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Evaluating vitamin C-related gene-environment and metabolite-environment interaction effects on intraocular pressure in the Canadian Longitudinal Study on Aging

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Abstract

High intraocular pressure (IOP) is an important risk factor for glaucoma, which is influenced by genetic and environmental factors. However, the etiology of high IOP remains uncertain. Metabolites are compounds involved in metabolism which provide a link between the internal (genetic) and external environments. O-methylascorbate has been reported to be associated with IOP. In addition, researchers have identified several genetic variants which are associated with metabolite concentrations, including O-methylascorbate and another vitamin C related metabolite, ascorbic acid 2-sulfate. We aimed to understand how O-methylascorbate and ascorbic acid 2-sulfate, or genetic variants associated with these metabolites, modify the associations between dietary environmental variables and IOP. We used data from 8060 participants of the Canadian Longitudinal Study on Aging. Using linear models adjusted for relevant covariates, we tested for interactions between six genetic variants previously found to be associated with O-methylascorbate and ascorbic acid 2-sulfate and four environmental variables related to diet (alcohol consumption frequency, smoking status, fruit consumption, and vegetable consumption). We also tested for interactions between serum concentrations of O-methylascorbate and ascorbic acid 2-sulfate and these environmental factors. We used a False Discovery Rate approach to correct for the 32 interaction tests performed. One interaction was suggestively significant after multiple testing correction (adjusted P-value < 0.1): rs8050812 and alcohol consumption frequency. Understanding how genetic variants and metabolites interact with the environment could shed light on biological pathways controlling IOP and lead to improved prevention and treatment of glaucoma.

Keywords CLSA, Gene-environment, Intraocular pressure, Metabolites

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Introduction

Glaucoma is one of the leading causes of irreversible blindness in the world [1]. One of the most important risk factors for glaucoma is elevated intraocular pressure (IOP) [2, 3], which is heritable [4, 5]. Investigation of the etiology of elevated IOP may be key to preventing glaucoma [6]. Several studies have investigated the genetic and environmental components that contribute to variation in IOP [7]. A recent genome-wide association study identified over 300 genetic loci associated with IOP [8]. However, there is still a large proportion of IOP variance which is unexplained [7, 8].

The exposome represents the totality of environmental exposures over the lifetime [9, 10]. One way to quantify a component of the exposome is through measured metabolite levels [11]. Metabolites are compounds involved in metabolism as intermediates or end-products. They can also help provide a link between the molecular/genetic environment and the external environment [11]. Past research by Hysi et al. investigated the influence of metabolites on IOP and found that O-methylascorbate, a vitamin C metabolite, was most associated [12]. While there is global concern about vitamin C status [13], there is conflicting evidence about the association between glaucoma and vitamin C [14, 15]. In addition, there is little research on the effect of vitamin C on IOP.

Other studies have identified genetic factors that are associated with metabolite levels [16, 17]. These genetic factors can be used when assessing the effect of metabolites on phenotypes in certain study designs, such as Mendelian Randomization [16] and could be helpful to detect interactions with environmental factors acting on metabolic pathways. We previously investigated genetic factors associated with O-methylascorbate and ascorbic acid 2-sulfate, which are both vitamin C related, and found several associations which had also been found in previous research [16, 18]. We identified three genetic variants which were independently significantly associated with each metabolite at the genome-wide significance level (P-value $< 5 \times 10^{-8}$). The variants identified were rs144009214, rs12414734 and rs8050812 for ascorbic acid 2-sulfate, and rs165879, rs4680, and rs61484427 for O-methylascorbate [18].

Furthermore, several environmental risk factors may interact with vitamin C in their effect on IOP and glaucoma. Some environmental risk factors relevant to vitamin C that were previously found to be associated with glaucoma include diet (including vitamin consumption) [19–21], smoking [22, 23] and alcohol [24, 25]. Smoking and alcohol are also associated with IOP [23–28].

There is still a large proportion of IOP variance which is not explained by genetic or environmental factors alone. By investigating the interplay between genetic variants, the metabolome, and the environment we can better understand IOP variation and glaucoma etiology. In this study, we investigated interactions between environmental risk factors relevant to vitamin C and metabolic/genetic factors affecting IOP in the Canadian Longitudinal Study on Aging (CLSA).

Materials and methods

Study population and design

We carried out a cross-sectional analysis using data from the Comprehensive Cohort of the Canadian Longitudinal Study on Aging (CLSA) [29]. The Comprehensive Cohort includes 30 097 Canadians between 45 and 85 years at recruitment with baseline data collected between 2012 and 2015. Participants in the Comprehensive Cohort underwent in-home interviews, in-depth clinical examinations and some provided biological samples at CLSA data collection sites located in Victoria, Vancouver, Surrey, Calgary, Winnipeg, Hamilton, Ottawa, Montreal, Sherbrooke, Halifax, and St. John's, Canada. Participants were included if they were community dwelling at baseline, not cognitively impaired at baseline, and able to respond in English or French. Full-time members of the Canadian Armed Forces, those residing on a federal First Nations reserve or settlement, residents in the three territories and some remote regions, those living in a long-term care institution at baseline, were excluded.

Of those in the Comprehensive Cohort, 26 622 individuals were genotyped [30], 9992 participants had their metabolite levels quantified [31], and around 9000 participants had both. Participants were then further excluded if they were not of European ancestry, were related to other participants, had missing or outlier IOP values or had missing confounder covariate information, leading to a final sample of 8060 individuals. Reporting of this research was informed by the Strengthening the Reporting of Genetic Associations (STREGA) guideline [32]. Written informed consent was obtained for all participants, and research ethics board approval was obtained for all CLSA affiliated sites. The analysis presented here was approved by the University of Ottawa research ethics board.

Genetic data

Consenting individuals from the Comprehensive Cohort provided blood samples which were stored at -80 °C before being shipped to a genomics facility and stored at -20 °C. 26 622 individuals in the CLSA were genotyped from the Comprehensive Cohort using the Affymetrix Axiom Array, leading to 794 409 variants genotyped [30]. We inferred genetic ancestry from genomic data by the CLSA to restrict the sample to European ancestry [30]. Genotyped data was used for imputation using the TOPMED reference panel, resulting in ~308 million imputed variants. For this analysis, we used the

imputed genetic data from six genetic variants of interest (rs144009214, rs12414734, rs8050812, rs165879, rs4680, and rs61484427) which were found in our previous research to be associated with either ascorbic acid 2 sulfate or O-methylascorbate. Genetic data were coded based on the number of minor alleles in an individual's genotype.

Metabolic data

Ten thousand participants were selected for metabolomics quantification. Among those, 3000 were selected from a group of participants who had fasted for over 5 h while 7000 were selected from the rest of the cohort. Sample selection was made to reflect the distribution of the Comprehensive Cohort by data collection site, age and sex. This process resulted in 9992 consenting participants from the Comprehensive Cohort having their metabolite levels quantified by an untargeted approach [31]. Metabolite levels were measured using mass spectrometry, followed by identification using the Metabolon Discovery HD4TM LC-MS platform. After quality control checks, 1314 identified metabolites were included in the final dataset. For our analysis, we focused on two metabolites: 2-O-methylascorbate and ascorbic acid-2-sulfate. We used measurements provided by the CLSA which were batch normalized. Metabolite values were log-transformed, extreme outliers (more than 3 SD away) were removed, and then the values were normalized to a mean of 0 and SD of 1, as done in previous research [16].

Ocular data

The outcome of interest for this analysis was intraocular pressure (IOP). IOP was measured in mmHg using a Reichart Ocular Response Analyzer in the baseline examination in the Comprehensive Cohort. Participants with an eye infection, who reported that they had eye surgery in the three months prior to examination, or who reported a detached retina in the three months prior to examination. Participants with measurements in both eyes had their IOP levels averaged. For participants with only one eye measurement, that value was used. We used IOP measurements that were adjusted for corneal mechanic properties, i.e. corneal compensated IOP (IOPcc). Instead of using current IOP, we estimated pre-treatment IOP. Participants were asked to bring all medications. For participants taking medications with a Drug Identification Number indicative of an IOP-lowering eye drop at the time of baseline examination, IOPcc was divided by 0.7 to account for the average medication effect, as done previously [27, 33, 34]. Three participants with outlier IOP levels, defined as >60 mmHg [27], were removed.

Glaucoma was self reported by participants who were asked whether a physician had ever diagnosed them with glaucoma.

Environmental risk factors

Alcohol consumption

Participants were asked "Have you ever drunk alcohol?" and if they said yes were asked "About how often during the past twelve months did you drink alcohol?". Respondents were categorized as "Never", "Occasional", "Weekly" and "Daily" drinkers based on these responses.

Cigarette smoking

The smoking variable was coded using the questions "Have you smoked at least 100 cigarettes in your life?" and "At the present time, do you smoke cigarettes daily, occasionally or not at all?". Based on the responses, the participants were categorized as "Never" "Former" or "Current" smokers. If participants said no to smoking at least 100 cigarettes, they were considered as never smokers. Those who said they smoke cigarettes daily or occasionally were considered as current smokers. Those who said they had smoked 100 cigarettes, but currently not at all were considered former smokers.

Dietary variables

Participants were asked to fill in the validated 36-item Short Diet Questionnaire [35, 36]. For fruit consumption, participants were asked "How often do you eat fruit (fresh, frozen, canned)?" and "How often do you drink 100% fruit juices?". These responses were standardized to the number of reported servings per day and added together total fruit consumption per day. One participant was excluded from this analysis based on having an outlier value of 20.

For vegetable consumption, the frequency of the consumption of the following foods were added together: "How often do you usually eat green salad (lettuce, with or without other ingredients)?", "How often do you usually eat carrots (fresh, frozen, canned, eaten on their own or with other food, cooked or raw)?", and "How often do you usually eat other vegetables (except carrots, potatoes or salad)?". These responses were standardized to the number of reported servings per day.

To calculate total daily caloric intake, responses from the Short Diet Questionnaire were used with methods described previously [27, 36]. Briefly, we used the reported frequencies of each food item from the SDQ, using portion sizes from a full food frequency questionnaire [37] used previously in the NuAge Study [38], and a nutrient database from the 2015 Canadian Nutrient File.

Other covariates

Sex was determined based on chromosomal sex using the genetic data. Other demographic variables included age, province of residence, highest level of education and income and were based on the responses given in in-home interviews. Highest level of education was based on the questions: "What is the highest degree, certificate, or diploma you have obtained?" and "Have you received any other education that could be counted towards a degree, certificate, or diploma from an educational institution?". Participants were coded as: Less than a Bachelor's, a Bachelor's degree or higher than a Bachelor's. Total household income was categorized as follows: <$20 000, $20 000–50 000, $50 000-100 000, $100 000-150 000,>$150 000, Don't know/Missing/Refused.

Participants were also asked "In the last 24 hours, have you had any food or (excluding water) drink?" and respondents who said yes stated the last time they did so. This variable was included as the time since last meal or drink in order to determine fasting status which is relevant to interpreting metabolite data. Participants were also asked if they had received a physician's diagnosis of diabetes or high blood pressure. Blood pressure was measured six times in a single session for each participant, and the average of the last five readings was used as the blood pressure measurement. We defined hypertension as self-reported physician diagnosis of high blood pressure or an average systolic blood pressure ≥ 130 mmHg or a diastolic blood pressure ≥ 80 mmHg. BMI was categorized as underweight (< 20 kg/m^2), normal (20–24.9 kg/m^2), overweight (25–29.9 kg/m^2) and obese (≥ 30.0 kg/m^2) similar to past studies [39].

Statistical analysis

First, we used descriptive statistics and graphs to understand the distribution of the variables and check necessary assumptions such as normality and linearity. Next, we assessed the association of each environmental, genetic, and metabolic variable individually with IOP using linear models adjusted for age, sex, income, province, education level, BMI, alcohol frequency, hypertension, diabetes, smoking status, total daily caloric intake, first ten genetic principal components (genetic models only), and hours since last meal or drink (metabolite models only). Environmental variables included: total fruit consumption, total vegetable consumption, alcohol consumption, and smoking status. Genetic variables included the six genetic variants associated with metabolites (rs144009214, rs12414734 and rs8050812 for ascorbic acid 2-sulfate and rs165879, rs4680, and rs61484427 for O-methylascorbate). Metabolic factors included O-methylascorbate and ascorbic acid 2-sulfate measured levels.

To assess the gene-environment and metabolite-environment interactions, we first fit linear models with and without gene-environment or metabolite-environment interaction terms. Each model was adjusted for the following potential confounders: age, sex, income, province, education level, hypertension, diabetes, total daily caloric intake, BMI, alcohol frequency, and smoking status. The gene-environment models were additionally adjusted for the first 10 principal components or genetic variation to account for confounding by ancestry. The metabolite-environment models were additionally adjusted for the hours since last meal or drink to reduce metabolite measurement bias. We conducted a likelihood ratio test for each gene/metabolite environment pair to assess the significance of the interaction term(s) in the model. We performed a complete case analysis, resulting in each model using slightly different sample sizes based on the number of missing values for the included genetic variables, metabolite levels, and fruit and vegetable consumption values. The total number of participants for each model is stated in Table S1 and ranged from 7722 to 8050. We ran a total of 32 interaction tests and adjusted for multiple testing using a False Discovery Rate approach [40] using the p.adjust function in R. Suggestive interactions were visualized by creating interaction plots using the R package sjPlot v. 2.8.15 [41]. All analyses were performed in R v.4.3.1 [42].

Results

Study sample description

The genetic and environmental variables of the 8060 participants from the CLSA Comprehensive Cohort are described in Table 1. The mean age of the cohort was 63 years (SD: 10.1) and 51.1% of the cohort were females. The mean IOP for the sample was 16.32 mmHg (SD: 3.95). The average number of servings of fruit consumed per day was 1.81 (SD: 1.14) and of vegetables was 1.91 (SD: 1.07). In the cohort, 46.3% of participants were never smokers, 44.7% were former smokers and 9.1% were current smokers. For alcohol consumption, 1.8% of participants were never drinkers, 39% were occasional drinkers, 42.7% were weekly drinkers and 16.5% were daily drinkers.

Single factor models

We examined each genetic, metabolic, and environmental factor in single factor models without interaction. Of the factors, total vegetable consumption, O-methylascorbate, and ascorbic acid 2-sulfate were statistically significantly associated with IOP. None of the genetic factors were significantly associated with IOP. The coefficient estimates and 95% confidence intervals for each model are shown in Fig. 1.

Table 1 Descriptive characteristics of sample ($N = 8060$)

Variable		*N* (%) or Mean (SD)
Intraocular Pressure		16.32 (3.95)
Self-reported Glaucoma		357 (4.4%)
IOP-lowering Treatment		166 (2.1%)
Female Sex		4120 (51.1%)
Age, Years		63 (10.1)
Low education	Less than a Bachelor's	1678 (20.8%)
	Bachelor's Degree	1855 (23.0%)
	University Degree above Bachelor's	4527 (56.2%)
Smoking Status	Never Smoker	3728 (46.3%)
	Former Smoker	3599 (44.7%)
	Current Smoker	733 (9.1%)
Alcohol Frequency	Never	147 (1.8%)
	Occasionally	3141 (39.0%)
	Weekly	3442 (42.7%)
	Daily	1330 (16.5%)
Fruit Consumption, # servings per day		1.81 (1.14)
Vegetable Consumption, # servings per day		1.91 (1.07)
Province	Alberta	801 (9.9%)
	British Columbia	1650 (20.5%)
	Manitoba	837 (10.4%)
	Newfoundland and Labrador	585 (7.3%)
	Nova Scotia	834 (10.3%)
	Ontario	1758 (21.8%)
	Quebec	1595 (19.8%)
Income	<$20 000	385 (4.8%)
	$20 000-$50 000	1718 (21.3%)
	$50 000-$100 000	2680 (33.3%)
	$100 000-$150 000	1546 (19.2%)
	>$150 000	1256 (15.6%)
	Missing/Refused/Don't Know	475 (5.9%)
Hypertension		4530 (56.2%)
Diastolic Blood Pressure (mmHg)		74.23 (9.87)
Systolic Blood Pressure (mmHg)		121.61 (16.72)
Diabetes		1368 (17.0%)
Daily Caloric Intake		1516.5 (466.3)
BMI	Underweight	219 (2.7%)
	Normal Weight	2165 (26.9%)
	Overweight	3289 (40.8%)
	Obese	2387 (29.6%)
rs4680	AA	2143 (26.6%)
	AG	4036 (50.1%)
	GG	1871 (23.2%)
rs8050812	CC	4141 (53.5%)
	CT	3033 (39.2%)
	TT	570 (7.4%)
rs165879	GG	7212 (91.3%)
	GA	664 (8.4%)
	AA	19 (0.2%)
rs144009214	CC	7744 (96.6%)
	CT	272 (3.4%)
	TT	3 (0.0%)

Table 1 (continued)

Variable		*N* (%) or Mean (SD)
rs61484427	$(TCT)_2$	5622 (72.0%)
	$(TCT_{)2}$/delTCT	2007 (25.7%)
	delTCT/delTCT	179 (2.3%)
rs12414734	GG	3043 (38.5%)
	GA	3776 (47.7%)
	AA	1093 (13.8%)

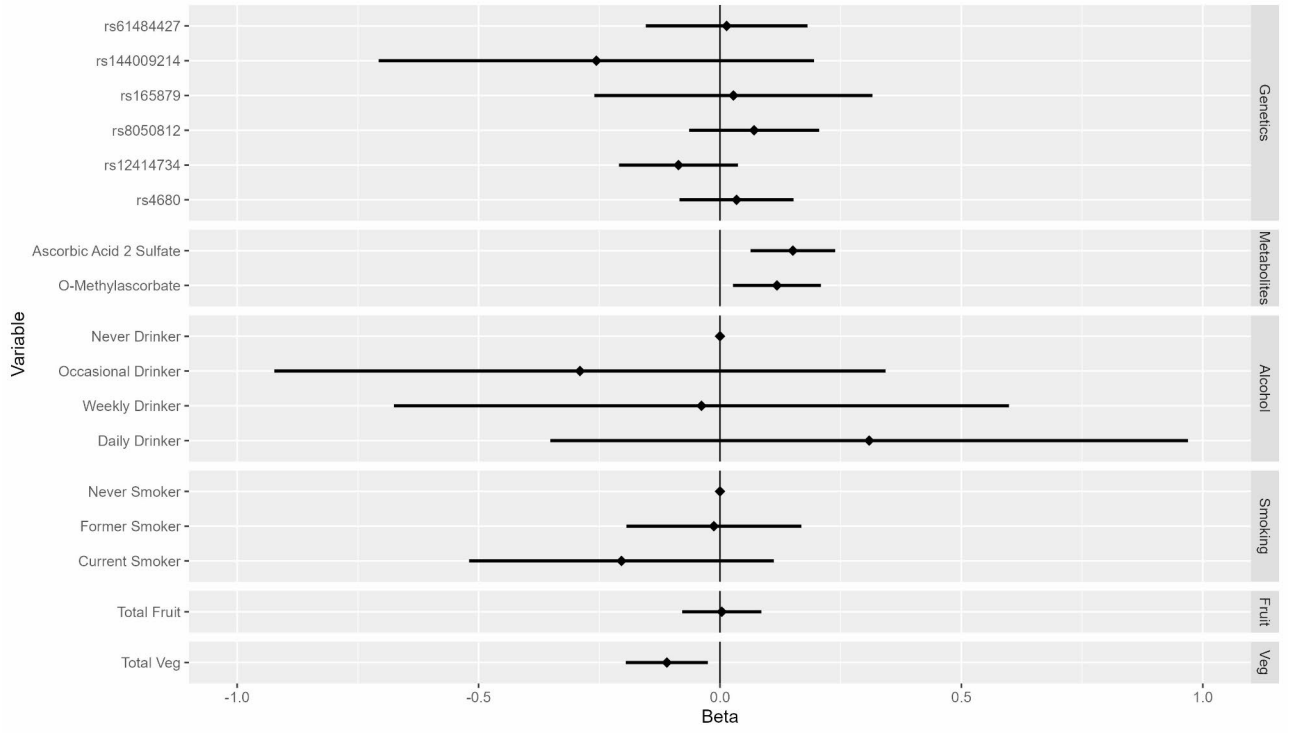

Fig. 1 Simple linear regression coefficient estimates (beta) and 95% confidence intervals for each individual genetic, metabolic, and environmental variable

Interaction results

Next, we examined the interactions between the metabolic/genetic factors and the environmental variables. Results from all the models are detailed in Additional File 1. Of the interactions evaluated, one was suggestively significant (P-value < 0.1) after correction for multiple testing, visualized in Fig. 2. This interaction was between alcohol consumption frequency and a genetic variant associated with ascorbic acid 2-sulfate: rs8050812.

The suggestive interaction between rs8050812 and alcohol consumption frequency (adjusted P-value = 0.094) is displayed in Fig. 2. The trend in Fig. 2 suggests that predicted values of IOP do not differ greatly by genotype in those that consume alcohol but are different among non-drinkers. In those that have the TT genotype, the predicted IOP value is lower.

Discussion

In the present analysis, we identified one gene-environment interaction associated with IOP that was suggestive after correcting for multiple testing. This interaction was between a genetic variant associated with ascorbic acid 2-sulfate (rs8050812) and alcohol consumption frequency.

To better understand how environmental variables are influenced by the genome and metabolome, we also looked more closely at the gene-environment interactions themselves using interaction plots. For the interaction between rs8050812 and alcohol consumption, those in the never drinker category had lower IOP predicted levels with an increasing number of minor alleles. This could suggest a protective effect of that genotype which could be inhibited by alcohol consumption.

While we were able to identify a suggestive gene-environment interaction, and while both metabolites were

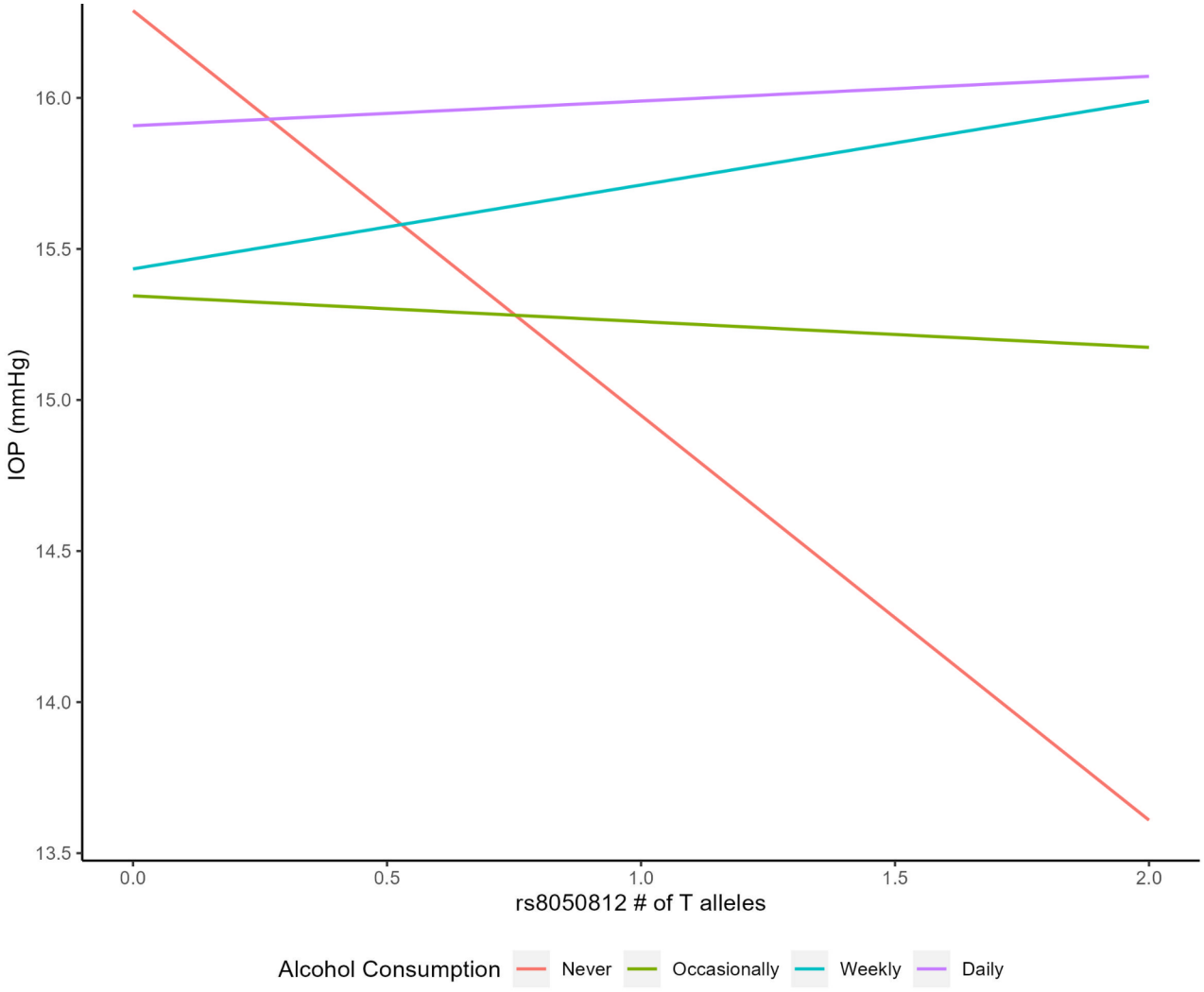

Fig. 2 Interaction plot for the suggestive interaction between rs8050812 and alcohol consumption frequency

associated with IOP in the single factor models, none of the metabolite-environment interactions were statistically significant. This casts doubt on whether the identified effect of the genetic variant on IOP is acting through the metabolite or whether the variant is acting through another pathway. Metabolite levels are influenced by many factors, which could make the identification of an interaction more difficult.

We considered pathways other than the vitamin C metabolic pathway through which the genetic variant may be interacting with the environmental variable. In our previous research, we identified genes which were mapped to genetic variants associated with ascorbic acid 2-sulfate using the platform FUMA [18]. Some of the genes mapped included *MAPK3* and *PTEN*. *MAPK3*, also known as extracellular signal-regulated kinase 1 (*ERK1*) is involved in the *MAPK* signaling pathway which has several functions [43]. Some research has also investigated the effect of alcohol on this signaling pathway [44]. For example, one study noted that alcohol inhibited the *MAPK* signaling pathway during the differentiation of liver cells [45]. Phosphatase and tensin homolog (*PTEN*) is a tumor suppressor gene which has been implicated in glaucoma pathogenesis and other markers such as visual acuity [46–48]. Other research has discussed the effects of alcohol on *PTEN* activity in the context of other diseases such as alcoholic liver disorder and ostopenia [49, 50]. These genes may suggest other pathways for impacting IOP that are not directly through the ascorbic acid 2-sulfate metabolic pathway.

The current analysis has many strengths, including the use of a large, high quality data sample of environmental, genetic, and metabolic data. There are also some limitations. One limitation is that only European-descent participants were included in the analysis due to the small percentage of participants of non-European ancestry

in the sample. Therefore, our findings would need to be investigated in other ancestry groups. Another limitation is that for some of the genetic variables, the number of participants with certain genotypes were very low. For example, the number of homozygotes for the minor alleles were three and 19 for the rs144009214 and rs165879 variants, respectively. No significant gene-environment interactions were detected with these variants, which could be due to a lack of power in these analyses. In addition, the number of participants who reported never drinking alcohol was low ($n = 147$, 1.8%), which affected our power to detect a statistically significant rather than suggestive interaction. Power limitations could thus explain the absence of significant findings.

Conclusions

In conclusion, we found suggestive evidence of gene-alcohol consumption interaction effect on IOP involving a metabolite-associated variant. This analysis would need to be reproduced in other samples with larger sample sizes to confirm these findings and to better understand the effect of the interactions. As well, future studies are needed to understand the role that metabolites play in these interactions.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12863-025-01301-w.

Supplementary Material 1

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Author contributions

RL was responsible for preparing the dataset, conducting data analysis, interpreting and visualizing the results, and preparing the manuscript. MR helped with results validation and support with data analysis. JL, PH, MHRG and EF helped with securing funding for the project and project conceptualization. MHRG and EF supervised the project completion and provided resources to aid in project completion. MHRG also helped with feedback on the methodology, analysis and results interpretation. All authors aided in reviewing the manuscript and providing final approval.

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Data availability

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. Code is made available on GitHub (https://github.com/Roy-Gagnon-lab).

Declarations

Ethics approval and consent to participate

The analysis presented here was approved by the University of Ottawa research ethics board.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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