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Epigenetic associations of *GPNMB* rs199347 variant with alcohol consumption in Parkinson's disease

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Introduction: Alcohol consumption can induce a neuroinflammatory response and contribute to the progression of neurodegeneration. However, its association with Parkinson's disease (PD), the second most common neurodegenerative disorder, remains undetermined. Recent studies suggest that the glycoprotein non-metastatic melanoma protein B (GPNMB) is a potential biomarker for PD. We evaluated the association of rs199347, a variant of the *GPNMB* gene, with alcohol consumption and methylation upstream of *GPNMB*.

Methods: We retrieved genetic and DNA methylation data obtained from participants enrolled in the Taiwan Biobank (TWB) between 2008 and 2016. After excluding individuals with incomplete or missing information about potential PD risk factors, we included 1,357 participants in our final analyses. We used multiple linear regression to assess the association of *GPNMB* rs199347 and chronic alcohol consumption (and other potential risk factors) with *GPNMB* cg17274742 methylation.

Results: There was no difference between the distribution of *GPNMB* rs199347 genotypes between chronic alcohol consumers and the other study participants. A significant interaction was observed between the GPNMB *rs199347* variant and alcohol consumption ($p = 0.0102$) concerning cg17274742 methylation. Compared to non-chronic alcohol consumers with the AA genotype, alcohol drinkers with the rs199347 GG genotype had significantly lower levels (hypomethylation) of cg17274742 ($p = 0.0187$).

Conclusion: Alcohol consumption among individuals with the rs199347 GG genotype was associated with lower levels of cg17274742 methylation, which could increase expression of the *GPNMB* gene, an important neuroinflammatory-related risk gene for PD.

KEYWORDS

Parkinson disease, DNA methylation, epigenesis, genetic, alcohol-related disorders, GPNMB

Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disorder, affecting >1% of the worldwide population ≥65 years of age, and is characterized primarily by slow movement, tremors, and rigidity (1, 2). The etiology of PD remains unknown, although it is believed to result from a combination of genetic, environmental, and behavioral factors by progressive loss of dopaminergic neurons in the substantia nigra and accumulation of α-synuclein within Lewy bodies (3–5). Pathophysiologically, PD has been associated with α-synuclein misfolding and aggregation, mitochondrial dysfunction, impairment of protein clearance, neuroinflammation, and oxidative stress (6–8). Epigenetic modifications, which can connect environment or behavior factors with the genetic changes underlying disease (9, 10), have been shown to modulate the immune systems of patients with PD (11–22).

Glycoprotein non-metastatic melanoma protein B (GPNMB), a type I transmembrane protein involved in immune cell maturation and activation (23), has been shown to be a promising biomarker of PD risk (24–27). *GPNMB* is highly expressed in macrophages and microglia, which play a significant role in neuroinflammation, and was shown to be upregulated in disease-associated microglia in neurodegenerative animal models (28, 29). A prominent single-nucleotide polymorphism (SNP) of *GPNMB*, rs199347, plays a significant role in modulating systemic immune response, especially neuroinflammation (21–26, 30). The rs199347 variant genotype and minor allele involves a guanine instead of the reference alanine (31) and has been identified as highly associated with PD risk (24). Recently, GPNMB was shown to promote the toxic aggregation of the alpha-synuclein protein in substantia nigra, which is believed to contribute to neurodegeneration, and could be a potential target for PD treatment (24, 32). Furthermore, recent proteomic analyses of cerebrospinal fluid have identified GPNMB as a primary causal protein in PD emphasizing its role in the disease's heterogeneity and causality (33). Notably, studies involving animal models have demonstrated that overexpression of GPNMB can mitigate degeneration of dopaminergic neurons and provide anti-neuroinflammatory benefits (34, 35).The differences among the rs199347 genotypes (AA, AG, and GG) are significantly associated with *GPNMB* expression in the brain as well as whole blood (24, 25, 27, 36–39). Moreover, large, integrated GWAS on methylation data from brain samples of patients with PD found that the association between *GPNMB* and PD could be regulated by DNA methylation (25, 27, 39). Indeed, hypomethylation at cg17274742, which is proximal to the 7p15 chromosomal region in which *GPNMB* is located, is associated with increased *GPNMB* expression in PD patients (25, 27).

Alcohol consumption can affect epigenetic modification and gene expression (40–42), and associated DNA methylation changes have been observed in both the peripheral and central nervous systems (43, 44). Chronic alcohol consumption can trigger neuroinflammation resulting in central nervous system injury and possibly neurodegeneration (45–48). High alcohol consumption has been shown to increase *GPNMB* levels (49, 50) suggesting an involvement with PD. However, the connection between alcohol drinking and PD remains poorly understood, and epidemiological studies show contradictory data (51–55). Whether PD is caused by chronic alcohol consumption remains unclear (53–56), although chronic alcohol consumption has been found to drive biological mechanisms with significant changes to DNA methylation in serum and brain tissues (40, 41, 43, 44).

To date, no study has evaluated the role of alcohol intake on the expression of *GPNMB*, and specifically the PD-associated *GPNMB* SNP rs199347, and DNA methylation. Identifying methylation patterns associated with alcohol consumption and *GPNMB* expression could help elucidate the influence of alcohol consumption on the risk of PD.

Methods

Participants and data source

Data were obtained from the Taiwan Biobank (TWB), an ongoing prospective cohort study of more than 150,000 participants. The TWB contains demographic and whole-genome sequencing data of Taiwanese (99% Han Chinese) without cancer aged 20 to 70 years, with lifestyle information captured through individual interviews (57, 58).

We enrolled all 2,352 individuals aged 20–70 years in the TWB with DNA methylation data. Using previously published epidemiology studies (59–63), we developed a list of potential risk factors for PD as variables for consideration: sex, age, body mass index, cigarette smoking, alcohol drinking, exercise, coffee drinking, uric acid levels, and hypertension. We excluded 995 individuals with incomplete or missing information, and the remaining 1,357 participants with complete information were included in our final analysis.

This study was approved by the Institutional Review Board of Chung Shan Medical University Hospital (CS1–20009).

DNA methylation assessment

The TWB assessed DNA methylation from whole blood using the Infinium MethylationEPIC BeadChip Kit (Illumina Inc, San Diego, CA, USA) (64–66). Cell-type heterogeneity was adjusted using the Reference-Free Adjustment for Cell-Type composition (ReFACTor) method (67). Methylation levels were quantified using beta values (0–1), which roughly correspond to the percent methylation at a particular site.

Genetic variant assessment

Genotyping was performed at Academia Sinica in Taiwan using a customized Axiom Genome-Wide Array Plate (Affymetrix, Santa Clara, CA, USA), and SNP information was obtained after imputation methods (68). All SNPs with minor allele frequency (MAF) <1% were excluded.

Covariate analysis

Chronic alcohol consumption was defined as drinking more than 150 ml of alcohol-containing beverage(s) per week for ≥6 months prior to enrollment in the TWB. Body mass index (BMI) was calculated (kg/m^2) and categorized as underweight (BMI < 18.5), normal weight (18.5 ≤ BMI < 24), overweight (24 ≤ BMI < 27), and obese (BMI ≥ 27). Current cigarette smokers were those who smoked continuously for ≥6 months, and former smokers were those who previously smoked but quit for ≥6 months. Participants who exercised were those reporting a regular habit of exercising ≥30 min each three times per week. Hypertension was classified by patients self-reporting a diagnosis to a physician.

Statistical analysis

We used multiple linear regression to evaluate the association of *GPNMB* rs199347 and alcohol drinking with *GPNMB* cg17274742 methylation, as well as the interaction between *GPNMB* rs199347 and alcohol drinking. The differences between variables were calculated using a t-test for continuous variables and a chi-square test for categorical variables. Statistical significance was defined as $\alpha < 0.05$. All analyses were conducted using PLINK version 1.9 beta (69) and SAS version 9.4 (SAS Institute Inc, Cary, NC, USA).

Results

Overall, 1,357 participants comprising 648 men and 709 women were included in the study (Table 1). The mean *GPNMB* cg17274742 methylation levels (beta values) for individuals with chronic alcohol consumption was 0.9484 ± 0.0009 (mean ± standard error), compared to 0.9484 ± 0.0003 for those who did not chronically consume alcohol. For the non-chronic group, the number (percentage) of participants with the *GPNMB* rs199347 AA, AG, and GG genotypes was 633 (51.72%), 511 (41.75%), 80 (6.54%), respectively. For the chronic alcohol-drinking group, the number (percentage) with the GPNMB rs199347 AA, AG, and GG genotypes were 66 (49.62%), 53 (39.85%), and 14 (10.53%), respectively. The methylation levels and genotype distribution did not differ significantly between the chronic alcohol consumers and the other study participants.

The *GPNMB* rs199347 variant and alcohol consumption was not significantly associated with the methylation of cg17274742 (Table 2). However, men had significantly lower levels of cg17274742 methylation compared to women (β = –0.00302, p = 0.0169), while hypertensive patients had significantly higher levels of cg17274742 methylation compared to non-hypertensive patients (β = 0.00208, p = 0.0171).

GPNMB rs199347 variant and alcohol drinking had a significant interaction (p = 0.0102) with the methylation of cg17274742 (Table 3). After stratifying by alcohol consumption, rs199347 was not significantly associated with the methylation of cg17274742 in the control group. However, compared to the rs199347 AA genotype, the GG genotype was significantly associated with lower levels of methylation at cg17274742 (β = –0.00635, p = 0.0366).

TABLE 1 Demographic characteristics of the study participants.

	Control group (n = 1,224)	Chronic alcohol consumers (n = 133)	p-Value
Beta value of cg17274742 methylation (mean ± SE)	0.9484 ± 0.000319	0.9484 ± 0.000935	0.9739
GPNMB rs199347 (n, %)			0.2272
AA	633 (51.72)	66 (49.62)	
AG	511 (41.75)	53 (39.85)	
GG	80 (6.54)	14 (10.53)	
Sex (n, %)			**<0.0001**
Female	693 (56.62)	16 (12.03)	
Male	531 (43.38)	117 (87.97)	
Age (years)	49.1 ± 0.3	51.6 ± 0.9	0.0127
Body mass index (n, %)			**<0.0001**
Normal weight	611 (49.92)	41 (30.83)	
Underweight	37 (3.02)	1 (0.75)	
Overweight	339 (27.70)	57 (42.86)	
Obesity	237 (19.36)	34 (25.56)	
Cigarette smoking (n, %)			**<0.0001**
Never	978 (79.90)	50 (37.59)	
Former	147 (12.01)	43 (32.33)	
Current	99 (8.09)	40 (30.08)	
Exercise (n, %)			0.9680
No	688 (56.21)	75 (56.39)	
Yes	536 (43.79)	58 (43.61)	
Coffee intake (n, %)			**0.0152**
No	793 (64.79)	72 (54.14)	
Yes	431 (35.21)	61 (45.86)	
Uric acid level (mg/dl)	5.4238 ± 0.0396	6.4609 ± 0.1343	**<0.0001**
Hypertension (n, %)			**0.0060**
No	1078 (88.07)	106 (79.70)	
Yes	146 (11.93)	27 (20.30)	

Continuous data are displayed as mean ± standard error (SE) and categorical data as numbers (percentages).
Bold values means p value <0.05.

After combining the rs199347 genotypes and alcohol consumption (Table 4), cg17274742 was significantly hypomethylated among chronic alcohol drinkers with the rs199347 GG genotype compared to the control group with the AA genotype (β= –0.00654, p = 0.0187).

TABLE 2 Association of rs199347 and alcohol consumption with cg17274742 methylation.

	β	p-Value
GPNMB rs199347 (ref: AA)		
AG	0.00067276	0.2425
GG	0.00047793	0.6683
Chronic alcohol consumption (ref: no)		
Yes	−0.00042063	0.6713
Sex (ref: female)		
Male	−0.00302	**0.0169**
Age (years)	0.00005373	0.1891
Body mass index (ref: normal weight)		
Underweight	−0.00305	0.0742
Overweight	0.00046711	0.4880
Obesity	−0.00052500	0.5004
Cigarette smoking (ref: never)		
Former	0.00002321	0.9788
Current	−0.00005172	0.9594
Exercise (ref: no)		
Yes	−0.00105	0.0839
Coffee intake (ref: no)		
Yes	0.00044856	0.4366
Uric acid level (mg/dl)	0.00011273	0.6367
Hypertension (ref: no)		
Yes	0.00208	**0.0171**

β, beta coefficient.
Bold values means p value <0.05.

Discussion

Using data from a large, national prospective cohort study, we identified an association between cg17274742 methylation status, the *GPNMB* rs199347 polymorphism, and alcohol consumption. Our findings suggest that carriers of the rs199347 GG genotype and those who chronically consume alcohol have significantly more cg17274742 hypomethylation, which may result in increased *GPNMB* expression. This is the first study to find a relationship between a specific *GPNMB* polymorphism and alcohol-associated methylation changes suggesting that a lifestyle change, particularly a reduction in alcohol consumption for carriers of the rs199347 GG genotype, could reduce *GPNMB* expression, which is highly correlated with the risk of PD.

Recent studies highlight the importance and benefits of involving diverse and multiethnic populations in genetic studies (70, 71), including more accurately representing the risks of genetically associated diseases in different populations (72). Most published GWAS and methylomic studies involving the *GPNMB* rs199347 variant were conducted in majority-white study populations (36–39, 73, 74). Our study uses information obtained from the Taiwan Biobank (TWB), the largest biobank in East Asia with dense SNP array data, and leverages its high-coverage whole-genome sequencing and DNA methylation data in a population of Han Chinese (58). Notably, the rs199347 genotype frequencies observed in our study (A = 0.72, G = 0.28) are roughly equivalent to those reported by the Allele Frequency Aggregator Project in East Asia (A = 0.716, G = 0.283), which is different from the frequencies reported in white individuals (A = 0.59, G = 0.41) (31). While the TWB provides a robust dataset for understanding genetic associations in the Han Chinese population, it is crucial to discuss the generalizability of our findings beyond this group. The genetic and lifestyle diversity across different populations may impact the observed associations. Therefore, further studies are needed to explore how genetic differences between populations might influence the relationship between the GPNMB rs199347 variant, alcohol consumption, and Parkinson's disease risk. Addressing these potential impacts would enhance the broader applicability of our findings and contribute to a more comprehensive understanding of genetic risk factors across diverse populations.

A novel integrative approach has been developed to align expression quantitative trait loci (eQTL) with genome-wide association study (GWAS) signals in Parkinson's disease (PD), utilizing an updated PD GWAS dataset. This strategy highlighted the methylation site cg17274742 within the GPNMB gene, a site of interest for Coloc analysis, which investigates common causal variants shared between eQTL and GWAS data. Methylation at this specific locus not only affects gene expression but also influences splicing activities at the GPNMB/NUPL2 locus facilitated by robust protein–protein interactions. These interactions may connect to genes associated with either Mendelian or sporadic forms of PD (25). The presence of PD-associated variants at this methylation site reveals critical molecular pathways influencing the pathogenesis of PD, thereby emphasizing the significant role of both genetic and epigenetic factors in its development. In our study, we found that males had more cg17274742 hypomethylation, which may increase *GPNMB* expression. After correcting for age, the prevalence of PD in men is approximately 1.4 times higher than that in women (75), although a 2014 meta-analysis suggests that this difference is evident only in the 50- to 59-year age group (59). Additionally, we found that hypertension was associated with cg17274742 hypermethylation, which could decrease *GPNMB* expression and potentially reduce PD risk (24, 36). Published evidence connecting hypertension and PD diagnosis is contradictory (76–81), and different effects of hypertension have been observed in white versus Asian populations (76–79). Moreover, some antihypertensive medications may contribute to PD risk; inhibitors of the renin–angiotensin–aldosterone system may delay proinflammatory effects, and alpha-1-adrenergic receptor antagonists can enhance glycolysis and could reduce PD risk (82–87).

Chronic alcohol consumption can damage the central nervous system (45), and a recent translational study suggests a strong correlation between alcohol use disorder and the inflammatory response in the brain (45). Chronic alcohol consumption can trigger pro-inflammatory cytokines by activating peripheral macrophages and microglia in the central nervous system, which may alter the

TABLE 3 Association of rs199347 with methylation of cg17274742 stratified by alcohol consumption.

	Control group (n = 1,224)		Chronic alcohol consumers (n = 133)	
	β	p-Value	β	p-Value
GPNMB rs199347 (ref: AA)				
AG	0.00081125	0.1804	-0.00152	0.4276
GG	0.00186	0.1236	-0.00635	**0.0366**
Sex (ref: female)				
Male	−0.00258	**0.0497**	−0.01076	**0.0488**
Age (years)	0.00006865	0.1141	−0.00007678	0.5481
Body mass index (ref: normal weight)				
Underweight	−0.00338	0.0513	0.01736	0.1111
Overweight	0.00031558	0.6586	0.00313	0.1606
Obesity	−0.00065127	0.4299	0.00065871	0.7934
Cigarette smoking (ref: never)				
Former	−0.00025122	0.7933	0.00356	0.1233
Current	−0.00008491	0.9409	0.00169	0.4856
Exercise (ref: no)				
Yes	−0.00107	0.0967	−0.00296	0.1258
Coffee intake (ref: no)				
Yes	0.00034271	0.5748	0.00266	0.1489
Uric acid level (mg/dl)	0.00017402	0.4992	0.0000587	0.9315
Hypertension (ref: no)				
Yes	0.00258	0.0061	−0.00044093	0.8616

Interaction (rs199347 * Alcohol consumption) p-value = 0.0102.
β, beta coefficient.
Bold values means p value <0.05.

neuroinflammatory state in the brain (46, 47). Previous studies have noted the importance of different biological processes and functions of GPNMB, including cell differentiation and development, inflammation and immune response, progression, and neurodegeneration deterioration (23). Additionally, the Genotype-Tissue Expression (GTEx) project has identified that the GPNMB rs199347 variant significantly influences gene expression in various brain tissues, including the basal ganglia, cerebellum, and frontal cortex (88, 89). These regions are closely linked to neural mechanisms, particularly the dopaminergic pathways, which are crucial in the pathophysiology of PD (3, 6). The impact of this variant on these brain regions underscores its potential role in the clinical manifestations of PD. Our finding suggests that lifestyle changes, particularly a reduction in alcohol consumption for carriers of the rs199347 GG genotype, could modulate GPNMB expression, thereby identifying a potentially modifiable risk factor in Parkinson's disease.

Our study was limited by the uncertainty around the amount of alcohol consumption. The TWB survey quantifies alcohol consumption in milliliters (ml), not milligrams (mg), and is therefore difficult to standardize across the study population. Additionally, data on alcohol consumption were self-reported and are subject to recall bias. Another limitation of our investigation concerns our inability to comprehensively account for all potential confounding variables, including dietary habits, lifestyle choices, and environmental exposures. Nevertheless, we mitigated this by controlling for a range of well-documented risk and protective factors, such as age, body mass index (BMI), smoking status, alcohol consumption, coffee intake, serum uric acid levels, and hypertension, all of which have been extensively studied in PD epidemiological research.

Ultimately, our results provide insight into the genetic and lifestyle factors associated with *GPNMB* expression, which is a potential biomarker and therapeutic target of PD. Future experiments using animal models or human cell lines to examine the underlying mechanisms behind this potential neuroinflammatory association in the central nervous system are warranted.

Conclusion

In summary, we found that having the GG genotype of GPNMB rs199347 with drinking habits decreases GPNMB methylation among Taiwan Biobank participants. These results provide

TABLE 4 Cg17274742 methylation based on rs199347 and alcohol consumption.

	β	*p*-Value
GPNMB rs199347 and alcohol consumption (ref: AA and non-chronic alcohol consumers/control)		
AG and control	0.00080588	0.1813
GG and control	0.0018	0.1343
AA and chronic alcohol consumer	0.00108	0.4274
AG and chronic alcohol consumer	0.00039023	0.7921
GG and chronic alcohol consumer	−0.00654	**0.0187**
Sex (ref: female)		
Male	−0.00299	**0.0178**
Age (years)	0.00005587	0.1711
Body mass index (ref: normal weight)		
Underweight	−0.003	0.0784
Overweight	0.00048126	0.4739
Obesity	−0.00061427	0.4298
Cigarette Smoking (ref: never)		
Former	0.00003035	0.9722
Current	−0.0001492	0.8831
Exercise (ref: no)		
Yes	−0.0011	0.0698
Coffee Intake (ref: no)		
Yes	0.00046963	0.4143
Uric acid level (mg/dl)	0.00016297	0.4950
Hypertension (ref: no)		
Yes	0.00219	**0.0119**

β, beta coefficient.
Bold values means p value <0.05.

information on the genetic and lifestyle factors that contribute to the expression, which is a PD potential biomarker and therapeutic target of PD, and could be used as a reference for experimental, longitudinal, or intervention studies evaluating the disease and its associated variables and mechanisms.

Data availability statement

The access to and use of the Taiwan Biobank data in the present work was approved by the Ethics and Governance Council (EGC) of Taiwan Biobank (approval number: TWBR10907-05) and the Institutional Review Board (IRB) of National Health Research Institutes, Taiwan (approval number: EC1090402-E). The data collection of Taiwan Biobank was approved by the Ethics and Governance Council (EGC) of Taiwan Biobank and the Department of Health and Welfare, Taiwan (Wei-Shu-I-Tzu NO.1010267471). The data utilized in this study are sourced from the Taiwan Biobank, accessible at (https://www.twbiobank.org.tw/). Due to restrictions, the data are not publicly available and were used under license specifically for this study. However, these data can be obtained from the corresponding author, Yung-Po Liaw, upon reasonable request and with permission from the Taiwan Biobank.

Ethics statement

Our study involves patients enrolled in the Taiwan Biobank (TWB). TWB was approved by the Institutional Review Board on Biomedical Science Research/IRB-BM, Academia Sinica and by the Ethics and Governance Council of Taiwan Biobank, Taiwan. Written informed consent was obtained before data collection from each participant in accordance with institutional requirements and the principles of the Declaration of Helsinki. This study was approved by the Institutional Review Board of the Chung Shan Medical University Hospital (CS1–20009). Participants in the Taiwan Biobank provided their written informed consent during enrollment. All methods were performed according to the relevant guidelines and regulations.

Author contributions

Y-CC: Conceptualization, Investigation, Validation, Visualization, Writing – original draft. Y-CL: Methodology, Validation, Writing – review & editing. ON: Investigation, Methodology, Supervision, Validation, Writing – review & editing. C-HH: Data curation, Formal analysis, Methodology, Writing – review & editing. J-HZ: Data curation, Formal analysis, Methodology, Writing – review & editing. S-LW: Conceptualization, Investigation, Validation, Writing – review & editing. Y-PL: Conceptualization, Funding acquisition, Investigation, Resources, Validation, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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