Association between rs1229984 in *ADH1B* and cancer prevalence in a Japanese population

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Abstract. Alcohol consumption is an established risk factor for cancer, but little is known regarding the effect of genetic polymorphisms in alcohol metabolism genes on alcohol-related cancer risk in the Japanese population. Associations between the *ADH1B* gene (alcohol dehydrogenase 1B), single nucleotide polymorphism (SNP) rs1229984 and cancer have been extensively studied yet evidence is inconsistent. This population-based case-control study primarily aimed to clarify any association between SNP rs1229984 in both overall and specific cancer risk in a Japanese population. The functional non-synonymous SNP rs1229984 (Arg48His) was genotyped using DNA samples from 1,359 consecutive autopsy cases registered in The Japanese Single Nucleotide Polymorphisms for Geriatric Research database. Medical and pathological record data from this database were used to categorise cases and controls. Results included 1,359 participants, 816 cases and 543 controls. Multinomial logistic regression analyses showed no significant association between rs1229984 presence and overall cancer risk in both dominant and recessive genetic inheritance models [Arg/Arg+Arg/His vs. His/His: Adjusted odds ratio (OR)=0.66 (95% CI=0.39-1.13; P=0.129), Arg/Arg vs. Arg/His+His/His: OR=0.95 (95% CI=0.75-1.20; P=0.657)]. However, results showed those homozygous for rs1229984 (genotype His/His) were at significantly decreased odds of lung cancer than other genotypes [recessive model: OR=0.64 (95% CI=0.44-0.93; P=0.020]. In conclusion, there was no significant association between rs1229984 and odds of overall or specific cancers except in lung cancer where His/His genotype decreased odds. To the best of our knowledge, the association between His/His and decreased odds of lung cancer is a novel finding. These findings require further validation in larger studies.

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Introduction

According to the World Health Organisation (WHO), cancer is the 2nd leading cause of death worldwide causing 9 million deaths in 2016, 0.4 million of which were attributable to alcohol consumption (4.2% of all cancer deaths) (1). The cancer burden is rising with an incidence of 18.1 million in 2018 and a predicted increase to an incidence of 29.5 million in 2040 (2). Japan must also tackle this increasing cancer burden with a reported 361,400 deaths attributable to cancer in 2014 alone (3). The highest incidence rates amongst men for lung cancer are seen in Eastern Asia, including Japan with rates above 40 per 100,000 (4). Incidence rates for known alcohol-related cancers such as stomach, liver and colorectal are markedly elevated in Eastern Asia (4). Researching the effect of genetic polymorphisms in alcohol-related cancer risk may help to tackle this global burden, as acknowledged by the WHO. Thus, research moves forward with the anticipation that SNP investigation may create genetic screening strategies identifying individuals at risk of cancer to provide appropriate lifestyle and clinical advice (5).

SNP rs1229984 is a missense variant located on exon 3 chr4:99318162 (GRCh38.p12) in the *ADH1B* gene on chromosome 4q23. It involves a single substitution of nucleotide cytosine (C) to thymine (T), resulting in an amino acid change from arginine to histidine in the β subunit of the *ADH* enzyme, hence this SNP is also referred to as Arg48His mutation. Those with SNP rs1229984 can be said to have mutant 'His allele', with the wild-type classified as 'Arg allele'. Individuals with SNP rs1229984 (His allele) metabolise ethanol to acetaldehyde 70- to 80-fold faster than individuals without due to increased enzymatic function (6). Acetaldehyde is a carcinogen that can promote cancer development through multiple mechanisms, thus any mutation that increases levels

of acetaldehyde, such as rs1229984, may confer increased cancer risk (7). Previous evidence explores the diverse role of rs1229984 in alcohol metabolism, alcohol drinking behaviours and in cancer risk (8). With regard to the effect of rs1229984 on alcohol drinking behaviours, evidence suggests that SNP presence confers a strongly protective effect against alcohol dependence (9-11). However, conclusions about rs1229984 and associations with cancer are conflicting with some reporting SNP presence conferring increased risk, decreased risk or no association with overall cancer. This suggests a scientific need to clarify the role of rs1229984 in cancer risk.

When looking at global allelic distributions of rs1229984, research suggests that Arg allele is more prevalent than His allele. Contrastingly, in the Japanese population mutant His allele is more common conferring SNP rs1229984 presence. Overall, SNP rs1229984 is most prevalent in the Eastern hemisphere and rarer in Western populations. Thus, studies specifically in the Japanese sub-population may be important in establishing the effect of rs1229984 on cancer risk due to higher SNP prevalence here than in the global population (12).

In the present study, rs1229984 status was classified as genotypes Arg/Arg, Arg/His or His/His representing wild-type homozygous; heterozygous or mutant homozygous individuals respectively.

The primary aim of this study was to clarify any association between SNP rs1229984 and overall cancer in a Japanese population. Our secondary aim was to identify any associations between rs1229984 and specific cancer phenotypes.

Patients and methods

Study participants. Experiments were conducted through genotyping DNA samples from consecutive autopsy cases registered in the internet database of Japanese single-nucleotide polymorphisms for geriatric research (JG-SNP database). The JG-SNP database is a collection of pathological data and samples from consecutive autopsy cases employed in Tokyo Metropolitan Geriatric Medical Centre (Tokyo, Japan) since 1995 (13). Autopsies were performed on 40% of all patients who died at the Tokyo Metropolitan Geriatric Hospital between 1995-2004 (14). Genomic DNA was extracted from the kidney renal cortex of consecutive autopsy patients by phenol-chloroform methods. Genomic DNA was isolated from kidney cortex tissue by proteinase K digestion followed by phenol-chloroform extraction. All pathological assessments at autopsy and genotyping experiments were performed in a double-blind manner for both pathologists and clinicians. Autopsy studies included medical information, such as the presence of undiagnosed latent cancers, thus providing a unique database for research on genetic polymorphisms. At the time of sampling in 2004 approximately 1,800 participants were present in the database for use in this study. A total of 1,359 samples remained for use in this study after accounting for loss of samples, poor DNA quality (checked via gel electrophoresis) and contamination of samples. Totally, 1,359 consecutive autopsy cases were analysed.

Cancer phenotype data of participants was also extracted from the JG-SNP database. Patients were defined as 'cases' or 'controls' depending on cancer status. Participants were defined as 'cases' if 1 or more cancer sites were recorded or 'controls' if the recorded value was 0 where no cancer was identified. Cases and controls were unmatched.

The proportion of different diseases in our study participants did not greatly differ from those in the census data of Ministry of Health, Labour and Welfare (Tokyo, Japan) (15), allowing the geriatric autopsy samples to be validly used in genotyping analysis.

Informed consent for the use of autopsy samples and patient clinical data for this study was obtained from the family of study participants at the time of autopsy. Clinical data such as drinking and smoking habit were extracted from patient medical records with informed consent. The study protocol was approved by The Ethics Committees of Tokyo Geriatric Hospital and Tokyo Medical and Dental University and authorized by TMDU Research Ethics Committee (approval no. 2016-011-02).

Sample preparation and genotyping. All autopsy cases were eligible if genomic DNA samples were available of adequate quality. DNA quality was checked via gel electrophoresis and random sampling before genotyping. Since the concentration of the DNA can affect the genotyping results, we quantified the concentration using Thermo Scientific NanoDrop One™ for all the samples used in our study. In a pilot study, RT-PCR was performed on diluted stock DNA samples to assess different concentrations of DNA. This showed concentrations of 2.5-5 ng/μl yielded best genotyping results. All 1,359 samples were mapped to 384 plates and diluted samples were transferred using a multichannel pipette-man. This experiment used a dry genotyping protocol according to the manufacturer's guidelines (Thermo Fisher Scientific, Inc.) thus plates were dried overnight in a dehumidifier (16). The reaction mixture was made by adding TaqMan Drug Metabolism Genotyping Assay (ID C__2688467_20) to the 384 plates. This genotyping assay contained two TaqMan MGB probes and forward and reverse primers. Each probe had fluorescent reporter dye VIC or FAM attached to the 5'end and a non-fluorescent quencher and MGB molecule bound to the 3'end. The probe with VIC dye bound to the complimentary region on the target DNA strand for Arg allele/nucleotide C (wild-type). The probe with FAM dye bound to the complimentary region on the DNA strand for His allele/nucleotide T (SNP presence). This substitution polymorphism can be denoted as context sequence (VIC/FAM): GCCACTAACCACGTGGTCATCTGTG[C/T]GACAGATTCCTACAGCCACCATCTA. Further information regarding sequences of primers and probes was requested from manufacturers, who stated that these are commercial secrets and only the assay ID is available (assay ID: C-2688467_20; Thermo Fisher Scientific, Inc.). A total of 2.5 μl of TaqMan Universal PCR Master Mix, 0.25 μl of 20X SNP genotyping assay and 2.25 μl of DNase-free water was added to the dried 384 plates, creating total 5 μl reaction mixture. The samples were mixed using a microplate mixer and spun down in a centrifuge. Centrifuging was performed at room temperature at 1,500 rpm/min for 5 min. The reaction mixture is photosensitive, so light exposure was avoided. The plates were transferred to LightCycler 480 instrument (Roche Diagnostics, Penzberg, Germany) to run qualitative RT-PCR. PCR settings were specified in the manufacturer's guide for a

Table I. General demographics of study participants.

Characteristics	Total number (%)	Number of cases (%)	Number of controls (%)	P-value[a]
Number	1,359	816 (60)	543 (40)	
Age[b]	80.1 (±8.87)	79.7 (±8.46)	80.6 (±9.62)	0.11
Sex				
Male	732 (54)	462 (37)	270 (63)	
Female	627 (46)	354 (44)	273 (56)	0.01[a]
Smoking status				
Smoker	650 (48)	408 (50)	242 (45)	
Non-smoker	624 (46)	369 (45)	255 (47)	0.18
Unknown	85 (6)	39 (5)	46	
Alcohol consumption				
Drinker	452 (33)	296 (36)	156 (29)	
Non-drinker	823 (61)	480 (59)	343 (63)	0.01[a]
Unknown	84 (6)	40 (5)	44 (8)	

[a]P<0.05. P-values were calculated using either χ^2 test or ANOVA, according to variable type. [b]Presented as the mean (±SD).

total of 50 cycles as follows: i) Pre incubation: 90 degrees for 10 min; ii) denaturation: 92 degrees for 15 sec and iii) annealing and extension: 60 degrees for 1 min.

Qualitative results were generated by the thermocycler through detection of fluorescent light emitted by both reporter dyes VIC and FAM, where the wavelength was ~551 and ~517 nm, respectively. This allowed samples to be genotyped where green light emission (~551 nm) indicated the sample was of genotype CC, where blue was genotype TT (~517 nm) and where red was heterozygous CT (17). All 1,359 samples obtained were genotyped leading to a 100% genotyping success rate. All primers and probes were purchased from Thermo Fisher Scientific, Inc.

Statistical analysis. Statistical analysis was performed blinded post genotyping using SPSS version 19 (IBM Corp). Two statistical tests were used to compare baseline characteristics between cases and controls. Statistical significance in categorical variables was evaluated using the Chi-square test and continuous variables were evaluated using analysis of variance (ANOVA). The Bonferroni correction used to adjust for multiple testing was not used in statistical analysis due to its conservative nature (18). This correction creates a more stringent criterion for 'statistical significance' by adjusting probability values (P), aiming to reduce risk of type I error when performing multiple statistical analyses. This is done by adjusting the conventional P<0.05 for population size. However, use can eliminate important significant findings and lead to an increase in type II errors. Therefore, in this study statistical significance was defined as results with P<0.05 in accordance with conventional standards. Hardy-Weinberg Equilibrium (HWE) was calculated to determine if genotype frequencies observed in the study population differed from expected population frequencies. Odds ratios (OR) and 95% confidence intervals (CI) were calculated using multinomial regression analysis to determine any association between genotype status and odds of cancer, in both overall and specific cancers. Confounding factors such as sex, smoking status, drinking status, and age at death were adjusted for in regression analysis, generating crude and adjusted ORs.

Results

Patient demographics. General demographics of the study population including age at death, sex, smoking status and drinking status are described in Table I. The study population comprised of 816 (60%) cases and 543 (40%) controls. There were 732 (54%) males and 627 (46%) females with a mean age at death of 80.1 (±8.87) (Table I).

'Smokers' were defined as patients who have ever smoked, and 'non-smokers' as those who have never smoked. The number of smokers was 650 (48%) and number of non-smokers was 624 (46%). There was no significant difference in smoking status between cases and controls as P=0.1837 (Table I).

'Drinkers' were defined as those who have ever consumed alcohol and 'non-drinkers' as those who have never consumed alcohol. The number of drinkers was 452 (33%) and number of non-drinkers was 823 (61%). Results showed drinking status was significantly different between cases and controls as P=0.0122 (Table I).

Overall, sex (P=0.0125) and alcohol consumption (P=0.0122) were the only variables significantly different between cases and controls as shown in bold (Table I). All other variables showed no significant difference between cases and controls as P>0.05 (Table I).

Genotyping results and allelic counts. A typical TaqMan assay scatter plot for SNP rs1229984 is shown in Fig. 1 as obtained from the LightCycler after RT-PCR. Each dot represents one sample (an individual participant), and the X and Y axes equate to the levels of fluorescence of two dyes FAM and VIC for His allele (SNP presence) and Arg allele (wild-type), respectively. Identification of both alleles for each

Table II. Overall genotyping results and allelic counts for SNP rs1229984 in all participants.

SNP	Alleles		Genotypes			P-value (HWE)[a]
rs1229984	His	Arg	His/His	Arg/His	Arg/Arg	0.993
No (%)	1,042 (77)	317 (23)	799 (59)	486 (36)	74 (5)	

[a]P-value of HWE calculation. Values are rounded up to nearest whole percent. HWE, Hardy-Weinberg Equilibrium.

Table III. Multinomial logistic regression association analysis using dominant inheritance genetic model (Arg/Arg: Arg/His+His/His).

		Crude OR[a]		Adjusted OR[b]	
Cancer phenotype	Genotype	OR	P-value[c]	OR	P-value[c]
	Arg/Arg	1.00 (reference)	N/A	1.00 (reference)	N/A
Total Cancer	Arg/His+His/His	0.7 (0.42-1.15)	0.129	0.66 (0.39-1.13)	0.129
Gastric	Arg/His+His/His	1.78 (0.7-4.51)	0.231	1.77 (0.7-4.5)	0.231
Lung	Arg/His+His/His	0.96 (0.43-2.15)	0.708	0.85 (0.38-1.94)	0.708
Colon	Arg/His+His/His	0.62 (0.29-1.34)	0.480	0.74 (0.33-1.69)	0.480
Pancreatic	Arg/His+His/His	0.65 (0.23-1.87)	0.321	0.58 (0.2-1.69)	0.321
Liver	Arg/His+His/His	1.04 (0.31-3.43)	0.975	1.02 (0.31-3.4)	0.975

[a]Crude OR, OR adjusted for only age and sex; [b]Adjusted OR, OR adjusted for age, sex, smoking status and drinking status. 95% confidence intervals are indicated by parenthesis. [c]P-value, significance defined at P<0.05. Values are given to 3 significant figures. OR, odds ratio.

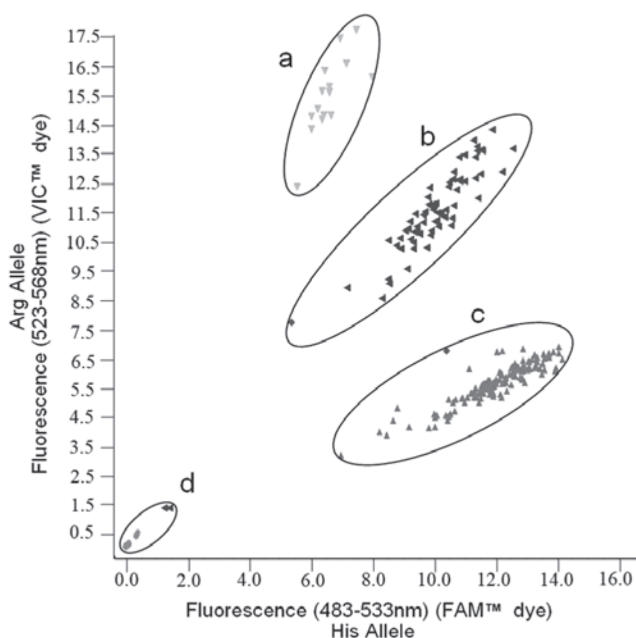

Figure 1. Endpoint fluorescence scatter graph. (a) Represents genotype Arg/Arg; (b) genotype Arg/His; (c) genotype His/His and (d) ambiguous samples which were repeated.

sample was calculated based on fluorescence levels from the LightCycler. Samples with only a substantial increase in FAM dye fluorescence conferred homozygosity for His allele. A substantial increase in only VIC dye fluorescence conferred homozygosity for Arg allele and substantial increase in both fluorescence signals conferred heterozygosity for alleles Arg/His. Thus, region A represents samples of alleles Arg/Arg (homozygous wild-type), region B of alleles Arg/His (heterozygous SNP) and region C of alleles His/His (homozygous SNP). Ambiguous samples or those that did not sufficiently fluoresce were repeated until clear separation was obtained, as represented by region D.

Information regarding overall genotyping results and allelic counts is provided in Table II. As expected, genotypes His/His and Arg/His were most prevalent in this Japanese population with overall genotype frequencies as follows-His/His: 799 (59%); Arg/His: 486 (36%); Arg/Arg: 74 (5%). Allele frequencies for His allele and Arg allele were calculated using the Hardy-Weinberg equations: $p^2 + 2pq + q^2=1$; $p + q=1$, where p is defined as the frequency of His allele and q as the frequency of Arg allele (since SNP rs1229984 is controlled by a pair of alleles). This showed that 1042 (77%) individuals had His allele and 317 (23%) had Arg allele. Our results matched allele frequency data in Japanese SNP database HGVD (Human Genetic Variation Database) (19). Results also suggest the study population is in Hardy-Weinberg Equilibrium (HWE) since the P-value was >0.05 so observed allele frequencies in this study population do not greatly differ from the expected frequencies. This suggests the distribution of alleles in the population is unlikely due to chance so further association analysis are interpretable (20). Overall, results were in accordance with previous data on allelic distributions of SNP rs1229984 that suggest reference His allele is more prevalent

Table IV. Multinomial logistic regression association analysis using recessive inheritance genetic model (Arg/Arg+Arg/His: His/His).

Cancer phenotype	Genotype	Crude OR[a]		Adjusted OR[b]	
		OR, (95% CI)	P-value[c]	OR, (95% CI)	P-value[c]
	Arg/Arg+Arg/His	1.00 (reference)	N/A	1.00 (reference)	N/A
Total Cancer	His/His	0.99 (0.79-1.23)	0.901	0.95 (0.75-1.20)	0.657
Gastric	His/His	1.21 (0.86-1.71)	0.264	1.16 (0.82-1.64)	0.399
Lung	His/His	0.7 (0.48-1.00)	0.051	**0.64 (0.44-0.93)**	**0.020**
Colon	His/His	1.1 (0.72-1.66)	0.662	1.08 (0.71-1.65)	0.724
Pancreatic	His/His	0.88 (0.49-1.59)	0.678	0.95 (0.51-1.77)	0.877
Liver	His/His	1.12 (0.63-1.99)	0.705	1.07 (0.6-1.91)	0.814

[a]Crude OR: OR adjusted for only age and sex. [b]Adjusted OR: OR adjusted for age, sex, smoking status and drinking status. [c]P-value: Significance defined at P<0.05. Significant values are in bold. Values are given to 3 significant figures. OR, odds ratio.

than alternate Arg allele in Japanese populations, differing from the worldwide distribution (12).

Association analysis using genetic models. Results of multinomial logistic regression analysis exploring the association between genotype status and odds of cancer are shown in Tables III and IV. Association analyses were performed for both overall cancer and all specific cancer phenotypes.

Results were calculated using dominant inheritance and recessive inheritance genetic models to account for different modes SNP of inheritance. Confounders were adjusted for in odds ratio (OR) calculations with adjustment for only age at death and sex in crude OR, and adjustment for age at death; sex; smoking status and drinking status in adjusted OR. Cancer phenotypes reported are shown in order of decreasing sample size. Further association analysis for cancer phenotypes with low prevalence have not been reported in detail due to low power.

Genotype homozygous Arg/Arg was used as the reference for comparison to other genotypes (Arg/His, His/His) in the dominant genetic model. Results show there was no significant difference in odds of overall cancer when comparing other genotypes (Arg/His, His/His) to reference Arg/Arg since Arg/Arg: Arg/His+His/His adjusted OR=0.66 (95% CI=0.39-1.13, P=0.129). Results for individual cancer risk in all cancer phenotypes similarly suggested there was no significant difference in odds of specific cancer when comparing Arg/Arg: Arg/His+His/His using a dominant model. The results for stomach, lung, colon, pancreatic and liver cancer are presented in further detail below for reference (Tables III and IV).

The genotypes Arg/Arg+Arg/His were used as the reference for comparison to genotype His/His in the recessive genetic model. Results show there is no significant difference in odds of cancer overall when comparing genotype His/His to reference Arg/Arg+Arg/His since Arg/Arg+Arg/His: His/His OR=0.95 (95% CI=0.75-1.20, P=0.657). However, adjusted results show there is a significant decrease in odds of lung cancer when comparing genotype His/His to reference Arg/Arg+Arg/His since Arg/Arg+Arg/His: His/His OR=0.64 (95% CI=0.44-0.93, P=0.020). This suggests that individuals who are genotype His/His (homozygous for rs1229984) may be at decreased risk of lung cancer compared to those of genotypes Arg/His or Arg/Arg. However, this result must be interpreted with caution due to a low number of lung cancer cases. No other significant associations with specific cancer phenotypes were found across all models (Tables III and IV).

In all examined models there was no significant association between rs1229984 and overall odds of cancer nor odds of specific cancer phenotypes, except in the case of lung cancer in a recessive genetic model where a significant decrease in odds was found in those homozygous for the SNP (genotype His/His).

Discussion

This study primarily aimed to clarify the association between single nucleotide polymorphism rs1229984 in the *ADH1B* gene and both overall and specific cancer risk in a Japanese population.

Detailed results for the following cancers were presented: total cancer, stomach, lung, colon, pancreatic and liver. Analyses of liver, pancreas and colon cancer were presented due to evidence that these are alcohol-related cancers (21). Stomach cancer is presented due to previous investigations into its association with rs229984 (22-25). Lung cancer is presented due to adequate sample size and significant findings. However, results for individual lung cancer sub-types have not been presented due to inadequate sample size and reduced power.

Overall, results showed no statistically significant association between SNP rs1229984 in both overall and specific cancer risk, except for lung cancer in a recessive genetic model where results suggested homozygous SNP presence (genotype His/His) may decrease cancer risk.

A recent meta-analysis found no significant association between rs1229984, colorectal, hepatocellular, stomach nor pancreatic cancer supporting our findings of no association between rs1229984 and specific cancer phenotypes in this study (26). However, lung cancer was not explored in this meta-analysis due to low reporting in studies suggesting research into associations with lung cancer require larger

sample sizes (26). The true effect of *ADH* gene SNPs on alcohol metabolism is debated. Birley *et al* suggest SNPs in the *ADH* region have lower effects on alcohol metabolism than previously expected (27) so the overall effect of rs1229984 on cancer risk may be minimal or non-existent. This evidence supports our finding of a lack of association between rs1229984 and overall and specific cancer risk but is rarely replicated in the literature perhaps due to publication bias.

Alternatively, this lack of association may be masked by the fact that single SNPs have little effect in the predisposition to complex traits, such as cancer, and that hundreds to thousands of loci are likely involved (8). Combining results for multiple loci may reveal the synergistic effect of multiple SNPs in alcohol metabolism and cancer risk. Indeed, the role of other *ADH1B* and *ALDH2* variants remains an area of interest (28). For example, the HapMap project estimates there are at least 449 polymorphic variants in the *ADH* region which may be involved in *ADH* gene expression, many of which have not been studied (29). Another author suggests rs1229984 may be in linkage disequilibrium with other unidentified *ADH* regions since it has previously been shown to be in linkage disequilibrium with *ADH1C* sites (30). This suggests multiple variants may influence the expression of rs1229984 and consequently affect cancer risk.

Evidence regarding the effect of rs1229984 on specific cancer risk has been contradictory. SNP presence may confer increased risk, decreased risk or no association cancer risk (31), whilst others argue the variant confers increased risk (32-34). As mentioned prior, the mechanism of how this variant may affect cancer risk is poorly understood but may be due to altered enzymatic function of *ADH1B* in individuals with rs1229984 (6). Some studies suggest that rs1229984 presence may confer a protective effect against only alcohol-related cancers, such as oesophageal cancer. In one study, Arg/Arg individuals (homozygous wild type) had a 3.99-fold increased risk of developing oesophageal cancer compared with His/His individuals (homozygous rs1229984) (35). This reduced risk may be explained by the theory that those with rs1229984 are more likely to be alcohol adverse or abstinent due to unpleasant side effects of acetaldehyde (36). Some suggest the interplay of *ADH1B* genotypes with levels of alcohol consumption may modulate oesophageal cancer risk (37). Despite evidence of this protective effect of rs1229984, findings have not been widely reproducible and meta-analyses are conflicting perhaps due to few robust studies and publication bias in the field (38).

The relationship between levels of alcohol consumption and cancer risk is complex. Epidemiological evidence has widely associated alcohol consumption with specific cancer risk with the International Agency for Research on Cancer (IARC) categorising the secondary metabolite acetaldehyde as 'carcinogenic to humans' in Group 1 (39). A dose-response relationship between alcohol consumption and cancer risk has been well established (21). However, in this study few samples had complete alcohol data and so drinking status was stratified only as 'drinkers' or 'non-drinkers', disallowing association analysis between levels of alcohol consumption, genotype and cancer risk. Thus, associations may have been missed as quantitative amount of drinking were not used in analyses potentially leading to exposure misclassification of participants.

Our finding that rs1229984 presence may confer a protective effect against lung cancer may be influenced by unknown factors such as levels of alcohol consumption. Indeed, results showed a significant difference in cancer prevalence between drinkers and non-drinkers suggesting levels of alcohol intake may be important (Table I). Evidence suggests that heavy drinkers may be at increased risk of lung cancer than moderate or never drinkers (21). The proposed mechanism is that high concentrations of ethanol exposure may cause decreased NK cell activity and immune suppression, leading to increased lung metastases (40). However, research into the association between levels of alcohol consumption and specifically lung cancer risk is limited. Overall, the effect of SNPs on specific cancer risk must be further researched before drawing conclusions.

This study had strengths and limitations. Selection bias may have been introduced as participants were consecutive autopsy cases meaning cause of admission or death may not be randomised. The contribution of any gene to cancer risk is modulated by many other social and environmental factors. Therefore, there may be uncontrolled confounders such as diet, levels of alcohol consumption, occupational status, socioeconomic status or ethnicity that may affect both the expression of rs1229984 and overall cancer risk. Indeed, rs1229984 has shown reduced cancer risk amongst Asians and mixed ethnicity groups, but increased risk amongst Caucasians suggesting there may be ethnic differences in alcohol metabolism and cancer risk (26). Measurement bias was reduced through machine genotype identification; however, autopsy practice may have differed between pathologists leading to potential misclassification of cancer outcomes. Lifestyle data was collected through a self-reported questionnaire from medical records leading to potential recall bias and inaccuracy of data. Cases were unmatched to controls meaning unknown confounders may not have been controlled for. Results may not be generalizable to other global populations or age at death groups, as this study focused on elderly Japanese population. Results may also not be generalizable to any association between rs1229984 and other diseases such as as alcohol use disorders (11) which has been extensively studied, or to non-alcohol-related disease (41). To our knowledge this is the first study looking more specifically at associations between rs1229984 in elderly Japanese population and cancer risk.

In conclusion, this study suggested there was no significant association between SNP rs1229984 and overall or specific cancer risk, except in the case of lung cancer where results suggested homozygous SNP presence (genotype His/His) decreased overall lung cancer risk.

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Availability of data and materials

All datasets used or analysed for this study are available from the corresponding author upon reasonable request.

Authors' contributions

The present study was designed by MM. Sample collection and preparation was performed by MS and TA. MS and TA conducted the autopsy and pathological analyses for all samples used. Experiments and statistical analyses were performed by PG and SP. The manuscript was written by PG with contributions from SP and MM. All authors read and approved of the final manuscript.

Ethics approval and consent to participate

Informed consent for the use of autopsy samples and patient clinical data for this study was obtained from the family of study participants at the time of autopsy. Clinical data such as drinking and smoking habit were extracted from patient medical records with informed consent. The study protocol was approved by The Ethics Committees of Tokyo Geriatric Hospital and Tokyo Medical and Dental University (Tokyo, Japan) and authorized by TMDU Research Ethics Committee (approval no. 2016-011-02).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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