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Alcohol drinking and brain morphometry in apparently healthy community-dwelling Japanese men

Ali Haidar Syaifullah [a, b], Akihiko Shiino [a, *], Akira Fujiyoshi [c, d], Aya Kadota [b, d], Keiko Kondo [d], Takahiro Ito [d], Hiroyoshi Segawa [b], Mohammad Moniruzzaman [b, d], Takashi Waki [e], Naoko Miyagawa [d, f], Ikuo Tooyama [g], Hirotsugu Ueshima [b, d], Katsuyuki Miura [b, d], for the SESSA Research Group[1], Co-chairpersons, Hirotsugu Ueshima [h], Katsuyuki Miura [h], Research members

[a] Biomedical MRI Science Center, Shiga University of Medical Science, Shiga, Japan
[b] Center for the Epidemiologic Research in Asia (CERA), Shiga University of Medical Science, Shiga, Japan
[c] Department of Hygiene, School of Medicine, Wakayama Medical University, Japan
[d] Department of Public Health, Shiga University of Medical Science, Shiga, Japan
[e] Department of Medical Statistics, Shiga University of Medical Science, Shiga, Japan
[f] International Center for Nutrition and Information, National Institute of Biomedical Innovation, Health and Nutrition, Shinjuku-ku, Tokyo, Japan
[g] Molecular Neuroscience Research Center, Shiga University of Medical Science, Shiga, Japan
[h] Shiga University of Medical Science, Otsu, Shiga, Japan

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ABSTRACT

The clinical implications of alcohol consumption have been extensively examined; however, its effects on brain structures in apparently healthy community-dwellers remain unclear. Therefore, we investigated the relationship between alcohol consumption and brain gray matter volume (GMV) in community-dwelling Japanese men using voxel-based morphometry (VBM). We recruited cognitively intact Japanese men, aged 40–79 years, from a population-based cohort in Shiga, Japan. Brain magnetic resonance imaging was performed, on average, 2 years after demographic and medical information was obtained in 2010–2014. A multivariable linear regression analysis of 639 men was conducted to elucidate the relationship between the amount of alcohol consumed and GMV. VBM statistics were analyzed by threshold-free cluster enhancement with a family-wise error rate of <0.05. The results obtained demonstrated that the amount of alcohol consumed was associated with lower GMV. The VBM analysis showed lower GMV within the parahippocampal, entorhinal, cingulate, insular, temporal, and frontal cortices and cerebellum in very heavy drinkers ($\geq$42 ethanol g/day) than in non-drinkers. Furthermore, alcohol consumption was associated with a higher white matter lesion volume. These results suggest subclinical structural changes similar to alcohol-related neurological diseases.

* Corresponding author. Biomedical MR Science Center, Molecular Neuroscience Research Center, Shiga University of Medical Science, Otsu, Shiga Prefecture, 520-2192 Japan. Telephone: +81 77 548 2943.
E-mail address: shiino@belle.shiga-med.ac.jp (A. Shiino).

[1] **Members of the SESSA Research Group:** *Co-chairpersons*: Hirotsugu Ueshima, Katsuyuki Miura (Shiga University of Medical Science, Otsu, Shiga); *Research members*: Minoru Horie, Yoshihisa Nakagawa, Takashi Yamamoto, Yasutaka Nakano, Emiko Ogawa, Hiroshi Maegawa, Katsutaro Morino, Itsuko Miyazawa, Yoshiyuki Watanabe, Kazuhiko Nozaki, Ikuo Tooyama, Akihiko Shiino, Akira Andoh, Teruhiko Tsuru, Hisakazu Ogita, Naomi Miyamatsu, Yasuyuki Nakamura, Aya Kadota, Keiko Kondo, Sayuki Torii, Takashi Kadowaki, Sayaka Kadowaki, Sentaro Suzuki, Takahiro Ito, Ayako Kunimura, Hiroyoshi Segawa (Shiga University of Medical Science, Otsu, Shiga), Akira Fujiyoshi, Aya Higashiyama (Wakayama Medical University, Wakayama), Tomonori Okamura, Koichiro Azuma (Keio University, Tokyo), Tatsuya Sawamura (Shinshu University, Matsumoto, Nagano), Michiya Igase (Ehime University, Toon, Ehime), Yasuharu Tabara (Kyoto University, Kyoto), Akira Sekikawa, Emma JM Barinas-Mitchell (University of Pittsburgh, Pittsburgh, PA, USA), Daniel Edmundowicz (Temple University, Philadelphia, PA, USA), Takayoshi Ohkubo (Teikyo University, Tokyo), Atsushi Hozawa (Tohoku University, Sendai, Miyagi), Yoshitaka Murakami (Toho University, Tokyo), Nagako Okuda (University of Human Arts and Sciences, Saitama), Hisatomi Arima, Atsushi Satoh (Fukuoka University, Fukuoka), Yoshikuni Kita (Tsuruga Nursing University, Tsuruga, Fukui), Takashi Hisamatsu (Okayama University, Okayama), Masahiko Yanagita (Doshisha University, Kyotanabe, Kyoto), Robert D. Abbott (Korea University, Seoul, Korea), Seiko Ohno (National Cerebral and Cardiovascular Center, Suita, Osaka), Naoyuki Takashima (Kindai University, Osakasayama, Osaka), Naoko Miyagawa (National Institute of Health and Nutrition, Tokyo), Maryam Zaid (Fudan University Shanghai, China), Yoshino Saito (Aino University, Ibaraki, Osaka).

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Introduction

Alcohol consumption has been increasing over the past decade (Poznyak & Rekve, 2018) and, in large amounts, is harmful to various organs, including the brain (Brust, 2010). Continuous consumption may lead to dependency, which is associated with neurological diseases, such as Wernicke-Korsakoff syndrome (Manzo et al., 2014), cerebellar ataxia (Shanmugarajah et al., 2016), and alcohol-related brain injury (Meda et al., 2018). Previous neuroimaging studies on alcohol-dependent individuals consistently reported a reduction of brain gray matter (GM) (Durazzo et al., 2014; Grodin, Lin, Durkee, Hommer, & Momenan, 2013; Gropper et al., 2016; Sinforiani et al., 2011; Wang et al., 2016), the part of the brain that houses neuronal cell bodies and is closely related to cognitive functions (Schmidt-Wilcke, Poljansky, Hierlmeier, Hausner, & Ibach, 2009). However, evidence of subclinical effects on GM in apparently healthy adults is limited (Fukuda et al., 2009; Paul et al., 2008; Preti et al., 2014; Sachdev, Chen, Wen, & Anstey, 2008; Taki et al., 2006; Topiwala et al., 2017). Previous studies on apparently healthy individuals showed inconsistent findings regarding regions of the brain affected by alcohol (Brust, 2010; Verbaten, 2009). The direction of association between alcohol consumption and GM volume (GMV) remains unclear, with positive (Sachdev et al., 2008), negative (Taki et al., 2006; Topiwala et al., 2017), or no association (Preti et al., 2014) being reported. A reason for this inconsistency may be that the majority of previous studies did not differentiate between past drinkers and non-drinkers (Fukuda et al., 2009; Sachdev et al., 2008; Topiwala et al., 2017); therefore, the misclassification of alcohol exposure may have distorted the estimated relationship between alcohol and GM (Fillmore, Stockwell, Chikritzhs, Bostrom, & Kerr, 2007). Furthermore, limited information is currently available on the Asian population. Therefore, an Asian population-based study with a representative sample is essential because East Asians have a higher prevalence of allele variant, such as aldehyde dehydrogenase (ALDH)2 (Eng, Luczak, & Wall, 2007), and a different drinking culture (Marugame et al., 2007) than Westerners, and alcohol consumption (Poznyak & Rekve, 2018) and neurological disorder burden (GBD 2016 Neurology Collaborators, 2019) are increasing.

Previous studies on apparently healthy adults reported discordant findings due to different brain analytical approaches. Over the past decade, voxel-based morphometry (VBM) has been the standard for objective neuroimaging studies. A VBM analysis uses two main approaches to assess magnetic resonance imaging (MRI) scans: a voxel-by-voxel analysis and volumetric analysis (Ashburner & Friston, 2000; Kurth, Gaser, & Luders, 2015). These approaches are considered to be complementary to each other and, thus, are used in tandem (Testa et al., 2004). However, some studies only employed one approach (Sachdev et al., 2008), which resulted in dubious interpretations. Furthermore, the traditional VBM procedures used by the majority of previous studies (Preti et al., 2014; Sachdev et al., 2008; Taki et al., 2006) have caveats, such as tissue misclassification and arbitrary cluster-forming threshold (Levy-Cooperman, Ramirez, Lobaugh, & Black, 2008; Smith & Nichols, 2009), and lower sensitivity than a more up-to-date method (Smith & Nichols, 2009). These caveats may be minimized by the application of a specific VBM methodology (Egger et al., 2017; Smith & Nichols, 2009).

In the present study, we aimed to clarify the subclinical relationship between alcohol consumption and brain structures using voxel-by-voxel and volumetric analyses in a population of randomly sampled, apparently healthy, community-dwelling Japanese men. To increase the accuracy of VBM analyses, we adapted an automated white matter lesion (WML) correction and applied a more sensitive, yet powerful statistical inference method (Smith & Nichols, 2009). We hypothesized that alcohol consumption is associated with lower GMV.

Materials and methods

Study participants and sample selection

We analyzed randomly sampled healthy Japanese men aged 40 years or older from the Shiga Epidemiological Study of Subclinical Atherosclerosis (SESSA). Detailed methods of enrollment in SESSA have been reported elsewhere (Hisamatsu et al., 2016; Ueshima et al., 2016). In brief, SESSA is a population-based cohort that was established between 2006 and 2008 and conducted in Kusatsu city, Shiga Prefecture, Japan. Among 2379 randomly selected Japanese men aged 40–79 years, 1094 participated in baseline examinations. Between 2010 and 2014, 853 participants were followed up for routine examinations and cognitive testing using the Cognitive Abilities Screening Instrument (Fujiyoshi et al., 2020). Two years later (between January 2012 and February 2015), 740 subjects underwent brain MRI using a 1.5-T scanner (Signa HDxt 1.5 T, GE Healthcare; Milwaukee, Wisconsin, United States) at the Shiga University of Medical Science Hospital. We herein analyzed the follow-up data of participants who underwent brain MRI. To avoid other causes of changes in brain MRI, we excluded 70 participants with a history of stroke, cognitive impairment (Cognitive Abilities Screening Instrument score < 74 [Teng et al., 1994]), and/or missing variables of interest (see Supplementary Table S1 online). To focus on current and non-drinkers while reducing potential misclassification (Fillmore et al., 2007), we excluded past drinkers (n = 31). Therefore, 639 participants were ultimately analyzed. Written informed consent was obtained from all study participants. The study protocol was reviewed and approved by the Institutional Review Board of the Shiga University of Medical Science and followed the Code of Ethics of the World Medical Association (Declaration of Helsinki).

Alcohol consumption assessment

We estimated alcohol consumption using a self-administered and structured questionnaire administered at the follow-up examination (2010–2014). Responses were confirmed by a trained nurse. Participants were asked whether they currently drank alcohol; those who responded “yes” were considered to be current drinkers; those who answered “no” were asked whether they used to drink regularly in the past. A participant was considered to be a past drinker if he responded “yes” and a non-drinker if he responded “no”. Current drinkers, but not past drinkers, were asked to estimate the frequency and amount of alcohol they consume during a typical week or month. The amount of alcohol was estimated by asking the size and number of containers or traditional units they typically consume on one occasion: “go” for Sake (Japanese traditional rice wine), bottles for beer, glasses for wine, and shots for whisky. One “go”, a traditional Japanese unit of volume, is 180 mL containing 23 g of ethanol (Okamura et al., 2004), similar to two glasses (220 mL) of wine, one medium bottle (500 mL) of beer, or two shots (70 mL) of whisky. Volumes were converted to the estimated amount of ethanol consumed in grams per week. Daily alcohol consumption was estimated from weekly alcohol intake divided by 7.

MRI image acquisition, pre-processing, and analysis method

Two certified neurosurgeons (KN, AS) independently assessed each MRI scan separately, without being informed of participant characteristics, to identify and exclude abnormal findings. High-

resolution 3D T1-weighted spoiled gradient recalled (SPGR) brain MRI acquisition parameters were as follows: TR = 13.5 ms, TE = 5.8 ms, thickness = 1.6 mm, with a 0.8-mm overlap, FA = 15°, frequency encoding = 288, 256 × 256 matrix. Images were converted into the Neuroimaging Informatics Technology Initiative (NIFTI) file format before the VBM analysis.

MRI scans were processed by the Brain Anatomical Analysis using Diffeomorphic deformation (BAAD) software (version 4.3), which enables the VBM procedure (http://www.shiga-med.ac.jp/~hqbioph/BAAD(English)/BAAD.html). The details of the standard VBM procedure have been described elsewhere (Kurth et al., 2015). All parameters in the pre-processing step were set to the default settings. In brief, images were set around AC-PC lines and resampled with a voxel size of 1 mm^3. Tissue segmentation and the intensity of non-uniformity removal were performed using Computational Anatomy Toolbox 12, developed by the Structural Brain Mapping Group at the University of Jena, Germany. Since WMLs exert a similar signal intensity to GM on T1-weighted images, we performed an automated WML correction using Lesion Segmentation Toolbox (LST) software on fluid-attenuated inversion recovery (FLAIR) images (Schmidt et al., 2012). FLAIR image acquisition parameters were as follows: TR = 8000 ms, TE = 120 ms, TI = 2000, ETL = 20, frequency encoding = 256, phase encoding = 192, thickness = 4 mm, FOV = 22 cm. Before GM/WM segmentation, we masked WML by the averaged signal intensities of surrounding normal WM. WML was divided into periventricular and deep white matter regions, and both volumes were calculated from FLAIR images using BAAD software. Coordinate transformation from a native space to the Montreal Neurological Institute space was conducted using Diffeomorphic Anatomical Registration Through Exponentiated Lie Algebra algorithm (Ashburner, 2007). Templates for this algorithm were made from 550 healthy control subjects of the IXI database. The total intracranial volume (TIV) and GMV values were extracted for use in the main analysis. TIV was calculated as the sum of GM, WM, and cerebrospinal fluid (CSF) volumes.

MRI scans were analyzed using two methods, voxel-by-voxel and volumetric analyses. The voxel-by-voxel analysis directly compares each voxel between brain MRI scans; voxel intensity is used to estimate the volume or density of specific brain tissue. Conversely, the volumetric analysis extracts and uses the value of the estimated brain tissue volume, either the total or a specific region of interest (ROI), to perform the analysis without considering each voxel. The voxel-by-voxel analysis retains the spatial information of the brain and is more sensitive for capturing significant differences in volume than the automated volumetric analysis (Voormolen et al., 2010). However, the results of the volumetric analysis are easier to interpret because it shows a quantified volume instead of a statistical map image. Therefore, the use of both methods in tandem is recommended (Testa et al., 2004).

Acquisition of other parameters

Demographic information, tobacco smoking status, education, cognitive screening test, and other medical history and lifestyle information were obtained at the follow-up examination of SESSA (from March 2010 to August 2014) using a self-administered questionnaire that was further confirmed by a trained nurse. The smoking status was categorized into current, never, and former smokers, i.e., participants who had smoked in the previous 30 days, had never smoked before, or had stopped smoking at least 30 days before the data were obtained, respectively. Average daily number of cigarettes smoked and smoking duration in years was taken and used to calculate lifetime amount of smoking as pack-years of smoking.

Blood pressure was measured after 5 min of rest in a sitting position and taken twice on the right arm of each participant while seated using an automated sphygmomanometer (BP-8800, Omron Health Care Co. Ltd.; Tokyo, Japan). Blood was drawn after 12-h fasting and used for laboratory testing (Shiga Laboratory, MEDIC; Shiga, Japan). Lipid concentration was measured using a standardized protocol according to the Cholesterol Reference Method Laboratory Network/United States Center for Disease Control and Prevention. Triglyceride and total cholesterol concentrations were measured using enzymatic assays, whereas high-density lipoprotein cholesterol (HDL-C) measurement was done using a direct method. Hemoglobin A1c (HbA1c) concentration was measured using latex agglutination immunoassay (Kyowa Medix; Tokyo, Japan) in accordance with either the protocol of the Japan Diabetes Society (JDS) or that of the National Glycohemoglobin Standardization Program (NGSP) (Kashiwagi et al., 2012). We converted JDS values to NGSP values using the formula recommended by the JDS (Kashiwagi et al., 2012). Participants with a fasting blood glucose level of ≥126 mg/dL or HbA1c (NGSP) value of ≥6.5%, and/or using antidiabetic medication were categorized as diabetic.

Statistical analyses

Statistical analyses of descriptive data and total GMV were performed using Statistical Analysis Software 9.4 (SAS; Cary, North Carolina, United States). Characteristic data of participants were separated by the alcohol consumption category as detailed below and assessed for differences using ANOVA. Data are presented as means (standard deviations), numbers (percentages), or medians (interquartile ranges) according to the respective variable types and distributions.

The main analysis comprised unstratified and stratified analyses. The unstratified analysis consisted of average weekly alcohol consumption as the independent continuous variable (g ethanol/week), with non-drinkers as 0, and total/local GMV as the dependent variable. The stratified analysis was performed to examine potential GMV differences across the following five alcohol consumption categories as indicator variables: non-drinkers (reference group), light drinkers (≥0.1 and < 14 ethanol g/day), moderate drinkers (≥14 and < 28 ethanol g/day), heavy drinkers (≥28 and < 42 ethanol g/day), and very heavy drinkers (≥42 ethanol g/day). These categories are similar to the US dietary guidelines (U.S. Department of Health and Human Services and U.S. Department of Agriculture, 2015). Total GMV was extracted with BAAD and used in the volumetric analysis using SAS; local GMV was analyzed using a VBM toolbox to enable the voxel-by-voxel analysis between MRI scans.

A multivariable linear regression model was used to assess the relationship between alcohol consumption and GMV in the volumetric and voxel-by-voxel analyses. The analysis of total GMV was performed using the volumetric analysis in two models. To select adjusting covariates, we initially constructed a prediction model by adding all covariates of interest (see Supplementary Table S1 online) in the model using the stepwise regression method in IBM SPSS version 25 (https://www.ibm.com/analytics/us/en/technology/spss/). The following covariates were selected because they remained significant in the relationship with total GMV and, thus, were added to Model 1: TIV, age (years), smoking amount (pack-years), fasting blood glucose (mg/dL), and total cholesterol (mg/dL). Model 2, an exploratory analysis, was further adjusted for available potential mediators and/or confounders based on previous studies (Brust, 2010; Enzinger et al., 2005): education (years), systolic blood pressure (mmHg), hypertensive medication, type 2 diabetes mellitus, HbA1c (%), HDL-C (mg/dL), and triglycerides (mg/dL). Following the total GMV analysis, we applied the voxel-by-

Table 1
Participant characteristics by alcohol consumption levels (n = 639, aged 40–79 years).

Variable	Drinking status				
	Non-drinkers n = 98	Light drinkers n = 207	Moderate drinkers n = 119	Heavy drinkers N = 96	Very heavy drinkers N = 119
Age (years)	68.3 ± 8.0	67.5 ± 9.0	68.2 ± 8.0	67.6 ± 8.4	65.9 ± 7.1
Body mass index (kg/m^2)	23.4 ± 3.0	23.2 ± 3.0	22.8 ± 3.0	23.2 ± 2.6	23.7 ± 2.9
Ethanol (g/week)[a,b]	0	25.0 (48.6)	138.2 (64.0)	241.8 (37.0)	392.0 (181.6)
Education (years)	12.7 ± 2.2	13.1 ± 2.4	13.1 ± 2.4	13.1 ± 2.4	12.8 ± 2.4
Total intracranial volume (mL)	1596 ± 101	1591 ± 103	1602 ± 103	1618 ± 125	1622 ± 115.7
Gray matter volume (mL)	578 ± 50	583 ± 48	575 ± 52	589 ± 55	580.9 ± 48.6
Smoking amount (pack-years)[a,b]	22.5 (39.5)	14.5 (35.0)	18.5 (34.5)	22.8 (34.8)	32.0 (33.6)
Systolic blood pressure (mmHg)[a]	129.1 ± 15.2	129.3 ± 16.0	130.1 ± 15.8	134.1 ± 17.5	136.7 ± 17.8
Total cholesterol (mg/dL)	200.0 ± 32.0	202.4 ± 31.5	201.2 ± 34.6	204.3 ± 40.4	205.8 ± 34.6
Triglyceride (mg/dL)[b]	103.0 (60.0)	99.0 (76.0)	91.0 (66.0)	110.5 (58.5)	123.0 (104.0)
HDL-C (mg/dL)[a]	54.5 ± 13.2	57.1 ± 14.2	62.9 ± 18.0	62.6 ± 18.6	63.9 ± 17.4
HbA1c (%)	6.0 ± 0.7	6.0 ± 1.0	5.9 ± 0.7	5.9 ± 0.7	5.84 ± 0.7
Fasting blood glucose (mg/dL)	100.7 ± 20.5	102.1 ± 24.0	102.8 ± 19.6	100.1 ± 21.3	106.0 ± 24.8
White matter lesion volume (mL)[b]	3.6 (6.0)	3.52 (7.9)	5.8 (11.0)	3.9 (8.8)	5.0 (9.2)

Non-drinkers (0 ethanol g/day), light drinkers (≥0.1 and < 14 ethanol g/day), moderate drinkers (≥14 and < 28 ethanol g/day), heavy drinkers (≥28 and < 42 ethanol g/day), and very heavy drinkers (≥42 ethanol g/day).
Abbreviations: HDL-C, high-density lipoprotein cholesterol; HbA1c, glycated hemoglobin A1c.
[a] Differently distributed ($p < 0.05$ by ANOVA).
[b] Shown as median (interquartile range).

voxel analysis and used Model 1 to confirm the results obtained in local GMV.

Statistical inferences for the voxel-by-voxel analysis were performed using a permutation test followed by a threshold-free cluster enhancement with a threshold for significance of $p < 0.05$, corrected for the family-wise error rate (Smith & Nichols, 2009). Cluster-based thresholding is considered to be more sensitive for identifying the true signal than voxel-based thresholding; however, arbitrary cluster-forming and spatial pre-smoothing thresholds strongly influenced the outcome. To avoid this limitation, the threshold-free cluster enhancement applied an algorithm that enhances the areas of signals in cluster-like regions to maintain the benefits of cluster-based thresholding, while avoiding the limitation described above. As a result, threshold-free cluster enhancement provides richer and more interpretable results (Smith & Nichols, 2009). Results on the statistical T-map were smoothed with an 8-mm full-width at half maximum kernel, rendered in 3D, and then presented in axial/coronal slices using MRIcroGL (mccauslandcenter.sc.edu/mricrogl/).

As a supplementary analysis, we examined the relationship between WML volumes and weekly alcohol consumption (g ethanol/week) using an ROI volumetric analysis. WML volumes were classified as three variables: total WML (tWML), periventricular WML (pvWML), and deep WML (dWML). The analysis was performed using a model similar to that used in the main analysis, except that total WM volume was used as a covariate instead of TIV. Since the WML volume distribution was skewed, we conducted additional analyses using log-transformed WML volumes. As a *post hoc* analysis of the voxel-by-voxel analysis, we extracted the volume of the significant region from the voxel-by-voxel analysis and performed a volumetric analysis using the same multivariable regression model as the total GMV analysis ("ROI volumetric analysis") to confirm the results obtained. This type of *post hoc* analysis is recommended because it is complementary to the voxel-by-voxel results (Voormolen et al., 2010). Additionally, we divided the number of significant voxels to the corresponding ROIs to show the proportion of affected region using BAAD. ROIs were obtained from Automated Anatomical Labelling Atlas integrated in BAAD.

Table 2
Slope of total gray matter volume per gram of alcohol consumed weekly (n = 639, aged 40–79 years).

	Ethanol consumption (g/week)		
	tGMV (mL)	(95% CI)	*p* value
Model 1	−0.02	(-0.03, −0.01)	<0.01
Model 2	−0.02	(-0.03, −0.01)	<0.01

Model 1 adjusted for total intracranial volume, age, total cholesterol, fasting blood glucose, and pack-years of smoking Model 2 further adjusted for years of education, triglyceride, high-density lipoprotein cholesterol, HbA1c, diabetes mellitus, systolic blood pressure, and hypertensive medication(s).
Abbreviations: tGMV, total gray matter volume; CI, confidence interval; HbA1c, glycated hemoglobin A1c.

Results

Participant characteristics

Among the 639 men analyzed, non-drinkers were the oldest, while heavy drinkers were the youngest (Table 1). The mean (standard deviation) age of and daily amount of alcohol consumed by all participants were 67.5 (8.3) years and 22.4 (25.1) g/day, respectively. Education duration, body mass index, HbA1c, and fasting blood glucose were distributed similarly between the non-drinker group and all drinking groups. Among drinkers, the amount of smoking was higher in heavy and very heavy drinkers, and was similar between non-drinkers and heavy drinkers. Systolic blood pressure and total cholesterol, triglyceride, and HDL-C levels were higher in heavy and very heavy drinkers than in all other groups. Overall participant characteristics are summarized in Supplementary Table S2 online.

Alcohol consumption and brain structure

Using a multivariable linear regression model, the volumetric analysis showed that alcohol consumption was inversely associated with total GMV in models 1 and 2 (Table 2, Supplementary Table S3). This relationship remained significant ($p = 0.0008$) after the exclusion of non-drinkers (see Supplementary Table S4 online).

The voxel-by-voxel analysis revealed an inverse association (family-wise error rate [FWER] < 0.05) between alcohol consumption and local GMV (Fig. 1). Significantly atrophied (lower volume) regions were observed in the posterior cingulate, the lateral lower temporal and lower parietal, and the insular and

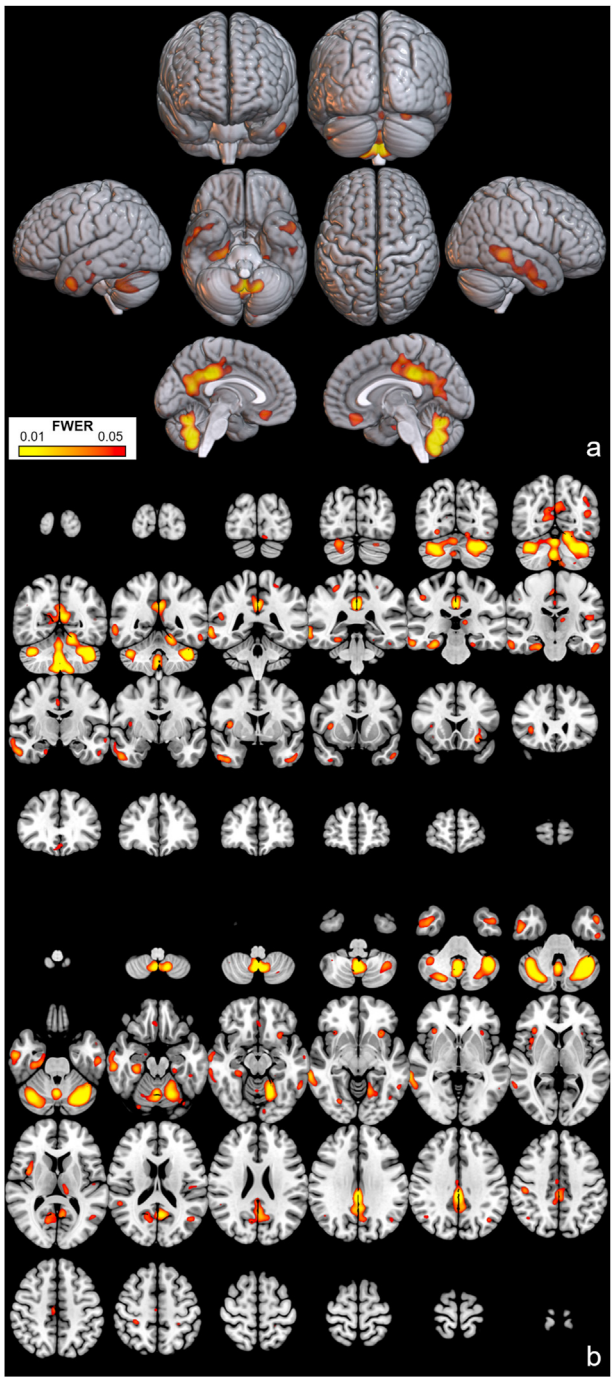

Fig. 1. Voxel-based morphometry analysis of alcohol consumption in all participants (N = 639). The amount of alcohol consumed was regarded as a continuous variable. The amount of alcohol consumed by non-drinkers was considered to be 0. (**a**) Results were adjusted for total intracranial volume, age, total cholesterol, fasting blood glucose, and pack-years of smoking. Highlighted areas show regions with significantly lower gray matter volumes (corrected for threshold-free cluster enhancement family-wise error rate [FWER] < 0.05). (**b**) Coronal and axial sections of voxel-based morphometry analysis showing regional changes in lower gray matter volume in association with alcohol consumption.

medial frontal cortices. The GMV of the parahippocampal region and entorhinal cortex also decreased with increases in alcohol consumption. In the cerebellum, the upper cerebellar vermis and tonsil were smaller. Unsmoothed (T-map) results are shown in Supplementary Figure S1 online. Total GMV results in drinkers were consistent with the results of the ROI volumetric analysis (see Supplementary Table S5 online). Proportion of significant voxels relative to the corresponding ROIs can be found in the supplementary article (see Supplementary Table S6 online).

When stratified by alcohol consumption levels, the volumetric analysis showed that total GMV was significantly lower in very heavy drinkers than in non-drinkers (Table 3). Similarly, the voxel-by-voxel analysis showed local GMV was lower in very heavy drinkers only and mostly in the posterior cingulate, frontal, temporal, parietal, and insular cortices and cerebellum (Fig. 2), which is consistent with the unstratified results (Fig. 1). An overlap figure of voxel-by-voxel results from the unstratified and stratified analyses is shown in Supplementary Figure S2 online. We also found that higher alcohol consumption was associated with greater volumes in three WML outcomes, namely, total, periventricular, and deep white matter, in all models (see Supplementary Tables S7 and S8 online).

Discussion

Principal findings

In the present study, alcohol consumption was significantly associated with lower total and local GMV after multivariable adjustments. These results suggest that chronic alcohol consumption induces asymptomatic GM alterations, independent of potential confounding factors, such as blood pressure (Taki et al., 2006). In the stratified analysis, total and local GMV were lower in very heavy drinkers, defined as ≥42 g of ethanol per day, than in non-drinkers. No significant differences were observed in other drinking groups.

Comparison to previous studies

According to the WHO global status report on alcohol and health 2018 (Poznyak & Rekve, 2018), Japanese men drank 29.3 g of pure ethanol daily, which was slightly higher than in the present study. However, population-based studies (Lin et al., 2005; Marugame et al., 2007; Nakamura et al., 2007) previously showed that older adult Japanese men consumed smaller amounts of alcohol than younger people did, and their average alcohol consumption was similar to that in the present study.

GM atrophy is commonly detected in alcohol-dependent subjects; however, limited information is currently available on the relationship between alcohol consumption and GMV in a non-alcoholic population. In the present study, we examined apparently healthy participants and found a relationship between alcohol consumption and lower GMV, especially in very heavy alcohol drinkers. Previous studies on non-dependent alcohol drinkers reported conflicting findings between GMV and alcohol consumption; some showed lower GMV (Fukuda et al., 2009; Taki et al., 2006; Topiwala et al., 2017; Verbaten, 2009), whereas others found higher GMV (Sachdev et al., 2008). In addition, studies on Asian populations, particularly VBM-based assessments, were limited. A non-VBM study on community-dwelling Japanese men and women found a significantly lower brain volume in moderate drinkers than in non-drinkers (Fukuda et al., 2009). However, their findings did not distinguish between the sexes. Since alcohol consumption by men is generally higher than that by women, the results obtained may be inconclusive and their interpretation is difficult. A VBM study on apparently healthy Japanese men failed to show an independent relationship between total GMV and alcohol (Taki et al., 2006). This study also had potential limitations, including the use of a lower resolution image (0.5 T) than that in the present study (1.5 T) and uncertainty regarding TIV adjustments.

A potentially serious limitation in some studies that assessed alcohol exposure is the inclusion of past drinkers as non-to-light

Table 3
Differences in adjusted total gray matter volume between current drinkers and non-drinkers (n = 639, aged 40–79 years).

Gray matter volumes (mL)	Alcohol consumption group				
	Non-drinkers	Light drinkers	Moderate drinkers	Heavy drinkers	Very heavy drinkers
	n = 98	n = 207	n = 119	n = 96	n = 119
		Diff. (95% CI)	Diff. (95% CI)	Diff. (95% CI)	Diff. (95% CI)
Model 1	Ref	3.2 (−2.9, 9.4)	−5.0 (−11.8, 1.8)	1.8 (−5.4, 9.0)	−10.2 (−17.2, −3.3)[a]
Model 2	Ref	3.0 (−3.2, 9.2)	−5.1 (−12.1, 1.9)	2.2 (−5.2, 9.6)	−9.4 (−18.8, −2.0)[a]

Non-drinkers (0 ethanol g/day), light drinkers (≥0.1 and < 14 ethanol g/day), moderate drinkers (≥14 and < 28 ethanol g/day), heavy drinkers (≥28 and < 42 ethanol g/day), and very heavy drinkers (≥42 ethanol g/day).
Model 1 adjusted for total intracranial volume, age, total cholesterol, fasting blood glucose, and pack-years of smoking.
Model 2 further adjusted for years of education, triglyceride, high-density lipoprotein cholesterol, HbA1c, diabetes mellitus, systolic blood pressure, and hypertensive medication(s).
Abbreviations: CI, confidence interval; Diff., difference in adjusted total gray matter volume compared to non-drinkers; HbA1c, glycated hemoglobin A1c.
[a] $p < 0.05$.

drinkers (Fukuda et al., 2009; Sachdev et al., 2008). Since past drinkers may have very heavily consumed alcohol in the past and stopped for some reason, their inclusion may distort the true relationship between alcohol consumption and brain structures, including atrophy (Fillmore et al., 2007). The regenerative potential of brain tissue is limited; once alcohol-related volume loss (or structural change) occurs, it may continue for a long time, even after the consumption of alcohol has stopped (Zahr & Pfefferbaum, 2017). Therefore, misclassification may have occurred, leading to erroneous estimations. This scenario is particularly problematic in studies using average alcohol consumption, such as grams/week, as the main exposure of interest (Fukuda et al., 2009; Sachdev et al., 2008; Topiwala et al., 2017). One approach to avoid this issue is the use of lifetime alcohol consumption as an exposure measure (Taki et al., 2006). However, we addressed this potential limitation by excluding past drinkers from our analysis. This exclusion was partly because we did not assess the amount of alcohol consumed by past drinkers. To the best of our knowledge, this is the first VBM study on apparently healthy Asian community-dwellers to have investigated the relationship between alcohol consumption and GMV while addressing potential misclassification bias.

A recent study by Topiwala and co-workers found that alcohol consumption (>112 g/week or >16 g/day) was associated with GM atrophy among apparently healthy civil servants in the United Kingdom (Topiwala et al., 2017). A hippocampal atrophy analysis based on a visual rating was performed and revealed higher odds of hippocampal atrophy in a dose-dependent manner, at least in moderate alcohol drinkers (7–14 units or roughly 8 g alcohol/day). We speculate that the discrepancy in GMV in moderate drinkers is based on differences in the population demography and analysis approach. The present study consisted of Japanese men with a higher variance in the amount of alcohol consumed and population age. It is important to note that their study did not perform a voxel-by-voxel or volumetric analysis between different drinking groups, which limited the interpretation of their VBM results. A voxel-by-voxel comparison is important in a VBM study because of its strengths over a volumetric analysis based on visual rating. It is not affected by the bias introduced by human involvement because it is automated and, thus, easier to replicate (Ashburner & Friston, 2000). A voxel-by-voxel analysis is more sensitive than a volumetric analysis while maintaining spatial information brain images (Ashburner, 2009; Ashburner & Friston, 2000); the combination of both resulted in more accurate and interpretable data (Testa et al., 2004). Among VBM studies analyzing apparently healthy participants (Sachdev et al., 2008; Taki et al., 2006; Topiwala et al., 2017), none performed a voxel-by-voxel analysis to investigate differences in local GMV between drinking groups (Preti et al., 2014; Sachdev et al., 2008; Taki et al., 2006; Topiwala et al., 2017). The present study is the first to report a lower GMV in very heavy drinkers than in non-drinkers using both volumetric and voxel-by-voxel analyses.

Clinical implications

GM regions associated with alcohol-induced alterations are located in the posterior cingulate, frontal, temporal, parietal, and insular cortices and cerebellum. These regions are important structures for integrating motor, behavior, and cognitive neural pathways (van Dun, Bodranghien, Marien, & Manto, 2016). The structural changes we observed in apparently healthy community-dwelling participants were similar to those previously reported in alcohol-dependent individuals and neurological diseases that rarely occurred in alcohol dependency. The insula cortex, for example, is an important brain region for self-awareness and emotion (Droutman, Read, & Bechara, 2015). This region is associated with addiction, including alcohol dependence, and a reduction in its volume suggests potentially addictive behavior (Droutman et al., 2015). Lower GMV in the cerebellum, mainly around the vermis, was previously detected in alcohol-dependent subjects (De Bellis et al., 2005) and also in individuals who developed diseases such as Wernicke-Korsakoff syndrome (Manzo et al., 2014) and cerebellar ataxia (Shanmugarajah et al., 2016). Atrophy of the parahippocampal gyrus and neighboring structures, traditionally related to dementia (Echavarri et al., 2011), was associated with alcohol consumption in studies on alcohol-dependent (Meda et al., 2018) and non-dependent (Topiwala et al., 2017) subjects. Hippocampal atrophy was not observed in the present study, which may be due to the exclusion of subjects with cognitive impairment. Atrophy of the posterior cingulate cortex, cuneus, and medial prefrontal cortex has been reported in alcohol-dependent subjects (Rando et al., 2011) and is indicative of the long-term effects of alcohol consumption on the structure of the default mode network (Buckner, Andrews-Hanna, & Schacter, 2008), as demonstrated by functional MRI (Wang et al., 2016). Additionally, frontal cortex atrophy is regarded as one of the common findings in alcohol dependency and other substance addictions (Abernathy, Chandler, & Woodward, 2010; Oscar-Berman & Marinkovic, 2003). Similarities between these clinical studies and the present results suggest that brain structure alterations occur prior to the development of related symptoms.

Our analyses also reveal that higher alcohol consumption was associated with a larger WML volume. The effects of alcohol on the white matter have been observed at the macroscopic and microscopic levels through multiple possible pathways, such as myelin degeneration by thiamine deficiency and ethanol neurotoxicity (de la Monte & Kril, 2014). The present results were consistent with previous findings using manual measurements (den Heijer et al.,

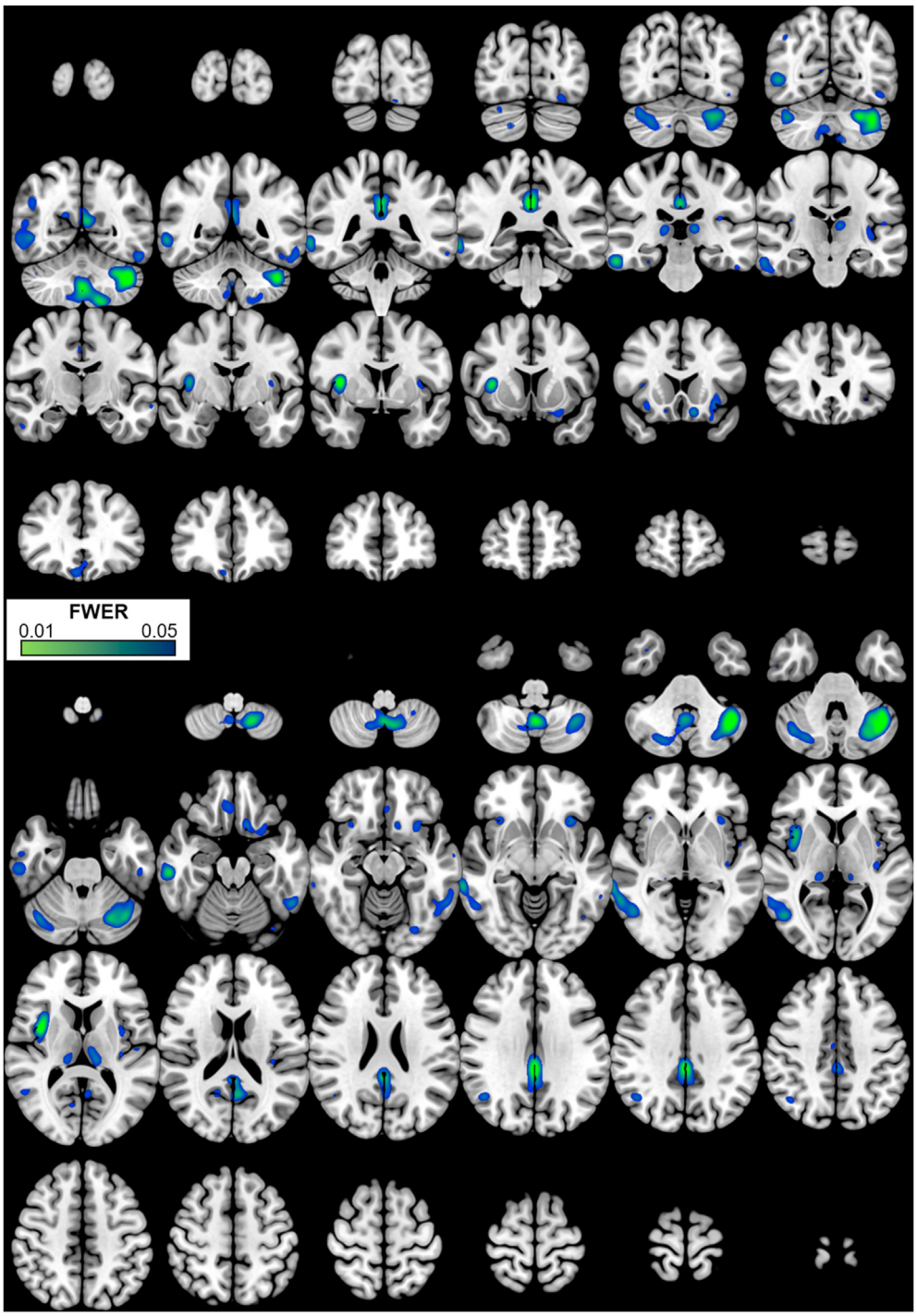

Fig. 2. Voxel-based morphometry subgroup analysis between very heavy drinkers (n = 119) and non-drinkers (n = 98). The drinking group was considered to be an index variable. Results were adjusted for total intracranial volume, age, total cholesterol, fasting blood glucose, and pack-years of smoking. Highlighted areas show regions with significantly lower gray matter volumes (corrected for threshold-free cluster enhancement family-wise error rate [FWER] < 0.05) in very heavy drinkers than in non-drinkers.

2004; Fukuda et al., 2009). Therefore, the automated WML correction and volume estimation used here may be applicable to future research.

Strength and limitations

The present study has several limitations. Since it was a cross-sectional study, we cannot infer longitudinal relationships between alcohol consumption and GMV. Our estimate of alcohol consumption was self-reported, which may be prone to bias and misclassification. Nevertheless, one of our previous studies (Sumi et al., 2019) indirectly suggested that our estimates were reasonable by confirming the expected positive correlations between estimated alcohol amount and concentrations of HDL-C and gamma-glutamyl transferase. Demographic and drinking variables were collected 2 years before MRI scans and a change in drinking habits over this period may have affected our estimate of this relationship. However, our participants showed consistent drinking

habits between the baseline (2006–2008) and follow-up (2010–2014) examinations (Supplementary Figure S3) with a correlation coefficient of 7.4. Therefore, our inference was less likely to be markedly distorted due to a potential change in alcohol drinking habits over the 2-year period. The exclusion of past drinkers may be viewed as a limitation. However, they were excluded to minimize potential confounding factors introduced by the inclusion of this group (Fillmore et al., 2007). The use of the lifetime drinking amount, such as the amount consumed multiplied by the period of drinking, may be another approach to address confounding factors. However, this information was not available in our data. The population used in the present study was exclusively composed of Japanese men; therefore, the results obtained may not be generalized to women and other races or ethnicities.

The main strengths of the present study were the large randomly selected population-based sample combined with the exclusion of individuals with a low cognitive status and those with a history of stroke, which enhanced the generalizability of the results obtained to healthy Japanese men or, to some extent, other East Asians, in contrast to the smaller number of participants and alcohol consumption variance in other VBM studies (Sachdev et al., 2008; Taki et al., 2006; Topiwala et al., 2017). The application of a specific VBM methodology (Smith & Nichols, 2009) and robust volume analysis added validity and significance and contributed to avoiding false positives. We circumvented SPM mis-segmentation for the elderly population (Levy-Cooperman et al., 2008) by applying WML removal. An additional strength of this study was the use of a standardized protocol in the acquisition of all variables.

Conclusion

A volumetric analysis showed an independent relationship between very heavy drinkers (≥42 g ethanol/day) and lower GMV among apparently healthy Japanese men. A voxel-by-voxel analysis showed that the relationship between alcohol consumption and lower GMV affected parahippocampal, entorhinal, cingulate, insular, temporal, and frontal cortices, and cerebellum, which are commonly atrophied regions in alcohol-related neurological diseases. These results suggest the presence of subclinical brain changes and will potentially promote healthier drinking habits in the general population. Additionally, few studies have been conducted on the relationship between alcohol consumption and brain structures in apparently healthy individuals, particularly in Asian populations; therefore, further information on Japanese men is needed. However, we did not observe any relationship in light-to-moderate drinkers, in contrast to previous studies on non-Asian individuals, a discrepancy that warrants further investigation.

Author contributions

A.S., A.F., K.M, H.U: conceptualization, methodology, and supervision. M.M., T.W., N.M., A.K., K.K., and T.I.: data curation, writing-reviewing and editing. A.H.S: investigation, writing-original draft, writing-reviewing and editing, visualization. All authors reviewed and approved the manuscript.

Declaration of competing interest

The authors do not have any conflicts of interest to disclose.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.alcohol.2020.11.006.

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