetically-inferred ancestry in the UKBB), prior history of smoking, slightly lower cholesterol levels, higher body mass index (BMI), higher hemoglobin A1c (HbA1c), higher IOP, thinner macular retinal nerve fiber layer (mRNFL) thickness, higher caffeine intake, higher alcohol intake, higher oral steroid use, diabetes, and coronary artery disease.

Plasma metabolites marginally improve glaucoma risk prediction

We first sought to determine whether the addition of metabolites could improve the prediction of glaucoma from basic demographics, clinical variables, and genetic data. We created four logistic regression models, each with increasing predictive variables considered, to evaluate their added utility for predicting glaucoma (see Methods and **Supplemental Table 2** for model construction details). We analyzed two sets of metabolite variables: a comprehensive set of 168 metabolites measured by nuclear magnetic resonance (NMR) spectroscopy included in the UKBB dataset and a limited set of 27 metabolites approved by the European Union (EU) for *in vitro* diagnostic use.

To evaluate the performance of each model, we plotted receiver operating characteristic (ROC) curves and calculated the area under the curve (AUC) metrics. In model 1, which utilized only metabolite data, the highest performance was achieved using the full panel of 168 metabolites, yielding an AUC value of 0.602 (95% CI=0.592-0.612) compared to 0.579 (95% CI=0.569-0.589; P=0.0003) using the 27 metabolites (**Figure 3**). Model 2, which incorporated demographic information, and model 3, which also included clinical variables, exhibited similar performance before the addition of metabolite data. In model 2, the addition of the 168 metabolite panel demonstrated the best performance, with an AUC value of 0.670 (95% CI=0.660-0.680) compared to 0.664 (95% CI=0.654-0.674) for the model without metabolites (P=0.002) and 0.666 (95% CI=0.656-0.676) for the limited panel of 27 metabolites. This

performance trend persisted in model 3 with the inclusion of 168 metabolites, producing an AUC of 0.680 (95% CI=0.660-0.700), representing an increase in AUC (P=0.02) from model 3 with the exclusion of metabolites (AUC 0.670; 95% CI=0.650-0.690). We subsequently examined whether integrating metabolite data into a PRS-based model could improve glaucoma prediction algorithms. In model 4, the panel of 168 metabolites yielded the best performance with an AUC value of 0.806 (95% CI=0.796-0.816), while the model without metabolites (AUC=0.801, 95% CI=0.791-0.811; P=0.004), as well as the model with a limited panel of 27 metabolites (AUC=0.802, 95% CI=0.792-0.812; P=0.006) both had lower AUC values. Thus, these findings suggest that the addition of metabolite data marginally enhances the prediction of glaucoma beyond the use of demographic and genetic data.

We then investigated whether the inclusion of the 168 metabolites could improve the predictive value for glaucoma by stratifying patients into specific subgroups and calculating AUC curves for each stratum. Accordingly, we found that metabolites showed a modestly improved predictive value for glaucoma among people of White ethnicity (P<0.001), those aged 55 and older (P=0.002), and males (P=0.002) (**Table 2**).

Metabolites associated with resilience to a high glaucoma polygenic risk score

As metabolite data only marginally augmented clinical or PRS predictions of glaucoma, we hypothesized that metabolites might provide utility for differentiating patients within risk groups. Specifically, we studied participants who possessed a high glaucoma PRS but did not have glaucoma. For this study, we labeled them resilient while recognizing there are many reasons they may not have glaucoma. Participants were stratified into PRS deciles, and metabolite signatures were identified to differentiate patients with and without glaucoma in the top decile (N=11,770) and the bottom half (N=58,358) of the glaucoma PRS distribution (**Figure**

4). Within the top decile of glaucoma PRS, compared to participants without glaucoma, participants with glaucoma were more likely to be older, male, of White ethnicity, and were prior smokers. Glaucoma participants with the highest PRS also had higher BMI, higher HbA1c, higher spherical equivalent, consumed more caffeine and alcohol, and were more likely to have diabetes and coronary artery disease (**Table 3**). Among participants in the bottom half of glaucoma PRS, participants with glaucoma were also more likely to be older, male, of Black and Asian ethnicity, prior smokers, had higher BMI and higher HbA1c, and were more likely to have diabetes and coronary artery disease (**Table 4)**. As expected, participants with glaucoma in both bins of glaucoma genetic risk had higher IOP and thinner mRNFL thickness.

Our multivariable-adjusted analysis revealed that higher probit-transformed levels of lactate (adjusted P_{NEF}=8.8E-12), pyruvate (adjusted P_{NEF}=2.9E-10), and citrate (adjusted P_{NEF}=0.018) were independently associated with no glaucoma in the top decile of glaucoma PRS (**Table 5**). In addition, lower levels of triglycerides and higher levels of selected HDL analytes had an adjusted $P_{NEF} < 0.2$ and were associated with no glaucoma among participants with high genetic risk. Among the bottom half of the PRS distribution, higher levels of albumin were associated with no glaucoma (adjusted P_{NEF}=0.047), while higher levels of small HDL, omega-3 fatty acids, docosahexaenoic acid, lactate, and citrate were associated with no glaucoma, albeit with an adjusted $P_{NEF} < 0.2$.

Interaction of metabolic and genetic biomarkers

The three metabolites (lactate, pyruvate, and citrate) for which higher levels were associated with reduced glaucoma prevalence in individuals with high genetic susceptibility had no statistically significant relationship with glaucoma for participants in the lower half of the PRS distribution at the adjusted $P_{NEF} < 0.05$ level. Thus, we hypothesized that there may be an

interaction between these metabolites and genetic risk. Such an interaction would suggest that these glycolysis/tricarboxylic acid (TCA) cycle metabolites are relevant primarily in the setting of high PRS, establishing them as resilience factors against elevated genetic risk of glaucoma. Indeed, we observed a significant interaction between elevated levels of total lactate, pyruvate, and citrate with glaucoma PRS quartile for predicting the risk of glaucoma ($P_{interaction}$=0.011; **Figure 5A**).

To confirm and visualize this interaction, we calculated and plotted the glaucoma odds ratio (OR) as a function of PRS quartile with each genetic predisposition bin stratified by the sum of the resilience-metabolite probit score quartiles (**Figures 5A and 5B**). Notably, high levels of resilience-associated metabolites were significantly associated with lower odds of glaucoma, particularly within the higher PRS quartiles (Q3 and Q4). For example, for individuals in PRS Q3 with total resilience-associated metabolite sum Q3, the glaucoma OR was 0.78 (95% CI=0.65-0.93; P=0.0059), using metabolite sum Q1 as the reference. In the same PRS Q3 with a higher quartile of total resilience-associated metabolite sum (Q4), the OR was 0.74 (95% CI=0.62-0.88; P=0.0009). The lowest OR of glaucoma was observed in participants with both the highest PRS (Q4) and highest resilience-associated metabolite sum (Q4), with an OR of 0.71 (95% CI=0.64-0.80; P<0.001). Although both age and PRS were significant predictors of glaucoma prevalence (both are strongly associated with increased risk), there was no evidence of a significant three-way interaction between the resilience-associated metabolite sum, PRS, and age ($P_{3\text{-}way\text{-}interaction}$=0.65).

Next, we investigated whether a holistic metabolic risk score (MRS) incorporating resilience metabolites and other measured metabolites (full panel of 168 metabolites; see Methods and **Supplemental Table 3** for the beta coefficients used in MRS construction) could

be used in conjunction with a glaucoma PRS to improve risk prediction. To quantify the degree of interaction of glaucoma PRS and MRS, we plotted glaucoma OR at various quartiles of glaucoma PRS and MRS compared to the first MRS quartile within each PRS quartile. We found a significant interaction between glaucoma MRS built from 168 metabolites and PRS ($P_{interaction}$=0.0012; see **Supplemental Figure 1A)**.

In stratified analyses by quartile of PRS, compared to the first quartile of MRS, higher MRS was associated with increased glaucoma prevalence across most PRS quartiles, with the most pronounced effect observed in individuals in the highest PRS and MRS quartile with an OR of 2.14 compared to those in the highest PRS and lowest MRS quartile (**Supplemental Figure 1B**). Among individuals in the highest MRS quartile (Q4), PRS Q1 had an OR of 1.66 (95% CI=1.15-2.42; P=0.0086), PRS Q2 had an OR of 2.08 (95% CI=1.62-2.67; P<0.001), PRS Q3 had an OR of 2.20 (95% CI=1.83-2.65; P<0.001), and PRS Q4 had an OR of 2.14 (95% CI=1.91-2.40; P<0.001). This suggests potential synergistic effects of genetic and metabolite risk factors on glaucoma risk that transcend the impacts of the resilience metabolites.

Most importantly, using individuals in the lowest quartiles of PRS and MRS (PRS Q1, MRS Q1) as the reference group for the entire population (n=117,698), we determined glaucoma OR as a function of PRS quartile, with further stratification by holistic MRS in each PRS bin (**Figure 6**). The OR for PRS Q1 with MRS Q4 relative to PRS Q1 with MRS Q1 was modest (OR=1.66; 95% CI= 1.15–2.42; P=0.0086). However, those with PRS Q4 (highest genetic risk) and MRS Q1 (lowest metabolic risk) had an OR of 11.7 (95% CI=8.72-16.0; P<0.001) compared to PRS Q1 MRS Q1. This illustrates the importance of genetics in impacting glaucoma risk. Strikingly, the glaucoma OR further increased to 25.1 (95% CI=18.8-34.1; P<0.001) for those with PRS Q4, MRS Q4 (combination of highest genetic and highest metabolic risk;

$P_{interaction}$=0.019). Altogether, this demonstrates the strong prognostic utility of combining both PRS and MRS measurements for assessing glaucoma risk, particularly in individuals with a high genetic predisposition.

Pyruvate supplementation lessens intraocular pressure and glaucoma

To functionally test the association between higher levels of pyruvate and resilience to glaucoma, we experimentally tested whether treatment with pyruvate induces resilience to IOP elevation and glaucoma in a human-relevant mouse model. Common variants in *LMX1B* are associated with IOP variation and the most common form of human glaucoma, primary open-angle glaucoma (POAG).[24–29] Rare Mendelian variants in *LMX1B* can produce early-onset ocular hypertension and open-angle glaucoma (OAG).[30] We have previously demonstrated that mice with a dominant mutation in *Lmx1b* (*Lmx1b*$^{V265D/+}$) develop IOP elevation and glaucoma.[30,31] Depending on the genetic background, this *Lmx1b*^{V265D} mutation induces either early-onset or later glaucoma[31]. Mutant mice with a C57BL/6J strain background develop severe, early-onset IOP elevation and glaucoma. As pyruvate and its metabolites were associated with no glaucoma despite strong genetic predisposition in the UKBB cohort (highest decile of PRS), we tested the ability of dietary pyruvate to induce resilience against the *Lmx1b*^{V265D}-induced glaucoma on this C57BL/6J genetic background. Pyruvate supplementation through drinking water substantially protected mice from IOP elevation and glaucoma. Pyruvate significantly protected against both anterior chamber deepening (ACD), a consequence of IOP elevation in mouse eyes (**Figure 7A-B**), and IOP elevation itself (**Figure 7C**). Importantly, pyruvate treatment protected against glaucomatous optic nerve degeneration (**Figure 7D**). Together, our findings strongly support the role of endogenous pyruvate in conferring resilience against glaucoma even countering strong

genetic predisposition. Further, they show that pyruvate can act as a potent resilience factor against IOP elevation and glaucoma when delivered orally.

Discussion

In this large cohort study, we found that the inclusion of metabolite data from an NMR platform only marginally improved various glaucoma prediction algorithms **(Figure 3)**. Nonetheless, using an agnostic approach, we found that lactate, pyruvate, and citrate levels collectively were associated with a 29% reduced risk of glaucoma among those in the top quartile of glaucoma genetic predisposition **(Figure 5B)**. We showed that pyruvate supplementation reduced glaucoma incidence in a human-relevant genetic mouse model (**Figure 7**). Furthermore, the interaction between glaucoma PRS and an MRS based on 168 metabolites provided synergistic predictive information regarding glaucoma risk (**Figure 6**). The statistical interaction we demonstrate between metabolite scores and PRS-derived glaucoma risk helps identify individuals who, despite high genetic risk, are less likely to develop glaucoma due to favorable metabolic profiles or, on the contrary, are more likely to develop glaucoma and should be monitored at an earlier age. Overall, these findings indicate that an MRS may provide clinically useful measures of metabolic status and improve patient risk stratification in glaucoma.

While earlier studies[32–34] showed modest discriminatory powers for a glaucoma PRS, recent larger genome-wide association studies reported significantly improved risk prediction.[7,35–37] However, no studies have examined the utility of incorporating metabolite data into glaucoma prediction algorithms combined with a PRS. Future work is needed to understand how non-genetic factors, known to alter glaucoma risk or IOP levels, like air pollution, psychological stress, physical activity, and dietary factors,[14] could change the MRS and alter high glaucoma genetic predisposition.

Previous studies have explored metabolomics to identify biomarkers for OAG. A systematic review analyzing 13 studies identified a total of 144 metabolites, of which 12 were associated with OAG.[22] Among studies using plasma samples, four metabolic pathways were significantly enriched: sphingolipid metabolism, arginine and proline metabolism, and beta-alanine metabolism. In another study across three US cohorts and the UKBB, higher levels of plasma diglycerides and triglycerides were adversely associated with glaucoma.[38] This is consistent with our observation that lower triglyceride levels were associated with glaucoma resilience, albeit with less significance compared to the three glycolysis-related metabolites. The biological mechanism is suggested to be the association of hypertriglyceridemia with increased blood viscosity and elevated IOP.[39–42] Consistent with this, a meta-analysis reported that individuals with glaucoma had a mean triglyceride level 14.2 mg/dl higher than those without the disease.[41]

While studies have identified biomarkers associated with glaucoma, to the best of our knowledge, there has been no investigation on biomarkers that are associated with being resilient to a high glaucoma genetic predisposition. We identified elevated levels of lactate, pyruvate, and citrate among individuals without glaucoma despite high genetic risk. The retina commonly utilizes energy produced from glycolysis and oxidative phosphorylation. Therefore, glycolysis end products (pyruvate and lactate) are important energy sources for retinal ganglion cells (RGCs).[43] RGCs express monocarboxylate transporters, which facilitate the uptake of extracellular pyruvate and lactate, improving energy homeostasis, neuronal metabolism, and survival.[44,45] Lactate is critical for RGC survival during periods of glucose deprivation,[46,47] and bioenergetic insufficiency contributes to RGC loss in glaucoma.[48–51] Finally, a study found that

baseline lactate levels were significantly decreased in patients with normal-tension glaucoma compared to non-glaucomatous controls.[52]

Pyruvate plays a central role in energy metabolism by linking glycolysis to the TCA cycle, ultimately driving ATP production by the electron transport chain. Pyruvate is converted into acetyl-CoA, which fuels the TCA cycle, and into lactate, a key molecule that regulates metabolism and serves as an important energy source.[53] The TCA cycle is tightly regulated to balance biosynthesis (producing metabolic precursors for lipids, carbohydrates, amino acids, nucleic acids, and co-factors) and energy production, adjusting dynamically to cellular needs.[54] Beyond its metabolic functions, pyruvate also acts as an antioxidant, scavenging reactive oxygen species (ROS), induceing the expression of the antioxidant response control gene *Nrf2*, and promoteing beneficial autophagy.[55–57] Its neuroprotective effects on RGCs have been demonstrated both in cell culture and in an induced model of glaucoma in rats.[58] Additionally, pyruvate combined with nicotinamide has been shown to enhance optic nerve protection in mice.[59] Importantly, the current study extends this evidence by demonstrating that pyruvate alone provides strong protection against glaucoma in a mouse model carrying a mutation in the ortholog of a human POAG gene. Further, it extends pyruvate's protective effects to the ocular drainage tissues, mitigating IOP elevation. Notably, although our validation data demonstrates the neuroprotective effects of exogenous pyruvate, it remains possible that endogenously produced pyruvate within ocular tissues may also contribute to RGC protection. A randomized Phase II clinical trial that tested pyruvate and nicotinamide in POAG patients found improved visual function in glaucoma patients compared to the placebo group.[60] Together, both preclinical and clinical evidence support the role of pyruvate as a protective factor against glaucoma.

Citrate is a key intermediate in the TCA cycle and is important for aerobic energy production. Elevated citrate levels might reflect enhanced energy supply and antioxidative capacity of ocular tissues, potentially protecting RGCs against oxidative stress.[50,61–64] Decreased levels of citrate may indicate increased usage or mitochondrial dysfunction.[65] Studies have found lower plasma citrate levels in glaucoma patients among adults and children.[66,67] A study measuring plasma citrate with a cutoff of 110 μmol/L to detect glaucoma had a sensitivity of 66.7% and a specificity of 71.4%.[66] These findings corroborate the protective effects of citrate in conferring resilience to glaucoma. Similar to pyruvate, plasma citrate may also play a role in retinal homeostasis, highlighting the need to disentangle serum-derived from intraocularly synthesized metabolites.

We also identified several lipoproteins associated with resilience, most notably cholesteryl esters in small high-density lipoprotein (HDL) and medium HDL. Although these lipoproteins did not reach an adjusted P-value<0.05 and thus remain suggestive biomarkers rather than definitive, this is consistent with studies describing the neuroprotective effect of HDL in the glaucoma pathophysiology.[68,69] Cholesteryl esters and triglycerides make up the core of lipoproteins, which transport lipids in the blood. Lipoproteins are characterized by their size, where HDL ranges from 8-12 nm in diameter, and low-density lipoprotein (LDL) ranges from 18-25 nm in diameter. The retinal pigment epithelium can take up lipoproteins,[70,71] and dysregulation of lipid metabolism can result in the accumulation of lipids and oxidative stress in the retina, resulting in RNFL thinning.[38,72–75] Thus, HDL has antioxidant properties to protect RGCs by facilitating cholesterol efflux from the retina.[76,77] Furthermore, as HDL analytes can cross blood-ocular barriers,[78] there is a plausible route for serum-derived HDL to influence RGC homeostasis. Nonetheless, the relative contributions of circulating lipoproteins versus local

synthesis within ocular tissues remain unclear and warrant further investigation. Notably, genes implicated by large cross-ancestry and European subset GWAS meta-analyses of POAG risk are enriched in processes related to apolipoprotein binding and lipid transport.[26,78] Incorporating ocular physiology and blood-retinal barrier considerations when interpreting lipoproteins as potential resilience biomarkers will be critical for future studies aimed at understanding and therapeutically targeting increased glaucoma risk.

This study had several strengths. First, the UKBB is a large cohort study with detailed covariate information on demographics, clinical characteristics, and glaucoma endophenotypes, allowing for better glaucoma risk profiling. Second, we used a PRS calculated from the large (~600k individuals) multi-trait analysis of genome-wide association studies on glaucoma and endophenotypes, including optic nerve head structural features and IOP data.[7] Finally, we supported our biobank analyses with functional tests in a disease-relevant mouse model, identifying a protective effect of pyruvate supplementation in animals susceptible to glaucoma. The data showing that pyruvate protected against IOP elevation (a key causative risk factor for glaucoma) and glaucomatous nerve damage in a mouse model, as well as pyruvate's biochemical relatedness to lactate and citrate regarding energy metabolism, mitigate against concerns about false discovery, uncontrolled confounding, reverse causation, and collinearity among biomarkers.

A major limitation of our study is that it includes a predominately European population, with an underrepresentation of African, Asian, and mixed American genetic ancestral groups; nonetheless, we chose to analyze Europeans and non-Europeans together to make the results as generalizable as possible. A second limitation is that glaucoma ascertainment in the UK Biobank is based on self-reported diagnoses and hospital records rather than comprehensive

ophthalmologic examinations. Nonetheless, it is reassuring that the prevalence of glaucoma in our sample (~4%) is similar to a directly performed disease burden estimate in a comparable, albeit slightly older, United Kingdom sample (2.7%).[79] In addition, a subset of UKBB participants have received glaucoma treatment, which could alter metabolite levels and could lead to an underestimate of the protective effects of metabolites. Moreover, the UKBB study includes a limited set of 168 metabolites, which is not inclusive of the comprehensive set of metabolites, known and unknown. This study also only focuses on plasma metabolites and does not account for ocular metabolite profiles. Due to the cross-sectional design of our study, establishing temporality is challenging. As a result, it remains unclear whether the observed metabolic changes contribute to the onset of glaucoma or arise as a consequence of the disease. However, the mouse data support a causal role for pyruvate as a factor that mitigates high genetic risk. This study's exploratory approach– investigating metabolite associations in an agnostic, hypothesis-free manner– underscores the need for replication. Regarding the mouse studies, we performed ad-lib pyruvate dosing and examined only a single human-related mutation, whereas individuals with a genetic predisposition to glaucoma typically carry a high cumulative burden of multiple common glaucoma-associated variants. Lastly, neither the mouse studies, nor the human data accurately inform the exact dosing of TCA cycle biomarkers needed to mitigate glaucoma risk. We find considerable overlap between TCA cycle biomarkers between those with and without glaucoma in the top decile of glaucoma risk (**Supplementary Table 1** and **Supplementary Figure 2**).

Overall, we have shown that plasma metabolites marginally enhance the predictive power of a glaucoma PRS and may have a role in identifying those who may be resilient to glaucoma despite high genetic predisposition. Our study identified protective metabolites linked to

glycolysis and mitochondrial function, suggesting important pathophysiology in glaucoma. One of these metabolites of significance is pyruvate. Oral pyruvate supplementation substantially protected against IOP elevation and conferred resilience against glaucoma. Thus, these results open new avenues for therapeutic strategies of pyruvate and its metabolites as resilience factors against glaucoma in genetically predisposed individuals.

Methods

Study Design

Our initial human analysis was conducted in three steps. First, we assessed whether plasma metabolite data alone can predict glaucoma risk with receiver operating characteristic (ROC) curves in the UK Biobank. We then integrated the metabolite data into a polygenic risk score (PRS)-based glaucoma risk assessment model to see if metabolites could enhance glaucoma prediction. Second, we focused on identifying a metabolomic signature of resilience to high glaucoma PRS by comparing plasma metabolites in glaucoma cases versus participants without glaucoma (henceforth referred to as resilient participants) from the top 10% of the glaucoma PRS. Third, we explored the interactions between metabolic scores and PRS in modifying glaucoma risk.

Study population and data collection

The UKBB is a prospective cohort study of over half a million participants aged 37 to 73 years at recruitment across the United Kingdom from 2006 to 2010. Participants were recruited through the National Health Service registers from 22 assessment centers, where they signed electronic informed consent to participate. Participants then completed in-depth touchscreen questionnaires and trained staff-led interviews, performed body measurements, and provided biological samples.[80] Additional outcomes are available from data linkage to hospital episode

statistics, the death register, and primary data.[80–82] Biological samples collected from these participants, including blood, urine, and saliva specimens, were used to generate genetic, metabolomic, and proteomic data.[83] Details of the UKBB study design and population are described online (https://www.ukbiobank.ac.uk). The UKBB study received approval from the National Health Service North West Multicentre Research Ethics Committee (reference number 06/MRE08/65) and the National Information Governance Board for Health and Social Care. This research, conducted under UK Biobank application number 36741, adhered to the tenets of the Declaration of Helsinki.

The UKBB included 502,613 participants, of which 173,679 participants had self-reported glaucoma data, ICD-coded, or previous glaucoma laser/surgical therapy (34.6%) at baseline. A subset consisting of 117,698 participants with metabolomic data and genetic profiling comprise the study population (**Figure 1)**. The glaucoma cases were defined based on those with self-reported glaucoma, in which participants selected “glaucoma” when they completed a touchscreen questionnaire with the question, "Has a doctor told you that you have any of the following problems with your eyes?". Glaucoma cases also included participants who reported a history of glaucoma surgery or laser therapy on the questionnaire or if an International Classification of Diseases (ICD) code for glaucoma (ICD 9th revision: 365.* [excluding 365.0]; ICD 10th revision: H40.* [excluding H40.0] and H42.*) was carried in linked Hospital Episode Statistics before the baseline assessment. The described approach to identifying glaucoma has been strongly supported by prior research publications.[27,28]

Metabolite Profiling

In the UKBB, a high-throughput nuclear magnetic resonance (NMR)-based biomarker platform (Nightingale Health Ltd; Helsinki, Finland) was used to measure the metabolomic

profile in the randomly selected non-fasting EDTA plasma samples from a subset of participants.[84] In contrast to liquid chromatography/mass spectroscopy, NMR spectroscopy produces distinctive spectral shapes for molecules containing hydrogen atoms.[85] The areas under the curve (AUC) are proportional to the concentration of each molecule based on chemical shifts and J coupling split patterns derived from quantum mechanics.[86,87] The NMR platform contains data on 249 metabolic biomarkers (168 absolute levels and 81 ratio measures), including a subset of 36 biomarkers (27 absolute levels and 9 ratio measures) certified for broad diagnostic use by the European Union (EU).[84] In this study, we focused on the 168-metabolite and the 27-metabolite sets for analysis (for a complete list of quantified metabolites, refer to the Supplementary File). Individual metabolite values were transformed to probit scores to standardize their range and reduce the impact of skewed distributions.

Construction of PRS for glaucoma

The glaucoma PRS used in this study was from a multi-trait analysis of GWAS (MTAG) on glaucoma.[7] This PRS was derived based on GWAS data from glaucoma (7,947 cases and 119,318 controls) and its endophenotypes: optic nerve head structure using vertical cup-disc ratio (VCDR) (including additional data from 67,040 UKBB and 23,899 International Glaucoma Genetics Consortium (IGGC) participants), and intraocular pressure (IOP, including additional data on 103,914 UKBB and 29,578 IGGC participants). The glaucoma PRS was constructed using single nucleotide polymorphisms (SNPs) with MTAG P-values ≤ 0.001, resulting in 2,673 uncorrelated SNPs after LD-clumping at $r^2 = 0.1$, and was found to have a predictive ability with an area under the curve (AUC) of 0.68 (95% confidence interval, CI, [0.67-0.70]) and 0.80 accounting for age, sex, family history, and PRS tested in the Australian and New Zealand Registry of Advanced Glaucoma cohort.

Model building/covariates

We first assessed whether the inclusion of probit-transformed metabolite data enhances glaucoma prediction algorithms. We adjusted for factors involving major determinants of variability in metabolites, POAG-established and suspected risk factors, and other comorbidities. In model 1, we considered metabolites only. Model 2 incorporated additional demographic covariates, including age (years), sex, genetic ancestry, season, time of day of specimen collection (morning, afternoon, night), and fasting time (hours). Model 3 incorporated covariates in model 2 and additional variables accounting for comorbidities potentially related to glaucoma including smoking status (never, past, and current smoker), alcohol intake (g/week),[10] caffeine intake (mg/day),[88] physical activity (metabolic equivalent of task [MET], hours/week), body mass index (kg/m^2), average systolic blood pressure (mm Hg), history of diabetes, hemoglobin A1C (HbA1c, mmol/mol), self-reported history of coronary artery disease, systemic beta-blocker use, oral steroid use, and mean spherical equivalent refractive error (diopters) across both eyes. Model 4 incorporated covariates in model 3 and a glaucoma PRS. We compared these models with and without the metabolites to determine the predictive ability of the metabolites above and beyond a glaucoma PRS and known risk factors. Missing values were imputed with medians for numeric variables and modes for factor variables.

Statistical analysis

Metabolite-Based Predictive Modeling

Multivariable logistic regression models with regularization (implemented with *glmnet*) were built to investigate the associations of metabolic biomarkers and the risk of glaucoma. We used four sequential models, adjusting for covariates as described above. For each model, we examined three groupings: no metabolites, the 27 metabolites corresponding to EU-stamped

validated markers, and 168 metabolites corresponding to all the measured plasma metabolites captured in the UKBB. We used regularization to address collinearity, which reduced the set of metabolites and other covariates considered in each model (reported in **Supplementary Table 2**). To examine model accuracy for predicting glaucoma prevalence, we utilized ROC curves and associated AUC measurements as a metric for model performance. We performed five-fold cross-validation and split data into 80% used for training and 20% used for evaluation. All metabolites' values were probit transformed and used as continuous variables (per 1 standard deviation (SD) increase). Additionally, we stratified glaucoma by age, ethnicity, and sex. The DeLong test was utilized to examine the statistical significance of AUC differences between models, with a threshold of $P<0.05$ as statistically significant.

Identifying resilience metabolites to high glaucoma polygenic risk scores

To identify individual metabolites associated with resilience to a high glaucoma PRS, we first performed a logistic regression model to obtain metabolite residuals from probit-transformed concentration data adjusting for the following variables: age, age-squared, time since the last meal/drink (≤4, 5–8, and 9+ hours), sex, ethnicity (Asian, Black, White, and mixed/other), season, time of day of specimen collection, smoking status (never, past, and current smoker), alcohol intake, caffeine intake, physical activity (metabolic equivalent of task [MET] hours/week), body mass index (kg/m^2), average systolic blood pressure (mm Hg), history of diabetes, HbA1c (mmol/mol), history of coronary artery disease, systemic beta-blocker use, oral steroid use, and spherical equivalent refractive error (diopters). Metabolites associated with glaucoma were nominated by performing a t-test comparing residuals in participants with and without glaucoma to identify metabolites significantly associated with glaucoma in both the top 10% and the bottom 50% of glaucoma PRS score. This stratification balanced case counts to

enable comparison of groups with adequate sample size (780 glaucoma cases in the bottom 50% of PRS score and 1,693 glaucoma cases in the top 10% of PRS score). To account for covariance in metabolite abundance, we corrected for multiple comparisons using the number of effective tests (NEF) method.[89] First, a correlation matrix was generated using pairwise complete observations to address any missing values. Eigenvalue decomposition was performed on the correlation matrix to capture the principal components. Principal components contributing more than 1% of the total variance were calculated, yielding 9 significant components, capturing 91.6% of the variance. Thus, we applied a Bonferroni adjustment based on the number of significant components (n=9) to calculate the adjusted p-values accounting for multiple hypotheses. P-values are considered statistically significant if the NEF-adjusted P-value was <0.05.[89] Given this was an exploratory analysis, NEF-adjust P-value <0.2 were considered worthy of additional analysis.

Construction of Metabolite Risk Scores

We constructed metabolite risk scores (MRS), which were calculated as a weighted sum of each metabolic biomarker from coefficients in the 168-metabolite base model. Specifically, we utilized a logistic regression model (implemented with a *binomial glm*) using probit transformed metabolite values to predict the prevalence of glaucoma using five-fold cross-validation. The resulting beta values were extracted and are reported in **Supplementary Table 3**. To determine if there is an interaction between glaucoma MRS and PRS, we utilized extracted model coefficients and corresponding significance from a binomial generalized linear model. We fit this model using the formula *Glaucoma ~ PRS * MRS + PRS + MRS + Covariates.* We classified participants into 16 groups by quartiles of MRS and PRS. Within each PRS quartile, we used MRS quartile 1 as the reference for comparison. Additionally, we used the group in both

PRS quartile 1 and MRS quartile 1 as the overall reference group for all comparisons among the groups. A chi-squared test was used to assess statistical significance between odds ratios (ORs) with 95% confidence intervals (CIs).

To investigate whether age modifies the associations of resilience metabolites with glaucoma, we conducted a stratified analysis and assessed a three-way interaction term of age (≥ or <58 years old, based on median age), the sum of resilience metabolite levels, and PRS using a Wald test for individuals in the top 10% PRS. All statistical analyses and plots were produced using R version 4.2.1 (R Foundation for Statistical Computing, Vienna, Austria). All statistical tests were two-sided.

Animal husbandry and ethics statement

Experimental mice had a C57BL/6J (Jackson Laboratory Stock #000664) genetic background. The *Lmx1b*^{V265D} (alias *Lmx1b*^{Icst}) mutation is previously reported to cause high IOP and glaucoma in mice, with IOP becoming elevated in some eyes during the first months after birth.[30,31,90] The mutation was backcrossed to the C57BL/6J strain for at least 30 generations. All mice were treated per the Association for Research in Vision and Ophthalmology's statement on the use of animals in ophthalmic research. The Institutional Animal Care and Use Committee of Columbia University approved all experimental protocols performed. Mice were maintained on PicoLab Rodent Diet 20 (5053, 4.5% fat) and provided with reverse osmosis-filtered water. Mutant and control littermates were housed together in cages containing ¼-inch corn cob bedding, covered with polyester filters. The animal facility was maintained at a constant temperature of 22°C with a 14-hour light and 10-hour dark cycle.

Genotyping mice

Lmx1b^{V265D} and *Lmx1b*$^{+}$ genotypes were determined by direct Sanger sequencing of a specific PCR product. Genomic DNA was PCR amplified with forward primer 5′-CTTTGAGCCATCGGAGCTG-3′ and reverse primer 5′-ATCTCCGACCGCTTCCTGAT-3′ using the following program: (1) 94 °C for 3 min; (2) 94 °C for 30 s; (3) 57 °C for 30 s; (4) 72 °C for 1 min; (5) repeat steps 2–4 35 times; and (6) 72 °C for 5 min. PCR products were purified and sequenced by the Genewiz (Azenta Life Sciences).

Slit-lamp examination

Anterior segments were examined approximately every 2 weeks between 1 month and 3 months of age and examined at monthly intervals between 3 months of age and harvest age (6 months). Balanced groups of males and females were examined as previously published.[30,31] Photographs were taken with a 40× objective lens. Anterior chamber deepening, a sign of exposure to high IOP, was graded based on a semiquantitative scale of no deepening (not present), mild, moderate, or severe, as previously published.[31] Under examination by either a slit-lamp or dissection scope (during IOP procedure), anterior chamber deepening was only detected in *Lmx1b* mutant groups. Groups were compared statistically by Fisher's exact test. n > 40 eyes were examined in each group.

Intraocular pressure measurement

IOP was measured with the microneedle method as previously described in detail.[91,92] Before cannulation, mice were acclimatized to the procedure room and anesthetized via an intraperitoneal injection of a mixture of ketamine (99 mg/kg; Ketlar, Parke-Davis, Paramus, NJ, USA) and xylazine (9 mg/kg; Rompun, Phoenix Pharmaceutical, St Joseph, MO, USA) immediately prior to IOP assessment, a procedure that does not alter IOP in the experimental

window.[92] IOP was measured at both 5-6 weeks and 8-10 weeks of age in WT and *Lmx1b*$^{V265D/+}$ eyes. Balanced groups of males and females were examined. During each IOP measurement period, the eyes of independent WT B6 mice were assessed in parallel with experimental mice as a methodological control to ensure proper calibration and equipment function. *Lmx1b* mutant groups were compared by ANOVA followed by Tukey's honestly significant difference. n > 30 eyes were examined in each *Lmx1b*^{V265D} mutant group and n > 20 eyes were examined in WT groups.

Pyruvate administration

Ethyl pyruvate (Sigma-Aldrich, St. Louis, Missouri) was dissolved in the standard institutional drinking water to a dose of 2000 mg/kg/day for adult mice based on the average volume mice consume. The mothers consumed this dose and delivered it to their suckling pups at an unknown dose throughout the first 3 to 4 weeks of life. At 4 weeks of age, the mice were weaned into separate cages. From 4 weeks on, the young mice consumed a dose of approximately 2000 mg/kg/day based on their water consumption and body weight over weekly intervals. Untreated groups received the same drinking water without pyruvate. The water was changed once per week. Treatment was started on postnatal day 2. Births were checked daily between 9 am and 12 pm to determine the pups' age.

Optic nerve assessment

We analyzed *Lmx1b*$^{V265D/+}$ and WT controls optic nerve for glaucomatous damage at 6 months of age (5.3-5.9 month range, majority are 5.8-5.9 months, sex balanced). Intracranial portions of optic nerves were dissected, processed, and analyzed as previously described.[48,93,94] Briefly, optic nerve cross-sections were stained with para-phenylenediamine (PPD) and examined for glaucomatous damage. PPD stains all myelin sheaths, but differentially darkly

stains the myelin sheaths and the axoplasm of damaged axons. This allows for the sensitive detection and quantification of axon damage and loss. Optic nerves were prepared for analysis with a 48-h fixation in 0.8% paraformaldehyde and 1.2% glutaraldehyde in 0.08 M phosphate buffer (pH 7.4) at 4 °C followed by overnight post-fix in osmium tetroxide at 4 °C. Nerves were washed twice for 10 min in 0.1 M phosphate buffer, once in 0.1 M sodium-acetate buffer, and dehydrated in graded ethanol concentrations. Tissues were then embedded in Embed 812 resin (Electron Microscopy Sciences, Fort Washington, PA, USA), and 1 μm-thick sections were stained in 1% PPD for ∼40 minutes. Stained sections were compared using a damage scale that is validated against axon counting.[93,95] Multiple sections of each nerve were considered when determining damage level. Nerves were determined to have one of 4 damage levels: 1) No glaucoma (NOE) – less than 5% axons damaged. This level of damage is seen in age- and sex-matched non-glaucomatous mice and is not due to glaucoma. We named this level no or early stage as some have early molecular changes when assessed with transcriptomics, but they cannot be distinguished from control by morphology;[50,96] 2) Moderate damage (MOD) – average of 30% axon loss; 3) Severe (SEV) – greater than 50% axonal loss and extensive axon damage; and 4) Very severe (V. SEV) – glial scar over the vast majority of nerve with few remaining axons.

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UK Biobank Eye and Vision Consortium

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acquisition, Conceptualization. All authors were involved in the interpretation of the results, and the critical review and approval of the manuscript.

Data Availability

Data from the UK Biobank cannot be shared due to our Material Transfer Agreement. To request access to the UK Biobank data, please make requests directly to the UK Biobank via https://www.ukbiobank.ac.uk/enable-your-research/apply-for-access.

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Figures

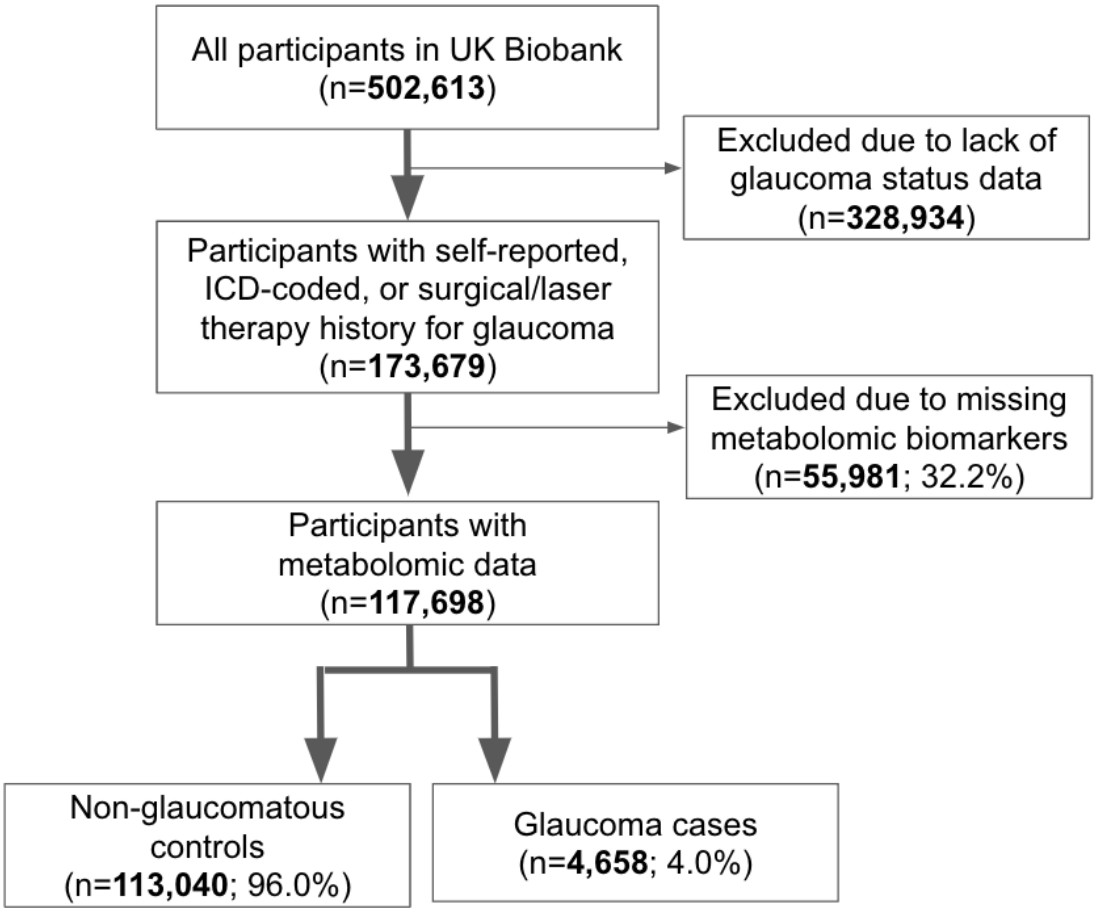


Figure 1. Participant flow chart describing inclusion and exclusion criteria from the UK Biobank

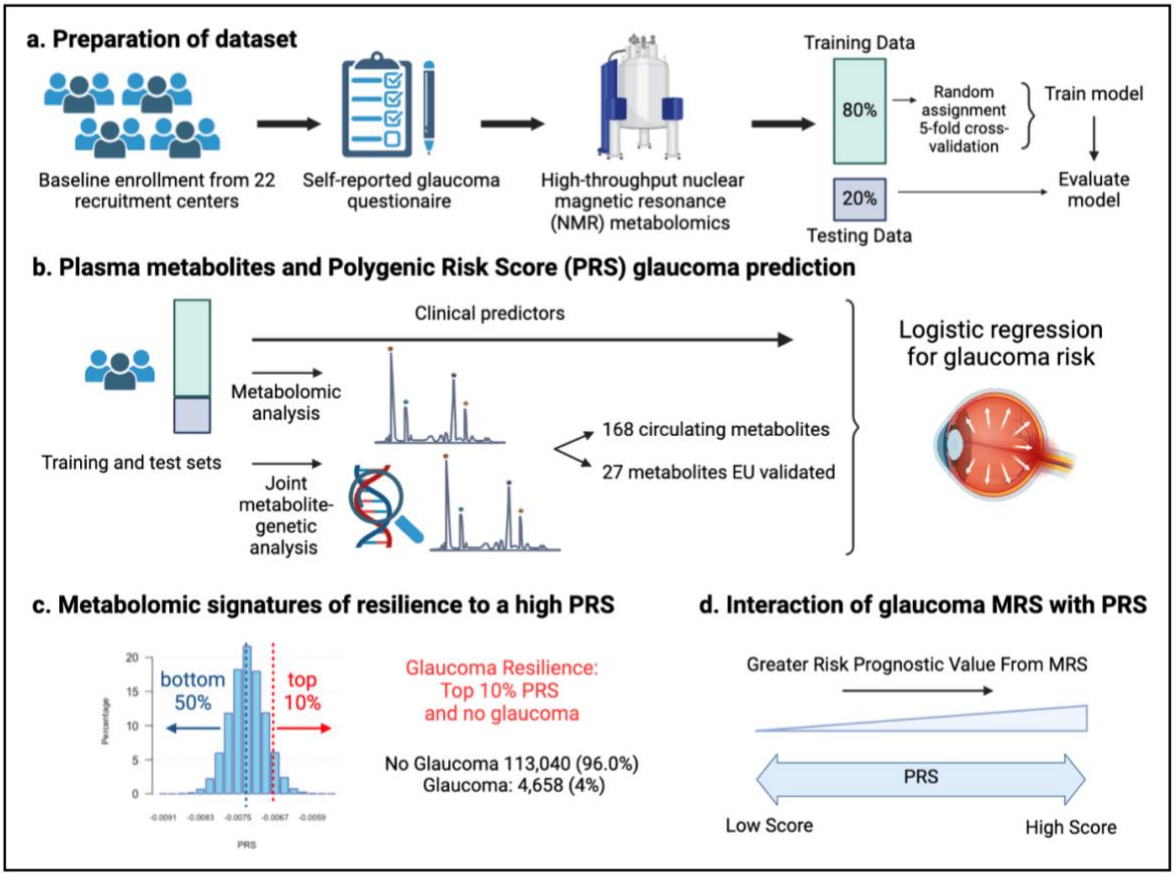


Figure 2. Study design from the UK Biobank. (a) 117,698 individuals had metabolomics data available from the UK Biobank, which was divided into a training and test set to formulate a metabolic risk score (MRS) model. (b) The inclusion of metabolites (either 168 metabolites on the NMR platform or a subset of 27 metabolites with European Union (EU) certification) in relation to prevalent glaucoma risk prediction was studied. (c) A histogram showing the polygenic risk score (PRS) distribution is shown. Overall, 4,658 cases and 113,040 individuals without glaucoma are available for analysis. The metabolomic signature of resilience to the top 10% of glaucoma PRS was assessed among 1,693 cases (14.4%) and 10,077 individuals without glaucoma (85.6%). (d) Interactions of prevalent glaucoma with MRS and PRS quartiles were examined.

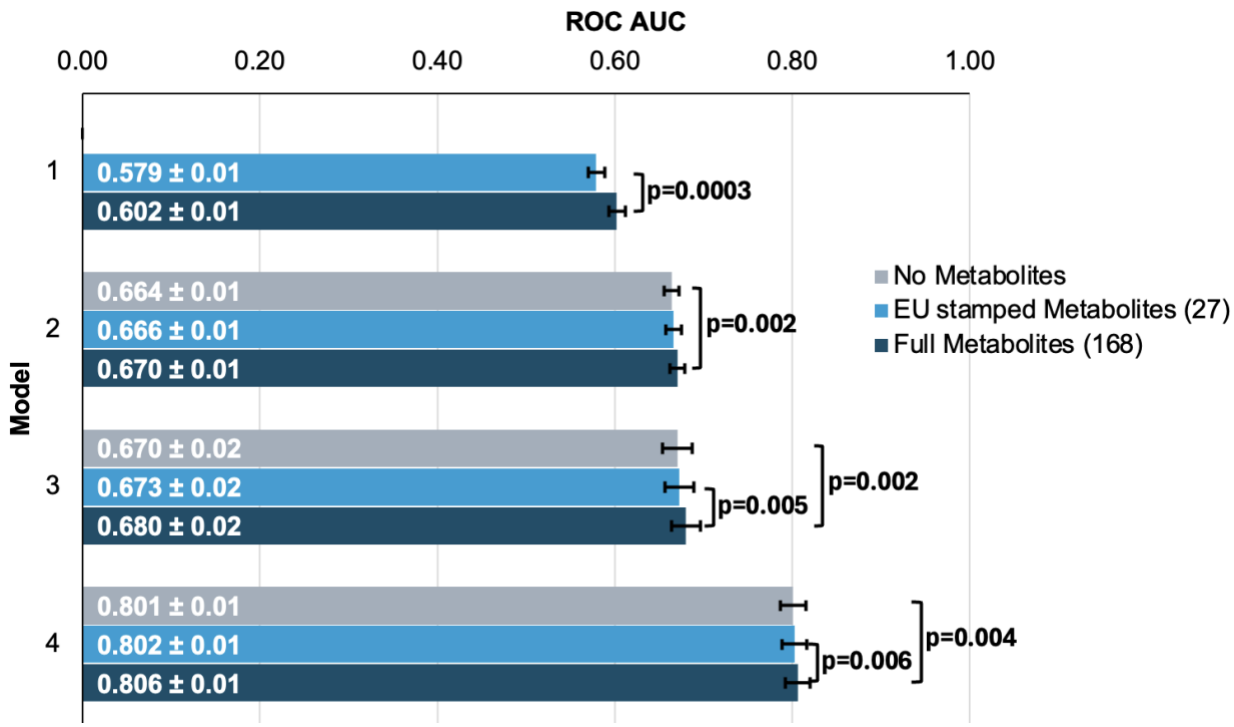


Figure 3. Inclusion of metabolite data into glaucoma prediction algorithms. Model 1 includes metabolites only; Model 2 incorporates additional covariates including age, sex, genetic ancestry, season, time of day of specimen collection, and fasting time; Model 3 incorporates covariates in Model 2 and smoking status (never, past, and current smoker), alcohol intake (g/week), caffeine intake (mg/day), physical activity (metabolic equivalent of task [MET], hours/week), body mass index (kg/m^2), average systolic blood pressure (mm Hg), history of diabetes, HbA1c (mmol/mol), history of coronary artery disease, systemic beta-blocker use, oral steroid use, and spherical equivalent refractive error (diopters); Model 4 incorporates covariates in model 3 and a glaucoma polygenic risk score (PRS). Each color represents a different panel of metabolites (grey = no metabolites; light blue = 27 metabolites; and dark blue = 168 metabolites). The white text represents the AUC ± 95% confidence interval.
Abbreviations: ROC, receiver operator curve; AUC, area under the curve; EU, European Union.

A.

	Bottom 50%	Top 10%
PRS	[-0.0090,-0.0073]	[-0.0068,-0.0055]
n	58,358	11,770
Glaucoma cases	780 (1.3%)	1,693 (14.4%)
No Glaucoma	57,578	10,077

B.

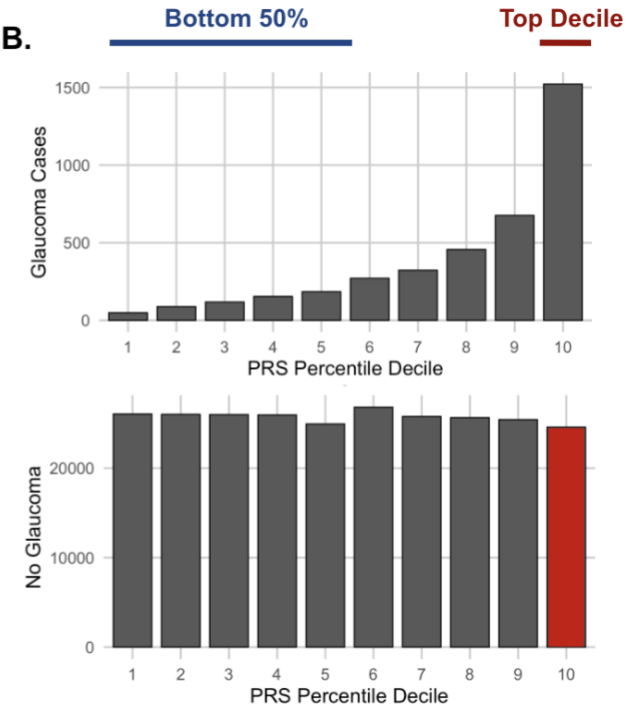
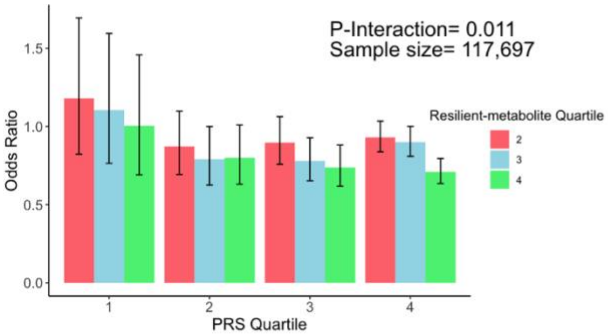


Figure 4. The distribution of glaucoma cases and no glaucoma stratified by polygenic risk score (PRS) deciles. Participants were divided into the bottom 50% and top 10% based on their glaucoma PRS, where the prevalence of glaucoma cases from (A) the bottom 50% (n=58,358) was 1.3% and from the top 10% (n=11,770) was 14.4%. (B) Box plot illustrating the distribution of participants with glaucoma (top) and no glaucoma (bottom) as a function of PRS decile. The blue line denotes participants at the bottom 50% of glaucoma PRS, the red line highlights the participants at the top decile of glaucoma PRS, and the red box represents the participants resilient to glaucoma despite high PRS.

A.

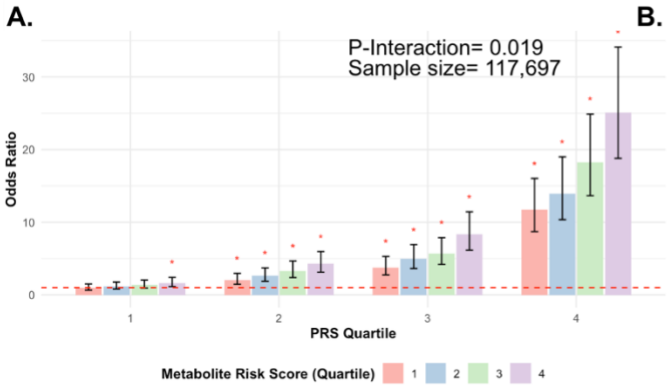


	Quartiles			
	Q1	Q2	Q3	Q4
PRS	-0.0090, -0.0075	-0.0075, -0.0073	-0.0073, -0.0070	-0.0070, -0.0055
Metabolite sum	-8.2, -1.5	-1.5, -0.2	-0.2, 1.3	1.3, 32.2

B.

	Glaucoma cases	Glaucoma Metabolite Reference	Odds Ratio (95% CI)	p-value
PRS Q1				
Metabolite sum Q2	66	55	1.18 (0.82, 1.69)	0.42
Metabolite sum Q3	61	55	1.1 (0.77, 1.60)	0.66
Metabolite sum Q4	57	55	1.0 (0.69, 1.46)	1.00
PRS Q2				
Metabolite sum Q2	138	161	0.87 (0.69, 1.10)	0.27
Metabolite sum Q3	128	161	0.79 (0.63, 1.00)	0.057
Metabolite sum Q4	127	161	0.80 (0.63, 1.01)	0.069
PRS Q3				
Metabolite sum Q2	266	297	0.90 (0.76, 1.06)	0.23
Metabolite sum Q3	228	297	0.78 (0.65, 0.93)	0.0059
Metabolite sum Q4	219	297	0.74 (0.62, 0.88)	0.0009
PRS Q4				
Metabolite sum Q2	750	793	0.93 (0.84, 1.03)	0.19
Metabolite sum Q3	733	793	0.90 (0.81, 1.00)	0.054
Metabolite sum Q4	579	793	0.71 (0.64, 0.80)	<0.0001

Figure 5. Interaction of three putative resilient metabolites (lactate, pyruvate, and citrate) and polygenic risk score (PRS) on glaucoma risk. (A) The bar chart shows the interaction of resilient probit-transformed metabolite sum with glaucoma genetic predisposition in each PRS quartile. In each glaucoma PRS quartile, the lowest metabolic sum quartile (Q1) is the metabolite reference group used to calculate the odds ratios. Each color represents resilient-metabolite sum quartiles (red = second quartile; blue = third quartile; and green = fourth quartile). Error bars show 95% confidence interval (CI). The table under the bar chart shows the ranges for the PRS and metabolite sum value quartiles. (B) The table shows odds ratios for glaucoma by PRS and putative resilient metabolite sum within various quartiles. The number of glaucoma cases within each resilient metabolite sum quartile and the number of glaucoma cases in the first quartile of resilient metabolite sum (Q1, labeled as glaucoma metabolite reference) are used to calculate the odd ratios. This analysis is adjusted for time since the last meal/drink (hours), age, age-squared, sex, ethnicity (Asian, Black, White, and other), season, time of day of specimen collection (morning, afternoon, night), smoking status (never, past, and current smoker), alcohol intake, caffeine intake, physical activity (metabolic equivalent of task [MET] hours/week), body mass index (kg/m^2), average systolic blood pressure (mm Hg), history of diabetes (yes or no), HbA1c (mmol/mol), history of coronary artery disease, systemic beta-blocker use, oral steroid use, and spherical equivalent refractive error (diopters).



	Quartiles			
	Q1	Q2	Q3	Q4
PRS	-0.0090, -0.0075	-0.0075, -0.0073	-0.0073, -0.0070	-0.0070, -0.0055
MRS	0.0000, 0.0316	0.0316, 0.0384	0.0384, 0.0462	0.0462, 0.5185

B.

	Glaucoma cases	Odds Ratio (95% CI)	p-value
PRS Q1			
MRS Q1	46	reference	1
MRS Q2	56	1.20 (0.81, 1.79)	0.40
MRS Q3	64	1.39 (0.95, 2.04)	0.11
MRS Q4	73	1.66 (1.15, 2.42)	0.0086
PRS Q2			
MRS Q1	94	2.06 (1.45, 2.96)	0.0001
MRS Q2	119	2.62 (1.87, 3.72)	<0.0001
MRS Q3	149	3.32 (2.40, 4.67)	<0.0001
MRS Q4	192	4.29 (3.12, 5.98)	<0.0001
PRS Q3			
MRS Q1	171	3.79 (2.75, 5.30)	<0.0001
MRS Q2	220	4.98 (3.65, 6.92)	<0.0001
MRS Q3	254	5.70 (4.19, 7.90)	<0.0001
MRS Q4	365	8.33 (6.17, 11.44)	<0.0001
PRS Q4			
MRS Q1	495	11.71 (8.72, 16.00)	<0.0001
MRS Q2	582	13.90 (10.36, 16.03)	<0.0001
MRS Q3	747	18.27 (13.66, 24.89)	<0.0001
MRS Q4	1031	25.08 (18.80, 34.12)	<0.0001

Figure 6. Interaction of the holistic metabolite risk score (n=168 metabolites) and polygenic risk score (PRS) on glaucoma risk. (A) The bar chart plots the odds ratio of glaucoma as a function of holistic probit-transformed MRS quartile with further stratification by glaucoma PRS in each MRS bin. The lowest quartile of glaucoma PRS and MRS is the reference group (see dotted red line) for the entire population. Each color represents the MRS quartiles (red = first quartile; blue = second quartile; green = third quartile; and purple = fourth quartile). Error bars show 95% confidence interval (CI). The table under the bar chart shows the ranges for the PRS and MRS quartiles. (B) Table showing odds ratios for glaucoma by polygenic risk score (PRS) and MRS within various quartiles. The number of glaucoma cases within each MRS and the number of glaucoma cases in PRS Q1 and MRS Q1 are used to calculate the odds ratios. This analysis is adjusted for time since the last meal/drink (hours), age, age-squared, sex, ethnicity (Asian, Black, White, and other), season, time of day of specimen collection (morning, afternoon, night), smoking status (never, past, and current smoker), alcohol intake, caffeine intake, physical activity (metabolic equivalent of task [MET] hours/week), body mass index (kg/m^2), average systolic blood pressure (mm Hg), history of diabetes (yes or no), HbA1c (mmol/mol), history of coronary artery disease, systemic beta-blocker use, oral steroid use, and spherical equivalent refractive error (diopters).

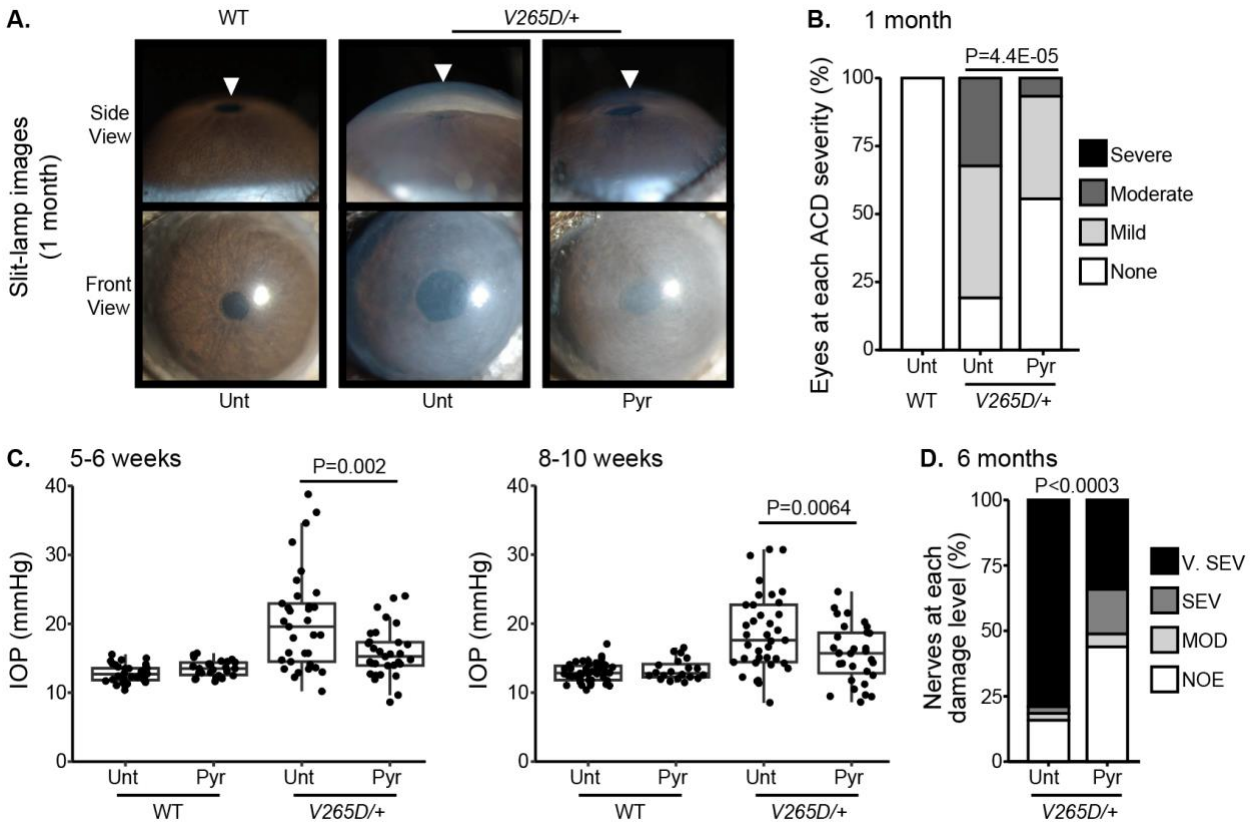


Figure 7. Pyruvate treatment protects from IOP elevation and glaucoma. (A) Representative photos of eyes from mice of the indicated genotypes and treatments (Unt = untreated, Pyr = pyruvate treated). *Lmx1b* is expressed in the iris and cornea, so *Lmx1b*^{V265D} mutant eyes have primary abnormalities of the iris and cornea. This includes corneal haze, which is present before IOP elevation in many eyes and likely reflects a direct transcriptional role of LMX1B in collagen gene expression. *Lmx1b*^{V265D} mutant eyes also develop anterior chamber deepening (ACD), a sensitive indicator of IOP elevation in mice. The WT and pyruvate-treated mutant eyes have shallow anterior chambers, while the untreated mutant eye has a deepened chamber (arrowheads). (B) Distributions of ACD are based on a previously defined scoring system.[31] Groups are compared by Fisher's exact test. n > 30 eyes were examined in each group. (C) Boxplots of IOP (interquartile range and median line) in WT and mutant eyes. Pyruvate treatment significantly lessens IOP elevation in mutants compared to untreated mutant controls. Groups were compared by ANOVA followed by Tukey's honestly significant difference. n > 30 eyes were examined in each *Lmx1b*^{V265D} mutant group, and n > 20 eyes were examined in WT groups. (D) Distributions of damage based on analysis of PPD-stained optic nerve cross sections from 6-month-old mice (Methods). Pyruvate treatment lessened the incidence of glaucoma (Fisher's exact test). No glaucoma was found in WT mice. Geno = genotype. n > 40 nerves examined per group. NOE = no glaucoma, MOD = moderate. SEV = severe, and V. SEV = very severe (see Methods).

Tables

Table 1. Demographic and clinical characteristics of the UK Biobank study population assessed in 2006-2010

Characteristic	No Glaucoma	Glaucoma	P-value
Sample Size (%)	113,040 (96.0)	4,658 (4.0)	
Sex - Male (%)	52,497 (46.4)	2,493 (53.5)	<0.001
Age at recruitment (mean (SD))	56.7 (8.0)	60.9 (6.6)	<0.001
Ethnicity (%)			0.0010
White	105,912 (94)	4,331 (93)	
Asian	2,977 (2.6)	127 (2.7)	
Black	2,228 (2.0)	130 (2.8)	
Other	1,923 (1.7)	70 (1.5)	
Genetic Ancestry (%)			<0.001
African	1,911 (1.7)	123 (2.7)	
AMR	261 (0.2)	2 (0.0)	
Asian	3,125 (2.8)	134 (2.9)	
European	96,991 (86.6)	4,001 (86.7)	
Smoking Status (%)			<0.001
Never	63,240 (56)	2,339 (52)	
Prefer not to answer	430 (0.4)	16 (0.4)	
Previous	38,926 (34)	1,732 (39)	
Current	10,605 (9.4)	410 (9.1)	
Total Cholesterol, mmol/l (median [IQR])	4.6 [3.98, 5.22]	4.5 [3.88, 5.18]	<0.001
Physical Activity, MET-minutes per week (mean (SD))	2,465 (2430)	2,458 (2,438)	0.84
Body Mass Index, kg/m^2 (mean (SD))	27.4 (4.8)	27.7 (4.7)	<0.001
HbA1c, mmol/mol (mean (SD))	36.0 (5.8)	37.4 (6.8)	<0.001
Spherical Equivalent, diopter (mean (SD))	-0.1 (2.1)	-0.1 (1.9)	0.67
Intraocular pressure, mmHg (mean (SD))	15.9 (2.6)	17.8 (4.5)	<0.001
mRNFL thickness, µm (mean (SD))	28.7 (1.8)	28.5 (1.3)	<0.001
Beta blocker use (%)	8,398 (7.4)	447 (9.6)	<0.001
Caffeine intake, mg/day (mean (SD))	165 (67)	168 (59)	0.0020
Alcohol intake, g/week (median [IQR])	84 [40.3, 146.9]	84 [48.0, 154.6]	<0.001
Diabetes (%)	6,512 (5.8)	463 (9.9)	<0.001
Oral steroid use (%)	3,043 (2.7)	184 (4.0)	<0.001
Coronary Artery Disease (%)	5,153 (4.6)	377 (8.1)	<0.001

Abbreviations: SD, standard deviation; IQR, interquartile range; AMR, mixed American; MET metabolic equivalent of task mRNFL, macular retinal nerve fiber layer

Table 2. Stratification of glaucoma by ethnicity, age, and gender for predictive assessment with and without using metabolite data.

Stratification	Sample size	Glaucoma cases	No Metabolites (AUC)	Metabolites (AUC)	P-value
Ethnicity					
White	110,243	4,331	0.675	0.686	<0.001
Asian	3,104	127	0.780	0.768	0.062
Black	2,358	130	0.704	0.706	0.52
Age					
<55 years	43,648	788	0.576	0.566	0.52
≥55 years	74,050	3,870	0.569	0.596	0.002
Gender					
Female	62,708	2,165	0.689	0.696	0.25
Male	54,990	2,493	0.659	0.673	0.002

The area under the curve (AUC) of the receiver operating characteristic (ROC) curve was calculated for each demographic stratification to evaluate the predictive performance of models both with and without metabolite data. Differences in model AUC were tested using the DeLong test, and p-values were reported. For models excluding metabolite data, the predictors include as appropriate, age, sex, genetic ancestry, season, time of day of specimen collection, fasting time (hours), smoking status (never, past, and current smoker), alcohol intake (g/week), caffeine intake (mg/day), physical activity (metabolic equivalent of task [MET], hours/week), body mass index (kg/m^2), average systolic blood pressure (mm Hg), history of diabetes, HbA1c (mmol/mol), history of coronary artery disease, systemic beta-blocker use, oral steroid use, and spherical equivalent refractive error (diopters). Models including metabolite data incorporated the same predictors with the addition of the 168 metabolite measurements.

Table 3. Demographic and clinical characteristics in 2006-2010 of participants among the top 10% of glaucoma polygenic risk score (PRS).

Characteristic	No Glaucoma	Glaucoma	P-value
Sample size (%)	10,077 (85.6)	1,693 (14.4)	
Sex - Male (%)	4,667 (46.3)	893 (52.7)	<0.001
Age at recruitment (mean (SD))	56.3 (8.0)	61.0 (6.3)	<0.001
Ethnicity (%)			<0.001
White	9,256 (91.9)	1,636 (96.6)	
Asian	279 (2.8)	24 (1.4)	
Black	345 (3.4)	20 (1.2)	
Other	197 (2.0)	13 (0.8)	
Smoking Status (%)			0.0030
Never	5,806 (57.6)	899 (53.1)	
Prefer not to answer	48 (0.5)	5 (0.3)	
Previous	3,402 (33.8)	640 (37.8)	
Current	821 (8.1)	149 (8.8)	
Physical Activity, MET-minutes per week (mean (SD))	2,439 (2,352)	2,514 (2,480)	0.23
Body mass index, kg/m^2 (mean (SD))	27.4 (4.7)	27.7 (4.8)	0.0080
HbA1c, mmol/mol (mean (SD))	36.00 (5.9)	37.0 (6.1)	<0.001
Spherical Equivalent, diopter (mean (SD))	-0.3 (2.2)	-0.2 (1.9)	0.0090
Intraocular pressure, mmHg (mean (SD))	17.2 (3.0)	18.3 (5.0)	<0.001
mRNFL thickness, µm (mean (SD))	28.7 (1.8)	28.5 (1.2)	<0.001
Beta blocker use (%)	719 (7.1)	179 (10.6)	<0.001
Caffeine intake, mg/day (mean (SD))	164 (65)	170 (60)	0.0010
Alcohol intake, g/week (median [IQR])	84 [38.6, 145.3]	84 [51.5, 155.3]	0.002
Diabetes mellitus (%)	607 (6.0)	151 (8.9)	<0.001
Oral steroid use (%)	292 (2.9)	59 (3.5)	0.22
Coronary Artery Disease (%)	443 (4.4)	148 (8.7)	<0.001
Total cholesterol, mmol/l (median [IQR])	4.61 [4.00, 5.25]	4.54 [3.90, 5.21]	0.006
Lactate, mmol/l (median [IQR])	3.96 [3.27, 4.78]	3.79 [3.16, 4.58]	<0.001
Pyruvate, mmol/l (median [IQR])	0.080 [0.06, 0.10]	0.077 [0.06, 0.09]	<0.001
Citrate, mmol/l (median [IQR])	0.065 [0.06, 0.07]	0.065 [0.06, 0.07]	0.87
Cholesteryl Esters in Small HDL, mmol/l (median [IQR])	0.33 [0.30, 0.36]	0.33 [0.30, 0.36]	0.005
Triglycerides in Very Large VLDL, mmol/l (median [IQR])	0.091 [0.05, 0.15]	0.10 [0.05, 0.17]	<0.001
Alanine, mmol/l (median [IQR])	0.29 [0.24, 0.35]	0.29 [0.24, 0.35]	0.58
Triglycerides in Chylomicrons and extremely Large VLDL, mmol/l (median [IQR])	0.088 [0.03, 0.18]	0.10 [0.04, 0.21]	<0.001
Acetoacetate, mmol/l (median [IQR])	0.010 [0.01, 0.02]	0.011 [0.01, 0.02]	<0.001
Cholesteryl Esters in Medium HDL, mmol/l (median [IQR])	0.41 [0.34, 0.48]	0.40 [0.34, 0.47]	0.003
Triglycerides in Large VLDL, mmol/l (median [IQR])	0.15 [0.09, 0.22]	0.16 [0.10, 0.23]	<0.001
Cholesterol in Medium HDL, mmol/l (median [IQR])	0.49 [0.42, 0.58]	0.48 [0.41, 0.57]	0.005

Abbreviations: SD, standard deviation; IQR, interquartile range; MET, metabolic equivalent of task HbA1c, hemoglobin A1C; mRNFL, macular retinal nerve fiber layer; HDL, high-density lipoprotein; VLDL, very low-density lipoprotein

Table 4. Demographic and clinical characteristics in 2006-2010 of participants among the bottom 50% of glaucoma polygenic risk score (PRS).

Characteristic	No Glaucoma	Glaucoma	P-value
Sample size (%)	57,578 (98.7)	780 (1.3)	
Sex - Male (%)	26,887 (46.7)	409 (52.4)	0.002
Age at recruitment (mean (SD))	57 (8.0)	60 (7.0)	<0.001
Ethnicity (%)			<0.001
White	55,034 (96)	690 (89)	
Asian	1,129 (2.0)	41 (5.3)	
Black	574 (1.0)	27 (3.5)	
Other	841 (1.5)	22 (2.8)	
Smoking Status (%)			0.67
Never	200 (0.3)	4 (0.5)	
Prefer not to answer	31,593 (54.9)	418 (53.6)	
Previous	20,276 (35.2)	287 (36.8)	
Current	5,509 (9.6)	71 (9.1)	
MET, minutes per week (mean (SD))	2,483 (2,450)	2,612 (2,562)	0.14
Body mass index kg/m^2 (mean (SD))	27.4 (4.8)	27.8 (4.7)	0.021
HbA1c, mmol/mol (mean (SD))	35.9 (5.7)	38.1 (7.8)	<0.001
Spherical Equivalent, diopter (mean (SD))	-0.03 (2.1)	0.01 (1.9)	0.63
Intraocular pressure, mmHg (mean (SD))	15.4 (2.4)	17.1 (3.9)	<0.001
mRNFL thickness, µm (mean (SD))	28.7 (1.7)	28.6 (1.3)	0.02
Beta blocker use (%)	4,322 (7.5)	71 (9.1)	0.11
Caffeine intake, mg/day (mean (SD))	1,66.3 (67.4)	166.6 (61.5)	0.90
Alcohol intake, g/week (median [IQR])	84 [41.7, 149.3]	84 [42.5, 145.9]	0.91
Diabetes (%)	3209 (5.6)	104 (13.3)	<0.001
Oral steroid use (%)	1,538 (2.7)	39 (5.0)	<0.001
Coronary Artery Disease (%)	2,656 (4.6)	64 (8.2)	<0.001
Total cholesterol, mmol/l (median [IQR])	4.59 [3.98, 5.22]	4.49 [3.76, 5.13]	0.001
Lactate, mmol/l (median [IQR])	3.95 [3.24, 4.75]	3.84 [3.21, 4.69]	0.16
Concentration of Small HDL Particles, mmol/l (median [IQR])	0.0097 [0.0089, 0.011]	0.0095 [0.0088, 0.010]	0.002
Cholesteryl esters in small HDL, mmol/l (median [IQR])	0.33 [0.30, 0.36]	0.32 [0.30, 0.36]	0.001
Albumin, mmol/l (median [IQR])	39.4 [37.3, 41.45]	38.9 [36.7, 40.8]	<0.001
Total lipids in small HDL, mmol/l (median [IQR])	1.16 [1.06, 1.26]	1.15 [1.05, 1.25]	0.018
Citrate, mmol/l (median [IQR])	0.065 [0.057, 0.074]	0.065 [0.057, 0.073]	0.52
Pyruvate, mmol/l (median [IQR])	0.080 [0.06, 0.10]	0.079 [0.061, 0.098]	0.14
Alanine, mmol/l (median [IQR])	0.29 [0.24, 0.35]	0.29 [0.24, 0.35]	0.95
Phospholipids in Small HDL, mmol/l (median [IQR])	0.66 [0.60, 0.72]	0.66 [0.60, 0.71]	0.033

Abbreviations: SD, standard deviation; MET, metabolic equivalents; HbA1c, hemoglobin A1C; mRNFL, macula region retinal nerve fiber layer; HDL, high density lipoprotein; VLDL, very low density lipoprotein

Table 5. Metabolites associated with glaucoma among participants in the top decile and the bottom half of glaucoma polygenic risk score.

Top 10% of glaucoma polygenic risk score			
Metabolites (Probit score)	**Glaucoma**	**No Glaucoma**	**Adjusted P-value (NEF)**
Lactate	-0.146	0.0239	8.8E-12
Pyruvate	-0.137	0.0117	2.9E-10
Citrate	-0.0693	0.0079	0.018
Triglycerides in Very Large VLDL	0.0606	-0.0043	0.10
Triglycerides in Chylomicrons and Extremely Large VLDL	0.0572	-0.0076	0.11
Acetoacetate	0.0744	0.0011	0.11
Cholesteryl Esters in Small HDL	-0.0239	0.0376	0.12
Cholesteryl Esters in Medium HDL	-0.0261	0.0276	0.13
Alanine	-0.0612	-0.0006	0.14
Triglycerides in Large VLDL	0.0629	0.0005	0.15

Bottom 50% of glaucoma polygenic risk score			
Metabolites (Probit score)	**Glaucoma**	**No Glaucoma**	**Adjusted P-value (NEF)**
Albumin	-0.110	-0.0099	0.047
Concentration of Small HDL Particles	-0.0943	-0.0062	0.071
Cholesteryl Esters in Small HDL	-0.0925	-0.0055	0.085
Cholesterol in Small HDL	-0.0919	-0.0063	0.088
Omega-3 Fatty Acids	-0.0896	-0.0052	0.092
Docosahexaenoic Acid	-0.0876	-0.0061	0.10
Total Lipids in Small HDL	-0.0844	-0.0051	0.12
Lactate	-0.0823	0.0031	0.12
Citrate	-0.0882	-0.0023	0.16
Alanine	-0.0766	0.0011	0.18

Potential confounders adjusted by regression include time since the last meal/drink (hours), age, age-squared, sex, ethnicity (Asian, Black, White, and other), season, time of day of specimen collection (morning, afternoon, night), smoking status (never, past, and current smoker), alcohol intake, caffeine intake, physical activity (metabolic equivalent of task [MET] hours/week), body mass index (kg/m^2), average systolic blood pressure (mm Hg), history of diabetes (yes or no), HbA1c (mmol/mol), history of coronary artery disease, systemic beta-blocker use, oral steroid use, and spherical equivalent refractive error (diopters).

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Conflict of Interest:

The authors declare the following financial interests/personal relationships that may be considered potential competing interests: LRP reports consulting honoraria from Twenty Twenty. JLW reports consulting fees from Editas and CRISPR Therapeutics. NZ reports consulting fees from Character Biosciences. APK has acted as a paid consultant or lecturer to Abbvie, Aerie, Allergan, Google Health, Heidelberg Engineering, Novartis, Reichert, Santen, Thea and Topcon. NV has acted as a paid consultant or lecturer to Santen, Thea, NovaEye, Glaukos, and Elios. RD reports being a scientific co-founder, consultant, and equity holder for Pensieve Health (pending) and being a consultant for Variant Bio and Character Bio. SWMJ is founder of Myco Advising LLC, a founder of Qura Inc and advises Starlight Bio Inc with no overlap to the currently presented research. SWMJ and NT are inventors on patents/patent applications held by The Jackson Laboratory and Columbia University on the use of pyruvate to treat glaucoma. No other disclosures were reported.