

Regular Article

A Genome-Wide Association Study Predicts the Onset of Dysgeusia Due to Anti-cancer Drug Treatment

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Received August 29, 2021; accepted October 11, 2021; advance publication released online October 16, 2021

Dysgeusia is a major side effect of anti-cancer drug treatment. Since dysgeusia significantly lowers the patient's QOL, predicting and avoiding its onset in advance is desirable. Accordingly, aims of the present study were to use a genome-wide association study (GWAS) to identify genes associated with the development of dysgeusia in patients taking anti-cancer drugs and to predict the development of dysgeusia using associated single nucleotide polymorphisms (SNPs). GWAS was conducted on 76 patients admitted to the Department of Hematology, Tokushima University Hospital. Using Sanger sequencing for 23 separately collected validation samples, the top two SNPs associated with the development of dysgeusia were determined. GWAS identified rs73049478 and rs41396146 SNPs on the retinoic acid receptor beta (*RARB*) gene associated with dysgeusia development due to the administration of anti-cancer drugs. Evaluation of the two SNPs using 23 validation samples indicated that the accuracy rate of rs73049478 was relatively high (87.0%). Thus, the findings of the present study suggest that the rs73049478 SNP of *RARB* can be used to predict the onset of dysgeusia caused by the administration of anti-cancer drugs.

Key words genome-wide association study; dysgeusia; anti-cancer drug; hematopoietic tumor; single nucleotide polymorphism

INTRODUCTION

Dysgeusia is a major side effect of anti-cancer drug treatment, which develops as early as 2–3 d and more often three weeks after the treatment commences. Symptoms of dysgeusia that persist throughout the anti-cancer drug treatment include difficulty in identifying taste, loss of taste, a constant bitter or metallic taste, and a feeling of chewing sand. Dysgeusia due to anti-cancer drugs occurs in approximately 67% of total cases and 38% of moderate to severe cases.^{1,2)} Anti-cancer drug treatment causes dysgeusia by reducing the number and damaging the cellular structure of normal taste receptor cells and causing neuropathy in the glossopharyngeal and facial nerves, which are the taste conduction pathways.³⁾ The papillae on the human tongue contains taste receptors called taste buds. Taste buds are cell aggregates consisting of approximately 30–70 cells, and taste receptors are expressed during the process of cell differentiation in these taste buds. Humans recognize taste by activating the receptors contained in the taste buds and generating taste signals, which are transmitted to the taste nerves and centers *via* synapses.⁴⁾ Zinc, which is essential for taste cell regeneration, forms a chelate with anti-cancer drugs and consequently is excreted from the body, resulting in zinc deficiency, affecting taste cell turnover, and causing dysgeusia. No direct treatment for dysgeusia has been established currently, and supportive care such as zinc administration is used.³⁾ However, a report demonstrated that there

was no statistically significant difference in the incidence of dysgeusia between the zinc-administered and placebo groups (73% in the zinc-administered group, 84% in the placebo group, *p* value = 0.16).⁴⁾

Dysgeusia occurs frequently but never leads to death; therefore, it is not deemed clinically important. However, dysgeusia dramatically reduces the patients' QOL, because diet plays an important role in not only nutrition and health but also social contexts such as communication through food and enjoyment of taste.⁵⁾ Therefore, prediction and avoidance of the onset of dysgeusia caused by anti-cancer agents are essential.

At present, it is expected that tailor-made medical care that is optimal for each patient can be realized at an early stage after considering individual differences in the patients. Tailor-made medical care is a next-generation medical system that is based on the patient's genomic information obtained from genetic diagnosis, unlike conventional medical care for the average population. The realization of tailor-made medical care will make treatment more effective for individual patients and avoid serious side effects. Despite the accumulation of vast amounts of genomic information due to recent advances in genome sequencing and analysis, personalized treatment is rarely provided to patients in Japan.

Genome-wide association studies (GWAS) can genotype more than 500000 single nucleotide polymorphisms (SNPs) and statistically investigate the association between SNP frequency and disease or genetic traits. GWAS involves not only

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the analysis of a specific narrow region but also a macroscopic view of the human genome. To date, GWAS in the Japanese population have identified SNPs in five novel candidate loci associated with trastuzumab-induced cardiotoxicity, and these have been used to develop a trastuzumab-induced cardiotoxicity risk prediction model.⁶⁾ Thus, GWAS is also useful for identifying markers for predicting the occurrence of side effects of anti-cancer drugs.

For humans, food not only serves as nutrition, but also functions as a means to enjoy taste and communicate; therefore, the occurrence of dysgeusia significantly reduces a patient's QOL. Being unable to taste deprives the patient of the joy and enjoyment of eating, consequently decreasing the patient's willingness to undergo treatment. Therefore, if side effects can be predicted, dysgeusia can be avoided, and the patient's mental distress due to anti-cancer drug treatment can be alleviated. Therefore, the aim of the present study was to test the hypothesis that SNPs identified by GWAS as strongly associated with the onset of dysgeusia could be used to predict and prevent the onset of side effects in patients taking anti-cancer drugs.

MATERIALS AND METHODS

Subjects This study was approved by the Human Genome and Genetic Analysis Research ethics committees of the Tokushima University (Approval Reference No. H26-29, date: January 5, 2015), and Clinical Research Ethics Committee of the Tokushima University Hospital (Approval Reference No. 2175, date: January 26, 2015). All participants provided written informed consent.

The present study included 99 patients admitted for chemotherapy against acute myeloid leukemia, diffuse large B-cell lymphoma, myelodysplastic syndrome, and acute promyelocytic leukemia to the Department of Blood Medicine at the Tokushima University Hospital from January 2015 to December 2019. Of the 99 patients, 76 were used for GWAS, and 23 were used for obtaining validation samples. The occurrence of taste disorders was self-reported.

Genotyping Genomic DNA was extracted from the saliva samples collected from the subjects using Oragene OG-500 Saliva collection kits (DNA Genotek Inc., Canada). Seventy-six samples were genotyped for 659184 markers using the Illumina Asian Screening Array V1.0 Kit (Illumina, San Diego, CA, U.S.A.), following the manufacturer's instructions. For the 23 validation samples, SNPs were genotyped using Sanger sequencing.

Statistical Analysis Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated for the dominant model using logistic regression analysis in the PLINK

version 1.07 software package (<http://pngu.mgh.harvard.edu/~purcell/plink/>).⁷⁾ The Manhattan plots were generated using the qqman package for R software (version 3.5.0, (<http://www.R-project.org/>)). Significant expression quantitative trait loci (eQTL) analysis of the identified SNPs were conducted using GTEx Portal database (<http://www.gtexportal.org/home/>),⁸⁾ and HaploReg V.4.1 (<http://archive.broadinstitute.org/mammals/haploreg/haploreg.php>) was used for the functional annotation of nucleotide variants.⁹⁾ Finally, the sensitivity, specificity, positive and negative predictive values, and accuracy were evaluated using the 23 validation samples.

RESULTS

The 76 hospitalized patients were treated with cytarabine, vincristine, bortezomib, fludarabine phosphate, melphalan, methotrexate, azacitidine, and/or rituximab. Dysgeusia was confirmed in 49 of the 76 patients following anti-cancer drug administration. The suspected anti-cancer drugs were vincristine and cytarabine in 26 and 16 cases, respectively; melphalan and bortezomib in 2 cases each; and fludarabine, methotrexate, and azacitidine in 1 case each. The dysgeusia-onset and non-dysgeusia-onset groups showed no difference in gender, age, and body mass index (Table 1).

We conducted a GWAS to identify the loci associated with the development of dysgeusia due to anti-cancer drug treatment. Manhattan plots of the GWAS are shown in Fig. 1. The loci rs73049478 and rs41396146 on chromosome 3 were suggestively associated (OR = 0.076, 95% CI = 0.022–0.257, $p = 3.5 \times 10^{-5}$ and OR = 0.090, 95% CI = 0.028–0.283, $p = 3.9 \times 10^{-5}$, respectively) with dysgeusia by anti-cancer drug treatment under the dominant model (Table 2). Both SNPs are located in the intron or upstream of the retinoic acid receptor beta gene (*RARB*). Neither of the SNPs was associated with a significant eQTL, and according to the HaploReg database, rs73049478 and rs41396146 resided in the regulatory motifs of 13 altered and Myf, respectively (Supplementary Table S1). Of the four SNPs in high linkage disequilibrium (LD) with rs73049478, two were associated with deoxyribonuclease (DNase) I hypersensitive regions, and three resided in the several motifs changed.

Furthermore, the ability of rs73049478 and rs41396146 to predict the development of dysgeusia was assessed using 23 validation samples. The dysgeusia-onset and non-dysgeusia-onset groups in validation samples also showed no difference in gender, age, and body mass index (Table 1). Typing results of the two SNPs for the validation samples are shown in Table 3. The accuracy rate of rs41396146 was relatively low (39.1%), whereas that of rs73049478 was relatively high at (87.0%; sensitivity, 88.2%; specificity, 88.2%; positive predictive value,

Table 1. Characteristics of Subjects

	GWAS (<i>N</i> = 76)			Validation (<i>N</i> = 23)		
	Control (<i>N</i> = 28)	Case (<i>N</i> = 48)	<i>p</i> -Value	Control (<i>N</i> = 7)	Case (<i>N</i> = 16)	<i>p</i> -Value
Age (years)	63.2 ± 11.9	59.7 ± 10.3	0.19	64.0 ± 8.0	58.1 ± 9.7	0.83
BMI (kg/m ²)	23.0 ± 2.7	22.1 ± 2.8	0.19	20.0 ± 3.4	20.4 ± 2.6	0.30
Male sex – no. (%)	19 (70.4)	25 (51.0)	0.16	4 (57.1)	6 (37.5)	0.34

Case refers to patients who developed dysgeusia due to anti-cancer drug treatment, and control refers to patients who did not. Data are presented as mean ± standard deviation. *p*-Values of age and body mass index (BMI) were obtained using unpaired Student's *t*-test, and *p*-values of the male sex were obtained using Fisher's exact test.

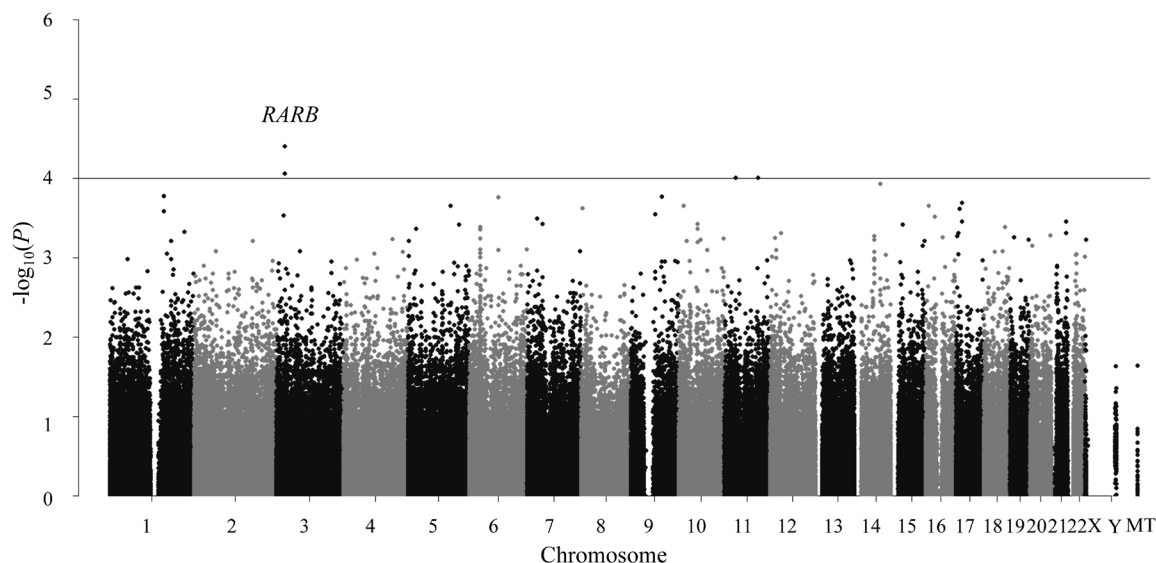


Fig. 1. Manhattan Plot from GWAS for Development of Dysgeusia Due to the Anti-cancer Drug Treatment

The negative \log_{10} -transformed p -values (y -axis) of SNPs are shown according to their position on the chromosome. The horizontal line represents suggestive association (p -value = 1×10^{-4}).

Table 2. The Top Two SNPs Identified in GWAS for Development of Dysgeusia Due to Anti-cancer Drug Treatment

Chr.	SNP	Gene locus	Allele	Control ($N = 28$)		Case ($N = 48$)		OR (95%CI)	p -Value
				Genotypes	AF	Genotypes	AF		
3	rs73049478	<i>RARB</i> intron or upstream	A/G	4/19/5	0.52	33/11/4	0.20	0.076 (0.022–0.26)	3.5×10^{-5}
3	rs41396146	<i>RARB</i> intron or upstream	T/C	5/19/4	0.48	34/10/4	0.19	0.090 (0.028–0.28)	3.9×10^{-5}

Chr, chromosome; SNP, single-nucleotide polymorphism; AF, allele frequency.

Table 3. Allele Frequency in the Validation Samples

SNP	Allele	Control ($N = 7$)		Case ($N = 16$)	
		Genotypes	AF	Genotypes	AF
rs73049478	A/G	5/0/2	0.29	15/0/1	0.06
rs41396146	T/C	4/1/2	0.36	10/6/0	0.19

SNP, single-nucleotide polymorphism; AF, allele frequency.

93.8%; negative predictive value, 71.4%).

DISCUSSION

In this study, we conducted a GWAS to identify genes related to dysgeusia caused by anti-cancer drug treatment. We identified that, in the dominant model, the loci rs73049478 and rs41396146 were suggestively associated with the development of dysgeusia caused by anti-cancer drug treatment. Patients with minor alleles of these SNPs had a reduced risk of developing dysgeusia compared to patients without minor alleles. These two loci are located in the intron of the *RARB* gene, which encodes the retinoic acid (RA) receptor. RA is the active form of vitamin A. RA signal transduction occurs *via* RA receptors, and various genes are regulated by RA signal transduction *in vivo*.¹⁰ It has been reported that RA is an important regulator of salivary gland morphogenesis in mammals, and inhibition of RA signaling interferes with salivary tissue epithelial growth and morphogenesis.¹¹ In addition, saliva transports taste substances to taste receptors and is known to

be involved in taste sensitivity by protecting the morphology of taste receptors.¹² These findings suggest that mutations in the *RARB* gene affect the development of salivary glands by inhibiting RA signaling and are associated with the development of dysgeusia by altering salivary secretion. However, these SNPs are located in the intron of the *RARB* gene, and it is unknown how this affects *RARB*. Regulatory motif changes in rs73049478 and rs41396146 SNPs or SNPs in high LD with rs73049478, as shown in the HaploReg database, could be involved in the regulation of *RARB* expression. Further studies are required to understand this phenomenon.

The abilities of rs73049478 and rs41396146 to predict the development of dysgeusia were evaluated using 23 validation samples, and the lead *RARB* SNP (rs73049478) yielded a relatively high accuracy rate (87.0%). Yoo *et al.* have reported that machine learning applied to clinical questionnaires and demographic, environmental, neuropsychological, genetic, and neuroimaging functions predicted sleep-related side effects after methylphenidate administration in young patients with attention-deficit/hyperactivity disorder (ADHD).¹³ The study included ADHD patients—83 subjects as a training dataset and 36 subjects as a testing dataset. The J48 algorithm predicted 86.1% (sensitivity 0.87; specificity 0.86; area under curve (AUC) 0.92) of the sleep side effect based on demographics (age, sex, intelligence quotient, height/weight), clinical information, and neuropsychological (continuous performance test, Stroop color-word test), genetic/environmental (*DATI*, *DRD4*, *ADRA2A*, and *SLC6A2* gene polymorphisms/lead, cotinine), and neuroimaging measures. In addition, Reinbolt *et al.* have

reported a cluster of 70 SNPs in aromatase inhibitor-treated patients with stage I–III breast cancer, where 695277 SNP genotyping of asymptomatic patients ($n = 39$) and those with clinically significant aromatase inhibitor-related arthralgia (AIA) resulting in aromatase inhibitor termination or therapy switch ($n = 123$) was performed.¹⁴ This SNP group predicted AIA occurrence with a maximum accuracy of 75.93%. In these studies, drug-induced side effects have been predicted by machine learning using information including gene polymorphisms with an accuracy rate in the range of 70 to 80%. Therefore, the accuracy rate of 87.0% in the current study was high, as were the positive and negative predictive values (93.8 and 71.4%, respectively). Positive and negative predictive values are important for understanding the specificity of a prediction. These are the percentage of people who actually develop side effects following prediction and the percentage of people who do not actually develop side effects although predicted, respectively.

This study had several limitations. In this study, many types of anti-cancer drugs were used, including antimetabolites and microtubule polymerization inhibitors. It will be a better approach to focus on one type of anti-cancer drug for analysis. In addition, there are electrogustometry, liquid tastant, and filter paper disc/strip methods that can be used to identify the onset of dysgeusia. However, even though such tests are useful for understanding the physiology of taste disorders, each method also has some limitations. For example, we previously determined that rates of dysgeusia measured using the paper-disc method were similar to those measured using self-reporting (unpublished). Therefore, in the present study, the onset of taste disorders was identified through face-to-face interviews. Previous studies have reported that the subjective evaluation of taste disorders subjectively using chemotherapy yields prevalence rates of 20 to 70%.¹⁵ In the present study, the taste disorder prevalence rate was 65%. The specific incidence of dysgeusia may depend on type of cancer and type of anti-cancer drugs used, as well as on assessment method. Therefore, internationally approved methodologies are needed to reduce artificial differences in incidence rates. In addition, the number of samples used in the present study was small, and rs73049478 of the validation control samples did not reach Hardy–Weinberg equilibrium. In future studies, biomarkers with a higher accuracy rate may be obtained by performing an analysis that unifies the number of samples and the types of anti-cancer drugs.

In conclusion, the *RARB* gene was identified by GWAS to be associated with the development of dysgeusia due to anti-cancer drug treatment. The discovery of preventive and therapeutic agents for taste disorders that target RARBs is expected. In addition, the lead *RARB* SNP (rs73049478) could be used to predict the occurrence of dysgeusia with an accuracy rate of 87.0%. We show that the onset of dysgeusia following the administration of anti-cancer drugs can be predicted using genetic information.

Acknowledgments We thank Prof. Aiko Yamauchi from Tokushima University for her useful suggestions and discussions.

Conflict of Interest The authors declare no conflict of interest.

Supplementary Materials The online version of this article contains supplementary materials.

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