Ethnicity, Dietary Factors, Patterns and Gene-Diet Interactions and their association with Intraocular Pressure and Glaucoma: The Canadian Longitudinal Study on Aging

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PREFACE

This thesis is original work by Alyssa Grant in contribution to the PhD Epidemiology research requirements. The research that makes up this thesis received ethics approval by the University of Ottawa in October 2021. Chapter Five of this thesis includes three manuscripts with co-authors Marie-Hélène Roy-Gagnon, Joseph Bastasic, Akshay Talekar, Garfield Miller, Mahsa Jessri, Gisele Li, Ralf Buhrmann, and Ellen E. Freeman entitled “Alcohol consumption, genetic risk, and intraocular pressure and glaucoma: The Canadian Longitudinal Study on Aging”, “Understanding Ethnic and Racial Differences in Intraocular Pressure and Glaucoma: The Canadian Longitudinal Study on Aging”, and “Diet, Genetic Risk, and their Association with Intraocular Pressure and Glaucoma: The Canadian Longitudinal Study on Aging”.

ABSTRACT

Objectives: Our goal was to examine the associations of alcohol consumption, and dietary factors, patterns and supplements with intraocular pressure (IOP) and glaucoma and to assess whether any associations are modified by a glaucoma polygenic risk score (PRS). We also sought to identify whether race/ethnicity is associated with IOP and glaucoma and explore potential social, behavioral, genetic and health-related reasons.

Methods: Cross-sectional analysis of data from the Canadian Longitudinal Study on Aging Comprehensive (CLSA) Cohort, consisting of 30,097 adults ages 45 to 85 years, was done. Alcohol consumption frequency and type were measured by interviewer-administered questionnaire. Total alcohol intake (grams/week) was estimated. Nutrition was assessed using a validated 36-item Short Diet Questionnaire. Participants were asked to report if they took calcium or iron supplements in the last month. We scored participants according to their adherence to the Mediterranean-Style Dietary Pattern Score and to an antioxidant-rich dietary pattern score derived from CLSA data using weighted partial least squares. Race/ethnicity was obtained using an interviewer-administered questionnaire. IOP was measured in mmHg using the Reichert Ocular Response Analyzer. Participants reported a diagnosis of glaucoma from a doctor. A glaucoma PRS developed by Craig et al. was constructed using CLSA genomic data. Logistic and linear regression models were used to adjust for demographic, behavioral, and health variables.

Results: Daily drinkers had higher IOP compared to those who never drank (beta coefficient (β) =0.45, 95% confidence interval (CI): 0.05, 0.86). An increase in total weekly alcohol intake (per 5 drinks) was also associated with higher IOP (β=0.20, 95% CI: 0.15, 0.26). The association between total alcohol intake and IOP was stronger in those with a higher genetic risk of glaucoma (P for interaction term= 0.041). Consuming calcium supplements was associated with lower IOP (β=-0.16, 95% confidence interval (CI): -0.31, 0.00) and increased odds of glaucoma (OR (odds ratio)= 1.30, 95% CI: 1.08, 1.56). Supplementation with iron and adherence to a Mediterranean or antioxidant-rich diet were not associated with IOP and glaucoma. Black individuals had higher mean IOP levels (β= 1.46, 95% CI, 0.63, 2.30) while Chinese, Japanese and Korean (β = -1.00, 95% CI, -1.62, -0.38) and Southeast Asian and Filipino individuals (β = -1.56, 95% CI, - 2.68, -0.43) had lower mean IOP levels as compared to White individuals after adjustment for sociodemographic, behavioral, genetic, and health-related variables. Black people were more likely to report glaucoma as compared to White people after adjustment (OR = 2.43, 95% CI, 1.27, 4.64). Latin American people (OR = 2.64, 95% CI, 1.02, 6.82) were also more likely to report glaucoma but this association was no longer statistically significant after adjusting for the PRS (OR=2.39, 95% CI 0.93, 6.13).

Conclusions: Alcohol frequency and total alcohol intake were associated with elevated IOP but not with glaucoma. The PRS modified the association between total alcohol intake and IOP. Supplemental calcium is associated with reduced IOP but increased odds

of glaucoma. Racial and ethnic differences in IOP and glaucoma were also identified. Adjusting for sociodemographic, behavioral, genetic, and health-related variables did not fully explain these differences. Longitudinal research is needed to further explore the reasons for these differences, to understand their relevance to disease pathogenesis and progression and to further elucidate the interactions of dietary and genetic factors on their risk of disease.

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CHAPTER 1: INTRODUCTION

Glaucoma, estimated to affect 80 million people and identified as a leading cause of irreversible vision loss worldwide, refers to a group of optic neuropathies that are characterized by progressive degeneration of retinal ganglion cells[1,2]. Retinal ganglion cells are neurons that connect retinal input to visual processing centers within the central nervous system[3]. Degeneration of these cells results in cupping of the optic disk and characteristic visual field loss[4]. The pathogenesis of glaucoma is believed to result from multiple stresses and insults although the factors contributing to its development and progression have not been fully characterized[5]. Further, glaucoma progresses without causing symptoms until late in the disease process, and as a result, diagnosis is frequently delayed until substantial amounts of neural damage have occurred[1].

One of the factors leading to glaucoma is intraocular pressure (IOP). IOP refers to the fluid pressure inside of the eye and is determined by the balance between secretion of aqueous humor by the ciliary body and its drainage through the trabecular meshwork and uveoscleral outflow pahways[6]. Aqueous humor is produced continuously in the posterior chamber and drains into the anterior chamber of the eye. The majority of the aqueous humor then drains into the venous system through the trabecular meshwork while a minority drains through the uveoscleral pathway[6]. If drainage of the aqueous humor is lower than its secretion then IOP will rise, and vice versa.

Elevated IOP can result from an accumulation of aqueous humor in the anterior chamber due to increased outflow resistance by the trabecular meshwork[7,8]. With aging, exposure to pharmacologic agents or pathological processes, the trabecular meshwork undergoes several alterations, including increased stiffness, which may contribute to increased resistance to aqueous humor outflow and thus elevated IOP over time[9,10].

When IOP increases above the normal range, it induces mechanical stress and strain with consequent compression, deformation and remodeling of the posterior structures of the eye, most notably to the lamina cribrosa and adjacent tissues, resulting in mechanical axonal damage, disruption of axonal transport and visual field loss[11-13]. As a result, elevated IOP is a major risk factor for the development and progression of most cases of glaucoma[1]. Glaucoma can affect anyone, but certain groups are at higher risk. Risk factors include genetic, demographic, lifestyle, medical, and environmental factors[14]. While glaucoma can be treated, it cannot be cured with current treatment approaches.

My thesis focused on utilizing data from the Canadian Longitudinal Study on Aging to better understand the dietary and ethnic factors associated with elevated IOP and glaucoma taking into account genetic risk of disease. Chapter 2 of my thesis will review the literature on this topic. Chapter 3 will present my study aims. Chapter 4 will describe the Canadian Longitudinal Study on Aging. Chapter 5 will include three

manuscripts (1 published and 2 in submission). Chapter 6 will present supplementary analyses and chapter 7 will integrate the results together for discussion.

CHAPTER 2: LITERATURE REVIEW

My thesis will focus on extending the research on race/ethnicity, dietary factors, and their interplay with genetic factors in their relationship with IOP and glaucoma. The literature on IOP and glaucoma will be reviewed first followed by the literature on ethnicity, genetic and dietary factors.

2.1 Intraocular pressure

IOP is measured using tonometry. The Goldmann applanation tonometer (GAT or AT) is the current gold standard instrument for measuring IOP[15] although with improvements in technology, its accuracy is now being critically analyzed. GAT uses the Imbert-Fick law: P=F/S, where P is the amount of pressure (mmHg), S is the applanated area of the corneal surface, and F is the force needed to applanate that area of the cornea[16]. The Imbert-Fick law, however, does not take into account biometric properties of the cornea. For example, as central corneal thickness (CCT) increases, a larger force is required to applanate the corneal surface resulting in an overestimated measure of the true IOP[17] As such, the measurement of CCT is required to provide a more accurate AT IOP measure[18]. Further, due to individual variations in CCT and diurnal fluctuations of IOP, it is recommended to take multiple measurements in patients suspected of having elevated IOP[19].

2.2 Glaucoma

The two most common forms of glaucoma in the world are primary open-angle glaucoma (POAG) and primary angle-closure glaucoma (PACG)[20]. Patients with POAG

have increased resistance to aqueous outflow through the trabecular meshwork pathway whereas the iris in patients with PACG typically obstructs access to the drainage pathways[1]. POAG is generally diagnosed during routine eye examination, which includes fundoscopic assessment of the cup: disc ratio (CDR), evaluation of the retinal nerve fiber layer (RNFL), open anterior chamber angles on gonioscopy, and visual field evaluation using perimetry[4,21]. PACG diagnosis relies on gonioscopy and may be assisted by anterior segment optical coherence tomography and ultrasound biomicroscopy[22]. Normal tension glaucoma (NTG), a type of POAG, can also occur in individuals with apparently normal IOP levels due to abnormally low cerebrospinal fluid pressure in the optic nerve subarachnoid space, impaired microcirculation, altered immunity, excitotoxicity, oxidative stress or secondary neurodegeneration from primary neural pathological processes[1,23-25]. The histopathological changes in NTG are consistent with those found in POAG, apart from the absence of elevated IOP, and diagnosis is generally confirmed during routine eye testing[26,27].

2.3 Current treatment practices

Currently, reduction of IOP is the only method proven to treat PACG and POAG[28]. Management guidelines recommend lowering IOP towards a target level, established based on pretreatment pressure levels, severity of retinal damage, risk factors for disease progression, life expectancy, and potential treatment adverse effects[1,29]. Prostaglandin analogue eye drops are typically the first line of medical therapy; however, several other classes of pressure-lowering medications are available including

β-Adrenergic blockers, α-Adrenergic agonists, carbonic anhydrase inhibitors, and cholinergic agonists, which are used as second –line agents or when there is a contraindication to prostaglandin analogues[1,30]. Prostaglandin analogues reduce IOP by stimulating the synthesis of enzymes that dissolve the extracellular matrix of the ciliary muscle, thus increasing the uveoscleral outflow.[31] β-Adrenergic blockers reduce IOP by blocking sympathetic nerve endings in the ciliary epithelium, reducing aqueous production[32]. α-Adrenergic agonists increase uveoscleral outflow and decrease aqueous humor production through ciliary body vasoconstriction[33]. Carbonic anhydrase inhibitors reduce aqueous humor production by reducing the production of bicarbonate ions in the ciliary epithelium[34] . Finally, cholinergic agonists lower IOP by inducing pupil constriction, leading to higher rate of aqueous clearance[35]. If medical therapy fails to achieve sufficient IOP reduction with acceptable adverse effects, laser or surgical therapy is indicated[1]. Laser trabeculoplasty and trabeculectomy are commonly performed procedures that lower IOP by inducing changes in the trabecular meshwork to increase aqueous outflow[1], while laser iridotomy is often done to treat PACG.

2.4 Risk factors for Elevated IOP and Glaucoma

Several risk factors for elevated IOP have been identified, including older age, female sex, race/ethnicity, dietary factors, increased body mass index (BMI), higher systolic and diastolic blood pressure, thicker CCT, exposure to air pollution, and elevated glycosylated hemoglobin (HbA1C) levels[36-42].

Risk factors for POAG include an increased CDR, CDR asymmetry, disc hemorrhage, elevated IOP, a family history of disease, Black race, Latino ethnicity, older age, the use of systemic or topical corticosteroids, smoking, type 2 diabetes mellitus (DM), obesity, and atherosclerosis[43-46]. Risk factors for PACG include female sex, older age, Asian ethnicity, as well as certain biometric characteristics of the eye (short axial length, crowded anterior segment, thicker irises and greater lens vault)[1,30,47,48]. Certain genes have been identified that account for less than 10% of all glaucoma cases; however, POAG and PACG are considered to be distinct genetic entities as different genes have been identified to be associated with each disease[1,49].

The rest of this section will go into more detail on the risk factors that I will be examining for my thesis: ethnicity, diet, and genetic factors.

2.4a. Ethnicity, Intraocular Pressure and Glaucoma

Racial and ethnic differences in IOP levels have been identified in which individuals of African descent have higher reported IOP levels when compared to Non-Hispanic White, Asian, and Latino individuals[36-40]. The disease burden of each glaucoma subtype also varies significantly by region and ethnicity, with POAG being more frequent among Black and Latino individuals and PACG more frequent among Chinese, South Asian and Inuit individuals[50-58]. Several population-based studies have also documented greater risk of glaucoma among Black and Latino people as compared to White people[50-53]. These variations have been attributed to genetic, anatomical, behavioral, social and environmental factors[59,60]. POAG was estimated to affect 52.7 million people globally in

2020[61]. A recent systematic review and meta-analysis estimated the global prevalence of POAG to be 2.4% (95% CI: 2.0%, 2.8%) based on data from population-based studies published between 2000-2020[62]. Further, the population prevalence of POAG is highest in Black populations (5.2% at 60 years, 12.2% at 80 years), followed by Hispanic or Latino (2.7% at 60 years, 12.7% at 80 years)[63], Other/Mixed (2.3% at 60 years, 7.9% at 80 years), Southeast Asian (2.2% at 60 years, 5.1% at 80 years), South Asian (2.1% at 60 years, 5.9% at 80 years), East Asian (1.8% at 60 years, 3.8% at 80 years), and is lowest among White populations (1.4% at 60 years, 5.3% at 80 years)[63]. NTG comprises a higher proportion of POAG cases in Asian populations (52-92%)[64-67], compared to populations of African (57.1%)[68] and European descent (30-38%)[52,69], which may be related to different allele frequencies of IOP and glaucoma-related SNPs present in different ethnic populations[64]. For example, several genetic loci have specifically been associated with NTG in Asian populations, suggesting that POAG phenotype may be dependent on IOP-related SNPs[70]. The magnitude of the observed ethnic variations also appear to be highly dependent on age, in which age-specific increases in POAG prevalence (per decade of age) are highest in Hispanics (OR= 2.31; 95% CI: 2.12, 2.52) and White populations (OR= 1.99; 95% CI: 1.86 2.12), followed by East and South Asians (OR= 1.48; 95% CI: 1.39, 1.57, OR= 1.56, 95% CI: 1.31, 1.88, respectively) and are lowest in Black populations (OR= 1.59; 95% CI: 1.52, 1.67)[63].

Black individuals may exhibit earlier onset and increased POAG prevalence, as well as increased progression to blindness[51,71-75]. This may be related to the fact that Black populations have the highest prevalence of POAG from early middle life and

therefore have a longer exposure to disease relative to other racial groups[63,76]. Latino individuals also have increased POAG incidence as compared to White people[37,45,77].

PACG is estimated to affect over 21 million people with a global pooled prevalence of 0.6% (95% CI: 0.5–0.8%) in the last 20 years[60,61]. Asia has the highest prevalence of PACG (0.7%, 95% CI = 0.6–1.0%), followed by Africa (0.4%, 95% CI: 0.2-0.5%), and Europe (0.2%, 95% CI= 0.1-0.6%)[60]. The prevalence of PACG also increases steadily with age (0.1% at 40-49 years vs.2.8% at 80+ years)[60]. There are also gender differences in glaucoma, where men are more likely to have POAG (OR: 1.30; 95% CI: 1.22, 1.41)[63] and less likely to have PACG (risk ratio (RR) = 0.71; 95% CI: 0.53–0.93)[60], as compared with women.

The reasons underlying these racial/ethnic differences are not entirely clear although they may include different methods of glaucoma diagnosis ascertainment as well as social, behavioral, genetic, anatomical, health, and healthcare access factors that contribute to disease pathogenesis, progression, and severity. For example, despite being identified as high risk, Black and Latino patients have less consistent glaucoma follow-up relative to White patients[78]. Potential barriers to adherence with follow-up may include an absence of visual symptoms, the need for lifelong treatment, and lack of information regarding irreversible vision loss resulting from glaucoma[78]. These differences may also result from racial/ethnic differences in susceptibility to other systemic comorbidities and their precursors[63]. For example, comorbidities associated with vascular dysregulation, including arterial hypertension and hypotension, ischemic cardiac disease and peripheral vasospasm, were significantly associated with glaucoma

progression[79]. Notable racial and ethnic differences and disparities exist in ischemic heart disease, in which Black and Hispanics have higher risks of stroke[80] as well as stroke occurrences at earlier ages[81],and in coronary heart disease in which disparities in risk factors, treatment and outcomes exist for ethnic minorities[82]. Further, racial/ethnic differences have been reported in hypertension, which contributes significantly to the variation in IOP[83,84]. A higher prevalence of cardiovascular risk factors is further complicated by disparities in access to healthcare, as well as socioeconomic status, sociocultural factors, and racial discrimination within and outside of the health care system that can potentiate racial and ethnic health disparities[85].

Further, studies have identified structural and biometric parameters associated with POAG and its progression in patients of African descent as compared with those of European descent, including greater optic disc area, deeper maximum cup depth, and thinner corneas[86-90]. Glaucoma patients of African descent were found to have increased levels of oxygen in the anterior chamber as compared to patients of European descent, which may contribute to increased oxidative stress, IOP and cellular damage[91]. In fact, both in vitro and in vivo studies have shown increases in oxidative‐stress and inflammation levels in endothelial cells of African Americans compared with Caucasians[92]. Racial/ethnic differences have also been reported in oxidative stress mechanisms in subjects with a family history of cardiovascular disease[93]. Finally, studies have shown POAG patients of African descent to have significantly lower retrobulbar blood flow compared with patients of European descent indicating that ocular blood flow may contribute more significantly to the pathophysiology of glaucoma in patients

of African compared with European descent[94,95]. Prior studies have been limited in the number of ethnic groups that they have included. They have also failed to thoroughly investigate reasons for racial and ethnic differences in glaucoma and IOP.

2.4b. Dietary Factors and Patterns, Intraocular Pressure, and Glaucoma

Dietary factors have been examined as potentially being able to influence both IOP-dependent and non-IOP dependent mechanisms in glaucoma, thereby affecting disease incidence or progression[96]. Growing evidence supports the involvement of increased oxidative stress as a common etiology contributing to the pathogenesis of neurodegenerative diseases, including Parkinson's, Alzheimer's, and glaucoma [97,98]. In fact, glaucoma-related RGC death occurs by apoptosis which is triggered by oxidative stress induced mitochondrial damage, inflammation, vascular endothelial dysregulation and dysfunction, and hypoxia[99].

Generally, healthy cells have an endogenous antioxidant defense system comprised of antioxidant enzymes that scavenge and neutralize free radicals, including reactive oxygen species (ROS).[100] Endogenous antioxidants synthesized by cells, including glutathione, vitamins C and E and ubiquinol, and exogenous antioxidants ingested through the diet, including, vitamins C and E, curcumin, resveratrol, quercetin, beta-carotene and lycopene, can also help maintain redox homeostasis[101,102]. However, during aging or disease, oxidative stress results from an imbalance between ROS production and antioxidant defenses, resulting in molecular damage and interruption of cellular signaling [98,103]. Further, oxidative stress can be potentiated by an improper

diet[104]. Oxidative stress is implicated in elevated IOP and glaucoma at multiple levels and contributes to disruptions in mitochondrial defense systems, calcium homeostasis dysregulation and RGC degeneration[105,106]. More specifically, the intracellular accumulation of ROS results in an increased calcium (Ca(2+)) influx, which then causes Ca (2+) influx into mitochondria and nuclei[107]. In mitochondria, the influx of Ca(2+) accelerates and disrupts normal metabolism, ultimately leading to neuronal cell death[107].

In humans, ROS are produced from exogenous and endogenous sources (Figure 1). Exogenous sources include unhealthy lifestyle factors such as reduced physical activity, unhealthy diet, and nutritional deficiency, and environmental pollutant/ toxin exposure including ultraviolet light, ionizing radiation, cigarette smoke, alcohol, heavy metals, chemicals, and pesticides[100,108,109]. Endogenous sources involve cellular metabolism, genetic alterations, immunodeficiency, infection, and inflammatory diseases[109]. Aging, a major glaucoma risk factor, is also an endogenous source of ROS production in various pathways[109], including reduced levels of antioxidants and antioxidant enzymes and accumulation of damages to mitochondrial DNA[104]. In fact, aging and oxidative stress are the leading causes of RGC damage and dysfunction, the primary cellular site of injury in glaucoma[109]. While RGC loss is irreversible, there is increasing evidence to suggest that RGC functional recovery is possible if intervention to normalize IOP occurs early enough[110].

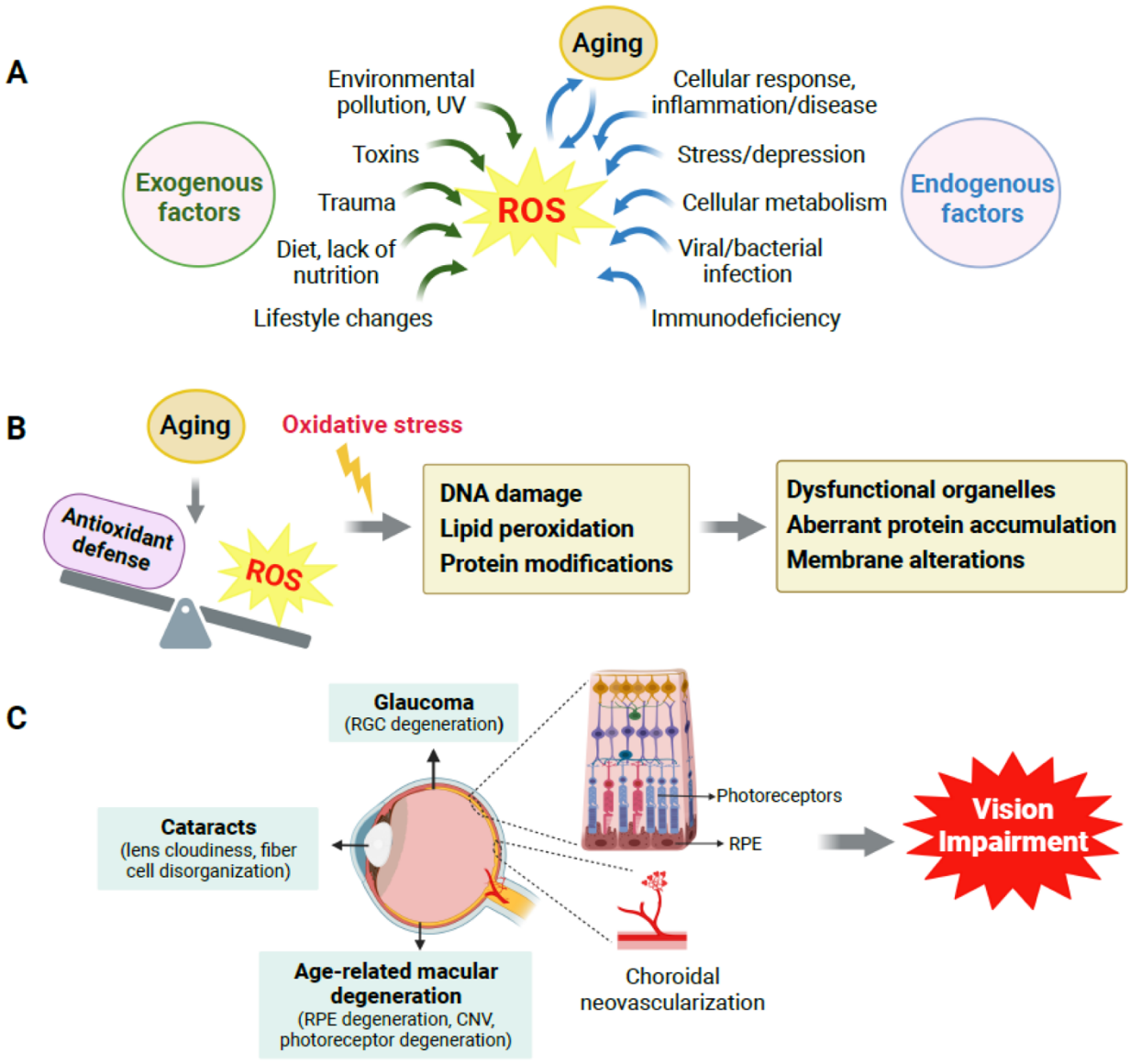

Figure 1. Aging, oxidative stress and their impact on age-related eye diseases. Figure from Kushwah et al[109].

Excess ROS can either oxidize biomolecules or structurally modify proteins and genes, thereby triggering downstream signaling cascades that may lead to the onset and progression of inflammatory diseases[111]. In fact, oxidative stress is known to mediate vascular dysregulation in cardiovascular diseases, including hypertension and

diabetes[112,113], suggesting inflammation-driven vascular dysregulation. Under normal physiological conditions, a low grade inflammation, known as para-inflammation[114], is mediated by complement and microglial activation and helps to repair minor tissue damage and restore function caused by endogenous stress. In glaucoma, para-inflammation is an important protective adaptive response in the RGC and optic nerve head[115]. With increased oxidative stress or reduced antioxidant defenses, due to increasing age, stress and/or disease, excessive uncontrolled complement or microglial activation may produce inflammatory responses with marked release of cytokines/chemokines causing irreversible damage to the RGC and optic nerve head[116]. Similarly, in the anterior chamber of the eye, para-inflammatory mechanisms may be involved in trabecular meshwork cell dysfunction and increased aqueous outflow resistance, ultimately leading to increased IOP[99].

In vascular endothelium, oxidative stress promotes systemic inflammation via activation of immune cells that migrate into the vasculature and release several factors including ROS, metalloproteinases, cytokines, and chemokines[117]. The release of these factors promote vascular damage and dysfunction, leading to vasoconstriction and vascular remodeling[118,119]. As healthy vision depends on RGC metabolic homeostasis which requires a well-regulated blood flow[120], vascular dysregulation may also affect glaucoma progression and vision recovery[120]. Impaired blood flow has been increasingly recognized to be implicated in the pathogenesis of neurodegenerative disease[121], as well as eye diseases, including age-related macular degeneration[122-124] and glaucoma[125,126] in humans. In fact , there is evidence of vascular dysregulation in the

choroid[127,128], optic nerve head[129,130], short posterior ciliary artery[131], and perifoveal macular capillaries[132] of patients with NTG and POAG, which may extend to the cerebral vasculature and contribute to an increased risk of cerebrovascular disorders[133]. Neurons of the retina (and brain) possess very high energy demands, and evidence suggests that impaired vascular autoregulation deprives RGC of oxygen and metabolites and the resulting energy insufficiency increases susceptibility of the optic nerve head to decreases in ocular perfusion pressure, increases in IOP, and increased local metabolic demands[120,134,135]. Ischemic damage, which may contribute to further impairment in autoregulation, results in changes to the optic nerve head, including axon and glial necrosis, consistent with glaucomatous optic neuropathy[135,136]. Excess ROS production within the vascular endothelium reduces the bioavailability of nitric oxide and thereby promotes vasoconstriction and subsequent vascular dysfunction[137]. It is therefore possible that oxidative stress, inflammation, and subsequent vascular dysregulation may mediate the associations of psychological disturbances such as stress, and other common physical comorbidities with glaucoma[120].

Dietary modifications such as increasing antioxidant intake can support vascular autoregulation and decrease redox state markers, maintaining cell and tissue homeostasis, thereby preventing inflammation [103,138]. Among dietary factors implicated in the pathogenesis of high IOP and glaucoma, the nutritional roles of alcohol, antioxidants, as well as certain dietary patterns and supplements have been mainly investigated.

2.4b1. Alcohol

First, alcohol has been studied in relation to IOP and glaucoma. Excessive alcohol consumption has previously been found to be associated with neurodegenerative diseases[139], which share common pathophysiological mechanisms with diseases like glaucoma[140]. Alcohol promotes the production of ROS and impairs the body's antioxidant defense mechanisms through numerous pathways, particularly in the liver[141]. First, alcohol metabolization in the liver results in the formation of acetaldehyde, a molecule whose further metabolism can lead to ROS formation[141]. Next, alcohol induces increased activity of the enzyme cytochrome P450, which metabolizes alcohol and produces ROS in the process[141]. Alcohol also induces increases in the cellular levels of free iron, which can facilitate ROS generation[141]. Further, alcohol can deplete the body's antioxidant levels, including glutathione and vitamin E[141].

In addition to oxidative stress mechanisms, alcohol may be implicated in vascular dysregulation pathways in glaucoma. A higher alcohol consumption has recently been found to be associated with more vascular dysfunction in the brain, retina, skin, kidney and in the blood[142]. For example, chronic alcohol consumption can compromise the structure and function of the kidneys and impair their ability to regulate electrolyte and fluid balance[143]. Chronic kidney disease has recently be demonstrated to be associated with glaucoma in a bidirectional manner[144], and may contribute to further vascular dysfunction and ischemia[145]. Increased alcohol intake has also been shown to be

associated with increased incidence of type 2 diabetes[146,147] and hypertension[148,149], diseases in which vascular dysfunction is a part of the pathogenesis[150,151] and putative risk factors for high IOP/glaucoma. It is therefore hypothesized that alcohol may also be implicated in the development of glaucoma. Studies reporting the associations of habitual alcohol consumption with IOP and glaucoma, however, have been inconsistent and the comparability of results is limited by the use of different alcohol measures across studies. A recent systematic review and meta-analysis found alcohol use to be associated with higher IOP and POAG in pooled analyses (OR= 1.18; 95% CI: 1.02-1.36); however, effect estimates were small and heterogeneity was considerable[152]. Alcohol types contain varying concentrations of polyphenols including flavonoids, which may exert neuroprotective effects on the retina[153]. Polyphenols represent the largest group of phytochemicals, with over 10,000 compounds, including phenolic acids, flavonoids, tannins, anthocyanidins, lignans and stilbenes[154]. These compounds have shown numerous biological benefits including: antioxidant, cardio-protective, anti-cancer, anti-inflammatory, antimicrobial, anti-ageing effects,[155] and protective actions against diabetes, obesity, cardiovascular and neurodegenerative diseases[156]. Many plants and foods including fruits, vegetables, tea, wine, flowers and microalgae are natural sources of polyphenols[157,158]. Studies have demonstrated antioxidant effects of polyphenols in RGC in vivo and vitro[159,160]. Specifically, many flavonoids were capable of increasing intracellular glutathione, reducing levels of ROS, preventing the influx of Ca(2+) and the loss of energy, despite high levels of ROS [159,160]. Polyphenols may also improve the release of nitric oxide by vascular endothelial cells, leading to vasorelaxation and

improved vascular autoregulation[161]. Some polyphenol sub-classes, including chalcones and flavonones, can be found mainly in beer while others, including tannins, stilbenes and proanthocyanidins are mainly present in wine[162]. Flavanols and flavan-3-ols are found in similar concentrations in beer and wine[162]. The mechanism of protection from oxidative stress and ischemia by polyphenols also appears to be highly specific for each compound[159,160]. Prior research has demonstrated that daily red wine and beer consumption are both associated with increases in systemic blood pressure[163], a risk factor for high IOP and glaucoma[84]. However, only one previous study examined associations of alcohol type (beer, wine, liquor, sherry) with IOP[164] and found no significant associations. ‘

2.4b2. Antioxidants

Observational research has demonstrated that higher intakes of fruits (including peaches[165] and oranges[166]), fruit juices[166], vegetables[167] (including carrots[165] and collard greens/kale[165,166]), and higher dietary nitrate and green leafy vegetable intakes[168] were associated with a reduced prevalence/incidence of glaucoma. Further, low intakes of vitamin A and vegetable fat were associated with elevated IOP and an increased risk of glaucoma[169]. Phytochemicals contained in plant foods, including carotenoids, polyphenols and flavonoids, are thought to play a key role in the protective effects exerted by a high intake of fruits and vegetables[170,171]. Polyphenols and carotenoids are antioxidant phytochemicals and play an important role in the prevention and treatment of chronic diseases caused by oxidative stress[156] Further, fruits and vegetables are high

in vitamin C, vitamin E, fiber, and magnesium; nutrients that have been associated with reduced oxidative stress[103,172,173].

Flavonoids

Flavonoids are a sub-class of polyphenols that are abundantly found in plant foods and beverages, including fruits, vegetables, tea, cocoa and wine[174]. Anthocyanins and other flavonoids can interact directly with the photoreceptor rhodopsin, enhancing its folding, stability, and regeneration[175]. Changes in retina undergoing rhodopsin degeneration include thinning of the outer nuclear layer and vision loss, specifically reduced contrast sensitivity[176]. In recent years, retinal thinning has been associated with pathology in neurodegenerative diseases, including Alzheimer's disease[177], Parkinson's disease[178] as well as IOP variability[179] and the risk of visual field progression in glaucoma patients[180]. Flavonoids may protect the ocular tissues through multiple pathways including decreasing oxidative stress-induced cell death[181], upregulating the endogenous antioxidant systems[182], inhibiting inflammatory pathways[181], enhancing mitochondrial[183] and anti-angiogenic functions[181], and modulating signaling pathways responsible for maintaining neuron function and survival[184].

Carotenoids

The macula of the eye concentrates three carotenoids: lutein, zeaxanthin, and *meso*-zeaxanthin, collectively referred to as macular pigment, that exert antioxidant and anti-inflammatory effects in the retina[185-187]. Carotenoids cannot be synthesized *in*

vivo by humans and must be obtained from dietary consumption[188]. Lutein and zeaxanthin can be obtained from green leafy vegetables as well as orange and yellow fruits and vegetables[189-192], while *meso*-zeaxanthin is believed to be formed at the macula by metabolic transformations of ingested lutein[193]. Depleted levels of these macular pigments, known as low macular pigment optical density, may be associated with an increased risk of retinopathy and/or visual impairment[194]. The macular pigment is believed to protect the retina, through three primary mechanisms: (1) by acting as a blue light filter; overexposure to blue light induces a significant increase in ROS production[195], the macular pigment screens out deleterious short-wavelength light[196]; (2) by limiting oxidative stress and inflammation induced by ROS[197]; and (3) by attenuating the deleterious effects of chronic inflammation in the macula[198-200]. Results from a recent systematic review demonstrate that greater dietary intakes of carotenoids are protective against the risk of glaucoma and that higher macular pigment optical density levels were associated with improved visual performance in glaucoma patients[194]. Like other phytochemicals, the antioxidant properties of carotenoids vary based on their chemical and physical properties[185]. Experimental rat and mouse retina models demonstrated that treatment with lutein was effective at preventing retinal damage induced by ischemia-reperfusion injury[201,202], inhibiting ischemic-induced cell death and improving endogenous glutathione levels[201].

Vitamin C and E

Vitamin C is a fat-soluble antioxidant commonly found in peppers, green leafy vegetables and in fruits such as kiwis, oranges, strawberries[203]. Vitamin C serves as an enzymatic cofactor for collagen synthesis and is effective at scavenging ROS[203]. Vitamin C protects RGC from injuries and helps restore visual function in glaucoma by lowering IOP, promoting a neuroprotective phenotype and increasing gene expression related to neurotropic factors, phagocytosis, and mitochondrial energy production[204]. After addressing heterogeneity, dietary intake vitamin C showed a beneficial association with POAG (OR= 0.39, 95% CI: 0.23–0.67) in a recent meta-analysis.[203]

Vitamin E is another fat-soluble antioxidant commonly found in nuts and seeds, avocados, and dark leafy vegetables[203,205]. Vitamin E inhibits oxidative modification of low-density lipoproteins (LDL) and prevents the formation of ROS[203]. Oxidized LDL produced by lipid peroxidation promotes pro-atherogenic changes through inflammatory and immunologic mechanisms[206]. Vitamin E deficiency may also result in peripheral neuropathy and retinopathy[203].

Fiber

Dietary fibers may also help to reduce the mediating pro-inflammatory processes involved in the pathogenic pathways of cardiometabolic diseases[207,208] and retinopathy[209] by improving gut microbial composition and function (improving food digestion and absorption), modifying diet and satiety (by lipid reduction and body weight reduction), improving lipid and glucose metabolism[210-212], and reducing intestinal[213] and systemic oxidative stress[214]. Higher dietary fiber intakes have been

associated with lower levels systemic inflammation markers in both healthy adults[215] and in those with age-related disease, including kidney disease[216] and diabetes[217].

Magnesium

Magnesium is an essential mineral that serves as a cofactor in over 300 enzymatic reactions, including those responsible for regulating blood pressure and mitochondrial energy production pathways[218,219]. Magnesium also acts as a calcium antagonist and participates in the regulation of calcium homeostasis[220]. Chronic magnesium deficiency results in excessive production of ROS and an exaggerated inflammatory response to immune and oxidative stress through the activation of neuroendocrinological pathways[218], and is commonly observed in cardiometabolic diseases including, heart failure, diabetes, hypertension and stroke[218,221]. This inflammatory response to magnesium deficiency predisposes to pro-atherogenic changes in lipoprotein metabolism, vascular endothelial dysfunction, thrombosis and hypertension[222]. As such, magnesium potentially has a major influence on the pathogenesis of cardiometabolic diseases[221,222]. In glaucoma, magnesium may protect RGC from oxidative stress by regulating voltage-dependent calcium channels, glutathione biosynthesis, lipid peroxidation, and maintaining the regulation of many enzymatic reactions[223].

Therefore, increasing antioxidant intake may improve vascular alterations associated with pro-inflammatory pathologies, including hypertension and diabetes[103], known risk factors for elevated IOP and glaucoma.

2.4b3. Dietary patterns

As individuals consume diets comprising several individual nutrients and food types in which interactions may occur, the use of dietary patterns to assess dietary exposures has become increasingly common in epidemiological studies. Conclusive evidence linking specific dietary patters with IOP and glaucoma, however, is lacking[18].

2.4b3.1. Mediterranean Diet

The Mediterranean diet, one of the most extensively studied dietary patterns, has been associated with reduced risks of type 2 diabetes[224], advanced age-related macular degeneration[225], and hypertension[226]. The Mediterranean diet is comprised largely of plant-based foods including vegetables, fruits, legumes, nuts, olive oil, and whole grains that are rich in antioxidant properties[227,228]. While higher adherence to a Mediterranean lifestyle, measured using a score of 10 habits including diet, was previously found to be associated with reduced glaucoma incidence[229], no studies to-date have found significant associations between adherence to the Mediterranean diet with IOP or glaucoma[230].

2.4b3.2. MIND Diet

The Mediterranean-Dietary Approach to Systolic Hypertension (DASH) diet intervention for neurodegenerative delay (MIND) diet is a hybrid of the Mediterranean and DASH diets, emphasizing food components such as green leafy vegetables, fish, nuts and berries, that are known for their antioxidant and neuroprotective properties[231,232]. Higher adherence to the MIND diet has been previously shown to be associated with slower rates of cognitive decline with age, and a reduced risk of Alzheimer's disease[231,233]. In the Rotterdam study, adherence to the MIND diet was recently found to be associated with a reduced risk of POAG in an IOP-independent manner[230].

2.4b4. Dietary Supplements

Finally, the relationship between dietary supplements and IOP/glaucoma has been studied. As the levels of oxidative markers are altered in patients with glaucoma[96], the use of dietary supplements rich in antioxidants may be beneficial in their management; however, there is currently a lack of supporting evidence. Further, as impaired calcium and iron regulation have been found in lamina cribrosa, retinal ganglion and trabecular meshwork cells of donors with glaucoma, it is believed that calcium and iron may play a role in glaucoma pathogenesis[234-236]. Excess cellular iron due to disease, diet, or supplementation increases the formation and release of ROS in tissues, leading to oxidative stress[237]. There are two forms of dietary iron: heme and non-heme. Meat, fish and poultry are rich in heme iron, which has a high bioavailability[238]. Vegetables and legumes are rich in non-heme iron, which has a reduced bioavailability[238]. The two dietary iron types have differing associations with

oxidative stress; non-heme iron decreases while heme iron increases oxidative stress, which highlights the effect of overall diet on oxidative stress and inflammation pathways[238]. The consumption of vegetables in legumes, rich in non-heme iron, is associated with a high-antioxidant dietary pattern which could reduce the negative effect of iron, whereas the consumption of heme containing meats is associated with a pro-oxidant diet[238]. Further, iron supplementation can increase hepcidin, a feedback regulated hormone that controls intestinal iron absorption and erythrocyte production[239], leading to lower iron absorption[240]. In fact, results from a randomized controlled trial demonstrated that healthy individuals had reduced non heme-iron absorption from food in response to iron supplementation[241]. Further, calcium supplementation may play a role in accelerating vascular calcification[242]. Vascular calcification is a chronic inflammatory process[243] resulting in arterial stiffness and is associated with higher blood pressure[244] and IOP[245]. Cross-sectional studies examining the association between the oxidants calcium and iron and glaucoma reported increased odds of glaucoma among participants who consumed ≥ 800 mg/d of supplementary calcium or ≥ 18 mg/d of supplementary iron[234] ,with high serum calcium levels[246], with high serum ferritin levels[247] or levels greater than 61 ng/mL[248], and higher total consumption of calcium[249] and iron[169,249]. A longitudinal study, however, reported no association with dietary calcium intake and incident glaucoma[250]. There is a need for more large studies examining the relationship between dietary factors and IOP/glaucoma.

2.4c. Genetic Factors, Intraocular Pressure, and Glaucoma

With heritability estimates ranging from 0.29 to 0.79, genetic factors are estimated to account for a large proportion of the variance in IOP[251-257]. Polymorphisms within the TMCO1 gene are associated with both IOP and POAG risk, suggesting partly shared genetic influences[251]. Genome wide association study (GWAS) meta-analyses have discovered over 100 genetic loci associated with IOP[258-261] and POAG[262-264] and polygenic risk scores (PRSs) derived from GWAS findings offer the prospect of performing genetic risk stratification for heritable traits and diseases. PRSs are commonly developed by summing the number of risk alleles carried by an individual, weighted by their magnitude of effect on the trait/disease of interest[265]. For example, a higher IOP polygenic risk score (PRS) has been reported to be associated with increased POAG risk[266]. However, these loci account for only a small proportion of the disease heritability[259] and biological mechanisms involved in the propensity to higher IOP and glaucoma remain largely unknown.

2.4d. Gene-Diet Interactions, Intraocular Pressure, and Glaucoma

Multiplicative interaction analysis is an important tool for understanding complex traits and multifactorial diseases. In general, multiplicative interaction is said to be present when the combined effect magnitude of two or more factors is statistically significantly different from the product of the effect magnitudes of the individual factors[267-269]. For complex traits and multifactorial diseases, assessing genetic influences without considering their interaction with environmental influences could be

misleading. The investigation of gene-environment interactions may thereby improve the ability to better predict IOP and glaucoma allowing the clinician to practice precision medicine[270]. Precision nutrition is a field of precision medicine in which individuals receive diets tailored to their personal biology, including their genomics[271]. As such, nutrigenetics may have the potential to help identify those at risk for high IOP/glaucoma and could therefore help in guiding diet. Nutrigenetic studies rely on GWAS to identify loci that are associated with various phenotypic traits and diseases[271]. As randomized controlled trials of diet interventions for IOP and glaucoma may not be feasible due to the slow progression of the disease, GWAS findings should then be validated in multiple cohorts and in functional studies prior to being incorporated into nutrigenetics[271]. For example, a gene-diet interaction was identified such that participants in the highest PRS quartile consuming the higher caffeine intake were more likely to have glaucoma and higher IOP[272]. In another study, stronger alcohol–IOP associations were observed in participants at higher glaucoma PRS quintiles ($P_{interaction} < 0.001$)[273]. The high PRS score may identify a potentially reduced reserve to withstand the frequent acute elevations of IOP associated with habitual caffeine or alcohol consumption [272,273]. A gene-diet interaction was also identified in which the odds of glaucoma was 3.87 times higher in the high-PRS group compared to the low-PRS group, but only among subjects with a low balanced diet intake[274]. Study authors hypothesized that a balanced diet intake may attenuate the genetic influence on glaucoma risk[274]. Further gene-diet interactions should be explored with IOP and glaucoma to better inform precision nutrition[275] guidance in those at high risk of glaucoma.

CHAPTER 3: Thesis Aims

Aim 1. Determine whether alcohol consumption frequency, total intake or type is associated with IOP and glaucoma.

Sub-Aim 1.1. Assess whether a glaucoma polygenic risk score (PRS) modifies the associations of the alcohol-related variables with IOP and glaucoma.

Aim 2. Determine whether dietary factors, patterns, and supplement use of calcium and iron are associated with IOP and glaucoma.

Sub-Aim 2.1. Assess whether a glaucoma PRS modifies the associations of the diet-related variables with IOP and glaucoma.

Aim 3. Identify whether ethnicity is associated with IOP and glaucoma, and if so, explore potential social, behavioral, genetic and health-related reasons.

CONCEPTUAL FRAMEWORK

The conceptual framework for aims 1-2 is shown in Figure 2. Confounding factors that have been identified a priori may bias the associations of baseline dietary factors with IOP and glaucoma, including non-time varying factors: age, sex, ethnicity, education and income [37-40]. Time varying factors including BMI, physical activity, tobacco/alcohol consumption, energy imbalance, eye care utilization and the presence of comorbid conditions[41-46] were also identified as potential confounders, however, the associations of time-varying covariates with baseline dietary factors, IOP, and glaucoma are time-dependent and the nature of the associations therefore depends on the time of measurement of each variable. For example, it is possible that certain factors may be classified as a confounder, intermediate variable, or a collider at different time points, depending on the time at which the covariates are measured. However, given the large sample size of the CLSA, we were not highly concerned of the potential increase in bias and decreased precision from the inclusion of variables that did not act as true confounders in the CLSA dataset and therefore included the time-varying factors in our original models. As gene-diet interactions have been identified for elevated IOP and glaucoma[272-274], interactions within the CLSA dataset were also evaluated. Specifically, the PRS may modify the associations between alcohol and dietary factors and the outcomes. The reason for this is that having a high number of genetic risk factors for elevated IOP/glaucoma may increase the susceptibility of a person to environmental risk factors for elevated IOP/glaucoma. As previous studies have reported differing

strengths of association between alcohol and IOP by sex[276,277], we also assessed whether sex modified the associations of alcohol with IOP and glaucoma.

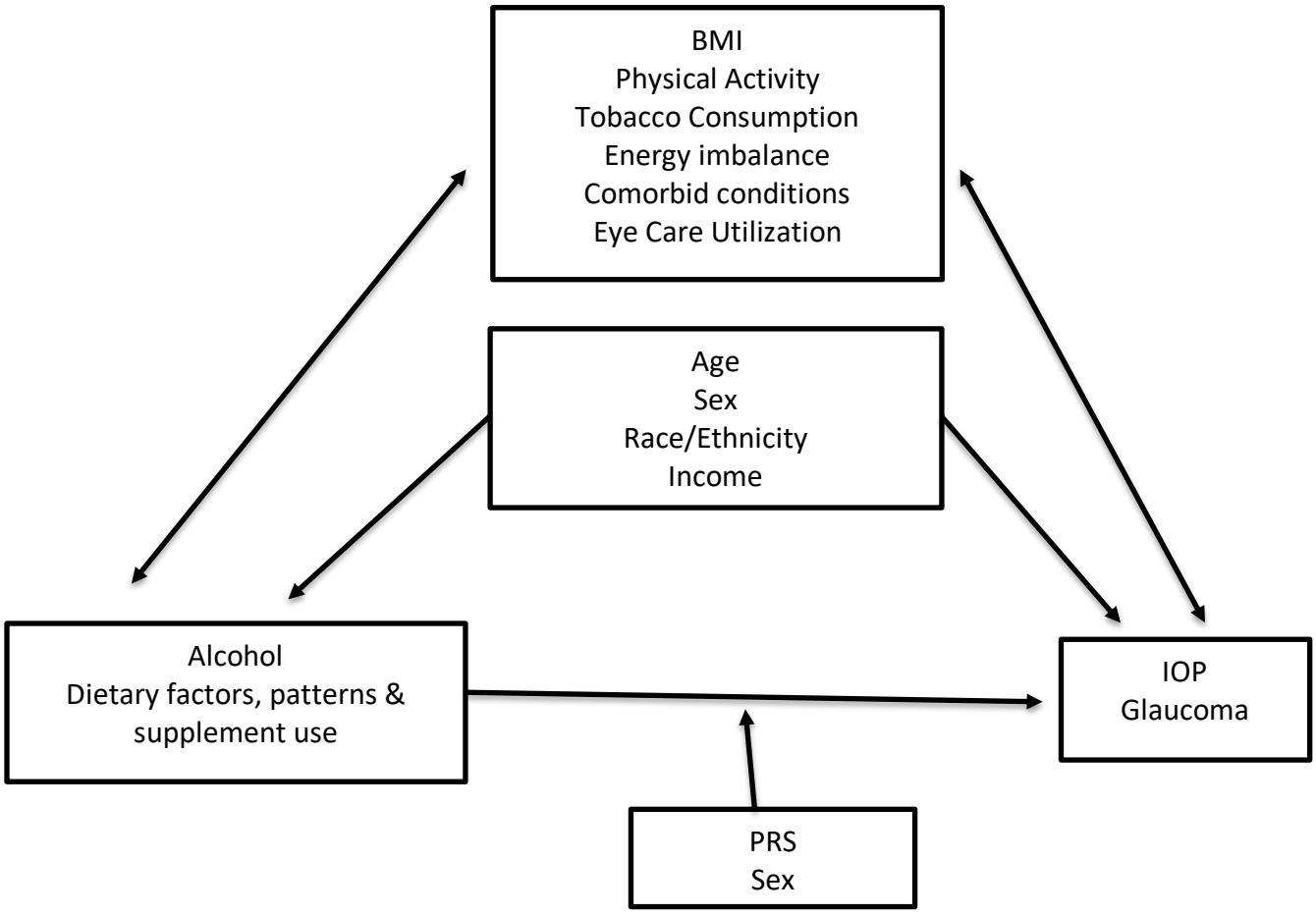

Figure 2. Conceptual Framework used to guide Aims 1 and 2

The conceptual framework used to formulate aim 3 and guide data analysis is presented in Figure 3. Based on our review of the literature, age, sex, education, income, BMI, physical activity, tobacco/alcohol consumption, as well as by the presence of comorbid conditions such as hypertension or diabetes, eye care utilization, and genetic factors[36-46] are risk factors for elevated IOP and glaucoma and therefore the

adjustment of these variables may improve the precision of the direct effect estimates of race/ethnicity on these outcomes. As previously mentioned, the direction of the associations between time-varying risk factors with IOP and glaucoma is dependent on the time of measurement of each variable, however these variables were included in our original models. We expected the glaucoma polygenic risk score (PRS) would confound the association between ethnicity and the outcomes because different ethnic groups may have different genetic risks and the PRS is a risk factor for both outcomes. Although socioeconomic factors such as education and income may mediate the associations, we were interested in examining the direct effect of race/ethnicity on IOP/glaucoma that does not pass through mediators and therefore adjusted for the potential mediators in analyses.

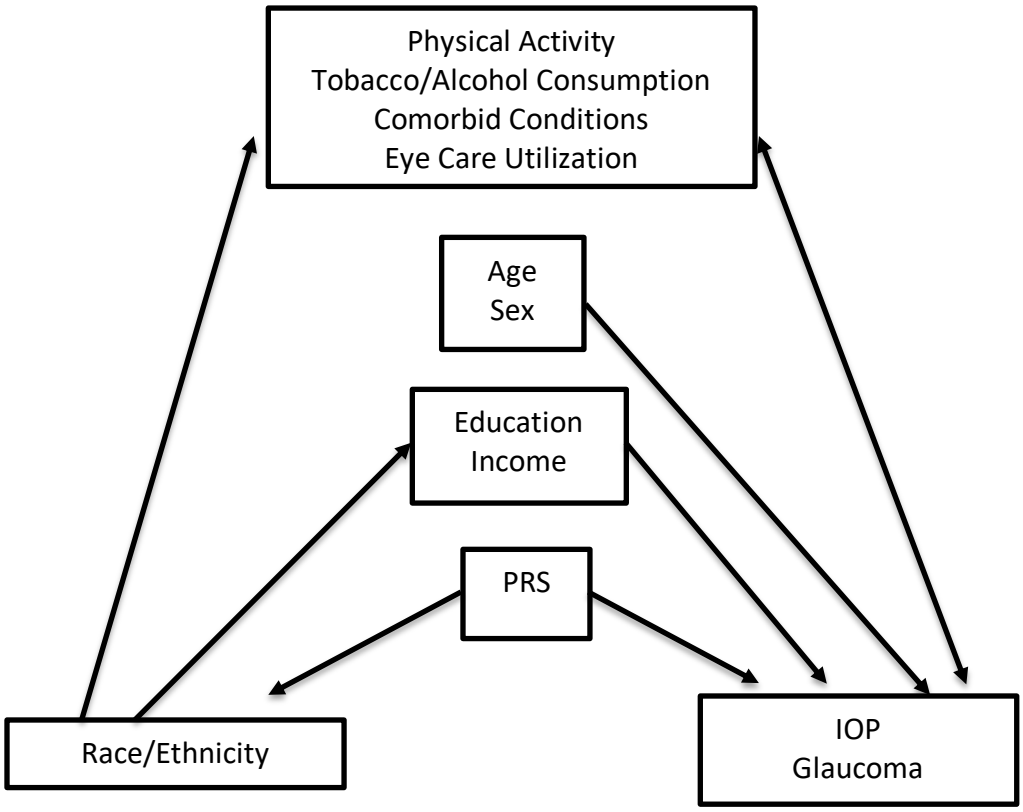

Figure 3. Conceptual Framework used to guide Aim 3

Directed Acyclic Graphs

An alternative to the conceptual frameworks above will also be presented and the results shown in supplementary analyses in Chapter 6. In observational studies, such as the CLSA, establishing causality is complicated by the presence of biases and confounders, some of which may be hidden or unmeasured. These issues are particularly salient when studying cross-sectional data, as there may be several possible explanations for the observed associations[278]. Therefore, the causal diagram framework or Directed Acyclic Graph (DAG) developed by Pearl[279] was used to explore the causal

relationships between dietary exposures and race/ethnicity with IOP and glaucoma in adults.

DAG models impose conditional independence constraints on a multivariate probability distribution and the acyclic path diagrams represent the assumed relationships between variables in a specified context[280,281]. Conditional independence implies that an exposure variable is independent of the outcome variable if all paths between the exposure and outcome are blocked by conditioning (or adjusting) on a set of variables. A confounder is a factor that causes both the exposure and outcome. In DAGs, confounding paths, colliding paths and mediators that can introduce bias can be visually depicted using DAGs. A confounding path is an open path between the exposure and outcome variables that passes through one or more confounders and can be closed by conditioning on one or more of the covariates on that path[281]. A colliding variable is a factor that both the exposure and outcome cause. A colliding path in a DAG is a closed path between the exposure and outcome that passes through one or more colliders[281]. These paths remain closed unless the colliders, or one of their descendants, are conditioned on. A mediator is a variable on the causal pathway between the exposure and outcome and conditioning on a mediating variable can induce a spurious association or eliminate an association between the exposure and outcome. A sufficient adjustment set is any set of variables that, if fully conditioned on, will provide an unbiased estimate for the exposure-outcome association of interest by closing all paths that are not causal and leaving all causal paths open[282].

To explain all potential pathways in which diet, alcohol, and ethnicity could be associated with IOP and glaucoma, DAGs were drawn using the online tool DAGitty (http://www.dagitty.net)[282]. The key covariates and their relationships with the exposures and outcomes were selected based on a review of the literature, presented in Chapter 2. Relevant variables were arranged in temporal order to establish whether they were confounders, colliders, or mediators of the primary relationships, Diet/ Alcohol → IOP, Glaucoma and Race/Ethnicity → IOP, Glaucoma.

Figures 4-6 present the final versions of the DAGs created to study relationships between dietary exposures and race/ethnicity with IOP and glaucoma. The DAGs depict assumptions about causal inference and competing exposures (confounders) and establish which of the exposures and confounders precede the outcomes of high IOP/glaucoma. These included paths through socioeconomic variables, health-related behaviours, biological measures, or a combination of the above.

Dietary exposures

Drinking excessive alcohol could cause biological changes such as oxidative stress, inflammation, and vascular dysregulation (Figure 4)[141,148,149]. These markers of biological stress could cause elevated IOP and glaucoma[109,120]. Other variables are expected to act as confounders or 'chance confounders' since they are expected to be either causally related to both alcohol and oxidative stress mechanisms influencing IOP and glaucoma (age, sex, education/income, physical activity, diet, smoking,

race/ethnicity, discrimination, diabetes) or causally or non-causally related to diet and causally related to glaucoma/IOP or to oxidative stress mechanisms influencing IOP and glaucoma (PRS). As the relationship between variables in DAGs must be directed, high blood pressure and chronic kidney disease (CKD) were not included due to potential bidirectional associations with vascular oxidative stress[283,284] and glaucoma[144], respectively. As energy imbalance may be influenced by both diet and physical activity levels, it was identified as a potential colliding variable. Furthermore, sex and the glaucoma PRS is expected to modify the association between alcohol and glaucoma/IOP. Age, sex, education, income, diabetes, diet, physical activity, smoking and race/ethnicity are considered the minimal sufficient adjustment set for estimating the total effect of alcohol on IOP and glaucoma.

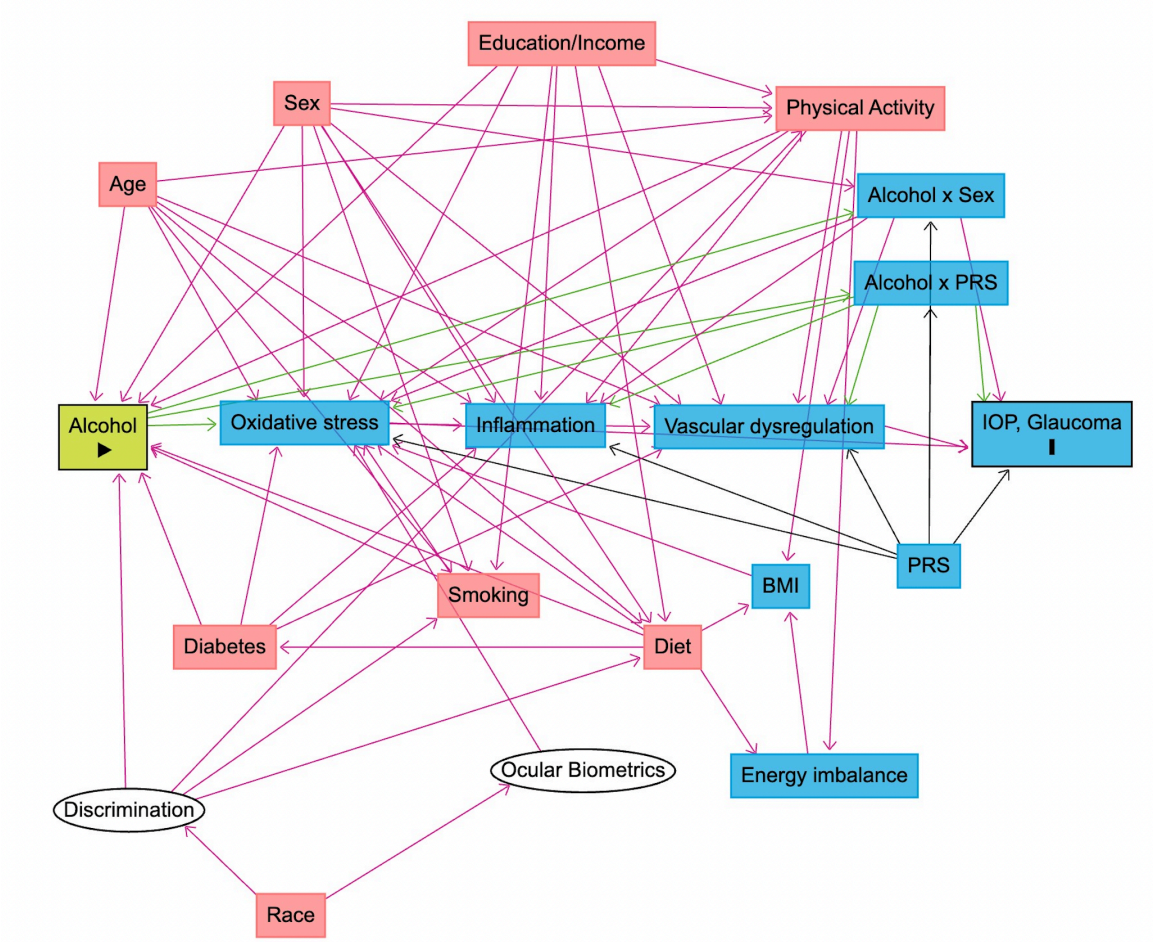

Figure 4. Directed acyclic graph (DAG) describing possible pathways through which alcohol use may lead to elevated IOP and glaucoma. IOP; intraocular pressure, PRS; polygenic risk score; Alcohol X Sex; Alcohol-sex interaction term, Alcohol X PRS; Alcohol-PRS interaction term.

Legend: The variable in green with the "<" symbol inside the rectangle is the exposure variable; the variable in blue with the letter "I" inside the rectangle is the outcome variable; variables in blue are the ancestors of the outcome; variables in red are ancestors of the outcome and exposure variables; variables in white circles are unobserved (latent); green arrows depict causal paths and pink arrows depict biasing paths.

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According to Figure 5, not eating a Mediterranean diet or a diet high in antioxidants could cause elevated BMI, high cholesterol, and diabetes which could then cause biological changes such as oxidative stress, inflammation, and vascular dysregulation[285]. These markers of biological stress could cause elevated IOP and glaucoma[109,120]. Other variables are expected to act as confounders or 'chance confounders' since they are expected to be either causally related to both diet and oxidative stress mechanisms influencing IOP and glaucoma (age, sex, education/income, physical activity, smoking, race/ethnicity, discrimination, diabetes) or causally or non-causally related to diet and causally related to glaucoma/IOP or to oxidative stress mechanisms influencing IOP and glaucoma (PRS). BMI and cholesterol were expected to mediate the associations of diet and oxidative stress and therefore the adjustment of these variables would bias the effect estimate of diet on IOP/glaucoma. High blood pressure due and CKD were excluded from the DAG due to their potential bidirectional associations with vascular oxidative stress[283,284] and glaucoma[144], respectively. Energy imbalance may also be a collider as it is influenced directly by both diet and physical activity levels. Furthermore, the glaucoma PRS is expected to modify the association between dietary patterns and glaucoma/IOP. The minimal sufficient adjustment set for estimating the total effect of diet on IOP and glaucoma is age, sex, education, income, smoking and discrimination. As racial discrimination and energy imbalance were not measured in the CLSA, race and total caloric intake were used as proxy variables in supplemental analyses.

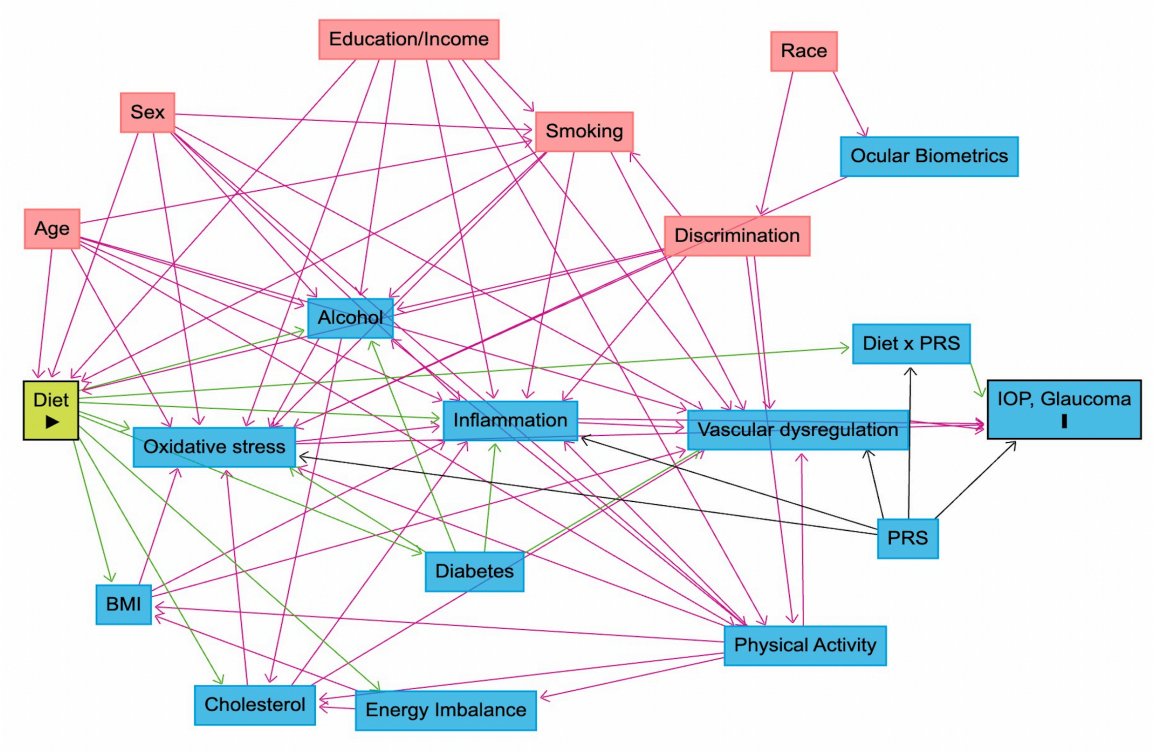

Figure 5. Directed acyclic graph (DAG) describing possible pathways through which dietary patterns may lead to elevated IOP and glaucoma. BMI; body mass index, IOP; intraocular pressure, PRS; polygenic risk score, Diet X PRS; Diet-PRS interaction term.

Legend: The variable in green with the "<" symbol inside the rectangle is the exposure variable; the variable in blue with the letter "I" inside the rectangle is the outcome variable; variables in blue are the ancestors of the outcome; variables in red are ancestors of the outcome and exposure variables; variables in white circles are unobserved (latent); green arrows depict causal paths and pink arrows depict biasing paths.

Race/ ethnicity

According to Figure 6, people of different races and ethnicities differ in the shape and size of the various structures of the eye (ocular biometrics) which over time could result in different amounts of oxidative stress, inflammation, and vascular dysregulation[91,92]. These markers of biological stress could cause elevated IOP and glaucoma[109,120]. The glaucoma PRS is expected to act as a confounder as it is causally

related to both race/ethnicity and IOP/glaucoma. Other variables may act as mediators since they are expected to be causally related to oxidative stress mechanisms influencing IOP and glaucoma and caused by race/ethnicity through discrimination (education/income, smoking, alcohol, physical activity, diet, diabetes, BMI). Age and sex are not-causally related to race/ethnicity but may confound the associations of diet, smoking, alcohol intake and physical activity with oxidative stress, inflammation, and vascular dysregulation. Due to the potential bidirectional associations of high blood pressure and oxidative stress[283,284] and CKD and glaucoma[144], these variables were excluded from the DAG model. While adjusting for mediating variables biases estimates of the total effect of the exposure on an outcome, mediator adjustment is reasonable in racial disparity studies in which you are interested in examining the direct effect of the exposure on the outcome that doesn't pass through the mediating variables. For example, adjusting for sociodemographic and health-related variables will allow us to isolate the effect of race/ethnicity on IOP/glaucoma that is not explained by the mediators. Therefore, while the minimal sufficient adjustment set for estimating the total effect of race/ethnicity on IOP and glaucoma is the glaucoma PRS, we also adjusted for age, sex, education/income, smoking, alcohol, physical activity, diet, diabetes, and BMI. A lack of eye care utilization may also be non-causally related to race/ethnicity and could directly cause glaucoma by leading to untreated elevated IOP. While eye care utilization could act as a 'chance confounder' the causal association of race/ethnicity with IOP and glaucoma, it could also be affected by having elevated IOP or glaucoma

since it must be managed through routine follow-up and was therefore excluded from the DAG.

The process of selecting the statistical models for analyses in the following chapters was informed by these conceptual frameworks.

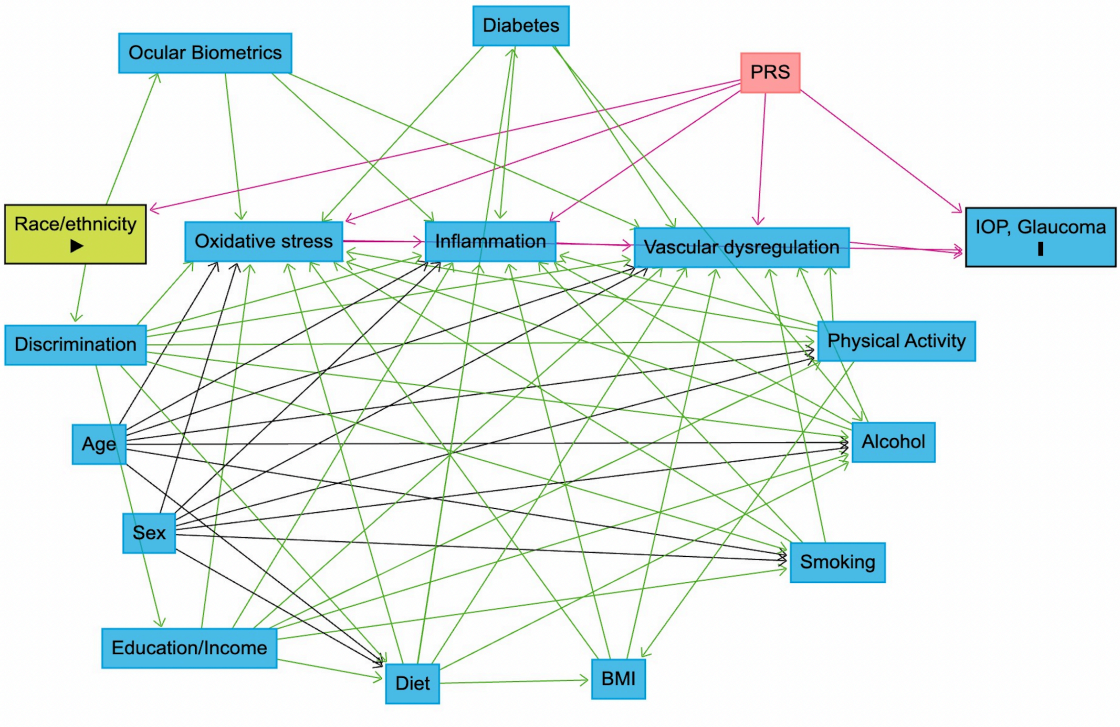

Figure 6. Directed acyclic graph (DAG) describing possible pathways through which race/ethnicity may lead to elevated IOP and glaucoma. IOP, intraocular pressure, PRS; polygenic risk score.

Legend: The variable in green with the "<" symbol inside the rectangle is the exposure variable; the variable in blue with the letter "I" inside the rectangle is the outcome variable; variables in blue are the ancestors of the outcome; variables in red are ancestors of the outcome and exposure variables; variables in white circles are unobserved (latent); green arrows depict causal paths and pink arrows depict biasing paths.

CHAPTER 4: METHODS

METHODS

Study Population and Design

The CLSA is a prospective cohort study of 30,097 adults from across Canada with data collected every 3 years[286]. A cross-sectional analysis was performed using the first round of data collection. We only used the first round of data collection because this allowed us to maximize statistical power by looking at glaucoma prevalence instead of incidence and because we did not expect IOP to change very much over a 3-year period[287]. The sample of the Comprehensive Cohort was obtained by utilizing stratified random sampling of provincial healthcare registration databases and random digit dialing of landline telephones. In the 2010 Statistics Canada Residential Telephone Service Survey, approximately 4% of households with adults aged 55 years and older used a cell phone exclusively and therefore exclusion of cell-phone only households was anticipated to have a modest impact on recruitment[288]. The overall CLSA Comprehensive Cohort response rate was 10%. Baseline demographic, lifestyle, social, health service and medication use data were obtained between 2012 and 2015 via 70-minute in-home interviews. Detailed physical assessments were performed within 2-3 weeks of the in-home interview at data collection sites within 25-50km of participant homes, which are located in Victoria, Vancouver, Surrey, Calgary, Winnipeg, Hamilton, Ottawa, Montreal, Sherbrooke, Halifax and St. John's at CLSA-affiliated universities.

To be included in the study, participants had to be aged 45-85 years, community dwelling, cognitively unimpaired, and speak English or French. Exclusion criteria

included being a full-time member of the Canadian Armed Forces, residing on a federal First Nations reserve or settlement, living in a long-term care institution, or not a permanent resident or Canadian citizen. These exclusion criteria were selected to coincide with the Canadian Community Health Survey (CCHS) sampling frame, as the CCHS Healthy Aging sample was used to recruit participants into the CLSA tracking cohort.

Ethics Approval and Data Access

The CLSA submitted a full-integrated protocol, including the Comprehensive Cohort protocol, in July 2010 to the affiliated universities and REB approval was received. Ethics approval for our analyses was obtained from the University of Ottawa in October 2021 (H-05-19-4466). We also received project approval from the CLSA.

DATA AVAILABILITY

Intraocular Pressure and Glaucoma

IOP was measured at the CLSA data collection sites using the Reichart Ocular Response Analyzer (Reichart Technologies, Depew, NY, USA). The average IOP of the right and left eyes was used to derive participant-level IOP values. If one eye had missing IOP data, then the IOP value of the other eye will be used. If a participant was taking medication with a drug identification number (DIN) indicative of an IOP-lowering eye drop, we imputed estimates of their pretreatment IOP by dividing their mean IOP by 0.7, which is the mean treatment effect of pressure-lowering eye drops[289]. We used corneal-

compensated IOP, which is adjusted for corneal mechanical properties. Participants were asked to report if they have ever had a physician diagnosis of glaucoma.

Race/Ethnicity

Race/ethnicity were reported using an interviewer-administered questionnaire at baseline. In order to avoid small numbers, participants were grouped into 1 of 8 racial/ethnic groups including 1) White, 2) Black, 3) Chinese, Japanese and Korean, 4) Southeast Asian and Filipino, 5) South Asian, 6) Arab and West Asian, 7) Latin American, and 8) Other.

Alcohol

Alcohol use was measured by interviewers during the in-home visit by asking participants "About how often during the past 12 months did you drink alcohol?". Participants were then categorized as never, occasional, weekly or daily drinkers. We defined occasional drinking as occurring 0-3 times per month and weekly drinking as 1-5 times per week. Daily drinking was defined as drinking 6 or more times per week.

Data on the type of alcohol consumed (white and red wine, beer, liquor, and other alcohol) were obtained in responses to the following questions: "In a typical week during the past 12 months, how many drinks of each of the following do you drink on weekends, that is, on Fridays and Saturdays?" and "In a typical week during the past 12 months, how many drinks of each of the following do you drink on weekdays, that is, from Sundays through Thursdays?". A drink was defined as 12 oz of beer, 5 oz of wine,

or 1.5 oz of liquor. Weekly consumption was then calculated for each alcohol type by adding weekday and weekend consumptions. People not currently drinking were assigned zero drinks of each type of alcohol. The weekly consumptions of each type of alcohol were added together. Total alcohol intake (grams/week) was then calculated by multiplying the total number of drinks per week by 13.45 grams. For more interpretable results, total alcohol intake was divided by 70 grams (~5-drink increase per week). Total alcohol values greater than 500g/week (~38 standard drinks) were considered probable reporting errors and were excluded[273].

Dietary Factors

Nutrition was assessed using a validated 36-item Short Diet Questionnaire (SDQ), designed to measure usual consumption (last 12 months) of total fat, fatty acids, cholesterol, trans fat, dietary fiber, calcium, vitamin D, and servings of fruit and vegetables[290]. As compared to the reference standard (means of three non-consecutive 24 hour diet recalls), Spearman correlations between the key nutrients and foods estimated by the SDQ are modest and significant ($p<0.01$)[290]. Responses are given in consumption frequencies, as the number of times food or beverage items were consumed per day, week or month. Dietary supplementation was measured by asking participants if they took calcium or iron supplements in the last month.

Dietary patterns

To assess the dietary features of our study population, we scored all participants according to their adherence to specific dietary patterns, including one of the most studied a priori dietary patterns (the Mediterranean-style diet), as well as a posteriori dietary pattern derived from CLSA data.

Mediterranean-Style Dietary Pattern

We constructed a modified version of the Mediterranean-Style Dietary Pattern Score (MSDPS)[291], developed by Rumawas et al. to measure adherence to a Mediterranean-style dietary pattern in non-Mediterranean populations. The MSDPS comprises of 13 components of the Mediterranean diet pyramid[292] including whole-grain cereals, fruits, vegetables, dairy, wine, fish, poultry, olives-legumes-nuts, potatoes, eggs, sweets, meats, and olive oil; each component except olive oil is scored from 0 to 10 depending on the level of adherence. The sum of the component scores ranges from 0-100 after standardization and is weighted by the proportion of energy consumed from Mediterranean diet foods. We modified certain components based on the data available in the CLSA, for example olive oil consumption was not measured in the SDQ and therefore was not included. Further, we included both cereals and bread products in the whole-grain component to more accurately reflect the standard Canadian diet[293]. As olive oil intake is not measured in the CLSA, this component was replaced by the ratio of monounsaturated (MUFA) to saturated (SFA) fatty acids, which has been used as an indirect parameter previously[294-298]. MUFA, SFA and total caloric intakes were calculated by methods previously described[290] using participants' reported frequencies of consumption of each SDQ item, for standard portion sizes estimated from a full food

frequency questionnaire[299] administered in the NuAge study[300], and a nutrient database based on the 2015 Canadian Nutrient File. A ratio of 1.5 or more of MUFA to SFA yielded 10 points, a ratio ≤ 1and <1.5 yielded 5 points and a ratio <1 yielded 0 points.

Hybrid method: wPLS (weighted partial least squares)

To define an "antioxidant-rich" dietary pattern, the total antioxidant intake (comprising of beta-carotene, vitamin C and Vitamin E) was used. Beta-carotene, vitamin C and vitamin E intakes were also calculated by methods previously described[290]. These nutrients were selected as they have been previously found to be associated with lower IOP and reduced risk of glaucoma[301] and had complete data available in the 2015 Canadian Nutrient File for the food items measured in the SDQ. For easier interpretation, all foods were aggregated into larger food categories based on nutrient profiles. Twenty-one food variables were selected as predictor variables in the wPLS model.

The wPLS antioxidant-rich dietary pattern score was calculated by summing the product of food intake and factor loading in the wPLS model of all predictor variables. To validate the derived dietary pattern from wPLS, random cross-split validation was performed five times. The Pearson correlations between the identified wPLS patterns for each half-split and the original sample was then examined.

Genetic Factors

The Affymetrix Axiom array was used to perform genome-wide genotyping of non-fasting blood samples from consenting participants of the CLSA Comprehensive

Cohort, resulting in 794,409 single nucleotide polymorphisms (SNPs) from 26,622 participants[302]. Release 3 of the CLSA genomic data was used and followed the marker- and sample-based quality control checks performed by the CLSA according to standard procedures[303]. Marker-based checks included checks for genotype consistency across genotyping batch, chromosomally defined sex, Hardy-Weinberg equilibrium, and discordance of genotyping across control replicates, while sample-based checks included checks for relatedness, heterozygosity, and genotype missingness. We excluded 15 individuals with extreme values of heterozygosity and genotype missingness and 1,666 related individuals. In addition, the CLSA genomic data release included genotype data imputed using the TOPMed reference panel at the University of Michigan Imputation Service, containing 97,256 reference samples at 308,107,085 genetic markers[303]. Both the genotyped and imputed SNP data were used to calculate a glaucoma polygenic risk score (PRS) for each CLSA participant with available genotype data that passed quality control checks. The PRS was developed by Craig et al.[304] based on 2,673 independent SNPs associated with glaucoma from their recent multitrait analysis of genome-wide association studies. After SNP alignment between the Craig et al. PRS SNPs and the CLSA data based on genome build GRCh38/hg38, 2,652 SNPs were available to calculate the PRS. Given the small proportion of missing SNPs, proxy SNPs were not selected to replace the missing ones. The PRS was calculated for each CLSA participant using a weighted sum of the 2,652 SNPs: $\sum_{i=1}^{2652} \hat{\beta}_i \times \text{SNP}_i$, where $\hat{\beta}_i$ is the estimated effect size of SNP_i on glaucoma from Craig et al. and SNP_i is the number of

copies of the effect allele in an individual genotype or the expected number of copies of the effect alleles for imputed genotypes (allelic dosage).

Covariates

We adjusted for potential confounders as shown in Figures 1 and 2. Demographic data including sex, age, education, and household income were collected in the CLSA questionnaire. Height and body mass were measured using standardized procedures at data collection site visits. BMI was calculated and classified according to the World Health Organization cut-points (underweight < 18.5 kg/m^2, normal weight 18.5–24.9 kg/m^2, overweight 25.0–29.9 kg/m^2, and obese ≥ 30.0 kg/m^2)[305]. Participants also reported whether they ever received a physician diagnosis of any one of 42 chronic conditions including diabetes and high blood pressure. Blood pressure was measured six times using the BpTru BPM200 blood pressure monitor (Medaval, Dublin, Ireland). The first reading was discarded, and the average of the subsequent five readings was used. Hypertension was defined if a participant reported a physician diagnosis of hypertension or if the average systolic blood pressure was 130 mmHg or higher or diastolic blood pressure was 80 mmHg or higher[306]. Nonfasting blood samples were collected from participants at data collection site visits. A complete lipid profile, including total cholesterol, and hemoglobin A1c (HbA1c) levels were measured by clinic analyzer (Roche Diagnostics Cobas 8000 series).

Lifestyle factors such as tobacco consumption (current and former smoking habits) were measured via questionnaire. Smoking status was classified as either

current, never, or former based on participant responses to the interview questions "Have you smoked at least 100 cigarettes in your life?" and "At the present time, do you smoke cigarettes daily, occasionally (at least once in last 30 days), or not at all (not in last 30 days)?" A current smoker was defined as a person who reported smoking at least 100 cigarettes and currently smokes daily or occasionally while a former smoker was someone who reported smoking at least 100 cigarettes in life but had not smoked in the last 30 days. For medication use, participants were asked if they have ever taken certain medications during data collection site visits. Medications were also reviewed in-person during the home visits in which interviewers recorded the generic (chemical) name of each medication the participants were taking as well as the dosage and the DIN. Self-reported physical activity (previous 7 days) was measured using the Physical Activity Scale for the Elderly (PASE). A total PASE score was computed by multiplying time spent in occupational, household and leisure activity types by empirically derived item weights and then summing overall activities[307].

Statistical Analysis

Preliminary exploratory data analyses were conducted using frequency tables, descriptive statistics, and graphical displays to assess the distributions of exposure and outcome variables. Logistic regression was used to assess the associations of race/ethnicity, dietary factors, patterns, and gene-diet interactions with glaucoma. Linear regression will be used to assess the association of these factors with IOP. We adjusted for the confounding variables that were identified in our conceptual frameworks while being careful not to adjust for overly correlated independent

variables. Further, we performed supplementary analyses (Chapter 6) in which we adjusted for the minimal sufficient adjustment sets identified in the DAG models in order to avoid bias induced by conditioning on mediating or colliding factors. Potential mediation was then assessed using Sobel-Goodman tests for linear regression models and by assessing the impacts of adjusting versus not adjusting for these variables in logistic regression models[308]. To account for the complex survey design, analytic weights and strata variables were used in all analyses. As recommended by the CLSA, a dummy variable for province was included in the regression models. Multiplicative interaction was assessed by adding interaction terms into the models and by stratifying regression models by PRS quartile; a P-value less than 0.05 was considered statistically significant. All analyses will be performed using Stata software Version 17 SE (College Station, Texas).

CHAPTER 5: MANUSCRIPTS

COVER PAGE

Manuscript #1 of the thesis follows. This manuscript is currently published at the journal *Investigative Ophthalmology and Visual Science*. My role was to design the analysis, derive all variables including the alcohol intake and type variables but not including the PRS variable, conduct the analyses, and prepare the manuscript under the supervision of Dr. Freeman and my TAC.

Alcohol consumption, genetic risk, and intraocular pressure and glaucoma: The Canadian Longitudinal Study on Aging

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ABSTRACT

Purpose: To examine the association of alcohol consumption with intraocular pressure (IOP) and glaucoma and to assess whether any associations are modified by a glaucoma polygenic risk score (PRS).

Methods: Cross-sectional analysis of data from the Canadian Longitudinal Study on Aging Comprehensive Cohort, consisting of 30,097 adults ages 45 to 85 years, was done. Alcohol consumption frequency (never, occasional, weekly and daily) and type (red wine, white wine, beer, liquor, other) were measured by interviewer-administered questionnaire. Total alcohol intake (grams/week) was estimated. IOP was measured in mmHg using the Reichert Ocular Response Analyzer. Participants reported a diagnosis of glaucoma from a doctor. Logistic and linear regression models were used to adjust for demographic, behavioral, and health variables.

Results: Daily drinkers had higher IOP compared to those who never drank (β=0.45, 95% confidence interval (CI): 0.05, 0.86). An increase in total weekly alcohol intake (per 5 drinks) was also associated with higher IOP (β=0.20, 95% CI: 0.15, 0.26). The association between total alcohol intake and IOP was stronger in those with a higher genetic risk of glaucoma (P for interaction term= 0.041). Alcohol consumption frequency and total alcohol intake were not associated with glaucoma.

Conclusion: Alcohol frequency and total alcohol intake were associated with elevated IOP but not with glaucoma. The PRS modified the association between total alcohol intake and IOP. Findings should be confirmed in longitudinal analyses.

INTRODUCTION

Alcohol consumption is a significant public health concern and has been recognized as a major modifiable risk factor for all-cause mortality[1] and age-related disease including cancer[2], liver disease[3], and diabetes[3]. Despite the disease burden, alcohol use remains highly prevalent. According to the 2017 Canadian Tobacco, Alcohol, and Drugs Survey, 21% of those who consumed alcohol exceeded Canada's Low-Risk Alcohol Drinking Guideline for chronic effects, which at the time was 10 drinks per week for women and 15 drinks per week for men[4].

Excessive alcohol consumption has previously been found to be associated with neurodegenerative diseases[3], including Alzheimer's disease, which shares common pathophysiological mechanisms with diseases like glaucoma[5]. Alcohol consumption is also associated with an increased risk of hypertension[6], itself a risk factor for high eye pressure. It was therefore hypothesized that alcohol may also be implicated in the development of glaucoma. Studies reporting the associations of habitual alcohol consumption with intraocular-pressure (IOP) and glaucoma, however, have been inconsistent. A recent systematic review and meta-analysis found alcohol use to be associated with higher IOP and open-angle glaucoma in pooled analyses [7]; however, effect estimates were small and heterogeneity was considerable. Some of this

heterogeneity may be explained by the lack of consideration of genetic factors in most previous studies. With heritability estimates ranging from 0.29 to 0.79, genetic factors may account for a large proportion of the variance in IOP[8-14] and in recent years a polygenic predisposition to elevated IOP/glaucoma has been identified[15-17]. For a disease like glaucoma, assessing genetic factors or environmental factors in isolation without considering their interaction could be misleading. In fact, interactions between genetic predisposition to IOP/glaucoma and lifestyle factors such as caffeine intake and diet have been reported previously[18,19]. To our knowledge, only one study has reported on the joint effects of polygenic risk and alcohol consumption in relation to IOP/glaucoma, and they found a stronger association between alcohol consumption and IOP in those with the most genetic risk[20]. Other studies reported sex-related differences in the association between alcohol and IOP[21,22].

As such, we used cross-sectional data from a large population-based sample of Canadian adults to examine associations of alcohol consumption with IOP and glaucoma and assessed whether associations were modified by a polygenic risk score or sex.

METHODS

Study Population and Design

We performed a cross-sectional analysis using the first round of data collection from the CLSA Comprehensive Cohort, which consists of 30,097 Canadian adults aged 45-85 years, with data collected every 3 years[23]. We only used the first round of data collection because not enough people have developed incident glaucoma to perform a longitudinal analysis at this time. The sample of the Comprehensive Cohort was

obtained by utilizing stratified random sampling of provincial healthcare registration databases and random digit dialing of landline telephones. Baseline data were obtained between 2012 and 2015 via in-home interviews and in-person physical examinations and biospecimen sample collections at CLSA data collection sites, which are located in Victoria, Vancouver, Surrey, Calgary, Winnipeg, Hamilton, Ottawa, Montreal, Sherbrooke, Halifax and St. John's. To be included in the study, participants had to be aged 45-85 years, community dwelling, cognitively unimpaired, and speak English or French. Exclusion criteria included being a full-time member of the Canadian Armed Forces, residing on a federal First Nations reserve or settlement, living in a long-term care institution, or not being a permanent resident or Canadian citizen.

Informed Consent and Ethics Approval

Written informed consent was obtained for all participants. Research Ethics Board approval was obtained for all CLSA affiliated sites in July 2010. Ethics approval for the present analysis was obtained from the University of Ottawa in October 2021.

Ocular Data

Participants were asked to report if they have ever had a physician diagnosis of glaucoma. IOP was measured at the CLSA data collection sites using the Reichert Ocular Response Analyzer (Reichert Technologies, Depew, NY, USA). The average IOP of the right and left eyes was used to derive participant-level IOP values. If one eye had missing IOP data, then the IOP value of the other eye was used. To estimate the pre-treatment

IOP, the IOP of those participants taking medications with a Drug Identification Number indicative of an IOP-lowering eye drop was divided by 0.7, which is the mean estimated treatment effect[24]. We used corneal-compensated IOP in our analyses, which is adjusted for corneal mechanical properties. IOP values greater than 60 mmHg were excluded, as these were considered probable measurement errors (n=3 people) based on expert opinion.

Alcohol

Alcohol use was measured by interviewers during the in-home visit by asking participants "Have you ever drunk alcohol" and "About how often during the past 12 months did you drink alcohol?". Participants were then categorized as never, occasional, weekly or daily drinkers. We defined occasional drinking as occurring 0-3 times per month and weekly drinking as 1-5 times per week. Daily drinking was defined as drinking 6 or more times per week.

Data on the type of alcohol consumed (white and red wine, beer, liquor, and other alcohol) were obtained in responses to the following questions: "In a typical week during the past 12 months, how many drinks of each of the following do you drink on weekends, that is, on Fridays and Saturdays?" and "In a typical week during the past 12 months, how many drinks of each of the following do you drink on weekdays, that is, from Sundays through Thursdays?". A drink was defined as 12 oz of beer, 5 oz of wine, or 1.5 oz of liquor. Weekly consumption was then calculated for each alcohol type by adding weekday and weekend consumptions. People not currently drinking were

assigned zero drinks of each type of alcohol. The weekly consumptions of each type of alcohol were added together. Total alcohol intake (grams/week) was then calculated by multiplying the total number of drinks per week by 13.45 grams, the number of grams of pure alcohol in a standard drink in Canada. Total alcohol values greater than 500g/week were considered probable reporting errors and were excluded, similar to other research[20]. For more interpretable regression results, total alcohol intake was divided by 70 grams (~5-drink increase per week).

Polygenic Risk Score

The Affymetrix Axiom array was used to perform genome-wide genotyping of non-fasting blood samples from consenting participants of the CLSA Comprehensive Cohort, resulting in 794,409 single nucleotide polymorphisms (SNPs) from 26,622 participants[25]. Release 3 of the CLSA genomic data was used and followed the marker- and sample-based quality control checks performed by the CLSA according to standard procedures[26]. Marker-based checks included checks for genotype consistency across genotyping batch, chromosomally defined sex, Hardy-Weinberg equilibrium, and discordance of genotyping across control replicates, while sample-based checks included checks for relatedness, heterozygosity, and genotype missingness. We excluded 15 individuals with extreme values of heterozygosity and genotype missingness and 1,666 related individuals. In addition, the CLSA genomic data release included genotype data imputed using the TOPMed reference panel at the University of Michigan Imputation Service, containing 97,256 reference samples at 308,107,085 genetic markers[26].

Both the genotyped and imputed SNP data were used to calculate a glaucoma polygenic risk score (PRS) for each CLSA participant with available genotype data that passed quality control checks. The PRS was developed by Craig et al.[27] based on 2,673 independent SNPs associated with glaucoma from their recent multitrait analysis of genome-wide association studies. After SNP alignment between the Craig et al. PRS SNPs and the CLSA data based on genome build GRCh38/hg38, 2,652 SNPs were available to calculate the PRS in the CLSA data, i.e., 0.8% SNPs were either not present in the CLSA data or were removed in the quality control steps. Given the small proportion of missing SNPs, proxy SNPs were not selected to replace the missing ones. The PRS was calculated for each CLSA participant using a weighted sum of the 2,652 SNPs: $\sum_{i=1}^{2652} \hat{\beta}_i \times \text{SNP}_i$, where $\hat{\beta}_i$ is the estimated effect size of SNP_i on glaucoma from Craig et al. and SNP_i is the number of copies of the effect allele in an individual genotype or the expected number of copies of the effect alleles for imputed genotypes (allelic dosage).

Demographic, Health, and Lifestyle Data

Demographic data including age, sex, race, education, and household income were collected during the in-home visit using an interviewer-administered questionnaire. Participants were grouped into White and Nonwhite race to have sufficient sample size for analysis. Height and weight were measured using standardized procedures at data collection site visits. Body mass index (BMI) was calculated and classified according to the World Health Organization cut-points

(underweight < 18.5 kg/m^2, normal weight 18.5–24.9 kg/m^2, overweight 25.0–29.9 kg/m^2, and obese ≥ 30.0 kg/m^2) [28].

Participants were asked to report whether they ever received a physician diagnosis of chronic conditions including diabetes and high blood pressure. Blood pressure was measured six times using the BpTru BPM200 blood pressure monitor (Medaval, Dublin, Ireland). The first reading was discarded, and the average of the subsequent five readings was used. Hypertension was defined if a participant reported a physician diagnosis of hypertension or if the average systolic blood pressure was 130 mmHg or higher or diastolic blood pressure was 80 mmHg or higher[29].

Smoking status was classified as current, never, or former based on participant responses to the interview questions "Have you smoked at least 100 cigarettes in your life?" and "At the present time, do you smoke cigarettes daily, occasionally (at least once in last 30 days), or not at all (not in last 30 days)?" A current smoker was defined as a person who reported smoking at least 100 cigarettes and currently smokes daily or occasionally while a former smoker was someone who reported smoking at least 100 cigarettes in life but had not smoked in the last 30 days.

Diet was assessed using a validated 36-item Short Diet Questionnaire (SDQ), designed to measure usual consumption (last 12 months) of total fat, fatty acids, cholesterol, trans fat, dietary fiber, calcium, vitamin D, and servings of fruit and vegetables [30,31]. Total caloric intake was calculated by methods previously described [30] using participants' reported frequencies of consumption of each SDQ item, for standard

portion sizes estimated from a full food frequency questionnaire[32] administered in the NuAge study[33], and a nutrient database based on the 2015 Canadian Nutrient File.

Statistical Analysis

Demographic, health, and behavioral factors were compared by alcohol consumption frequency. In separate multivariable analyses, linear regression was used to determine the relationship between alcohol consumption frequency, total alcohol intake, and alcohol type with IOP while logistic regression was used for glaucoma. We also ran regression models with all alcohol types entered together. Locally weighted scatterplot smoothing was used to graph IOP vs. predictor variables and linearity was checked by visual inspection. Possible non-linearity was examined by adding squared terms or spline terms and testing their statistical significance. Regression models were adjusted for potential confounding variables including age, sex, education, income, race, smoking, diabetes, systemic hypertension, BMI, , total caloric intake, and province. The variables were entered into the regression models either as continuous variables or categorical variables as they are shown in Table 1. Potential effect modification by the PRS was examined in two ways: by stratifying the regression models by PRS quartile and by fitting an interaction term between total alcohol intake and PRS quartile, entered as a linear term. Sensitivity analyses were done 1) using current IOP instead of pre-treatment IOP, 2) limiting IOP analyses to those without glaucoma. As recommended by the CLSA, sampling weights and strata variables were incorporated into all analyses using the SVY commands in Stata SE 16 (StataCorp, College Station, TX, USA).

RESULTS

Descriptive characteristics

Over 99% of participants in the CLSA Comprehensive Cohort (n=30,084) had complete alcohol consumption frequency data. Of the sample, 16% were daily drinkers, 41% were weekly drinkers, 40% were occasional drinkers, and 2% never drank. Participant characteristics by alcohol consumption frequency are presented in Table 1. As compared to never, occasional, and weekly drinkers, daily drinkers were older, more likely to be current or former smokers, and to have higher IOP. Daily drinkers were less likely to make less than $20,000 per year and to be obese. Never drinkers were more likely to be female, nonwhite, and to never smoke.

In Table 2, we compared those with and without a report of glaucoma. People with glaucoma were older, had higher pre-treatment and current IOP, were more likely to take ocular anti-hypertensive medications, and were more likely to be in the highest PRS quartile than those without glaucoma.

Alcohol and IOP

Higher alcohol consumption frequency was associated with higher IOP after adjustment for demographic, lifestyle, and health variables as shown in Table 3. As compared with never drinkers, daily (β=0.45, 95% CI: 0.05, 0.86) drinkers had higher IOP. Occasional and weekly drinkers did not have higher IOP compared to never drinkers ($P>0.05$). The total amount of alcohol consumed was also associated with IOP. An

increase in total alcohol intake of 5 drinks per week was associated with higher IOP after adjustment (β=0.20, 95% CI: 0.15, 0.26).

Associations between alcohol type and IOP are presented in Supplementary Table 1. Among alcohol types, a 5 drink per week increase in daily red wine (β=0.23, 95% CI: 0.15, 0.32), daily white wine (β=0.18, 95% CI: 0.06, 0.31), and beer (β=0.20, 95% CI: 0.10, 0.30) was associated with higher IOP whereas liquor (β=0.13, 95% CI: -0.01, 0.27), and other (β=0.10, 95% CI: -0.51, 0.71) alcohol types were not statistically significantly associated with IOP.

Alcohol and Glaucoma

In Table 4, no statistically significant associations were found between alcohol consumption frequency and glaucoma after adjustment for demographic, lifestyle, and health variables (P>0.05). Glaucoma was also not statistically significantly associated with alcohol type (Supplementary Table 2) or total alcohol intake (OR=1.01, 0.95, 1.08) (Supplementary Table 3) after adjustment for demographic, lifestyle, and health variables (P>0.05).

Investigation of Interaction by PRS

Analyses including the PRS were limited to those with genetic data. The PRS was very strongly associated with IOP with β values for each ascending quartile being 0.79, 1.38, and 2.04 in those per quartile of increasing genetic risk, respectively (P<0.001). These β values represent the difference in the mean IOP (in mmHg) for a 1-category

difference in the PRS. Total alcohol intake was more strongly associated with IOP in people in higher quartiles of genetic risk (Table 5). For example, the β values between alcohol and IOP in the four PRS quartiles were 0.13, 0.17, 0.20, and 0.25 per quartile of increasing genetic risk, respectively. This interaction was statistically significant (interaction term P=0.041).

By contrast, total alcohol intake was not associated with glaucoma in any of the PRS quartiles and there was no trend to indicate a stronger association in those with greater genetic risk of disease (interaction term P-value=0.654) (Supplementary Table 4).

Sensitivity Analyses

Given that we estimated pre-treatment IOP for people taking IOP-lowering medication to account for treatment effects, we did a sensitivity analysis using the current IOP values. The results from the sensitivity analysis were consistent with our main results with the exception that frequency of liquor consumption was now statistically significantly related to IOP (β=0.14, 95% CI 0.01, 0.28). Also, people with glaucoma were excluded to see if associations differed in those without glaucoma. After exclusion of those with glaucoma (n=1,103), daily drinking showed an attenuated relationship with IOP (β=0.35, 95% CI -0.06, 0.76), p=0.090). The association between total alcohol intake and IOP was unaffected (P<0.001). The interaction between alcohol intake and the PRS was very similar as the β values between alcohol and IOP in the four PRS quartiles were 0.12, 0.18, 0.18, and 0.22 per quartile of increasing genetic risk,

respectively. However, the loss of sample size led to an attenuated P-value for the interaction term (P=0.102).

DISCUSSION

Consuming an increased frequency and amount of alcohol, particularly red wine and beer, was associated with higher levels of IOP while it was not associated with the prevalence of glaucoma. Daily drinkers had an IOP that was 0.45 mmHg higher than people who never drank alcohol. Furthermore, the association between total alcohol intake and IOP was stronger in those with higher genetic risk of glaucoma.

The acute effect of alcohol (within 1-3 hours) is to lower IOP[34]. However, long-term, detrimental effects of chronic alcohol consumption on IOP are biologically plausible. Alcohol consumption can lead to dehydration by increasing urine production. Dehydration may cause increases in blood viscosity and flow resistance, which could impact IOP[35]. Chronic drinking also releases cortisol which can increase blood pressure[36], a risk factor for IOP. Furthermore, increased oxidative stress and DNA damage associated with chronic alcohol use may exacerbate and/or accelerate age-related changes [37] of the trabecular meshwork [38].

One would think that since alcohol was related to higher IOP, it would also be related to glaucoma. Perhaps explaining our finding that alcohol was associated with higher IOP but not glaucoma, certain types of alcohol like red and white wine and beer contain varying concentrations of polyphenols including flavonoids, which may exert neuroprotective effects on the retina [39]. In patients with glaucoma and ocular

hypertension, a systematic review and meta-analysis of 8 randomized controlled clinical trials found that dietary flavonoid interventions had statistically significant benefits on improving or maintaining visual field relative to a placebo but had no significant effects on IOP, systolic, or diastolic blood pressure suggesting that any potential mechanism of action of flavonoids may be IOP-independent [40]. Also, the use of the self-report of glaucoma, which may lead to misclassification, could explain the null finding. Finally, the use of cross-sectional data may have led to reverse causality such that people with glaucoma might have had higher alcohol consumption when younger, but after being diagnosed with glaucoma, they may have reduced their consumption. This would dilute any potential association.

Our findings coincide with several previous studies reporting positive associations between alcohol use and IOP[7,20-22,41,42]. We confirmed the finding by Stuart *et al* of an interaction between alcohol consumption and the same glaucoma PRS on IOP[20]. Stronger associations in those at higher genetic risk may indicate a reduced reserve to withstand elevations of IOP due to dietary exposures, as discussed in other studies that examined associations of habitual caffeine and alcohol consumption with IOP and glaucoma[19,20].

To our knowledge, only one previous study examined alcohol type (beer, wine, liquor, sherry) with IOP[43]. In contrast to our finding in which servings of red wine, white wine, and beer were positively associated with IOP, Ramdas et al found no association between alcohol type and IOP. Similar to our finding, prior research has demonstrated

that daily red wine and beer consumption are both associated with increases in systemic blood pressure[44].

Our results differ from the findings of a recent systematic review and meta-analysis which found a positive association of alcohol use and OAG[7]; however, the pooled effect size was small and of borderline statistical significance (OR=1.18, 95% CI: 1.02, 1.36). Further, we found no evidence of associations between any alcohol type and glaucoma and the glaucoma PRS did not significantly modify the associations of alcohol consumption frequency or total intake with glaucoma, which coincides with previous research findings[20].

A major strength of this study is the utilization of a large population-based sample that includes genetic data. Among the limitations, glaucoma was based on self-report and no information was available on severity or subtype. However, studies that did have information on glaucoma subtype found that alcohol was associated with POAG, which is the most common type of glaucoma in Canada [7]. Alcohol consumption was also based on self-report, which may have led to an under-report of drinking in some people due to social desirability. We did not have data on caffeine or sodium intake, which may have led to residual confounding. Also, data on retinal nerve fiber layer and macular thickness, which have been previously found to be adversely associated with alcohol consumption[45], were unavailable in the CLSA. Further, due to the cross-sectional design, we are unable to delineate temporality of the alcohol consumption and the onset of glaucoma/high IOP. Finally, the use of a PRS created from European-derived index variants, which sometimes do not replicate in non-European

samples[46], may not have captured the genetic risk of glaucoma as well in non-European samples. However, our results in the European sample were similar to our overall results.

Although the effect sizes in this research may seem small and not clinically significant, it is important to remember that our results compare average IOP between participants rather than within participants. It is possible that daily drinking in a particular individual, especially at high genetic risk, may lead to much higher elevations of IOP within that individual than what our study showed on average. It is possible that daily drinking may make it more difficult to achieve the target IOP with treatment.

Our research suggests that greater alcohol use and certain alcohol types are associated with elevated IOP but not with glaucoma. The association between total alcohol intake and IOP was stronger in those at higher genetic risk of glaucoma. Longitudinal research is needed to further understand the interaction of dietary and genetic factors on their risk of disease.

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Conflict of Interest

The authors have no financial or any other kind of personal conflicts with this paper.

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Table 1. Distribution of participant characteristics by alcohol consumption frequency

	Never n=713 (2.4%)	Occasional n=12,102 (40.2%)	Weekly n=12,424 (41.3%)	Daily n=4,845 (16.1%)
	Mean (SD) or %			
Age (years)	61.5 (11.2)	60.1 (10.1)	58.2 (9.6)	62.7 (11.2)
Sex				
Female	67.5%	59.1%	48.5%	59.5%
Male	32.5%	40.9%	51.5%	40.5%
Race				
White	73.7%	91.7%	96.0%	97.3%
Nonwhite	26.3%	8.3%	4.0%	2.7%
Education				
Less than Bachelor's degree	79.3%	81.3%	70.4%	73.1%
Bachelor's degree	9.7%	10.7%	15.5%	13.2%
University degree or certificate above	11.0%	8.0%	14.0%	13.7%
Household income				
$100,000 +	17.5%	25.5%	44.9%	36.2%
$50,000 - $100,000	25.1%	31.5%	31.9%	35.6%
$20,000 - $50,000	33.2%	26.8%	15.0%	20.0%
<$20,000	10.5%	9.5%	3.3%	2.6%
Refused/ Don't know	13.6%	6.7%	5.0%	5.5%
Smoking status				
Current	5.4%	13.0%	9.8%	14.2%
Former	13.5%	40.5%	46.5%	58.3%
Never	81.2%	46.5%	43.8%	27.5%
Diabetes				
None	76.3%	77.7%	86.9%	85.7%
Type 1	1.4%	0.8%	0.5%	0.6%
Type 2	14.3%	13.5%	5.9%	6.0%
Suspect/neither type	8.0%	8.0%	6.7%	7.8%
BMI				
Underweight	1.1%	0.8%	0.5%	0.9%
Normal weight	27.4%	24.3%	29.9%	33.8%
Overweight	34.7%	34.7%	42.4%	43.6%
Obese	36.8%	40.2%	27.3%	21.8%
Systemic hypertension				
Yes	57.2%	56.0%	49.9%	58.7%

No	42.8%	44.0%	50.1%	41.3%
Total Caloric Intake (kcal/day)	1485.4 (468.6)	1472.0 (472.4)	1493.2 (473.9)	1492.9 (495.9)
Pre-treatment IOP (mmHg)	16.2 (3.2)	15.8 (3.8)	16.0 (3.8)	16.7 (4.3)
Glaucoma				
Yes	5.6%	5.0%	3.3%	5.1%
No	94.4%	95.1%	96.7%	94.9%
Total Alcohol Intake (g/week)	N/A	8.8 (25.3)	89.6 (87.8)	206.9 (162.7)
Province				
Alberta	10.8%	10.0%	10.0%	8.3%
British Columbia	17.8%	19.2%	20.8%	26.3%
Manitoba	14.3%	12.0%	9.3%	7.6%
New&Lab.	11.1%	8.2%	7.1%	4.7%
Nova Scotia	9.1%	11.5%	9.5%	8.5%
Ontario	22.4%	19.9%	21.9%	24.0%
Quebec	14.4%	19.2%	21.4%	20.6%

New&Lab=Newfoundland and Labrador

Table 2. Characteristics of people with and without glaucoma

	Glaucoma (n=1,525) Mean (SD) or %	**No Glaucoma (n=28,408) Mean (SD) or %**
Age, Years	68.0 (10.7)	59.3 (10.0)
Pre-treatment IOP, mmHg	20.4 (7.2)	15.9 (3.6)
Current IOP, mmHg	17.5 (4.7)	15.8 (3.5)
% Taking >= 1 Glaucoma Med	38.1%	0.3%
Glaucoma PRS Quartile		
1 Low	15.0%	25.2%
2	19.6%	25.2%
3	22.3%	25.0%
4 High	43.0%	24.6%

Table 3. Linear regression analysis of the association of alcohol consumption frequency with IOP

Alcohol consumption frequency n=26,339	**IOP β***	**95% CI**
Never	0.00	
Occasional	-0.22	-0.59, 0.15
Weekly	0.11	-0.26, 0.49
Daily	0.45	0.05, 0.86

***Adjusted for age, sex, race, education, income, smoking, BMI, diabetes, systemic hypertension, total caloric intake, and province

Table 4. **Logistic regression analysis of the association of alcohol consumption frequency with glaucoma**

Alcohol consumption frequency n=27,588	**Participants with Glaucoma**	**Glaucoma OR***	**95% CI**
Never/rarely (2.4%)	48	1.00	
Occasional (40.2%)	672	1.26	0.78, 2.02
Weekly (41.3%)	497	1.13	0.69, 1.83
Daily (16.1%)	291	1.30	0.79, 2.14

***** Adjusted for age, sex, race, education, income, smoking, BMI, diabetes, systemic hypertension, total caloric intake, and province

Table 5. Investigation of interaction between PRS and alcohol with IOP

PRS Quartile	Total Alcohol intake	β *	95% CI
1 Low (n=5,473)	Per 70 gram (~5-drink) increase	0.13	0.03, 0.22
2 (n=5,485)	Per 70 gram (~5-drink) increase	0.17	-0.04, 0.31
3 (n=5,502)	Per 70 gram (~5-drink) increase	0.20	0.08, 0.31
4 High (n=5,512)	Per 70 gram (~5-drink) increase	0.25	0.12, 0.38

*Adjusted for age, sex, education, income, race, smoking, BMI, diabetes, systemic hypertension, total caloric intake, and province; P-value= 0.041 for interaction term between total alcohol intake and PRS quartile

Supplementary Table 1. Linear regression analysis of the association of alcohol type with IOP

Alcohol type (per 5 drinks/week) (n=26,339)	**IOP β***	**95% CI**
Red wine	0.23	0.15, 0.32
White wine	0.18	0.06, 0.31
Beer	0.20	0.10, 0.30
Liquor	0.13	-0.01, 0.27
Other	0.10	-0.51, 0.71

***Adjusted for each alcohol type, age, sex, race, education, income, smoking, BMI, diabetes, systemic hypertension, total caloric intake, and province

Supplementary Table 2. Logistic regression analysis of the association of alcohol type with glaucoma

Alcohol type (per 5 drinks/week) (n=27,588)	**Glaucoma OR***	**95% CI**
Red wine	1.07	0.96, 1.19
White wine	1.10	0.95, 1.27
Beer	0.95	0.83, 1.08
Liquor	0.93	0.81, 1.08
Other	0.85	0.48, 1.51

***Adjusted for each alcohol type, age, sex, race, education, income, smoking, BMI, diabetes, systemic hypertension, total caloric intake, and province

Supplementary Table 3. Logistic regression analysis of the association of total alcohol intake with glaucoma

n=27,588	**Glaucoma OR***	**95% CI**
Total alcohol intake, per 70 g/week (5 drink) increase	1.01	0.95, 1.08

***** Adjusted for age, sex, race, education, income, smoking, BMI, diabetes, systemic hypertension, total caloric intake, and province

Supplementary Table 4. Investigation of interaction between PRS and alcohol intake with glaucoma

PRS Quartile	Average Total Alcohol intake	OR*	95% CI
1 Low (n=5,707)	Per 70 gram (5-drink) increase	1.00	0.83, 1.20
2 (n=5,712)	Per 70 gram (5-drink) increase	1.09	0.94, 1.27
3 (n=5,732)	Per 70 gram (5-drink) increase	0.99	0.86, 1.14
4 High (n=5,740)	Per 70 gram (5-drink) increase	1.03	0.92, 1.15

*Adjusted for age, sex, race, education, income, smoking, BMI, diabetes, systemic hypertension, total caloric intake, and province; P-value= 0.654 for interaction term between total alcohol intake and prs quartile

COVER PAGE

Manuscript #2 of the thesis follows. This manuscript is currently under review at *the Journal of Glaucoma*. My role was to design the analysis, estimate nutrient intakes for the foods and beverages in the CLSA food frequency questionnaire, create the dietary pattern scores, derive variables except the PRS variable, conduct the analyses, and prepare the manuscript under the supervision of Dr. Freeman and my TAC.

Diet, Genetic Risk, and their Association with Intraocular Pressure and Glaucoma: The Canadian Longitudinal Study on Aging

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Précis

Supplemental calcium was associated with reduced intraocular pressure (IOP) but increased glaucoma prevalence, suggesting that calcium supplements may affect glaucoma pathophysiology via IOP-independent mechanisms.

ABSTRACT

Purpose: To investigate the associations between dietary supplements and patterns with intraocular pressure (IOP) and glaucoma and to assess whether associations were modified by a glaucoma polygenic risk score (PRS).

Methods: We performed a cross-sectional analysis using data from the Canadian Longitudinal Study on Aging Comprehensive Cohort, consisting of 30,097 adults ages 45 to 85 years. Nutrition was assessed using a validated 36-item Short Diet Questionnaire. Participants were asked to report if they took calcium or iron supplements in the last month. We scored participants according to their adherence to the Mediterranean-Style Dietary Pattern Score and to an antioxidant-rich dietary pattern score. IOP was measured in mmHg using the Reichert Ocular Response Analyzer. Participants reported a diagnosis of glaucoma from a doctor. Logistic and linear regression models were used to adjust for demographic, lifestyle, and health variables.

Results: Consuming calcium supplements was associated with lower IOP (β=-0.16, 95% confidence interval (CI): -0.31, 0.00) but an increased odds of glaucoma (OR (odds ratio)= 1.30, 95% CI: 1.08, 1.56). Supplementation with iron was not associated with IOP or glaucoma (P>0.05). IOP and glaucoma were not associated with adherence to a Mediterranean or antioxidant-rich diet after covariate adjustment. No interactions with the PRS were found. The 21 dietary components were not associated with IOP/glaucoma.

Conclusion: Supplemental calcium is associated with reduced IOP but increased glaucoma odds. Longitudinal research with detailed data on dosage and duration of

supplement use is needed to further understand the relationship between calcium supplements, IOP, and glaucoma.

Keywords: Diet, Dietary supplements, Mediterranean diet, Glaucoma, Intraocular pressure

INTRODUCTION

Glaucoma is a leading cause of irreversible vision loss worldwide characterized by progressive degeneration of retinal ganglion cells and estimated to affect 80 million people[1,2]. The pathogenesis of glaucoma is believed to result from multiple stresses and insults although the factors contributing to its development and progression have not been fully characterized[3]. Glaucoma progresses asymptomatically until late stages and as a result, diagnosis is frequently delayed until substantial amounts of neural damage have occurred[1,3]. Currently, reduction of intraocular pressure (IOP) is the only method proven to treat glaucoma[4].

Dietary factors have been examined as potentially being able to influence both IOP-dependent and non-IOP dependent mechanisms in glaucoma, thereby affecting disease incidence or progression[5]. In observational studies, participants consuming more servings of tea[6], fruits (including peaches[7] and oranges[8]), fruit juices[8], vegetables[9] (including carrots[7] and collard greens/kale[7,8]) and with higher dietary nitrate and green leafy vegetable intakes[10] were less likely to have prevalent/incident glaucoma. Further, higher total consumptions of calcium[11], iron[11,12], coffee[13-16], and alcohol[17] and low intakes of vitamin A and vegetable fat[12] were associated with increased risk of glaucoma and elevated IOP. While previous research suggests higher total consumptions of iron and calcium to be associated with an increased prevalence of glaucoma, a greater dietary rather than supplemental intake of calcium and iron was found to be associated with reduced prevalence of glaucoma[11]. Thus, it is possible that dietary and supplemental forms of these oxidants pose different biological effects.

As individuals consume diets comprising several individual nutrients and food types in which interactions may occur, the use of dietary patterns to assess defined food intake patterns has become increasingly common in epidemiological studies. Conclusive evidence linking specific dietary patters with IOP and glaucoma, however, is lacking[18]. For example, the Mediterranean diet, one of the most extensively studied dietary patterns, has been associated with reduced risks of type 2 diabetes[19], advanced age-related macular degeneration[20], and hypertension[21]; however, no studies to-date have found significant associations between adherence to the Mediterranean diet with IOP or glaucoma[22].

Previous studies examining associations of dietary factors/patterns with IOP and glaucoma were limited in that they did not account for genetic factors in their analysis. Genetic factors are estimated to account for a large proportion of the variance in IOP[23-29] and in recent years a polygenic predisposition to elevated IOP/glaucoma has been identified[30-32]. Interactions between genetic predisposition to IOP/glaucoma and dietary factors including caffeine, alcohol, and a balanced diet intake have been reported previously[33-35], though no studies to our knowledge have reported on the joint effects of polygenic risk and dietary pattern adherence in relation to IOP and glaucoma.

Using data from the Canadian Longitudinal Study on Aging (CLSA), we investigated hypotheses about 1) iron and calcium supplementation being associated with higher IOP/greater glaucoma, 2) the Mediterranean and antioxidant dietary patterns being associated with lower IOP/less glaucoma, and 3) interactions between

dietary patterns and the genetic risk of glaucoma. We also explored relationships between 21 dietary components and IOP/glaucoma.

MATERIALS AND METHODS

Study Population and Design

We performed a cross-sectional analysis using the first round of data collection from the CLSA Comprehensive Cohort, which consists of 30,097 Canadian adults aged 45-85 years, with data collected every 3 years[36]. The sample of the Comprehensive Cohort was obtained by utilizing stratified random sampling of provincial healthcare registration databases and random digit dialing of landline telephones. Baseline data were obtained between 2012 and 2015 via in-home interviews and in-person physical examinations and bio specimen sample collections at CLSA data collection sites, which are located in Victoria, Vancouver, Surrey, Calgary, Winnipeg, Hamilton, Ottawa, Montreal, Sherbrooke, Halifax and St. John's. To be included in the study, participants had to be aged 45-85 years, community dwelling, cognitively unimpaired, and speak English or French. Exclusion criteria included being a full-time member of the Canadian Armed Forces, residing on a federal First Nations reserve or settlement, living in a long-term care institution, or not a permanent resident or Canadian citizen.

Ethics Approval and Data Access

Research Ethics Board approval was obtained for all CLSA affiliated sites in July 2010. Ethics approval for the present analysis was obtained from the University of Ottawa in October 2021.

Intraocular Pressure and Glaucoma

Participants were asked to report if they have ever had a physician diagnosis of glaucoma. IOP was measured at the CLSA data collection sites using the Reichart Ocular Response Analyzer (Reichart Technologies, Depew, NY, USA). The average IOP of the right and left eyes was used to derive participant-level IOP values. If one eye had missing IOP data, then the IOP value of the other eye was used. To estimate the pre-treatment IOP, the IOP of those participants taking medications with a Drug Identification Number (DIN) indicative of an IOP-lowering eye drop was divided by 0.7, which is the mean estimated treatment effect[37]. We used corneal-compensated IOP in our analyses, which are adjusted for corneal mechanical properties. IOP values greater than 60 were excluded, as these were considered probable measurement errors.

Dietary Factors

Nutrition was assessed using a validated 36-item Short Diet Questionnaire (SDQ), designed to measure usual consumption (last 12 months) of total fat, fatty acids, cholesterol, trans fat, dietary fiber, calcium, vitamin D, and servings of fruit and vegetables[38,39]. Responses are given in consumption frequencies, as the number of times food or beverage items were consumed per day, week or month. Dietary supplementation was also measured by asking participants if they took calcium or iron supplements in the last month.

Dietary patterns

To assess the dietary features of our study population, we scored all participants according to their adherence to specific dietary patterns, including one of the most studied *a priori* dietary patterns (the Mediterranean-style diet), as well as *a posteriori* dietary pattern derived from CLSA data.

Mediterranean-Style Dietary Pattern

We constructed a modified version of the Mediterranean-Style Dietary Pattern Score (MSDPS) [40], developed by Rumawas et al. to measure adherence to a Mediterranean-style dietary pattern in non-Mediterranean populations. The MSDPS comprises of 13 components of the Mediterranean diet pyramid[41] including whole-grain cereals, fruits, vegetables, dairy, wine, fish, poultry, olives-legumes-nuts, potatoes, eggs, sweets, meats, and olive oil; each component except olive oil is scored from 0 to 10 depending on the level of adherence. The sum of the component scores ranges from 0-100 after standardization and is weighted by the proportion of energy consumed from Mediterranean diet foods. We modified certain components based on the data available in the CLSA. For example, olive oil consumption was not measured in the SDQ and therefore was not included. Further, we included both cereals and bread products in the whole-grain component to more accurately reflect the standard Canadian diet[42]. As olive oil intake is not measured in the CLSA, this component was replaced by the ratio of monounsaturated (MUFA) to saturated (SFA) fatty acids, which has been used as an indirect parameter previously[43-47]. MUFA, SFA and total caloric intakes were calculated by methods previously described[38] using participants' reported frequencies of consumption of each SDQ item, for standard portion sizes estimated from a full food

frequency questionnaire[48] administered in the NuAge study[49], and a nutrient database based on the 2015 Canadian Nutrient File. A ratio of 1.5 or more of MUFA to SFA yielded 10 points, a ratio ≤ 1and <1.5 yielded 5 points and a ratio <1 yielded 0 points.

Antioxidant-rich Dietary Pattern

To define an "antioxidant-rich" dietary pattern, the total antioxidant intake (comprising of beta-carotene, vitamin C and Vitamin E) was used. Beta-carotene, vitamin C and vitamin E intakes were also calculated by methods previously described[38]. These nutrients were selected as they have been previously found to be associated with lower IOP and reduced risk of glaucoma[50] and had complete data available in the 2015 Canadian Nutrient File for the food items measured in the SDQ. For easier interpretation, all foods were aggregated into larger food categories based on nutrient profiles. Twenty-one food variables were selected as predictor variables in the weighted partial least squares (wPLS) model and can be found in Supplementary Table 2.

The wPLS antioxidant-rich dietary pattern score was calculated by summing the product of food intake and factor loading in the wPLS model of all predictor variables[51]. To validate the derived dietary pattern from wPLS, random cross-split validation was performed five times. The Pearson correlations between the identified wPLS patterns for each half-split and the original sample was then examined.

Polygenic Risk Score

The Affymetrix Axiom array was used to perform genome-wide genotyping of non-fasting blood samples from consenting participants of the CLSA Comprehensive Cohort, resulting in 794,409 single nucleotide polymorphisms (SNPs) from 26,622 participants[52]. Release 3 of the CLSA genomic data was used and followed the marker- and sample-based quality control checks performed by the CLSA according to standard procedures[53]. Marker-based checks included checks for genotype consistency across genotyping batch, chromosomally defined sex, Hardy-Weinberg equilibrium, and discordance of genotyping across control replicates, while sample-based checks included checks for relatedness, heterozygosity, and genotype missingness. We excluded 15 individuals with extreme values of heterozygosity and genotype missingness and 1,666 related individuals. The CLSA genomic data release also included genotype data imputed using the TOPMed reference panel at the University of Michigan Imputation Service, containing 97,256 reference samples at 308,107,085 genetic markers[53]. Imputed SNP data were used to calculate a glaucoma polygenic risk score (PRS) for each CLSA participant with available genotype data that passed quality control checks. Out of 2,673 SNPs used to develop a PRS by Craig et al. [54], 2,652 were present in the CLSA genotyping data after SNP alignment. The PRS was calculated for each CLSA participant using a weighted sum of the 2,652 SNPs: $\sum_{i=1}^{2652} \hat{\beta}_i \times \text{SNP}_i$, where $\hat{\beta}_i$ is the estimated effect size of SNP_i on glaucoma from Craig et al. and SNP_i is the number of copies of the effect allele in an individual genotype or the expected number of copies of the effect alleles for imputed genotypes (allelic dosage).

Demographic, Health, and Lifestyle Data

Demographic data including age, sex, race, household income and education were collected during the in-home visit using an interviewer-administered questionnaire. Participants were grouped into White and Nonwhite to have an adequate sample size for analysis.

Body mass index (BMI) was calculated and classified according to the World Health Organization cut-points (underweight < 18.5 kg/m^2, normal weight 18.5–24.9 kg/m^2, overweight 25.0–29.9 kg/m^2, and obese ≥ 30.0 kg/m^2) [55].

Participant smoking status was classified as either current, never, or former based on participant responses to the interview questions "Have you smoked at least 100 cigarettes in your life?" and "At the present time, do you smoke cigarettes daily, occasionally (at least once in last 30 days), or not at all (not in last 30 days)?" A current smoker was defined as a person who reported smoking at least 100 cigarettes and currently smokes daily or occasionally while a former smoker was someone who reported smoking at least 100 cigarettes in life but had not smoked in the last 30 days. Blood pressure was measured 6 times using the BpTRU™ BPM200 Blood Pressure Monitor (Medaval, Dublin, Ireland). The first reading was discarded and the average of the subsequent 5 readings was used to derive participant-level values. Hypertension was defined if a participant reported a physician diagnosis of hypertension or if the average systolic blood pressure was 130 mmHg or higher or diastolic blood pressure was 80 mmHg or higher[56]. Participants were asked to report whether they ever received a physician diagnosis of diabetes. Physical activity level was evaluated using the Physical

Activity Scale for Elderly (PASE). A total PASE score was computed by multiplying time spent in occupational, household and leisure activity types by empirically derived item weights and then summing overall activities[57]. Participants were asked to report all prescribed medications that they were taking and to show the containers.

Statistical Analysis

Mean IOP levels were compared for demographic, health and lifestyle factors at baseline. In separate multivariable analyses, linear regression was used to determine the relationship between dietary factors and patterns with IOP and logistic regression was used for glaucoma. Regression models were adjusted for potential confounding variables including age, sex, race, education, income, smoking, diabetes, systemic hypertension, BMI, PASE score, and province. Potential effect modification by the PRS was examined in two ways: by fitting interaction terms and by stratifying the regression models by PRS quartile. For exploratory analyses, the Bonferroni correction was used to address multiple comparisons for the 21 dietary components and their association with IOP and glaucoma. The Bonferroni adjustment was performed by dividing the original α-level (0.05) by 21 to give 0.0024. As recommended by the CLSA, sampling weights and strata variables were incorporated into all analyses using the SVY commands in Stata SE 16 (StataCorp, College Station, TX, USA).

RESULTS

Descriptive characteristics

Participant characteristics by calcium and iron supplement use are presented in Table 1. Participants who reported calcium supplement use in the last month were

older, more likely to be female, have glaucoma, lower levels of education and income, had lower PASE scores and were less likely to be current smokers, overweight or obese, or have type 2 diabetes as compared to participants who did not report calcium supplement use. Participants who reported iron supplement use were more likely to be female, were less likely to be current smokers or have systemic hypertension, and were more likely to be underweight or normal weight, have type 2 diabetes as compared to those who did not.

Dietary pattern adherence, IOP and glaucoma

The mean MSDPS score was 26.6 (standard deviation (SD)= 8.1, range 0-64.4). Mediterranean and antioxidant-rich diet adherence were not associated with IOP after adjustment for demographic, lifestyle, and health variables as shown in Table 2 (P>0.05). Glaucoma was also not statistically significantly associated with adherence to a Mediterranean or antioxidant-rich diet after adjustment (P>0.05).

Dietary supplement use, IOP and glaucoma

Associations between iron and calcium supplement use with IOP and glaucoma are presented in Table 3. Participants who reported consumption of calcium supplements in the past month had lower IOP as compared to those who did not, after adjustment (β=-0.16; 95% CI: -0.31, 0.00). However, participants who reported consumption of calcium supplements in the past month had increased odds of glaucoma after covariate adjustment (OR=1.30, 1.08, 1.56). These associations remained

statistically significant after adjustment for possible confounding factors such as serum vitamin D levels, vitamin D supplementation, report of osteoporosis, and taking bisphosphonates (data not shown). Since calcium supplements were associated with glaucoma, we examined whether calcium channel blockers, often taken for blood pressure, were associated with glaucoma in the opposite direction. However, they were also associated with glaucoma (OR=1.45, 95% CI 1.12, 1.87) but not IOP (β=-0.21; 95% CI: -0.53, 0.11). The consumption of iron supplements (past month), however, was not statistically significantly associated with IOP (β=-0.16; 95% CI: -0.41, 0.10) or glaucoma (OR=0.89, 0.60, 1.31) after covariate adjustment.

Investigation of Interaction by PRS

Analyses including the PRS were limited to those with genetic data. The PRS was very strongly associated with IOP with β values for each ascending quartile being 0.79, 1.38, and 2.09 in those per quartile of increasing genetic risk, respectively (P<0.001).

Adherence to a Mediterranean or antioxidant-rich diet were not associated with IOP among participants in quartiles 1-3 of genetic risk (Table 4). Among participants in the fourth PRS quartile, those with a higher adherence to the Mediterranean diet had statistically significant (β=0.43; 95% CI:0.05, 0.80; Q3) and borderline statistically significant (β=0.37; 95% CI: -0.03, 0.77; Q4) higher IOP. Similarly, among participants in the fourth PRS quartile, those with a higher adherence to an antioxidant-rich diet had higher IOP (β=0.49; 95% CI: 0.03, 0.95; Q3). These interactions, however, were not statistically significant (interaction term P=0.199 and P=0.595, respectively).

Adherence to a Mediterranean or antioxidant-rich diet were also not associated with glaucoma in any quartile of genetic risk (Table 5). Further, the interaction terms were not statistically significant (interaction term P=0.300 and P=0.466, respectively).

Dietary components, IOP and glaucoma

Separate regression models exploring associations between 21 different dietary components with IOP and glaucoma are presented in Supplementary Table 1. Using the conventional level of Type 1 error (P=0.05), higher intakes of non-starchy vegetables and high sugar snacks were associated with lower IOP (β=-0.11; 95% CI:-0.17, -0.05 and β=-0.15; 95% CI:-0.27, -0.03, respectively) whereas higher intakes of high fat dairy products were associated with higher IOP after covariate adjustment (β=0.15; 95% CI:0.05, 0.25). However, these associations were no longer statistically significant after Bonferroni correction (p>0.0024). Further, no dietary components were statistically significantly associated with glaucoma (P>0.05).

Sensitivity Analyses

Given that we estimated pre-treatment IOP for people taking IOP-lowering medication to account for treatment effects, we conducted sensitivity analyses using the current IOP values (data not shown). The results from the sensitivity analyses were consistent with our main results. Another sensitivity analysis was done restricting the models using the PRS to those of European ancestry. The results were consistent with the full sample with the exception that two associations found in the full sample were

no longer statistically significant. Those in the 3rd quartile of the Mediterranean diet and the 4th quartile of genetic risk no longer had statistically significantly higher IOP (β=0.29, 95% CI -0.09, 0.68). Also, those in the 3rd quartile of the antioxidant diet and the 4th quartile of genetic risk no longer had statistically significantly higher IOP (β= 0.28, 95% CI -0.20, 0.75).

DISCUSSION

To our knowledge, this is the first study to examine associations of supplemental calcium and iron use with IOP. Participants who reported consuming calcium supplements had an IOP that was 0.16 mm Hg lower than those not consuming calcium supplements, but had an increased odds of glaucoma. Supplementation with iron was not associated with IOP or glaucoma. IOP and glaucoma were not associated with adherence to a Mediterranean or antioxidant-rich diet after adjustment and no interactions with the PRS were found. Further, in exploratory analyses, no dietary components were associated with IOP or glaucoma after covariate adjustment. Growing evidence supports the involvement of increased oxidative stress as a common etiology contributing to the pathogenesis of neurodegenerative diseases, including Parkinson's, Alzheimer's, and glaucoma [58,59]. Oxidative stress results from an imbalance between reactive oxygen species production and antioxidant defenses, resulting in molecular damage and interruption of cellular signaling[59,60]. Dietary modifications such as increasing antioxidant intake can decrease redox state markers, maintaining cell and tissue homeostasis, thereby preventing inflammation [60,61]. Further, as impaired calcium and iron regulation have been found in lamina cribrosa, retinal ganglion and trabecular

meshwork cells of donors with glaucoma, it is believed that calcium and iron may also play a role in glaucoma pathogenesis[62-65]. Dietary and supplemental calcium play critical roles in calcium homeostasis[66]. Serum calcium levels, however, are not only determined by exogenous calcium intake but are additionally hormonally regulated by the parathyroid hormone, vitamin D and calcitonin[67]. Any increased risks from calcium supplementation may be explained by the quick and sustained increases in serum calcium following calcium supplement ingestion[66]. In the eye, disturbances in calcium homeostasis of human trabecular meshwork cells may contribute to increased outflow resistance and thereby increased IOP[68]. Alterations of intracellular calcium in tissues are also associated with insulin resistance[69] and vascular resistance[70], risk factors for cardiometabolic diseases, which are also associated with IOP and glaucoma [71-74]. Further, calcium channel blocker (CCB) use is associated with higher parathyroid hormone levels, which could also alter calcium homeostasis[75]. For example, there has been evidence that CCBs increase blood flow to the optic nerve head and fovea[76]. It has also been found that a significant reduction in the rate of disc and field damage is observed in normal tension glaucoma (NTG) patients who receive CCBs[76-78]. However, in a systematic review and meta-analysis by our team, we found that CCBs were associated with a higher odds of glaucoma while they were not associated with IOP[79].

Contrary to our hypothesis, in our study, participants who reported consumption of calcium supplements in the past month had lower IOP as compared to those who did not. We are not sure how to explain this finding. One potential explanation is that 62% of participants taking calcium supplements had reported a diagnosis of osteoporosis. It's

possible that osteoporosis or its treatment may pose effects on IOP and/or glaucoma[80]. However, this association remained significant after adjustment for such factors as serum vitamin D levels, vitamin D supplementation, report of osteoporosis, and taking bisphosphonates. Our finding contrasts results from a single-center Asian study which found higher serum calcium levels to be associated with higher IOP in males (β=0.025, 95% CI:0.007-0.043) and females (β=0.050, 95% CI=0.030, 0.069) [71]. However, this difference may be explained by the fact that serum calcium levels may not only reflect exogenous calcium intake but also the ability to maintain homeostasis[81].

In line with our hypothesis, we found that people taking calcium supplements had a higher odds of glaucoma. This finding coincides with previous cross-sectional studies that reported increased odds of glaucoma among participants who consumed ≥ 800 mg/d of supplementary calcium (OR= 2.44, 95% CI: 1.25–4.76) and with a higher total consumption of calcium (*p*-trend <0.0001) [11,62]. Further, a recent retrospective cohort study found that patients who used bisphosphonates, a first line agent used to treat osteoporosis, had higher risks of angle-closure glaucoma (ACG) compared to non-users[82]. Therefore, calcium supplements, or treatments often taken with them, may affect glaucoma pathophysiology via IOP-independent mechanisms. However, adjustment for bisphosphonates did not change the association with glaucoma. Also, when CCBs were added to the model, they were also associated with glaucoma. Perhaps either too much or too little calcium can both increase the risk of glaucoma.

We did not find supplemental iron to be associated with higher IOP or the prevalence of glaucoma. Our null findings with iron contrast with previous studies that

found increased odds of glaucoma among participants who consumed ≥ 18 mg/d of supplementary iron[62], with high serum ferritin levels[83] or levels greater than 61 ng/mL[84], and higher total consumption of iron[11,12]. A potential reason we did not find an association may be due to heterogeneity in the iron supplement doses taken by the CLSA participants. As iron absorption from oral supplements tends to be low and the fractional iron absorption decreases with larger iron doses, it is currently recommended take 60-200 mg split in 2 or 3 daily doses[85]. Without data on dosage or duration of supplementation, we may be missing an association between higher dose or longer term iron supplementation and glaucoma.

Finally, Mediterranean and antioxidant-rich diet adherence were not associated with IOP or glaucoma and the PRS did not significantly modify the associations. Our results coincide with findings from the Rotterdam study, which did not find significant associations between adherence to the Mediterranean diet and IOP or incident open-angle glaucoma[22]. We do not know of any other studies that examined the Mediterranean diet with IOP or glaucoma. However, one Spanish study reported that higher adherence to a Mediterranean lifestyle, measured using a score of 10 lifestyle habits including the Mediterranean diet, was found to be associated with a lower risk of developing glaucoma[86]. However, adherence to the Mediterranean diet alone was not statistically significantly associated with the risk of developing glaucoma after covariate adjustment and authors hypothesized that adherence to a number of individually beneficial lifestyle habits may lead to a synergistic effect[86,87]. To our knowledge, no prior studies have examined adherence to an antioxidant-rich diet with IOP or glaucoma.

However, the Mediterranean-Dietary Approach to Stop Hypertension (DASH) intervention for neurodegenerative delay (MIND) diet is a hybrid of the Mediterranean and DASH diets, emphasizing food components such as green leafy vegetables, fish, nuts and berries, that are known for their antioxidant and neuroprotective properties[88,89]. Higher adherence to the MIND diet has been previously shown to be associated with slower rates of cognitive decline with age, and a reduced risk of Alzheimer's disease[88,90]. In the Rotterdam study, adherence to the MIND diet was recently found to be associated with a reduced risk of POAG in an IOP-independent manner [22]. We were unable to construct a MIND diet score so we cannot directly compare our results to those of the Rotterdam Study.

A major strength of this study is the utilization of a large population-based sample that includes genetic data and many confounding variables. Further, we used a validated food frequency questionnaire to derive dietary intakes. However, among the limitations, data on berry consumption was not available in the CLSA and we were therefore unable to construct the MIND diet score. Further, glaucoma was based on self-report and no information was available on severity or subtype. However, we had data on measured IOP. Dietary and alcohol intakes as well as physical activity levels were also based on self-report and may be subject to recall and social desirability biases. Our data on supplements lacked information on duration of use or dosage. Further, due to the cross-sectional design, we are unable to delineate temporality of the dietary exposures and the onset of glaucoma/high IOP. Further, the use of a PRS created from European-derived index variants may not have captured the genetic risk of glaucoma as

well in non-European samples [91]. However, our results were consistent when restricting to the European sample.

Our research suggests that supplemental calcium is associated with reduced IOP but increased glaucoma prevalence. Longitudinal research with more detailed dietary data is needed to further understand the interaction of dietary and genetic factors on the risk of disease.

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Conflict of Interest

The authors have no financial or any other kind of personal conflicts with this paper.

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Table 1. Baseline characteristics of CLSA participants, by dietary supplement use

	Dietary Supplement Mean (SD)/ %			
	Calcium		**Iron**	
	Yes (n=8,282)	No (n=20,449)	Yes (n=1,712)	No (n=26,997)
Age (years)	62.8 (10.6)	58.6 (9.7)	59.4 (10.7)	59.7 (10.1)
Sex				
Female	76.3	43.7	70.9	51.1
Male	23.7	56.4	29.1	48.9
Race				
White	94.2	93.9	92.7	94.0
Nonwhite	5.8	6.1	7.3	6.0
Education				
Less than Bachelor's degree	78.9	73.7	76.9	75.0
Bachelor's degree	11.2	14.1	12.1	13.4
University degree or certificate above	10.0	12.2	11.0	11.6
Income				
$100,000 +	26.6	38.4	33.8	35.3
$50,000 - $100,000	32.6	32.2	29.6	32.5
$20,000 - $50,000	26.0	19.1	22.1	20.9
<$20,000	7.1	5.0	6.5	5.5
Refused/ Don't know	7.8	5.3	8.0	5.8
Smoking status				
Current	7.9	12.2	8.7	11.2
Former	46.2	44.2	44.7	44.7
Never	45.9	43.6	46.6	44.1
Energy intake (kcal/day)	1513.9 (498.1)	1478.7 (469.1)	1505.8 (477.0)	1486.8 (477.3)
PASE score	137.9 (73.9)	156.0 (78.5)	148.8 (75.4)	151.4 (78.0)
BMI				
Underweight	1.0	0.6	1.5	0.7
Normal weight	34.7	25.6	34.2	27.6

Overweight	36.5	40.1	31.8	39.6
Obese	27.8	33.7	32.5	32.1
Systemic hypertension				
Yes	52.2	54.1	48.7	54.0
No	47.8	45.9	51.3	46.0
Diabetes				
None	84.2	82.2	79.3	83.0
Type 1	0.6	0.6	0.9	0.6
Type 2	8.4	9.5	11.8	9.0
Suspect/neither type	6.7	7.7	8.0	7.4
Pre-treatment IOP (mm Hg)	16.1 (4.2)	16.0 (3.7)	15.8 (3.6)	16.1 (3.9)
Glaucoma				
Yes	6.2	3.7	4.4	4.3
No	93.9	96.4	95.6	65.7

Table 2. Separate regression models showing relationships between dietary patterns with IOP and glaucoma

	IOP		Glaucoma	
	β*	95% CI	OR†	95% CI
Mediterranean	**(n=25,151)**		**(n=26,324)**	
Q1	0.00		1.00	
Q2	0.05	-0.13, 0.24	1.03	0.81, 1.30
Q3	0.11	-0.08, 0.29	0.94	0.75, 1.18
Q4	0.06	-0.13, 0.25	0.99	0.79, 1.26
Antioxidant rich	**(n=25,292)**		**(n=26,472)**	
Q1	0.00		1.00	
Q2	-0.04	-0.23, 0.14	0.94	0.73, 1.21
Q3	-0.03	-0.23, 0.16	0.96	0.73, 1.25
Q4	-0.11	-0.32, 0.11	1.03	0.78, 1.37

*****Adjusted for age, sex, race, education, income, smoking, BMI, diabetes, systemic hypertension, energy intake, PASE score, and province using linear regression

†Adjusted for the covariates above using logistic regression

Table 3. Separate regression models of the relationship between dietary supplements with IOP and glaucoma

	IOP n=25,230		**Glaucoma n=26,407**	
	β*	**95% CI**	**OR†**	**95% CI**
No iron supplements Consumption of iron supplements	0.00 -0.15	 -0.40, 0.10	1.00 0.89	 0.60, 1.31
No calcium supplements Consumption of calcium supplements	0.00 -0.16	 -0.31, 0.00	1.00 1.30	 1.08, 1.56

*****Adjusted for age, sex, race, education, income, smoking, BMI, diabetes, systemic hypertension, energy intake, PASE score, and province using linear regression

†Adjusted for the covariates above using logistic regression

Table 4. Investigation of interaction between PRS and dietary pattern with IOP

PRS Quartile	Dietary pattern	IOP β*	 95% CI
1 Low	Mediterranean		
	Q1	0.00	
	Q2	-0.24	-0.57, 0.09
	Q3	0.13	-0.22, 0.48
	Q4	-0.05	-0.39, 0.29
2	Mediterranean		
	Q1	0.00	
	Q2	0.08	-0.28, 0.44
	Q3	0.09	-0.28, 0.45
	Q4	-0.07	-0.43, 0.30
3	Mediterranean		
	Q1	0.00	
	Q2	0.22	-0.25, 0.69
	Q3	-0.20	-0.64, 0.24
	Q4	0.27	-0.20, 0.73
4 High	Mediterranean		
	Q1	0.00	
	Q2	0.01	-0.42, 0.44
	Q3	0.43	0.05, 0.80
	Q4	0.37	-0.03, 0.77
1 Low	Antioxidant rich		
	Q1	0.00	
	Q2	0.07	-0.27, 0.41
	Q3	-0.09	-0.44, 0.25
	Q4	-0.06	-0.46, 0.34
2	Antioxidant rich		
	Q1	0.00	
	Q2	-0.05	-0.40, 0.31
	Q3	0.03	-0.35, 0.41
	Q4	-0.13	-0.55, 0.28
3	Antioxidant rich		
	Q1	0.00	
	Q2	-0.17	-0.60, 0.27
	Q3	-0.26	-0.72, 0.20
	Q4	-0.13	-0.62, 0.36
4 High	Antioxidant rich		
	Q1	0.00	
	Q2	0.13	-0.29, 0.55
	Q3	0.49	0.03, 0.95
	Q4	0.27	-0.18, 0.73

*Adjusted for age, sex, race, education, income, smoking, BMI, diabetes, hypertension, energy intake, PASE score, and province

Table 5. Investigation of interaction between PRS and dietary pattern with glaucoma

PRS Quartile	Dietary pattern	Glaucoma OR*	95% CI
1 Low	Mediterranean		
	Q1	1.00	
	Q2	1.68	0.84, 3.36
	Q3	2.26	0.97, 5.22
	Q4	1.49	0.67, 3.29
2	Mediterranean		
	Q1	1.00	
	Q2	0.73	0.42, 1.27
	Q3	1.15	0.69, 1.90
	Q4	0.67	0.39, 1.17
3	Mediterranean		
	Q1	1.00	
	Q2	1.03	0.62, 1.72
	Q3	0.81	0.51, 1.30
	Q4	1.08	0.64, 1.84
4 High	Mediterranean		
	Q1	1.00	
	Q2	0.99	0.65, 1.51
	Q3	0.76	0.51, 1.12
	Q4	1.29	0.87, 1.91
1 Low	Antioxidant rich		
	Q1	1.00	
	Q2	1.44	0.64, 3.21
	Q3	1.05	0.47, 2.35
	Q4	1.69	0.70, 4.07
2	Antioxidant rich		
	Q1	1.00	
	Q2	0.50	0.28, 0.89
	Q3	0.63	0.36, 1.10
	Q4	0.69	0.35, 1.35
3	Antioxidant rich		
	Q1	1.00	
	Q2	0.94	0.52, 1.68
	Q3	0.94	0.50, 1.77
	Q4	1.25	0.66, 2.40
4 High	Antioxidant rich		
	Q1	1.00	
	Q2	1.03	0.68, 1.54
	Q3	1.09	0.68, 1.74
	Q4	0.94	0.60, 1.47

*Adjusted for age, sex, race, education, income, smoking, BMI, diabetes, systemic hypertension, energy intake, PASE score, and province

Supplemental Tables

S-Table 1. Separate regression models showing relationships between different dietary components with IOP and glaucoma

Per 1-serving increase (daily)	IOP β*	95% CI	Glaucoma OR†	95% CI
Fruit	-0.02	-0.10, 0.05	1.03	0.95, 1.12
Fruit juice	0.11	-0.02, 0.23	1.03	0.88, 1.19
Non-starchy vegetables	-0.11	-0.17, -0.05	0.99	0.91, 1.07
Legumes	0.04	-0.20, 0.29	0.96	0.72, 1.27
Nuts and seeds	0.04	-0.08, 0.16	0.96	0.84, 1.09
Whole grains	0.01	-0.09, 0.10	1.03	0.89, 1.20
Potatoes (non-fried)	0.09	-0.16, 0.34	0.96	0.75, 1.23
Potatoes (fried)	0.14	-0.47, 0.76	1.19	0.58, 2.46
Fish	0.30	-0.07, 0.68	1.42	0.90, 2.22
Poultry	0.19	-0.15, 0.53	1.18	0.80, 1.75
Processed meat	-0.17	-0.56, 0.23	0.81	0.53, 1.23
Red meat	-0.06	-0.29, 0.18	0.98	0.72, 1.34
Sauces and gravies	-0.08	-0.38, 0.23	1.02	0.69, 1.49
Butter and margarine	0.01	-0.09, 0.10	0.96	0.85, 1.08
High sugar snacks	-0.15	-0.27, -0.03	0.98	0.85, 1.13
Salty snacks	0.08	-0.16, 0.31	1.02	0.76, 1.37
High fat dairy	0.15	0.05, 0.25	1.00	0.88, 1.14
Low fat dairy	0.02	-0.04, 0.08	0.99	0.91, 1.08
Salad dressing	-0.03	-0.20, 0.14	0.96	0.77, 1.19
Eggs	0.13	-0.05, 0.31	0.92	0.70, 1.20
Calcium-fortified foods	-0.11	-0.24, 0.02	1.13	0.94, 1.36

*Adjusted for age, sex, race, education, income, smoking, BMI, diabetes, hypertension, energy intake, PASE score, and province using linear regression

†Adjusted for the covariates above using logistic regression

S-Table 2. Predictor variables in the weighted partial least squares (wPLS) models

Food variables derived from the 36-item food frequency questionnaire
Fruit
Fruit juice
Non-starchy vegetables[1]
Legumes
Nuts and seeds
Whole grains[2]
Potatoes (non-fried)[3]
Potatoes (fried)[4]
Fish
Poultry
Processed meat[5]
Red meat[6]
Sauces and gravies
Butter and margarine
High sugar snacks[7]
Salty snacks
High fat dairy[8]
Low fat dairy[9]
Salad dressing
Eggs
Calcium-fortified foods[10]

[1]Carrots, green salad, other; [2]whole wheat breads, high fibre breakfast cereals; [3]boiled, mashed or baked potatoes; [4]french fries, poutine or other fried potatoes; [5]Sausages, hot dogs, ham, smoked meat, bacon, patés, cretons, terrines; [6]beef, pork, veal, lamb, game; [7]chocolate, cakes, pies, doughnuts, pastries, cookies, muffins, ice-cream, frozen yogurt, milk-based desserts; [8]whole milk, regular yogurt, regular cheeses; [9]2%, 1%, or skim milk, low-fat yogurt, low-fat cheeses;[10]calcium fortified foods, calcium fortified juice, calcium fortified milk and other calcium fortified beverages

COVER PAGE

Manuscript #3 of the thesis follows. This manuscript is currently published at the journal *Heliyon*. My role was to design the analysis, derive all variables except the PRS variable, conduct the analyses, and prepare the manuscript under the supervision of Dr. Freeman and my TAC.

Exploring Ethnic and Racial Differences in Intraocular Pressure and Glaucoma: The Canadian Longitudinal Study on Aging

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ABSTRACT

Purpose: To determine whether self-reported race/ethnicity is associated with intraocular pressure (IOP) and glaucoma and to explore whether any associations are due to social, behavioral, genetic, or health differences.

Design: Cross-sectional analysis of population-based data

Methods: We used the Canadian Longitudinal Study on Aging Comprehensive Cohort, which consists of 30,097 adults aged 45-85 years. Race/ethnicity was self-reported. Corneal-compensated intraocular pressure (IOP) was measured in mmHg using the Reichert Ocular Response Analyzer. Participants were asked to report if they have ever had a diagnosis of glaucoma and whether they used eye care in the past year. A glaucoma polygenic risk score (PRS) was calculated. Logistic and linear regression models were used.

Results: Black individuals had higher mean IOP levels (beta coefficient (β) = 1.46; 95% confidence interval [CI], 0.62, 2.30) while Chinese, Japanese and Korean (β = -1.00; 95% CI, -1.63, -0.38) and Southeast Asian and Filipino individuals (β = -1.56; 95% CI, -2.68, -0.43) had lower mean IOP levels as compared to White individuals after adjustment for sociodemographic, behavioural, genetic, and health-related variables. Black people were more likely to report glaucoma as compared to White people after adjustment (odds ratio [OR] = 2.43; 95% CI, 1.27, 4.64).

Conclusion: Racial and ethnic differences in IOP and glaucoma were identified. Adjusting for sociodemographic, behavioral, genetic, and health-related variables did not fully explain these differences. Longitudinal research is needed to further explore the

reasons for these differences and to understand their relevance to disease pathogenesis and progression.

INTRODUCTION

Glaucoma, a leading cause of irreversible vision loss worldwide and estimated to affect 76 million people in 2020, consists of a group of optic neuropathies undergoing progressive degeneration of retinal ganglion cells[1-3]. Factors related to the development and progression of glaucoma have not been fully characterized but both genetic and environmental factors are important[1,4]. Currently, reduction of intraocular pressure (IOP) is the only method proven to treat glaucoma[5].

For reasons that we do not yet fully understand, type and frequency of glaucoma vary by ethnic background. People having European, African, and Latin American ancestry are much more likely to develop primary open-angle glaucoma (POAG) rather than primary angle-closure glaucoma (PACG) while people of Asian ancestry may develop either POAG or PACG [3,6]. Furthermore, Black Americans have a higher prevalence of glaucoma than White Americans [7] with the prevalence in Hispanic Americans falling between the two [8]. Research has suggested that Black Americans may have an earlier age of onset of glaucoma and an increased progression to blindness [9,10], although other studies have reported similar visual field progression between Black and White Americans [11] and the Ocular Hypertension Treatment Study found that the association between Black race and the incidence of glaucoma disappeared when adjusting for other factors like central corneal thickness [12]. In addition, The Barbados Eye Study reported that Black participants had higher IOP than mixed race or White participants [13].

Prior studies have been limited in the number of ethnic groups that they have included. They have also failed to thoroughly investigate reasons for racial and ethnic differences in glaucoma and IOP. Using the baseline data from the Canadian Longitudinal Study on Aging (CLSA) that included multiple racial and ethnic groups of sufficient size, we investigated the associations between ethnicity, IOP, and glaucoma and assessed whether the associations were due to social, behavioral, genetic, health, or healthcare access factors.

METHODS

Study Population and Design

A cross-sectional analysis was conducted using the first wave of data from the CLSA Comprehensive Cohort made up of 30,097 Canadian adults aged 45-85 years[14]. Stratified random sampling of provincial healthcare registration databases and random digit dialing of landline telephones was used to obtain the CLSA sample. The first wave of data was collected between 2012 and 2015 through in-home interviews and visits to data collection sites for physical examinations and biospecimen sample collections. These sites were located in cities across 7 provinces. Inclusion criteria were: participants had to be aged 45-85 years, living in the community, not cognitively impaired, and speak English or French. Exclusion criteria were: being a full-time member of the Canadian Armed Forces, living on a federal First Nations reserve or settlement, residing in a long-term care institution, or not a permanent resident or Canadian citizen.

Informed Consent and Ethics Approval

All participants gave written informed consent to participate and publish. Research Ethics Board approval was acquired for all CLSA sites in July 2010. The University of Ottawa Office of Research Ethics and Integrity gave approval for the present analysis in October 2021 (H-12-18-2153).

Ocular Data

Participants were asked to report any previous diagnosis of glaucoma by a doctor and whether they had visited an ophthalmologist or optometrist in the past year. Corneal-compensated IOP was evaluated using the Reichert Ocular Response Analyzer (Reichert Technologies, Depew, NY, USA). The IOP of the right and left eyes was averaged together for analysis. If one eye was missing IOP data, then the IOP value of the other eye was used. To estimate the pre-treatment IOP, the IOP of participants taking medications with a Drug Identification Number (DIN) of an IOP-lowering eye drop was divided by 0.7, which is the mean estimated treatment effect[15]. This approach has been used previously [16]. IOP values greater than 60 were treated as probable measurement errors and were excluded.

Race/Ethnicity

Race/ethnicity was self-reported using an interviewer-administered questionnaire at baseline. In order to avoid small numbers, participants were grouped into 1 of 9 racial/ethnic groups including 1) White, 2) Black, 3) Chinese, Japanese and

Korean, 4) Southeast Asian and Filipino, 5) South Asian, 6) Arab and West Asian, 7) Latin American, 8) Other, and 9) Mixed. People who reported being from more than one group were placed into the "Mixed" category.

Genetic Data

Genome-wide genotyping of non-fasting blood samples from consenting participating were done using the Affymetrix Axiom array resulting in 794,409 single nucleotide polymorphisms (SNPs) from 26,622 participants [17]. Release 3 of the CLSA genomic data was used. Marker- and sample-based quality control checks were performed by the CLSA using standard procedures [17]. Marker-based checks were done for the examination of genotype consistency across genotyping batch, chromosomally defined sex, Hardy-Weinberg equilibrium, and discordance of genotyping across control replicates. Sample-based checks were done for the examination of relatedness, heterozygosity, and genotype missing values. 15 individuals were excluded with extreme values of heterozygosity and genotype missingness while 1,666 individuals were excluded for relatedness. Release 3 also included genotype data imputed using the TOPMed reference panel at the University of Michigan Imputation Service. These imputed data contained 97,256 reference samples at 308,107,085 genetic markers [17].

A glaucoma polygenic risk score (PRS) was calculated for each CLSA participant having available genotype data that passed quality control checks. The PRS was previously developed by Craig et al. [18] using 2,673 independent SNPs associated with glaucoma from their multitrait analysis of genome-wide association studies. Using

genome build GRCh38/hg38, we had 2,652 SNPs available in the CLSA to calculate the PRS. Because there were so few missing SNPs (<1%), proxy SNPs were not chosen to replace the missing SNPs. The PRS was calculated for each CLSA participant with a weighted sum of the 2,652 SNPs: $\sum_{i=1}^{2652} \hat{\beta}_i \times \mathrm{SNP}_i$, where $\hat{\beta}_i$ is the effect size of SNP_i on glaucoma from Craig et al. and SNP_i is the number of copies of the effect allele in an individual genotype or the expected number of copies of the effect alleles for imputed genotypes. We standardized the PRS to have a mean of 0 and standard deviation (SD) of 1.

Principal component analysis was performed on the CLSA genotype data and the top four principal components were clustered, yielding six clusters reflecting ancestry [17]. Each ancestry cluster was named by cross tabulating it with self-reported ethnicity and finding the dominant group.

Demographic, Health, and Lifestyle Data

Age, sex, education and income were collected during the in-home visit via an interviewer-administered questionnaire. Participants had their height and weight measured using standardized procedures at data collection site visits. Body mass index (BMI) was calculated and categorized according to World Health Organization guidelines (underweight < 18.5 kg/m^2, normal weight 18.5–24.9 kg/m^2, overweight 25.0–29.9 kg/m^2, and obese ≥ 30.0 kg/m^2) [19].

Participants were asked if they had a physician diagnosis of diabetes or high blood pressure. Also, blood pressure was measured 6 times using the BpTRU™ BPM200

Blood Pressure Monitor (Medaval, Dublin, Ireland). Readings 2 through 5 were averaged. Hypertension was defined if: 1) a participant reported a physician diagnosis of hypertension or 2) if the average systolic blood pressure was 130 mmHg or higher or 3) diastolic blood pressure was 80 mmHg or higher[20].

Participants were asked "Have you smoked at least 100 cigarettes in your life?" and "At the present time, do you smoke cigarettes daily, occasionally (at least once in last 30 days), or not at all (not in last 30 days)?" Answers to these questions were used to determine if a person was a current, former, or never smoker. A current smoker reported smoking at least 100 cigarettes and currently smokes daily or occasionally. A former smoker reported smoking at least 100 cigarettes in their lifetime but had not smoked in the last 30 days. Questions were asked about alcohol consumption frequency and type of alcohol consumed during the in-home visit. Total alcohol intake (grams/week) was obtained by multiplying the weekly number of portions of each alcohol type by 13.45 grams (the total alcohol content of a standard portion size specified in the CLSA). Total alcohol intake was then divided into tertiles.

Statistical Analysis

Our two primary outcomes were pre-treatment IOP and glaucoma. Mean IOP levels were examined by racial/ethnic, demographic, health, and behavioral factors. The proportion of participants that used eye care in the past 12 months and the distribution of the mean PRS were also examined by racial/ethnic group. Differences were tested by the linear and logistic regression. To adjust for potential confounding variables

including age, sex, education, income, smoking, alcohol intake, diabetes, systemic hypertension, BMI, PRS, and province, linear regression was used for IOP while logistic regression was used for glaucoma. Given that race/ethnicity was self-reported, additional analyses were performed to examine PCA genetic ancestry clusters with IOP and glaucoma. Sensitivity analyses were done to examine current IOP instead of pre-treatment IOP and IOP in the left eye instead of mean iop. Sampling weights and strata variables were integrated into all analyses using the SVY commands in Stata SE 16 (StataCorp, College Station, TX, USA).

RESULTS

Descriptive Statistics

Our analysis sample consisted of 25,398 people (84% of the Comprehensive Cohort) who had complete genetic and IOP data with IOP measures within the accepted range (up to 60mmHg). Those missing IOP and/or genetic data (n=4,699) were very similar to those not missing data except they were older, more likely to be female, and drank less alcohol (Supplementary Table 1).

The mean IOP of participants by race/ethnicity, demographic, behavioral, health-related, and genetic factors is shown in Table 1. Participants who were Black, older, had lower incomes, drank more alcohol, had type 2 diabetes or high blood pressure, had higher BMI, used eye care, and had higher glaucoma PRS scores had higher mean IOP levels ($P<0.05$). Chinese, Japanese, and Korean, Southeast Asian and Filipino participants, and women had lower IOP values ($P<0.05$).

The proportion of participants who had contact with an ophthalmologist or optometrist in the past 12 months by racial/ethnic group is presented in Figure 1. Compared to White participants (55.3%), Arab and West Asian participants had the lowest proportion of eye care utilization (35.4%, P=0.002)), followed by Black participants (44.6%, P=0.043)). Southeast Asian and Filipino participants had the highest eye care utilization (62.2%) although it was not statistically significantly different from White participants (P=0.420).

The distribution of the standardized PRS by racial/ethnic group is shown in Table 2. A higher PRS may mean a higher genetic risk of glaucoma. The mean PRS differed significantly by racial/ethnic group. Black participants had the highest mean PRS (mean=0.46, P<0.001) compared to White participants followed by Chinese, Japanese, and Korean people (mean=0.37, P<0.001).

Regression Models with Self-Reported Ethnicity

In Table 3, linear regression was used to adjust the relationship between race/ethnicity and IOP for potentially confounding variables including age, sex, education, income, smoking, alcohol intake, BMI, diabetes, systemic hypertension, eye care utilization, and province. Model 1 adjusts for all variables except the PRS while Model 2 adjusts for all variables including the PRS. In Model 1, individuals of Black race had higher mean IOP levels (beta coefficient (β) = 1.83; 95% confidence interval [CI], 1.00, 2.66) while Chinese, Japanese, and Korean (β = -0.70; 95% CI, -1.32, -0.09) and Southeast Asian and Filipino individuals (β = -1.32; 95% CI, -2.52, -0.12) had lower mean

IOP levels as compared to White individuals after adjusting for sociodemographic, behavioral, and health-related variables. Adjusting for the PRS in Model 2 slightly attenuated the relationship between Black race and IOP (β= 1.46, 95% CI, 0.62, 2.30), although it remained statistically significant, while it increased the associations between Chinese, Japanese, and Korean ethnicity (β= -1.00, 95% CI, -1.63, -0.38) and Southeast Asian and Filipino ethnicity and IOP (β= -1.56, 95% CI, -2.68, -0.43).

The logistic regression results for glaucoma are shown in Table 4. In Model 1 of Table 4, individuals of Black race (odds ratio [OR] = 2.83; 95% CI, 1.47, 5.47) and Latin American ethnicity (OR = 2.64; 95% CI, 1.02, 6.82) were more likely to report glaucoma compared to White individuals after adjustment for demographic, behavioral, and health variables (Table 4). Further adjustment for the PRS (Model 2) somewhat attenuated the association between Black race and glaucoma (OR=2.43, 95% CI 1.27, 4.64) although it remained statistically significant. Adjustment for the PRS attenuated the association between Latin American ethnicity and glaucoma (OR=2.39, 95% CI 0.93, 6.14) such that it was no longer statistically significant.

Regression Models with Genetic Markers of Ancestry

Because self-reported race and ethnicity may not accurately reflect ancestry[21], especially in those with mixed ancestry , we conducted additional analyses instead using genetic markers of ancestry. Results were consistent with our main analyses using the self-report of race/ethnicity (Supplementary Table 2). Individuals of African descent had higher mean IOP (β=2.06, 95% CI 1.27, 2.85), while individuals of East or Southeast Asian

ancestry had lower mean IOP (β=-0.73, 95% CI -1.26, -0.21) as compared to individuals of European descent after covariate adjustment. As before, adjusting for the PRS somewhat attenuated the association between African ancestry and IOP while it strengthened the association between East or Southeast Asian ancestry and IOP.

Similarly, we examined the association between genetic ancestry and glaucoma (Supplementary Table 3). As with self-reported race/ethnicity, people of African descent were more likely to report glaucoma compared to individuals of European descent after adjustment (OR=2.70, 95% CI 1.42, 5.13). Adjustment for the PRS somewhat reduced the strength of the association.

In contrast to our findings using self-reported ethnicity, people with genetic Latin American ancestry were not more likely to report glaucoma compared to those of European descent before (OR=1.27, 95% CI 0.63, 2.55) or after PRS adjustment (OR=1.21, 95% CI 0.62, 2.36). On the other hand, genetic Arab or West Asian ancestry was associated with glaucoma both before (OR=1.55, 95% CI 1.03, 2.33) and after PRS adjustment (OR=1.59, 95% CI 1.06, 2.38).

Sensitivity Analyses

The results from the sensitivity analysis using current IOP measures were consistent with our main results (data not shown). Also, the results for IOP using data from only 1 eye are consistent with results using mean IOP (data not shown).

DISCUSSION

We attempted to understand whether racial and ethnic differences in IOP and glaucoma are due to social, behavioral, genetic, health, or healthcare differences. We found that Black participants had higher mean IOP levels while Chinese, Japanese, and Korean, and Southeast Asian and Filipino participants had lower mean IOP levels as compared to White participants after adjustment. The PRS sometimes acted as a positive confounder (adjusting for it attenuated the association) and sometimes acted as a negative confounder (adjusting for it exaggerated the association). Adjustment for the PRS somewhat reduced the strength of the association with IOP among Black participants while it increased the strength of the association for Southeast Asian and Filipino individuals.

Furthermore, we found that individuals of Black race and Latin American ethnicity were more likely to report glaucoma as compared to White individuals, after adjusting for sociodemographic, behavioral, and health-related variables. Adjustment for the glaucoma PRS reduced the strength of the associations among Black and Latin American participants. Our results using the self-report of race and ethnicity were largely confirmed using measures of genetic ancestry. An exception was that only self-reported Latin American ancestry was associated with glaucoma before adjustment for the PRS while genetic Latin American ancestry was not. Further, genetic Arab or West Asian ancestry was associated with glaucoma while self-reported Arab or West Asian ancestry was not.

Documenting racial or ethnic differences in health without attempting to explain them is of limited value. Researchers need to consider the conceptual framework

underlying their investigation into health disparities[22,23]. Too often, ethnic differences have been attributed to genetic causes without consideration of other social, behavioral, or health-related causes[24]. We identified several groups of factors that could potentially cause ethnic differences in IOP and glaucoma including social, behavioral, health, healthcare, and genetic factors. Despite adjusting for social factors like education and income, behavioral factors like alcohol consumption and smoking, health factors like diabetes and high blood pressure, and care factors like eye care in the last year, strong associations between race/ethnicity and IOP and glaucoma remained. Further adjustment for the PRS somewhat attenuated our findings in Black participants while it somewhat strengthened our findings with IOP in Chinese, Japanese and Korean and Southeast Asian and Filipino individuals. Ultimately, we were not able to explain the racial and ethnic differences in IOP and glaucoma.

The CLSA is fairly unique in its large numbers of racial and ethnic groups allowing comparison of IOP between groups within the same study. Another very large study with multiple ethnic groups, the UK Biobank, also found that Black participants had higher IOP (β=0.77, 95% CI 0.63, 0.90) and that Chinese participants had lower IOP (β= -0.74, 95% CI -1.10, -0.38) than White participants [25]. The Barbados Eye Study and South African Eye Study also found that Black participants had higher IOP than mixed race or White participants [13,26] and the Singapore Epidemiology of Eye Diseases Study reported that Chinese people had lower IOPs than Malay or Indian participants [27]. Our findings for glaucoma were also consistent with previous research. Several studies have

previously reported that Black participants [7,28-30] and Latin American participants [8,31] were more likely to have glaucoma than White participants.

We did not find that a polygenic risk score, demographic, behavioral, or health-related factors explained the relationship between ethnicity and IOP/glaucoma. However, it's possible that other biological factors are related to ethnic differences. For example, although we had data on corneal-compensated IOP, we did not have data on other ocular biometric parameters. Researchers have previously identified structural and biometric parameters associated with POAG and its progression in African-Americans as compared to those of European descent including larger optic disc area, deeper maximum cup depth, and thinner corneas[32-35]. Further, African-American glaucoma patients were found in prior research to have higher levels of oxygen in the anterior chamber as compared to patients of European descent, which may contribute to increased oxidative stress, IOP, and cellular damage[36]. Studies have also shown African-American glaucoma patients to have significantly lower retrobulbar blood flow compared to patients of European descent[37,38].

Consistent with previous study results[39], Black and Latin American participants reported less contact with ophthalmologists and optometrists relative to White participants, which could put them at greater risk of developing glaucoma if they have untreated ocular hypertension. Arab and West Asian participants also were much less likely to have seen an ophthalmologist or optometrist in the last year. However, we did not find that adjustment for recent eye care use diluted racial and ethnic differences.

The main strength of this study is the use of a large population-based sample of multiple racial/ethnic groups and data on social, behavioral, health-related, and genetic factors. Several limitations must be noted. First, glaucoma status was based solely by self-report with no information available on severity or subtype. However, our findings with glaucoma are consistent with many other studies[7,8,28-31]. Furthermore, data on retinal nerve fiber layer and macular thickness, structures implicated in glaucoma disease pathogenesis which have been previously found to vary according to race and ethnicity[40,41] , were unavailable in the CLSA. Next, the use of a PRS constructed from European-derived variants, which may not replicate in non-European samples[42], may mean that we were not able to adequately adjust for the genetic risk of glaucoma. Therefore, we cannot rule out that the racial and ethnic differences in IOP and glaucoma are due to genetic differences. Also, although the CLSA had several racial and ethnic categories of participants, most of the categories were of limited size giving reduced statistical power to detect differences. Finally, beyond education and income, we did not have extensive data on social factors like experience with discrimination that could cause health disparities[22].

CONCLUSION

Racial and ethnic differences in IOP and glaucoma were identified. Adjusting for sociodemographic, behavioral, genetic, and health-related variables did not explain these differences. Longitudinal research is needed to further explore the reasons for

these differences and to understand their relevance to disease pathogenesis and progression.

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Conflict of Interest

The authors have no financial or any other kind of personal conflicts with this paper.

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Data Availability Statement

Data are available from the Canadian Longitudinal Study on Aging (www.clsa-elcv.ca) for researchers who meet the criteria for access to de-identified CLSA data.

Ethics Declarations

This study was reviewed and approved by the University of Ottawa Office of Research and Integrity, with the approval number: H-12-18-2153 (October 2021).

All participants provided written informed consent to participate in the study and publish the results.

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Table 1. Average IOP by self-reported race/ethnicity, demographic, health, lifestyle, and genetic factors

n=25,398	IOP (mm Hg) Mean (SD)	P-value
Race/ethnicity		
White (n= 24,043)	16.1 (3.8)	ref
Black (n=160)	17.2 (4.3)	0.004
Chinese, Japanese and Korean (n=205)	15.0 (3.6)	<0.001
Southeast Asian and Filipino (n=80)	14.6 (3.2)	0.007
South Asian (n=209)	16.1 (3.6)	0.813
Arab and West Asian (n=99)	15.6 (3.6)	0.306
Latin American (n=82)	15.8 (4.0)	0.639
Other (n=152)	16.4 (3.3)	0.312
Mixed (n=364)	16.1 (3.8)	0.840
Age group (years)		
45-54 (n=6,452)	15.3 (2.7)	ref
55-64 (n=8,457)	16.2 (3.8)	<0.001
65-74 (n=6,202)	16.9 (4.9)	<0.001
75-85 (n=4,287)	17.1 (5.5)	<0.001
Sex		
Male (n=12,792)	16.3 (4.0)	ref
Female (n=12,606)	15.9 (3.7)	<0.001
Education		
University degree or certificate above (n=5,477)	16.0 (5.4)	ref
Bachelor's degree (n=6,014)	15.7 (5.0)	0.001
Less than Bachelor's degree (n=13,864)	16.1 (3.3)	0.141
Household income		
≥$100,000 (n=8,929)	15.7 (3.7)	ref
$50,000- $100,000 (n=8,422)	16.2 (4.0)	<0.001
$20,000- $50,000 (n=5,242)	16.3 (3.8)	<0.001
<$20,000 (n=1,228)	16.1 (3.6)	0.074
Refused/ Don't know (n=1,577)	16.0 (4.2)	0.036
Smoking status		
Current (n=2,145)	15.6 (3.2)	ref
Former (n=11,154)	16.2 (3.9)	0.001
Never (n=12,006)	16.0 (3.9)	<0.001
Alcohol intake (grams/week)		
T1 (n=9,979)	15.9 (3.7)	ref
T2 (n=7,739)	16.0 (3.9)	0.053
T3 (n=7,515)	16.3 (3.9)	<0.001
Diabetes		
None (n=20,916)	15.9 (3.8)	ref

Type 1 (n=136)	16.2 (4.4)	0.699
Type 2 (n=2,279)	16.8 (4.3)	<0.001
Suspect/ neither type (n=1,809)	16.2 (3.7)	0.017
High blood pressure		
No (n=11,348)	15.6 (3.7)	ref
Yes (n=14,050)	16.5 (3.9)	<0.001
BMI		
Underweight (n=169)	15.1 (3.8)	ref
Normal weight (n=7,406)	15.7 (3.9)	0.222
Overweight (n=10,383)	16.1 (3.9)	<0.001
Obese (n=7,358)	16.3 (3.6)	<0.001
Eye care utilization		
No (n=9,987)	15.8 (3.4)	ref
Yes (n=14,343)	16.3 (4.2)	<0.001
PRS		
Q1 (n=6,340)	15.0 (3.3)	ref
Q2 (n=6,352)	15.8 (3.6)	<0.001
Q3 (n=6,362)	16.4 (3.9)	<0.001
Q4 (n=6,344)	17.1 (4.1)	<0.001

*The following variables had missing data: race/ethnicity (n=4), education (n=43), smoking (n=93), alcohol (n=165), diabetes (n=258), BMI (n=82), eye care utilization (n=1,068); ref=reference category

Figure 1: Percent of participants who had contact with an ophthalmologist or optometrist in past 12 months by self-reported racial/ethnic group

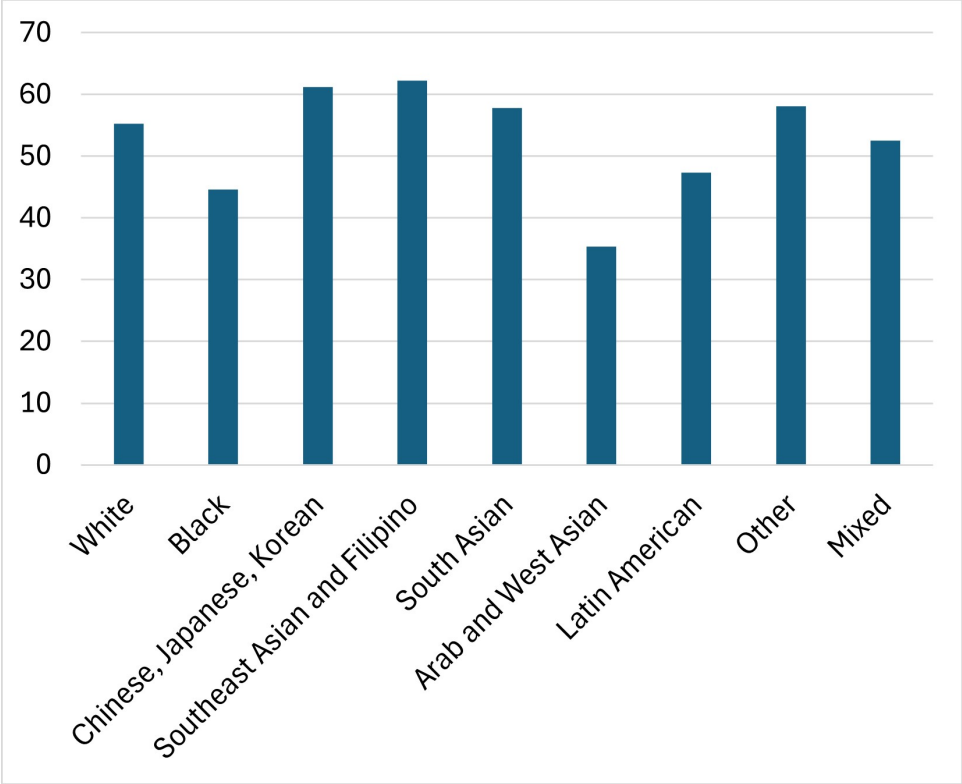

Table 2. Mean standardized PRS by self-reported racial/ethnic group

Race/ethnicity	PRS Mean (SD)	P-value
White	0.00 (1.00)	ref
Black	0.46 (0.70)	<0.001
Chinese, Japanese and Korean	0.37 (0.83)	<0.001
Southeast Asian and Filipino	0.23 (0.63)	0.029
South Asian	0.24 (0.91)	0.038
Arab and West Asian	-0.07 (0.87)	0.618
Latin American	0.28 (1.07)	0.103
Other	0.26 (0.95)	0.015
Mixed	-0.01 (0.86)	0.934

ref=reference category

Table 3. Linear regression analyses of the associations of self-reported race/ethnicity with IOP

	Model 1* **IOP n=22,205 β (95% CI)**	**Model 2†** **IOP n=22,205 β (95% CI)**
Race/ethnicity		
White	0.00	0.00
Black	1.83 (1.00, 2.66)	1.46 (0.62, 2.30)
Chinese, Japanese and Korean	-0.70 (-1.32, -0.09)	-1.00 (-1.63, -0.38)
Southeast Asian and Filipino	-1.32 (-2.52, -0.12)	-1.56 (-2.68, -0.43)
South Asian	0.56 (-0.09, 1.22)	0.37 (-0.34, 1.07)
Arab and West Asian	-0.02 (-0.88, 0.84)	0.08 (-0.71, 0.86)
Latin American	0.44 (-0.85, 1.73)	0.22 (-0.88, 1.33)
Other	0.57 (-0.13, 1.28)	0.34 (-0.38, 1.06)
Mixed	0.38 (-0.18, 0.94)	0.37 (-0.19, 0.93)
PRS	-	0.81 (0.74, 0.87)

*Adjusted for age, sex, education, income, smoking, alcohol intake, BMI, diabetes, high blood pressure, eye care utilization, and province

†Adjusted for all covariates in model 1 and PRS

Table 4. Logistic regression analyses of the associations of self-reported race/ethnicity with glaucoma

	Model 1* **Glaucoma n=23,123 OR (95% CI)**	**Model 2†** **Glaucoma n=23,123 OR (95% CI)**
Race/ethnicity		
White	1.00	1.00
Black	2.83 (1.47, 5.47)	2.43 (1.27, 4.64)
Chinese, Japanese and Korean	0.53 (0.15, 1.83)	0.46 (0.13, 1.62)
Southeast Asian and Filipino	2.63 (0.89, 7.78)	2.40 (0.82, 7.05)
South Asian	1.02 (0.49, 2.10)	0.98 (0.48, 1.98)
Arab and West Asian	1.63 (0.40, 6.59)	1.83 (0.46, 7.25)
Latin American	2.64 (1.02, 6.82)	2.39 (0.93, 6.14)
Other	2.20 (0.87, 5.54)	1.99 (0.78, 5.05)
Mixed	1.83 (0.74, 4.56)	1.91 (0.74, 4.90)
PRS	-	1.56 (1.42, 1.71)

*Adjusted for age, sex, education, income, smoking, alcohol intake, BMI, diabetes, high blood pressure, eye care utilization and province

†Adjusted for all covariates in model 1 and PRS

Supplementary Table 1. Demographic, health, lifestyle factors of those with and without missing data

	Missing IOP and/or genetic data (n=4,699) Mean (SD) or %	**Complete IOP and genetic data (n=25,398)** Mean (SD) or %
Self-reported race/ethnicity		
White	91.7	94.1
Black	1.4	0.7
Chinese, Japanese and Korean	1.1	0.8
Southeast Asian and Filipino	0.4	0.4
South Asian	1.0	0.8
Arab and West Asian	0.5	0.5
Latin American	0.6	0.3
Other	1.3	0.7
Mixed	2.0	1.7
Age group (year)		
45-54	38.5	38.7
55-64	28.5	32.1
65-74	18.5	18.4
75-85	14.4	10.8
Sex		
Female	59.6	51.2
Male	40.4	48.8
Education		
Less than Bachelor's degree	76.5	75.6
Bachelor's degree	12.0	13.2
University degree or certificate above	11.5	11.3
Household income		
≥$100,000	30.7	35.4
$50,000- $100,000	30.8	32.3
$20,000- $50,000	22.1	21.1
<$20,000	8.2	5.7
Refused/ Don't know	8.2	5.6
Smoking status		
Current	12.3	11.5
Former	43.1	44.9
Never	44.6	43.6
Alcohol intake (grams/week)	61.4 (98.6)	70.1 (105.7)
Diabetes		
None	81.8	82.7

Type 1	8.1	6.4
Type 2	10.9	9.1
Suspect/ neither type	6.5	7.6
High blood pressure		
No	47.1	45.9
Yes	52.9	54.1
BMI		
Underweight	1.1	0.6
Normal weight	30.0	27.6
Overweight	35.9	39.6
Obese	33.0	32.2
Glaucoma		
No	94.3	95.9
Yes	5.7	4.1
Eye care utilization		
No	42.5	44.8
Yes	57.5	55.2

Supplementary Table 2. Linear regression analyses of the associations of PCA genetic ancestry cluster with IOP

	Model 1* **IOP n=22,207 β (95% CI)**	**Model 2†** **IOP n=22,207 β (95% CI)**
PCA cluster		
European	0.00	0.00
African	2.06 (1.27, 2.85)	1.64 (0.83, 2.45)
Chinese, Japanese and Korean, Southeast Asian and Filipino	-0.73 (-1.26, -0.21)	-1.04 (-1.57, -0.50)
South Asian	0.09 (-0.54, 0.72)	-0.05 (-0.70, 0.60)
Arab and West Asian	0.11 (-0.20, 0.41)	0.16 (-0.13, 0.46)
Latin American	-0.13 (-0.91, 0.66)	-0.30 (-0.99, 0.40)
PRS	-	0.81 (0.74, 0.87)

*Adjusted for age, sex, education, income, smoking, alcohol intake, BMI, diabetes, high blood pressure, eye care utilization and province

†Adjusted for all covariates in model 1 and PRS

Supplementary Table 3. Logistic regression analyses of the associations of PCA genetic ancestry cluster with glaucoma

	Model 1* **n=23,126** **OR (95% CI)**	**Model 2†** **n=23,126** **OR (95% CI)**
PCA cluster		
European	1.00	1.00
African	2.70 (1.42, 5.13)	2.20 (1.18, 4.12)
Chinese, Japanese and Korean, Southeast Asian and Filipino	1.06 (0.46, 2.42)	0.93 (0.40, 2.16)
South Asian	0.99 (0.52, 1.87)	0.95 (0.51, 1.78)
Arab and West Asian	1.55 (1.03, 2.33)	1.59 (1.06, 2.38)
Latin American	1.27 (0.63, 2.55)	1.21 (0.62, 2.36)
PRS	-	1.56 (1.42, 1.71)

*Adjusted for age, sex, education, income, smoking, alcohol intake, BMI, diabetes, high blood pressure, and province

†Adjusted for all covariates in model 1 and PRS

CHAPTER 6: Supplementary Analyses

In supplementary tables 1-8, we present estimates of the associations of alcohol consumption frequency, alcohol types, total alcohol intake as well as interactions between sex and the glaucoma PRS and alcohol with IOP and glaucoma, adjusting for the minimal sufficient adjustment sets identified using Daggity. For the associations of alcohol with IOP and glaucoma, the minimally sufficient adjustment set to block all backdoor paths included age, sex, race/ethnicity, education, income, diet, diabetes, physical activity, and smoking. As the Mediterranean diet score includes wine intake, the antioxidant diet score was included in the regression models. As per CLSA guidelines, we included a dummy variable for province in all regression models. Results were mainly consistent with our primary analyses except for white wine which was no longer statistically significantly associated with IOP after adjusting for the minimal sufficient adjustment set. Further, when adjusting for the minimally sufficient adjustment set, an interaction was found between alcohol consumption and sex with IOP such that females had 0.14 mm Hg higher increases in IOP (95% CI: 0.05-0.24) per 5-drink increases in weekly alcohol intakes relative to males. However, no interaction was found between alcohol consumption and sex with glaucoma (data not shown). As the risks posed by the presence of clinical comorbidities, such a hypertension and diabetes, would differ among those controlled vs. not controlled with medication, we re-ran all models with additional adjustment for HbA1c. Hypertension was defined using a measure of blood pressure which was found to be associated with oxidative stress in a bidirectional manner. Therefore, we did not adjust for blood pressure or

antihypertensive medication use. Models with additional adjustment for HbA1c were mainly consistent, however, daily drinking was no longer associated with higher IOP (β =0.35, 95% CI: -0.09-0.79) while white wine was associated with higher IOP (β =0.22, 95% CI: 0.00-0.43).

Supplementary Table 1. Linear regression analysis of the association of alcohol consumption frequency with IOP

Alcohol consumption frequency n=25,360	**IOP β***	**95% CI**
Never	0.00	
Occasional	-0.21	-0.59, 0.17
Weekly	0.10	-0.28, 0.48
Daily	0.44	0.03, 0.85

***Adjusted for age, sex, race/ethnicity, education, income, diabetes, smoking, physical activity, diet and province

Supplementary Table 2. Logistic regression analysis of the association of alcohol consumption frequency with glaucoma

Alcohol consumption frequency n=26,564	**Glaucoma OR***	**95% CI**
Never/rarely	1.00	
Occasional	1.22	0.74, 2.02
Weekly	1.10	0.66, 1.84
Daily	1.25	0.74, 2.11

***Adjusted for age, sex, race/ethnicity, education, income, diabetes, smoking, physical activity, diet and province

Supplementary Table 3. Investigation of interaction between PRS and alcohol with IOP

PRS Quartile	**Total Alcohol intake**	**β ***	**95% CI**
1 Low (n=5,288)	Per 70 gram (~5-drink) increase	0.08	0.00, 0.14
2 (n=5,271)	Per 70 gram (~5-drink) increase	0.10	0.01, 0.20
3 (n=5,249)	Per 70 gram (~5-drink) increase	0.17	0.07, 0.28
4 High	Per 70 gram (~5-drink) increase	0.20	0.10, 0.30

(n=5,257)			

*Adjusted for age, sex, race/ethnicity, education, income, diabetes, smoking, physical activity, diet and province ; P-value= 0.007 for interaction term between total alcohol intake and PRS quartile

Supplementary Table 4. Linear regression analysis of the association of alcohol type with IOP

Alcohol type (per 5 drinks/week) (n=25,229)	**IOP β***	**95% CI**
Red wine	0.31	0.20, 0.41
White wine	0.19	-0.03, 0.41
Beer	0.18	0.07, 0.28
Liquor	0.08	-0.09, 0.24
Other	0.25	-0.61, 1.10

*****Adjusted for age, sex, race/ethnicity, education, income, diabetes, smoking, physical activity, diet and province

Supplementary Table 5. Logistic regression analysis of the association of alcohol type with glaucoma

Alcohol type (per 5 drinks/week) (n=26,246)	**Glaucoma OR***	**95% CI**
Red wine	1.06	0.94, 1.19
White wine	1.10	0.92, 1.31
Beer	0.91	0.79, 1.05
Liquor	0.89	0.75, 1.07
Other	0.81	0.37, 1.77

*****Adjusted for age, sex, race/ethnicity, education, income, diabetes, smoking, physical activity, diet and province

Supplementary Table 6. Linear regression analysis of the association of total alcohol intake with IOP

n=25,209	**IOP β***	**95% CI**
Total alcohol intake, per 70 g/week (5 drink) increase	0.13	0.08, 0.18

*****Adjusted for age, sex, race/ethnicity, education, income, diabetes, smoking, physical activity, diet and province

Supplementary Table 7. Logistic regression analysis of the association of total alcohol intake with glaucoma

n=26,406	**Glaucoma OR***	**95% CI**
Total alcohol intake, per 70 g/week (5 drink) increase	0.99	0.94, 1.04

***** Adjusted for age, sex, race/ethnicity, education, income, diabetes, smoking, physical activity, diet and province

Supplementary Table 8. Investigation of interaction between PRS and alcohol intake with glaucoma

PRS Quartile	**Average Total Alcohol intake**	**OR***	**95% CI**
1 Low (n=5,268)	Per 70 gram (5-drink) increase	0.98	0.84, 1.14
2 (n=5,251)	Per 70 gram (5-drink) increase	1.02	0.93, 1.11
3 (n=5,225)	Per 70 gram (5-drink) increase	1.04	0.91, 1.19
4 High (n=5,234)	Per 70 gram (5-drink) increase	0.98	0.89, 1.08

*Adjusted for age, sex, race/ethnicity, education, income, diabetes, smoking, physical activity, diet and province ; P-value= 0.877 for interaction term between total alcohol intake and prs quartile

In supplementary tables 9-13, we present estimates of the associations of dietary factors, patterns, supplements and the interactions between the glaucoma PRS

and dietary patterns with IOP and glaucoma, adjusting for the minimal sufficient adjustment sets. The minimally sufficient adjustment set identified using Daggity for the associations of diet with IOP and glaucoma included age, sex, race/ethnicity, education, income, and smoking. We also included a dummy variable for province in all regression models. Potential mediation by BMI and cholesterol were assessed using Sobel-Goodman tests for linear regression models and by assessing the impacts of adjusting versus not adjusting for these variables in regression models. Results were mostly consistent with our primary analyses. However, in contrast with our primary results, egg, fish and poultry consumption were associated with higher IOP, while high sugar snacks were associated with lower IOP after adjusting for the minimal sufficient adjustment set. However, these associations were no longer statistically significant after Bonferroni correction. Sobel testing confirmed a partial mediating role of BMI for the associations of Mediterranean and antioxidant dietary pattern scores with IOP ($p<0.01$ and $p<0.00$, respectively). Sobel testing did not confirm a mediating role for cholesterol ($p>0.233$). However, using the Baron and Kenney approach, BMI and cholesterol did not appreciably mediate associations of Mediterranean or antioxidant dietary pattern scores with IOP or glaucoma as adjusting versus not adjusting for cholesterol and BMI posed no substantial changes to the results (data not shown).

Supplementary Table 9. Separate regression models showing relationships between dietary patterns with IOP and glaucoma

	IOP		**Glaucoma**	
	β*	**95% CI**	**OR†**	**95% CI**
Mediterranean	**(n=26,906)**		**(n=28,225)**	

Q1	0.00		1.00	
Q2	0.08	-0.10, 0.26	1.05	0.84, 1.31
Q3	0.12	-0.05, 0.29	0.93	0.75, 1.15
Q4	0.14	-0.04, 0.33	0.98	0.85, 1.23
Antioxidant rich	**(n=27,057)**		**(n=28,384)**	
Q1	0.00		1.00	
Q2	-0.03	-0.21, 0.15	0.98	0.77, 1.24
Q3	-0.02	-0.20, 0.17	0.97	0.76, 1.24
Q4	-0.08	-0.27, 0.11	1.02	0.80, 1.29

***** Adjusted for age, sex, race/ethnicity, education, income, smoking and province using linear regression

†Adjusted for the covariates above using logistic regression

Supplementary Table 10. Separate regression models of the relationship between dietary supplements with IOP and glaucoma

	IOP n=27,086		**Glaucoma n=28,414**	
	β*	**95% CI**	**OR†**	**95% CI**
No iron supplements Consumption of iron supplements	0.00 -0.19	 -0.44, 0.06	1.00 0.97	 0.68, 1.37
No calcium supplements Consumption of calcium supplements	0.00 -0.18	 -0.33, -0.03	1.00 1.26	 1.05, 1.50

***** Adjusted for age, sex, race/ethnicity, education, income, smoking and province using linear regression

†Adjusted for the covariates above using logistic regression

Supplementary Table 11. Investigation of interaction between PRS and dietary pattern with IOP

PRS Quartile	Dietary pattern	IOP β*	95% CI
1 Low (n=5,603)	Mediterranean		
	Q1	0.00	
	Q2	-0.23	-0.54, 0.10
	Q3	0.15	-0.18, 0.48
	Q4	0.07	-0.25, 0.40
2 (n=5,606)	Mediterranean		
	Q1	0.00	
	Q2	0.12	-0.23, 0.46
	Q3	0.10	-0.25, 0.45
	Q4	-0.08	-0.43, 0.28
3 (n=5,618)	Mediterranean		
	Q1	0.00	
	Q2	0.27	-0.18, 0.71
	Q3	-0.11	-0.54, 0.31
	Q4	0.41	-0.08, 0.90
4 High (n=5,608)	Mediterranean		
	Q1	0.00	
	Q2	0.03	-0.39, 0.45
	Q3	0.35	-0.01, 0.71
	Q4	0.38	-0.02, 0.79
1 Low (n=5,641)	Antioxidant rich		
	Q1	0.00	
	Q2	0.12	-0.20, 0.45
	Q3	-0.12	-0.43, 0.20
	Q4	0.02	-0.34, 0.38
2 (n=5,635)	Antioxidant rich		
	Q1	0.00	
	Q2	-0.11	-0.45, 0.23
	Q3	0.10	-0.25, 0.46
	Q4	-0.12	-0.49, 0.26
3 (n=5,645)	Antioxidant rich		
	Q1	0.00	
	Q2	-0.01	-0.46, 0.45
	Q3	-0.20	-0.70, 0.17
	Q4	-0.07	-0.60, 0.31
4 High (n=5,639)	Antioxidant rich		
	Q1	0.00	

	Q2	0.01	-0.42, 0.43
	Q3	0.38	-0.07, 0.82
	Q4	0.04	-0.41, 0.48

* Adjusted for age, sex, race/ethnicity, education, income, smoking and province

Supplementary Table 12. Investigation of interaction between PRS and dietary pattern with glaucoma

PRS Quartile	Dietary pattern	Glaucoma OR*	95% CI
1 Low	Mediterranean		
(n=5,840)	Q1	1.00	
	Q2	1.60	0.82, 3.13
	Q3	2.03	0.96, 4.92
	Q4	1.21	0.56, 2.61
2	Mediterranean		
(n=5,844)	Q1	1.00	
	Q2	0.86	0.49, 1.52
	Q3	1.08	0.66, 1.78
	Q4	0.68	0.40, 1.13
3	Mediterranean		
(n=5,861)	Q1	1.00	
	Q2	1.17	0.73, 1.89
	Q3	0.90	0.56, 1.43
	Q4	1.12	0.68, 1.83
4 High	Mediterranean		
(n=5,845)	Q1	1.00	
	Q2	0.96	0.64, 1.44
	Q3	0.78	0.54, 1.13
	Q4	1.28	0.87, 1.90
1 Low	Antioxidant rich		
(n=5,881)	Q1	1.00	
	Q2	1.37	0.63, 2.99
	Q3	1.02	0.49, 2.11
	Q4	1.34	0.65, 2.76
2	Antioxidant rich		
(n=5,875)	Q1	1.00	
	Q2	0.68	0.36, 1.29
	Q3	0.72	0.42, 1.23
	Q4	0.82	0.46, 1.47
3	Antioxidant rich		
(n=5,888)	Q1	1.00	
	Q2	0.89	0.53, 1.51
	Q3	0.85	0.48, 1.48
	Q4	1.21	0.64, 2.02
4 High	Antioxidant rich		
(n=5,875)	Q1	1.00	
	Q2	1.13	0.76, 1.66
	Q3	1.19	0.75, 1.88

	Q4	1.01	0.68, 1.52

* Adjusted for age, sex, race/ethnicity, education, income, smoking and province

Supplementary Table 13. Separate regression models showing relationships between different dietary components with IOP and glaucoma

Per 1-serving increase (daily)	IOP β*	95% CI	Glaucoma OR†	95% CI
Fruit	-0.02	-0.08, 0.05	1.05	0.98, 1.13
Fruit juice	0.10	-0.01, 0.21	0.99	0.86, 1.14
Non-starchy vegetables	-0.11	-0.16, -0.05	0.98	0.91, 1.06
Legumes	0.04	-0.19, 0.26	1.00	0.78, 1.28
Nuts and seeds	0.02	-0.10, 0.13	0.97	0.86, 1.08
Whole grains	0.05	-0.02, 0.13	1.01	0.90, 1.12
Potatoes (non-fried)	0.11	-0.13, 0.35	0.95	0.76, 1.20
Potatoes (fried)	0.22	-0.34, 0.79	1.26	0.68, 2.34
Fish	0.37	0.02, 0.72	1.37	0.90, 2.08
Poultry	0.37	0.07, 0.68	1.14	0.80, 1.62
Processed meat	-0.01	-0.36, 0.35	0.90	0.64, 1.28
Red meat	0.10	-0.11, 0.31	0.94	0.71, 1.24
Sauces and gravies	0.10	-0.18, 0.39	1.05	0.74, 1.46
Butter and margarine	0.05	-0.04, 0.14	1.03	0.93, 1.14
High sugar snacks	-0.10	-0.20, -0.00	0.95	0.85, 1.06
Salty snacks	0.14	-0.08, 0.36	1.13	0.88, 1.44
High fat dairy	0.14	0.06, 0.23	0.99	0.89, 1.11
Low fat dairy	0.04	-0.01, 0.10	1.00	0.92, 1.07
Salad dressing	-0.04	-0.19, 0.12	0.94	0.77, 1.14
Eggs	0.18	0.01, 0.36	0.98	0.78, 1.23
Calcium-fortified foods	-0.12	-0.25, 0.00	1.12	0.94, 1.32

***** Adjusted for age, sex, race/ethnicity, education, income, smoking and province using linear regression

†Adjusted for the covariates above using logistic regression

In supplementary tables 14-17, estimates of the associations of race/ethnicity and genetic ancestry cluster with IOP and glaucoma are presented. As the goal of our analysis was to gain a better understanding of the reason for ethnic differences in IOP and glaucoma, we conditioned on strong predictors of the outcome, even if they are not considered true confounders (i.e. they were not causally associated with the exposure). As such, in addition to the PRS, we adjusted for age, sex, education, income, smoking, alcohol intake, BMI, diabetes, PASE score and province. As eye care use may be both a cause of high IOP and glaucoma and also caused by high IOP and glaucoma, we did not adjust for it in our supplementary regression models, as we had in the primary analyses. Further, as blood pressure was identified to have bidirectional associations with oxidative stress mechanisms, we examined the effects of removing it from the regression models. Potential mediation by education, income, alcohol intake, physical activity and smoking were assessed by assessing the impacts of adjusting versus not adjusting for these variables in regression models. Results were consistent with our primary analyses. Separately removing education, income, alcohol intake, and smoking status did not significantly change the associations of race/ethnicity with IOP indicating that mediation by these variables in unlikely. Removing PASE score, however, resulted in a smaller beta coefficient for the Southeast Asian and Filipino group which was no longer statistically significant. Separately removing education and alcohol intake from the regression models of race/ethnicity with glaucoma did not significantly alter results. However, removing income, smoking and physical activity in separate models all

resulted in reduced effect sizes for the Southeast Asian and Filipino group that no longer reached statistical significance. As the risks posed by diabetes would differ among those controlled vs. not controlled with medication, we also re-ran models with additional adjustment for HbA1c and results were unchanged (data not shown).

Supplementary Table 14. Linear regression analyses of the associations of race/ethnicity with IOP (n=28,301)

	Model 1* **β (95% CI)**	**Model 2†** **β (95% CI)**
Race/ethnicity		
White	0.00	0.00
Black	1.82 (0.98, 2.66)	1.46 (0.61, 2.21)
Chinese, Japanese and Korean	-0.72 (-1.34, -0.10)	-1.64 (-1.66, -0.39)
Southeast Asian and Filipino	-1.33 (-2.56, -0.09)	-1.57 (-2.72, -0.41)
South Asian	0.52 (-0.16, 1.20)	0.34 (-0.40, 1.08)
Arab and West Asian	-0.14 (-1.00, 0.72)	-0.04 (-0.83, 0.74)
Latin American	0.43 (-0.77, 1.63)	0.21 (-0.82, 1.25)
Other	0.53 (-0.18, 1.25)	0.31 (-0.43, 1.04)
Mixed	0.40 (-0.17, 0.96)	0.38 (-0.18, 0.94)
PRS	-	0.81 (0.74, 0.87)

*Adjusted for age, sex, education, income, smoking, alcohol intake, BMI, diabetes, PASE score, and province

†Adjusted for all covariates in model 1 and PRS

Supplementary Table 15. Logistic regression analyses of the associations of race/ethnicity with glaucoma (n=28,064)

	Model 1* OR (95% CI)	Model 2† OR (95% CI)
Race/ethnicity		
White	1.00	1.00
Black	2.30 (1.19, 4.43)	2.01 (1.07, 3.80)
Chinese, Japanese and Korean	0.60 (0.18, 2.05)	0.51 (0.14, 1.77)
Southeast Asian and Filipino	2.84 (1.01, 8.04)	2.56 (0.92, 7.12)
South Asian	1.07 (0.52, 2.20)	0.96 (0.45, 2.03)
Arab and West Asian	1.44 (0.37, 5.58)	1.54 (0.41, 5.86)
Latin American	2.64 (1.08, 6.49)	2.32 (0.96, 5.63)
Other	2.29 (0.90, 5.82)	2.12 (0.83, 5.42)
Mixed	1.84 (0.72, 4.74)	1.87 (0.70,4.97)
PRS	-	1.55 (1.41, 1.71)

*Adjusted for age, sex, education, income, smoking, alcohol intake, BMI, diabetes, PASE score, and province

†Adjusted for all covariates in model 1 and PRS

Supplementary Table 16. Linear regression analyses of the associations of PCA genetic ancestry cluster with IOP (n=28,303)

	Model 1* β (95% CI)	Model 2† β (95% CI)
PCA cluster		
European	0.00	0.00
African	2.05 (1.25, 2.85)	1.63 (0.82, 2.44)
Chinese, Japanese and Korean, Southeast Asian and Filipino	-0.71 (-1.24, -0.18)	-1.02 (-1.55, -0.48)
South Asian	0.01 (-0.65, 0.68)	-0.11 (-0.80, 0.58)
Arab and West Asian	0.10 (-0.22, 0.41)	0.15 (-0.15, 0.46)
Latin American	-0.16 (-0.93, 0.61)	-0.36 (-1.01, 0.36)
PRS	-	0.80 (0.74, 0.87)

*Adjusted for age, sex, education, income, smoking, alcohol intake, BMI, diabetes, PASE score, and province
†Adjusted for all covariates in model 1 and PRS

Supplementary Table 17. Logistic regression analyses of the associations of PCA genetic ancestry cluster with glaucoma (n=28,067)

	Model 1* OR (95% CI)	Model 2† OR (95% CI)
PCA cluster		
European	1.00	1.00
African	2.31 (1.22, 4.39)	1.94 (1.05, 3.58)
Chinese, Japanese and Korean, Southeast Asian and Filipino	1.20 (0.54, 2.68)	1.03 (0.45, 2.34)
South Asian	1.00 (0.53, 1.90)	0.92 (0.48, 1.78)
Arab and West Asian	1.53 (1.02, 2.30)	1.56 (1.04, 2.34)
Latin American	1.24 (0.63, 2.44)	1.19 (0.62, 2.30)
PRS	-	1.55 (1.42, 1.71)

*Adjusted for age, sex, education, income, smoking, alcohol intake, BMI, diabetes, PASE score, and province

CHAPTER 7: DISCUSSION

Summary of Overall Findings

This thesis examined risk factors of elevated IOP and glaucoma: race/ethnicity, alcohol consumption and dietary factors, patterns, and supplement use. In the CLSA Comprehensive Cohort, Black individuals had higher mean IOP levels (β = 1.46; 95% CI, 0.63, 2.30) while Chinese, Japanese and Korean (β = -1.00; 95% CI, -1.62, -0.38) and Southeast Asian and Filipino individuals (β = -1.55; 95% CI, - 2.68, -0.42) had lower mean IOP levels as compared to White individuals after adjustment for sociodemographic, behavioural, genetic, and health-related variables. Black people were more likely to report glaucoma as compared to White people after adjustment (OR = 2.43; 95% CI, 1.27, 4.64). Latin American people (OR = 2.64; 95% CI, 1.02, 6.82) were also more likely to report glaucoma but this association was no longer statistically significant after adjusting for the glaucoma PRS (OR=2.39, 95% CI 0.93, 6.13).

Next, daily drinkers had higher IOP compared to those who never drank (β=0.47, 95% CI: 0.05, 0.88). An increase in total weekly alcohol intake (per 5 drinks) was also associated with higher IOP (β=0.13, 95% CI: 0.07, 0.18). The association between total alcohol intake and IOP was stronger in those with a higher genetic risk of glaucoma (P for interaction term= 0.002). Alcohol consumption frequency and total alcohol intake were not associated with glaucoma.

Finally, consuming calcium supplements was associated with lower IOP (β=-0.16, 95% CI: -0.31, 0.00) and an increased odds of glaucoma (OR= 1.30, 95% CI: 1.08, 1.56), while supplementation with iron was not associated with IOP or glaucoma. No dietary

components were associated with IOP or the odds of glaucoma after covariate adjustment and Bonferroni correction. IOP and glaucoma were not associated with adherence to a Mediterranean or antioxidant-rich diet after covariate adjustment.

Ethnic and Racial Differences in IOP and Glaucoma

Documenting and investigating racial and ethnic differences in IOP and glaucoma can help to better understand the causes of glaucoma and to identify groups who may need more frequent eye exams. Our work was unique in its ability to examine multiple ethnic groups in the same study and to examine whether a wide variety of potential confounding variables explained any ethnic differences. No prior studies have examined whether a glaucoma polygenic risk score explained ethnic differences. We found that Black participants had higher mean IOP levels while Chinese, Japanese, and Korean, and Southeast Asian and Filipino participants had lower mean IOP levels as compared to White participants after covariate adjustment. Further, we found that individuals of Black race and Latin American ethnicity were more likely to report glaucoma as compared to White individuals, after adjustment. The use of the self-report of race and ethnicity can have measurement error. For example, self-reported race/ethnicity errors may occur when individuals are not fully aware of their true ethnicity or only know their recent ancestry or geographic origin[309]. People may also identify with one racial/ethnic group despite a mixed background[309]. Further, in admixed populations (including Latin American and African), self-reported race/ethnicity may not be accurate due to the high genetic, socioeconomic, cultural, and geographical heterogeneity within and across

population groups[310]. As such, population stratification (systemic ancestry differences between cases and non-cases) is common in ethnically admixed populations[311]. While genetic ancestry may contribute significantly to the pathogenesis of diseases, it provides less information on societal constructs such as economic, resource and health-related disparities[309]. Our results using the self-report of race and ethnicity were largely confirmed using measures of genetic ancestry, however, self-reported Latin American ancestry was associated with glaucoma while genetic Latin American ancestry was not while genetic Arab or West Asian ancestry was associated with glaucoma while self-reported Arab or West Asian ancestry was not. Genetic markers may provide more accurate information on potential population stratification which may explain these differences. After adjusting for sociodemographic, behavioral, genetic, and health-related variables the direct effects of race, ethnicity and ancestry remained associated with IOP and glaucoma. A potential explanation for our findings is that residual confounding in our analyses may be limiting our ability to fully explain the observed differences. For example, data on retinal nerve fiber layer and macular thickness, structures of the eye implicated in glaucomatous pathology and known to vary according to race and ethnicity[312], were not available in the CLSA. Extensive data on social factors (e.g. discrimination) that could cause health disparities were also unavailable. Lastly, the use of a PRS of European-derived index variants may mean that we were not able to adequately adjust for the genetic risk of glaucoma[313]. More work is needed to develop polygenic risk scores in non-European ethnic groups in order to more fully capture the genetic risk of glaucoma in all people[314].

Alcohol Findings

People often assume that drinking moderate amounts of alcohol is safe. However, we found that consuming an increased frequency and amount of alcohol, particularly red wine and beer, was associated with higher levels of IOP while it was not associated with the odds of glaucoma. Daily drinkers had an IOP that was 0.46 mmHg higher than people who never drank alcohol. Further, the association between total alcohol intake and IOP was stronger in females and those with higher genetic risk of glaucoma. One would think that since alcohol was related to higher IOP, it would also be related to glaucoma. Perhaps explaining our finding that alcohol was associated with higher IOP but not glaucoma is certain types of alcohol like red and white wine and beer contain varying concentrations of polyphenols including flavonoids, which may exert neuroprotective effects on the retina via IOP-independent pathways[153,315]. The use of the self-report of glaucoma, which may lead to misclassification, could also explain the null finding[316]. Although the effect sizes in this research may seem small and not clinically significant, it is important to remember that our results compare average IOP between participants rather than within participants. It is possible that daily drinking in a particular individual, especially at high genetic risk, may lead to much higher elevations of IOP within that individual than what our study showed on average. It is possible that daily drinking may make it more difficult to achieve the target IOP with treatment. Our work is only the second study to test for an interaction between alcohol consumption and a polygenic risk score. We have confirmed the interaction found in the UK Biobank.

Some studies have reported a stronger dose-dependent association between alcohol and IOP in men[276,277]; however, this finding may result from the smaller number of heavy female drinkers included in study samples. In our analytic sample, 50.9% of participants were female and we found that females had significantly higher IOP elevations per 5-drink increase in weekly alcohol intake as compared to males, when adjusting for the minimally sufficient adjustment set. However, no significant interaction was found between alcohol consumption and sex with glaucoma suggesting this sex-specific effect may be IOP-dependent. This finding is biologically plausible, for example sex-related differences in gastric oxidation of ethanol have been identified such that females have an increased bioavailability of ethanol following ingestion which could contribute to an increased vulnerability to the damaging effects of alcohol use[317].

Diet Findings

Participants who reported consuming calcium supplements had an IOP that was 0.16 mm Hg lower but had an increased odds of glaucoma compared to those who did not while supplementation with iron was not associated with IOP or glaucoma. Further, IOP and glaucoma were not associated with adherence to a Mediterranean or antioxidant-rich diet after adjustment. No interactions with the polygenic risk score were found. In exploratory analyses, no dietary components were associated with IOP or the odds of glaucoma after covariate adjustment and Bonferroni correction. Our findings may highlight important differences in supplemental and dietary calcium absorption and on impacts on calcium homeostasis and thereby glaucoma

pathophysiology[249,318,319]. A potential reason we did not confirm previously reported associations with iron, IOP and glaucoma may be due to heterogeneity in the iron supplement doses taken by the CLSA participants[320]. A potential explanation for our null findings of dietary pattern adherence and IOP/glaucoma was that overall adherence to a Mediterranean diet or antioxidant-rich diet among CLSA participants was quite low. Further, we expect that null findings of associations among dietary factors and patterns with the odds of glaucoma may result from the lack of data regarding glaucoma subtype and severity in the CLSA.

Strengths of the Research

The main strength of this study is the utilization of a large population-based sample of multiple racial/ethnic groups and data on social, behavioral, health-related, and genetic factors which allowed for comparisons between groups within the same study. A further strength of this dataset was that we were able to adjust for a large number variables known to be associated with race/ethnicity, alcohol intake, diet, IOP, and glaucoma, and to examine potential effect modification thereby furthering knowledge of the complex interplay of risk factors. Further, we used a validated FFQ to derive dietary intakes[290,321], had data on both current and corrected IOP as well as data on genetic ancestry clusters to supplement self-reports of race/ethnicity.

Risk of Bias

The main risk of bias of the present analysis is the use of self-reported data including the data on eye disease, demographic, lifestyle and health-related factors. There was no ophthalmological exam done as part of the CLSA confirming reports of eye disease. The use of self-reported eye disease is prone to misclassification due to a lack of patient recall, awareness and understanding of diagnoses[316]. However, some diseases may be more prone to misclassification than others. For example, glaucoma may be more prone to misclassification as many people with glaucoma do not know they have it until rather late in the disease process. As previously discussed, glaucoma is often asymptomatic in early stages, and reliance on IOP, visual field testing, evaluation of optic disc cupping and the RNFL, may delay diagnosis and treatment until irreversible damage to RGC may have already occurred[322]. Furthermore, patients may be confused by other conditions like ocular hypertension that require treatment similar to glaucoma leading to an over-estimation of the diagnoses. Those reporting a diagnosis of glaucoma may also be more prone to recalling alcohol use or unhealthy dietary behaviors than people who did not report glaucoma (i.e. recall bias). This differential misclassification of exposure by glaucoma status would lead to more cases to be considered exposed and could bias any associations between alcohol intake, unhealthy diet and glaucoma away from the null. However, we did not find any statistically significant associations between diet or alcohol and glaucoma. The misclassification of self-reported ethnicity, however, is unlikely to differ by glaucoma status and this non-differential misclassification measurement bias would bias toward the null. The misclassification of

glaucoma status, however, is likely differential by diet, alcohol and ethnicity. For example, those who drink less, eat healthier, or are White may more accurately reflect their glaucoma status due to more frequent preventative health service use. These individuals would also be less likely to have disease, leading to fewer exposed to unhealthy diet, alcohol or low socioeconomic status being considered to have glaucoma, which would bias the results towards the null. However, due to more frequent health service use, these healthier individuals may be more likely to be diagnosed with glaucoma in its earlier stages, resulting in more glaucoma cases being considered unexposed, which would again lead to a bias towards the null. The overall direction of bias induced by the misclassification of glaucoma status, however, would depend on the proportion of subjects misclassified. Monte Carlo sensitivity analyses could be used in future work to assess uncertainty due to the self-report of glaucoma by specifying plausible distributions of the sensitivity and specificity of self-reported glaucoma and then probabilistically sampling data to generate a range of bias-corrected estimates[323]. Our findings should be confirmed in studies that have diagnosed glaucoma and information on severity and subtypes.

Next, social-desirability bias may have led participants to under-report their chronic conditions or the frequency at which they engage in unhealthy behaviours such as smoking and drinking alcohol and over-report socially desirable behaviours such as physical activity and consuming healthy food such as fruits and vegetables[324]. However, CLSA interviewers were trained to ask questions in a nonjudgmental and standardized way. Future studies employing Mendelian randomization analyses may provide

association results with less bias (due to reverse causation, confounding, and self-report) and could help indirectly validate the alcohol and diet intake measures.

Despite the use of a validated food frequency questionnaire to derive dietary intakes, most nutrient intakes and the number of servings of fruit and vegetables derived from the SDQ administered in the CLSA have been previously found to be significantly lower as compared to those estimated from the means of three non-consecutive 24 hour diet recalls (24HR)[290] (the reference standard). Spearman correlations between the key nutrients and foods estimated by the SDQ and 24HR were low to moderate ($p<0.01$), ranging from 0.19 (cholesterol) to 0.45 (daily servings of fruits and vegetables)[290]. Given the reduced ability of the SDQ to accurately reflect consumption patterns of the target nutrients and foods it is possible the associations of dietary factors and patterns with IOP and glaucoma were underestimated in our study.

Further, the use of a PRS of European-derived index variants, which often do not replicate in non-European samples[313], may mean that we were not able to adequately adjust for the genetic risk of glaucoma. Therefore, we cannot rule out that the racial and ethnic differences in IOP and glaucoma are due to genetic differences.

Next, the CLSA baseline lacked data on retinal nerve fiber layer and macular thickness[312], social factors beyond education and income, dietary factors including caffeine[41,272,325] and sodium intakes[326], biomarkers of oxidative stress, vascular dysregulation and inflammation, as well as from calories in alcohol, caffeinated beverages or food items not included in the FFQ. For example, sociocultural factors (such as religion and spirituality, social networks, social support, and cultural norms) and

racial discrimination within and outside of the health care system can potentiate racial and ethnic health disparities[85] at multiple levels throughout the life course. These social factors may therefore account for a large proportion of the observed racial and ethnic differences observed in IOP and glaucoma. The lack of data on retinal nerve fiber layer, macular thickness, caffeine and sodium intakes as well as calories from alcohol, caffeinated beverages and food items not included in the FFQ may have contributed to further residual confounding.

Further, due to the cross-sectional design which is prone to reverse causation, we are unable to delineate temporality of the alcohol consumption and diet with the onset of glaucoma/high IOP. For example, if someone reduces their alcohol consumption or unhealthy dietary behaviors because they have been diagnosed with glaucoma, that would lead to an underestimate of any harmful association between drinking alcohol or unhealthy diet with glaucoma. Further, given that glaucoma and elevated IOP are latent conditions, disease latency bias could have affected the results where the conditions may have occurred prior to measurement of the exposures. For example, among those who reported a diagnosis of glaucoma, surgical or medical treatment may have lowered IOP to normal levels prior to measurement of dietary exposures. Therefore, the measured dietary exposures do not reflect those prior to the onset or during the latent period of their disease, leading to biased estimates of association. The ambiguous temporality of certain variables due to the measurement methods and the cross-sectional design may also increase the uncertainty of the

pathways depicted in the DAG models. Longitudinal data are needed to rule out reverse causality and disease latency bias.

Further, our original analyses may be subject to overadjustment bias due to adjusting for potential mediating factors, colliding variables, and descendants of the outcome. Conditioning on a factor that isn't a true confounder may result in reduced precision of the effect estimates. For associations of dietary patterns/factors with IOP and glaucoma, BMI and cholesterol are probably causally related to dietary exposures and, in turn, may contribute to the oxidative stress mechanisms implicated in elevated IOP and glaucoma, as depicted in the DAG in figure 3. Therefore adjustment for these factors may have introduced bias in the estimate for the total effect of diet on IOP and glaucoma[327]. Similarly, overadjustment for variables not included in the minimal sufficient adjustment sets, such as high blood pressure which is associated with oxidative stress mechanisms in a bidirectional manner and may act as a collider or mediating factor depending on the time the variables were measured, total caloric intake (proxy for energy imbalance) which was identified as a potential collider in the DAGs, or adjustment for eye care utilization, which can be caused by high IOP or a glaucoma diagnosis may have biased our effect estimates. In fact, removing PASE score from the associations of race/ethnicity with IOP and separately removing education, alcohol intake, and PASE score from models of the associations of race/ethnicity with glaucoma changed the significance of the findings in our supplemental analyses indicating potential overadjustment in primary analyses. While Sobel testing confirmed

a partial mediating role of BMI for the associations of Mediterranean and antioxidant dietary pattern scores with IOP, our supplementary analyses were mainly consistent with our primary analyses and therefore the potential overadjustment bias in our study did not pose meaningful effects on our results.

Generalizability of Research Findings

The CLSA study excluded those who could not speak English or French, those living on a First Nations reserve or in an institution, those in the military and those with overt cognitive impairment. Further, the individuals recruited via random digit dialing were required to have a landline telephone. Self-selection in the CLSA also may lead to recruitment of individuals of higher socioeconomic status and better health[286]. Therefore, generalizability of our results to more remote areas in Canada and from areas outside of the 11 data collection site locations as well as to groups other than those studied remains unknown. However, CLSA demographic and lifestyle measures were found to be comparable with Canadian census and CCHS estimates, which had high response rates[286].

Future Research

We demonstrated multiple racial and ethnic differences in IOP and glaucoma. Adjustment for sociodemographic, behavioral, genetic, and health-related variables including a glaucoma polygenic risk score and use of eye care in the last year did not

substantially alter the results. Our research suggests that greater alcohol use, certain alcohol types and dietary factors and supplements are associated with IOP but not with glaucoma. While our work has moved this field of research forward, further longitudinal research, including diverse genomic data, are needed to further explore the reasons for these differences and to understand their relevance to disease pathogenesis and progression. Future longitudinal research and mediation analyses with more complete dietary data (derived from a 24HR or a full FFQ), information of glaucoma subtype and severity, retinal nerve fiber layer and macular thickness, biometric parameters of the eye, and social factors beyond education and income are also needed to further understand the relationships between IOP, glaucoma and alcohol use, dietary factors, patterns and supplements and to further elucidate the interactions of dietary and genetic factors on their risk of disease.

Implications of our Findings

This study adds to the growing body of evidence implicating gene–diet interactions in high IOP [272-274], increasing the probability of precision nutrition and dietary recommendations based on genomic data in future glaucoma care. However, additional research is needed before interventions are developed and implemented into care. If our research is confirmed and greater knowledge is gained about underlying mechanisms, targeted lifestyle interventions could be developed and targeted towards the high risk groups. For example, ophthalmologists and optometrists could provide better nutrition guidance and discuss the impacts that factors such as diet and alcohol

use pose on IOP/glaucoma in healthy individuals and those with higher genetic risk prior to the onset of disease.

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