

# A Genome-wide Association Study on Confection Consumption in a Japanese Population- The Japan Multi-Institutional Collaborative Cohort study.

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**A Genome-wide Association Study on Confection Consumption in a Japanese Population- The Japan Multi-Institutional Collaborative Cohort study**

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**Keywords:** genome-wide association study, sweet food consumption, aldehyde dehydrogenase 2

(ALDH2), alcohol intake confounding

## Abstract

Differences in individual eating habits may be influenced by genetic factors, in addition to cultural, social, or environmental factors. Previous studies suggested that genetic variants within sweet taste receptor genes family were associated with sweet taste perception and the intake of sweet foods. The aim of this study was to conduct a genome-wide association study (GWAS) to find genetic variations that affect confection consumption in a Japanese population. We analyzed GWAS data on sweets consumption using 14,073 participants from the Japan Multi-Institutional Collaborative Cohort study. We used a semi-quantitative food frequency questionnaire to estimate food intake that was validated previously. Association of the imputed variants with sweets consumption was performed by linear regression analysis with adjustments for age, sex, total energy intake and principal component analysis components 1 to 3. Furthermore, the analysis was repeated adjusting for alcohol intake (g/day) in addition to the above-described variables. We found 418 single nucleotide polymorphisms (SNPs) located in 12q24 that were associated with sweets consumption. SNPs with the 10 lowest P-values were located on nine genes including at the *BRAP*, *ACAD10*, and *ALDH2* regions on 12q24.12-13. After adjustment for alcohol intake, no variant was associated with sweets intake with genome-wide significance. In conclusion, we found a significant number of SNPs located on 12q24 genes that were associated with sweets intake before adjustment for alcohol intake. However, all of them lost statistical significance after adjustment for alcohol intake.

## Introduction

Differences in individual eating habits may be influenced by genetic factors, in addition to cultural, social, or environmental factors. The sweet taste receptor gene family, including *TAS1R2* and *TAS1R3*, is located on chromosome 1 in humans [1-3]. Previous candidate gene studies suggested that genetic variants of this receptor gene family were associated with sweet taste perception and the intake of sweet foods [4-6]. Some of these studies showed the differential effects of SNPs on sucrose sensitivity and sugar intake depending on the presence or absence of being overweight [5, 6]. Other genetic variants that candidate gene studies reported to be associated with sweet consumption were *GNAT3*, (which encodes the G protein alpha subunit that plays a prominent role in bitter and sweet taste transduction as well as in umami taste transduction) [7], *GLUT2* (a glucose transporter gene) [8], and *FGF21* (a fibroblast growth factor gene) [9, 10]. All these variants suggested by candidate gene studies could not be replicated by subsequent GWAS in both samples of European ancestry and Japanese, probably because the previous candidate gene studies did not consider population stratification [11, 12]. Hwang et al., in a GWAS of the perception and intake of sweet substances, found rs11642841 within the *FTO* gene on chromosome 16 was strongly associated with the intake of total sugar in a UK Biobank sample [11].

Recently, the results of several GWAS on the dietary habits of the Japanese population have shown interesting pleiotropic effects of rs671 across dietary habits on foods and beverages and we and others found that an effective rs671 allele was inversely associated with fish consumption, and was directly associated with coffee consumption [12-15]. Kawafune, et al. found a strong direct association

between an effective rs671 allele and a five-point scale of sweet taste preference [12]. All these associations were profoundly attenuated by adjustment for alcohol drinking.

For more than a century, people in Japan have eaten two main types of confectionaries: traditional Japanese style confectionaries (*wagashi*), and European style confectionaries, including patisseries and *confiseries* (confectionaries), which arrived in Japan in the Meiji era. *Wagashi* are traditional Japanese confectioneries often served with green tea, and made of *mochi* (sticky rice cake), *anko* (*azuki* bean paste), and fruit. In the present GWAS, we took a semi-quantitative amount of *wagashi* and European style confectionaries as a more objective measure of sweets liking, rather than a self-assessed scale of sweet taste preference. The purposes of this study were (1) to replicate GWAS on sweets consumption performed in samples of European ancestry in a Japanese population, and (2) to use a more objective measure of sweets liking, rather than a self-assessed scale of sweet taste preference.

## Methods

### *Study population*

The study was conducted with participants aged from 35 to 69 years as a cross-sectional study within the Japan Multi-Institutional Collaborative Cohort (J-MICC) study, a large cohort study that was started in 2005 to investigate gene environment interactions in lifestyle-related diseases. The age range was set for the following reasons: The youngest age for inclusion was set at 35 years old, anticipating easier recruitment from health check examinees and others. Also, incidences of non-communicable



diseases, including cancer, are thought to increase after age 35. The oldest age for inclusion was set at 70 years old, anticipating an easier understanding of participants for genomic study. For this study, about 100,000 volunteers provided their blood and lifestyle data based on a questionnaire. The participants in the J-MICC study were recruited from 14 different areas throughout Japan between 2004 and 2014. Of 47,163 respondents enrolled between 2004 and 2013, 14,539 participants were randomly selected considering the distribution of study areas for the present GWAS. We used data ver. 20180112. Details of the J-MICC study were reported elsewhere [13, 14, 16]. Briefly, participants completed a questionnaire about lifestyle and medical information, and had blood sampled at the time of the baseline survey. The study participants lived in communities in four regions (Chiba, Daiko, Fukuoka, and Saga), were health check examinees in seven regions (Shizuoka-Sakuragaoka, Shizuoka, Okazaki, Iga, Takashima, Kagoshima, and KOPS), and first-visit patients at a cancer hospital in the Aichi Cancer Center region. In Kyoto and Tokushima regions, the study participants were inhabitants in communities and health check examinees as well as employees of companies or local governments. All participants in this study gave written informed consent prior to enrollment. The study protocol was approved by the ethics committees of Nagoya University Graduate School of Medicine, and other institutions participating in the J-MICC study. The present study was conducted according to the principles expressed in the World Medical Association Declaration of Helsinki.

Of 14,539 participants, 448 were excluded by the GWAS screening described below in the section on *Genotyping and quality control filtering*. Of the remaining 14,091 participants, 12 with daily

energy intake less than 500 kcal or greater than 5,000 kcal were excluded. As a result, we analyzed the data of 14,073 participants in the present study.

### *Questionnaire and measurements*

The questionnaire for the J-MICC studies included questions on medical history, height, weight, smoking and drinking habits, and dietary habits. The questionnaire was checked by experienced staff to confirm completeness and consistency. Height and weight measurements and blood sampling were conducted as part of a health checkup or for research purposes at the institutions participating in the J-MICC study [16]. Question items were collected using a scientifically validated self-administered questionnaire [17-21]. Body mass index (BMI) was calculated by dividing body weight in kilograms by the square of height in meters.

### *Dietary Assessment*

We used a semi-quantitative food frequency questionnaire (FFQ) to estimate food intake that was validated previously [17-21]. We chose 20 foods/food groups and beverages, the details of which are given in **Supplementary Information on FFQ**. Food intake frequencies were classified into eight categories (never or seldom, 1-3 times per month, 1-2 times per week, 3-4 times per week, 5-6 times per week, once a day, twice a day, and three or more times a day, which were converted to 0, 0.1, 0.2, 0.5, 0.8, 1, 2, and 3, respectively, before analysis). Confections in the questionnaire were grouped in two

categories as follows: 1. Western confections (cakes or cream puffs, etc.), 2. Japanese confections (manju [steamed sweet bean buns], etc.). For each category of confections, the frequency was multiplied by the portion size, and the total confections intake amount calculated. Unfortunately, we did not have any data on intakes of added sugar or sugar sweetened beverages. Total alcohol intake was estimated as the sum of pure alcohol intake. The frequency of alcohol intake was obtained using six categories (never or seldom, 1-3 times per month, 1-2 times per week, 3-4 times per week, 5-6 times per week, and every day). Total alcohol consumption (g/day) was estimated as the summed amount of pure alcohol consumption.

#### *Genotyping and quality control filtering*

Buffy coat fractions and DNA were prepared from blood samples and stored at  $-80^{\circ}\text{C}$  at the central J-MICC Study office. DNA was extracted from all buffy coat fractions using a BioRobot M48 Workstation (Qiagen Group, Tokyo, Japan) at the central study office. For the samples from two areas (Fukuoka and Kyushu and the Okinawa Population Study [KOPS]), DNA was extracted locally from samples of whole blood using an automatic nucleic acid isolation system (NA-3000, Kurabo, Co., Ltd, Osaka, Japan). The 14,539 study participants from the 13 areas of the J-MICC study were genotyped at the RIKEN Center for Integrative Medicine Sciences using a HumanOmniExpressExome-8v1.2 BeadChip array (Illumina Inc., San Diego, CA, USA). Twenty-six participants with inconsistent sex information between the questionnaire and the estimate from genotyping were excluded. The

identity-by-descent method in the PLINK 1.9 software [22, 23] identified 388 close relationship pairs ( $\pi\text{-hat} > 0.1875$ ) and one sample from each pair of the 388 was excluded. Principal component analysis (PCA) [24] with a 1000 Genomes reference panel (phase 3) [25] detected 34 participants whose estimated ancestries were outside the Japanese population [26]. These 34 participants were excluded. In the remaining 14,091 participants, SNPs with a genotype call rate  $< 0.98$  and/or a Hardy-Weinberg equilibrium exact test P-value  $< 1 \times 10^{-6}$ , a low minor allele frequency (MAF)  $< 0.01$ , or a departure from the allele frequency computed from the 1000 Genomes Phase 3 EAS samples were excluded. The quality control filtering resulted in 14,091 individuals and 570,162 SNPs.

#### *Genotype imputation*

Genotype imputation was performed using SHAPEIT [27] and Minimac3 software [28] based on the 1000 Genomes reference panel (phase 3) [25]. After genotype imputation, strict quality control filters were applied; namely, variants with an  $R^2 < 0.3$  were excluded, resulting in 12,617,547 variants. Finally, 4,112,564 variants with MAF  $< 0.01$  in patients were removed, resulting in 8,504,983 variants for the analysis. We used DosageConvertor software [29] to convert dosage files in VCF format from Minimac3 to PLINK formats.

#### *Power calculations to test of an association between confection intake and SNPs*

Statistical power to detect a true association was calculated by the method of DeLongchamp et al. [30], based on the number of participants and genetic data of the discovery phase J-MICC study. The required non-centrality parameter was obtained by the equation A4 listed in the Appendix A by Visscher et al. [31]. When then the number of participants is 14,073, with 14 covariates for adjustment, 0.8 for linkage disequilibrium (LD)  $R^2$ , 0.2 for MAF, 0.02 for squared standardized beta estimate, and 8,500,000 for variants analyzed, the statistical power is calculated as 0.991 according to the method proposed by Deongchamp et al. [30].

#### *Association analyses between genetic variants and confections intake*

Associations between all imputed variants and confections intake were analyzed by linear regression assuming the additive effects of the allele dosage on confections intake (g/day) adjusted for age, sex, total energy intake, and principal component analysis (PCA) components 1 to 3 using PLINK 1.9 software [22, 23]. Furthermore, the analysis was repeated adjusting for alcohol intake (ethanol g/day) in addition to the above-described variables. Variants achieving genome-wide significance ( $P < 5 \times 10^{-8}$ ) were considered to be confections intake-associated variants. An R package for creating a Q-Q plot, GWAS Tools was used [32]. For scatter plots of P-values derived from genome-wide scan results for confections intake, qqman software was used [33]. To visualize regions of interest, we used the LocusZoom program [34]. For sensitivity analyses, we performed GWAs on Western and Japanese confections intakes separately.

In addition, replication analysis adjusted for age, sex, total energy intake, and PCA components 1 to 3 using the J-MICC samples for 20 SNPs that were previously reported to be associated with sweet taste perception or confections intake [1-11] was performed.

Student's t-tests were used to compare means between men and women.

## Results

### *Baseline characteristics*

Baseline characteristics of the all, male and female participants are shown in Table 1. The mean age of the participants was  $54.8 \pm 9.4$  y and the percentage of women was 55.0%. The mean confections intake was  $198 \pm 142$  KJ/day. Total energy intake was  $7,135 \pm 1,490$  KJ/day. The means for protein, fat, carbohydrate (% of total energy), and alcohol intake (g/day) were  $12.7 \pm 2.0\%$ ,  $23.8 \pm 6.4\%$ ,  $55.8 \pm 6.2\%$ ;  $9.4 \pm 16.3$  g/day, respectively. The mean BMI level was  $23.1 \pm 3.3$  kg/m<sup>2</sup>. The mean age, total energy intake, %carbohydrate intake, alcohol intake, and BMI were significantly larger in men than in women. The mean confections intake, %protein and %fat intake were significantly smaller in men than in women.

### *Association analyses between confections intake and genetic variants*

Genome-wide analyses among the 8,504,983 variants adjusted for age, sex, total energy intake and PCA 1-3, identified 418 SNPs on chromosome 12 that were associated with confections intake with genome-wide significance ( $P < 5 \times 10^{-8}$ ). SNPs with the 10 lowest P-values that were associated with

confections intake are shown in Table 2. The quantile-quantile plot of the observed P-values is shown in Figure 1. The inflation factor of the genome-wide scan was 1.019 (95% confidence interval: 1.018-1.021), indicating that the population structure was well-adjusted. Figure 2 shows scatter plots of P-values derived from genome-wide scan results for confections intake, which found 418 SNPs at 12q24 with genome-wide significance ( $P < 5 \times 10^{-8}$ ). SNPs with the 10 lowest P-values were located on nine genes, namely the *BRAP*, *ACAD10*, *ALDH2*, *ADAM1A*, *MAPKAPK5*, *TMEM116*, *NAA25*, *TRAFD1*, and *HECTD4* regions (Supplemental Figure). After adjustment for alcohol intake (g/day), no variant was associated with confections intake with genome-wide significance ( $P < 5 \times 10^{-8}$ ). The quantile-quantile plot of the observed P-values is shown in Figure 3. The inflation factor of the genome-wide scan was 1.017 (95% confidence interval: 1.016-1.019), indicating that the population structure was well-adjusted. The results of sensitivity analyses on Western and Japanese confections intakes separately yielded similar results.

#### *Replication of previously reported SNPs*

The results of a replication study in our J-MICC GWAS data, which adjustment for age, sex, PCA 1 to 3, and total energy intake on the 20 SNPs that were previously reported to be associated with sweet taste perception or confections intake are shown in Table 3. None of the SNPs reported were statistically significant ( $P < 0.05/20 = 0.0025$ ) in our J-MICC data.

## Discussion

In the present GWAS adjusted for age, sex, total energy intake and PCA 1-3 in a Japanese population, we found 418 SNPs located on 12q24 genes; the *BRAP*, *ACAD10*, *ALDH2*, *ADAM1A*, *MAPKAPK5*, *TMEM116*, *NAA25*, *TRAFD1*, and *HECTD4* regions on 12q24.12-13 were significantly associated with confections intake before adjustment for alcohol intake. However, after adjustment for alcohol intake (g/day), no variants were associated with confections intake with genome-wide significance.

Previous studies suggested several SNPs within *TAS1R2*, *GNAT3*, *GLUT2*, *FGF21* and *FTO* were associated with the perception of confections and sugar [1-11]. *TAS1R2* (taste receptor type 1 member 2) is a protein encoded by the *TAS1R2* gene in humans. Sweet taste receptor gene family members *TAS1R2* and *TAS1R3* are G-coupled protein genes located on chromosome 1. However, from our GWAS results, no SNPs on chromosome 1, as well as others on chromosomes 3, 4, 7, 16, and 19 were significantly associated with confections intake in replication analysis. Regarding other genes found to be apparently associated with confections intake, analysis with adjustment for alcohol intake in addition indicated they could not be causal genes; descriptions are shown in **Supplementary**

### Information on Genes.

In our GWAS results, rs671 was strongly associated with confections intake, with the lowest P-value before adjustment for alcohol intake. Rs 671 is a missense mutation in the aldehyde dehydrogenase 2 (*ALDH2*) gene. *ALDH2* is an important enzyme in alcohol metabolism that catalyzes the reaction of acetaldehyde oxidization to acetic acid. In Asian populations, an rs671 mutation replaces



Glu at codon position 504 with Lys.[35] This mutation reduces the activity of ALDH2 [36-39]. In previous studies, ALDH2 was associated with ischemic stroke [40], chronic kidney diseases [41], blood pressure [42], primary biliary cirrhosis [43], esophageal cancer [37, 39], and hip fracture [44].

In the present study, most SNPs on chromosome 12 found to be associated with confections consumption were in strong LD with the *ALDH2* gene. After adjustment for alcohol intake (g/day), however, no variants were associated with confections intake with genome-wide significance ( $P < 5 \times 10^{-8}$ ). The fact that the associations between SNPs on chromosome 12 and confections intake were strongly confounded by alcohol drinking status implies the importance of gene-environmental factor interactions. The rs671 mutation directly affects the amount of alcohol consumed. Although previous studies reported genetic variants associated with sweet taste perception and the intake of sweet foods, the present study suggested that in Japanese alcohol drinking has a much larger influence on eating habits related to sweet foods than the taste receptor. Kawafune, et al. found a strong direct association between the effective allele of rs671 and a five-point scale of sweet taste preference. The relation was attenuated by adjusting for alcohol consumption. However, in that study, the association between rs671 and sweet taste preference was not significant among participants who hardly drank alcohol at all. If rs671 is the true causal SNP for sweet taste preference, the relationship should have been seen in non-drinkers. Interestingly, interaction between alcohol drinking and foods intake was noted in a European population. According to the study in a French population by Kesse, et al. increasing alcohol consumption was associated with a higher total energy intake, a higher percentage of energy intake as

protein and lipids. Conversely, energy provided by carbohydrates and intake of fruits decreased with increasing alcohol consumption [45].

*BRAP-ALDH2* loci, including rs671, were found to be associated with sweet foods consumption before adjustment for alcohol intake in the present study, and it has been reported that this region is among several identified loci with strong, and very recent (around the past 2000-3000 years), evolutionary selection signatures in Japanese people in a recent whole-genome sequencing study by Okada et al. [46]. Using high-depth whole genome sequence data of 2,234 Japanese individuals, they calculated singleton density score (SDS), which can detect the signatures of very recent natural selection in a single population. *BRAP-ALDH2* loci was the one of four loci in which genome-wide SDS P-values demonstrated significant natural selection pressure that satisfied the genome-wide significance threshold.

This study has several limitations. We did not perform a replication study in a different Japanese population because the present results were negative from a GWAS point of view. A replication study in a European population, however, would probably yield quite different results from those we found in the present study, since *ALDH2* polymorphism is restricted in Eastern Asian populations. Second, although we used a semi-quantitative FFQ to estimate food intake as reported previously [17-21], the number of sweet foods included in the FFQ was small, and data on the intakes of added sugar or sugar sweetened beverages, as well as sweetness intensity elicited by the "sweet foods" were not included, thus some difference from actual sweet foods intake is inevitable. Although

fruit intake may be of interest, a separate GWAS should be performed reflecting a number of cohort studies on the associations between fruit or vegetable consumption and outcomes, such as cancer or cardiovascular disease [47, 48]. Third, in the present study, confections contributed to 2.8% of the total calory intake. Although this contribution appears small, we know that previous observational studies of several foods and beverages that had small contribution to total calorie intake, such as sugar, fruit and vegetable, reported significant impact on outcomes [47-49]. Thus, we the significance of our findings is not small.

In conclusion, we found a significant number of SNPs located on 12q24 genes that were associated with confections intake before adjustment for alcohol intake. However, all lost significance after adjustment for alcohol intake.

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### **Conflict of Interest**

None of us have any conflict of interests.

### **Authorship**

The authors' responsibilities were as follows—TS, YaN, YK and KW: designed the research;

YaN, AS, NI, CG, KenM, AK, KatM, KT, MH, HI, MM, TT, DN, KeiM, IO, NK, EO, HM, YoN, MW,

SS, SKK, KA, KK, YM, MK, KT, YK and KW: conducted the research; TS, YaN, AN, AS and NI :

analyzed data and performed statistical analysis; TS, YaN and YD: wrote the manuscript and had

primary responsibility for final content; and all authors: read and approved the final manuscript.

**Supplementary material**

For supplementary material referred to in this article, please visit

[https://\\*\\*\\*\\*\\*](https://*****)

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**Table 1 Baseline characteristics of the study participants**

	J-MICC (all)	Men	Women	P
Number	14,073	6,329	7,744	
Women (%)	55.0			
Age (years)	54.8±9.4	55.4±9.3	54.3±9.4	<0.001
Confections intake (KJ/day)	198±142	179±162	243±177	<0.001
Total energy intake (KJ/day)	7,135±1,490	7,974±1,514	6,431±1,074	<0.001
Protein intake (% energy)	12.7±2.0	11.8±1.8	13.3±1.9	<0.001
Fat intake (% energy)	23.8± 6.4	20.5±5.4	26.6±5.9	<0.001

Carbohydrate intake (% energy)	55.8±6.2	56.8±6.7	54.9±5.6	<0.001
Alcohol intake (g/day)	9.4±16.3	17.3±20.2	2.9±7.6	<0.001
BMI (kg/m <sup>2</sup> )	23.1± 3.3	23.8±3.2	22.5±3.4	<0.001

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Values are shown as the mean ± SD, or as percentages. P values are by Student's t-tests.

BMI=Body Mass Index, J-MICC= the Japan Multi-Institutional Collaborative Cohort study

**Table 2 SNPs with the 10 lowest P-values that were associated with confections intake in discovery samples (J-MICC study, N=14,073), adjusted for age, sex, PCA.**

SNP	Chr	Position	Gene	EA	NEA	EAFR	BETA	SE	P
rs11066001	12	112119171	BRAP	C	T	0.2652	1.6672	0.1471	1.20E-29
rs11066015	12	112168009	ACAD10	A	G	0.2559	1.7044	0.1477	1.13E-30
rs4646776	12	112230019	ALDH2	C	G	0.256	1.6999	0.1476	1.43E-30
rs671	12	112241766	ALDH2	A	G	0.2563	1.7045	0.1474	<b>8.62E-31</b>
			ADAM1A/						
rs78069066	12	112337924	MAPKAPK5/	A	G	0.2604	1.6924	0.1492	1.09E-29
			TMEM116						
rs11066132	12	112468206	NAA25	T	C	0.2526	1.7512	0.1531	3.63E-30
rs116873087	12	112511913	NAA25	C	G	0.2562	1.7193	0.1524	2.12E-29

rs12231737	12	112574616	TRAFD1	T	C	0.2619	1.6963	0.1509	3.46E-29
rs144504271	12	112627350	HECTD4	A	G	0.2581	1.6797	0.1503	6.86E-29
rs2074356	12	112645401	HECTD4	A	G	0.2325	1.6933	0.1524	1.43E-28

Genome-wide analyses among the 8,504,983 variants adjusted for age, sex, total energy intake, and PCA 1-3, identified 418 SNPs on chromosome 12 that were associated with confections intake with genome-wide significance ( $P < 5 \times 10^{-8}$ ).

SNP=single nucleotide polymorphism, Chr=chromosome; Chromosomal position (GRCh37/hg19), EA=effect allele, NEA=non-effect allele, EA FR=effect allele frequency; Beta=effect size; SE=standard error of effect size, J-MICC= the Japan Multi-Institutional Collaborative Cohort study,



**Table 3 Replication analysis using the J-MICC samples for SNPs that were associated with confections intake in previous studies**

Gene	SNP	Chr:Posotion	EA	NEA	EAFR	BETA	SE	P
<i>TASIR2</i>	rs12033832	1:19166294	A	G	0.473	0.330	0.130	0.011
	rs3935570	1:19167371	T	G	0.066	-0.042	0.261	0.871
	rs35874116	1:19181393	C	T	0.113	-0.102	0.203	0.616
	rs121377303	NA						
	rs75346183	4:162614852	A	G	0.208	0.211	0.160	0.187
	rs97017963	NA						
<i>TASIR3</i>	rs307355	1:1265154	C	T	0.806	-0.117	0.161	0.467
	rs35744813	1:1265460	C	T	0.805	-0.120	0.161	0.457
<i>GNAT3</i>	rs7792845	7:80151369	C	T	0.785	0.228	0.163	0.163
	rs940541	7:80150594	G	A	0.828	0.076	0.170	0.653

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	rs1107660	7:80150131	G	T	0.821	0.061	0.167	0.713
	rs1107657	7:80150018	C	T	0.821	0.061	0.167	0.713
	rs1524600	7:80138303	A	G	0.114	0.006	0.202	0.975
	rs6467217	7:80138178	C	T	0.114	0.006	0.201	0.976
	rs6970109	7:80138074	A	C	0.114	0.006	0.201	0.976
	rs6975345	7:80123999	C	T	0.111	-0.047	0.203	0.817
	rs10242727	7:80119730	G	A	0.111	-0.042	0.204	0.837
	rs6467192	7:80107798	A	G	0.111	-0.037	0.204	0.856
	rs6961082	7:80100969	A	C	0.099	-0.001	0.214	0.995
<i>GLUT2</i>	rs5400	3:170732300	A	G	0.020	-0.712	0.455	0.118
<i>FGF21</i>	rs838133	19:49259529	G	A	0.973	-0.138	0.676	0.839
<i>FTO</i>	rs11642841	16:53845487	A	C	0.098	-0.016	0.217	0.940

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Accepted manuscript

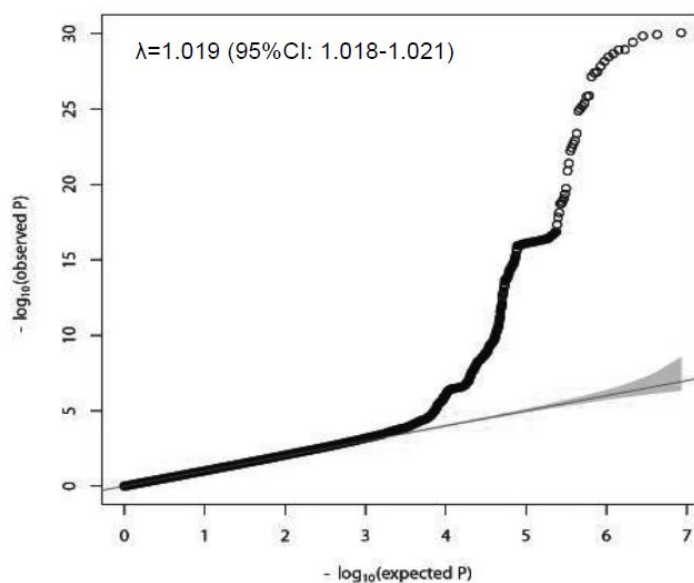
We carried out a replication study on the 20 identified loci (including sweet taste receptor gene family) associated with sweets intake.

SNP=single nucleotide polymorphism, Chr=chromosome, Position=chromosomal position (GRCh37/hg19), EA=effect allele, NEA=non-effect allele,

EAF=effect allele frequency, Beta=effect size, SE=standard error of effect size

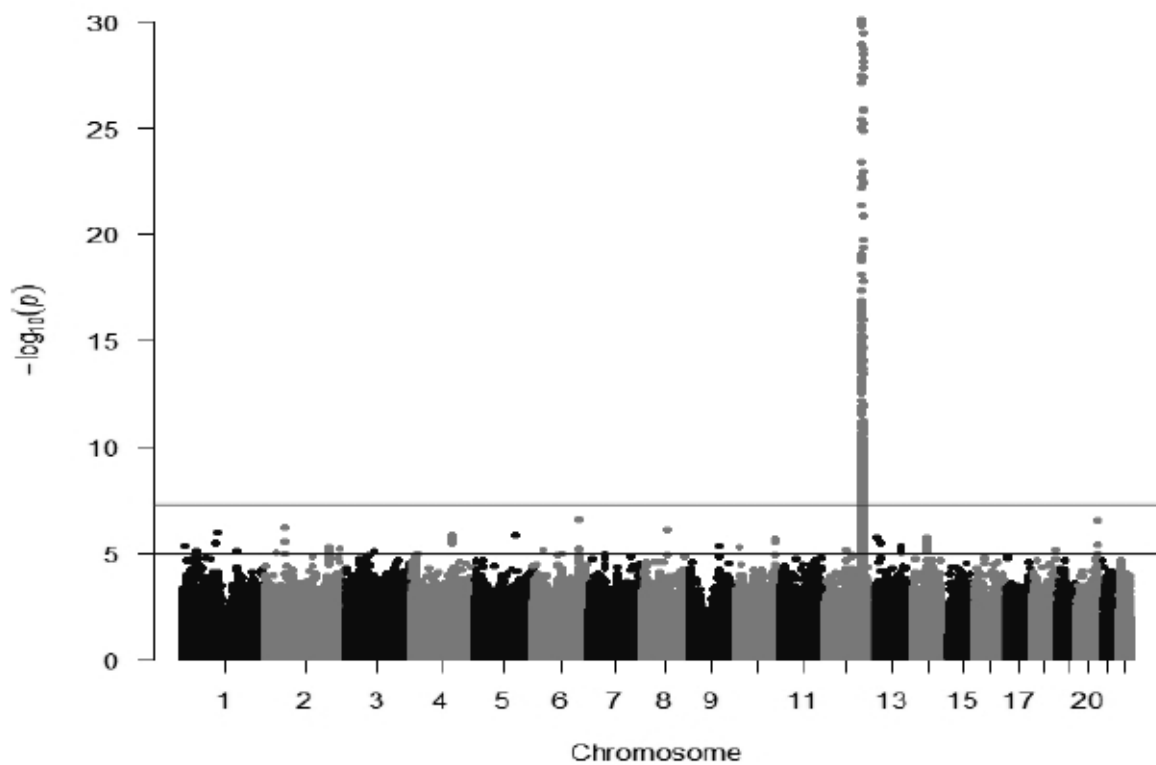
**Figure legends****Figure 1.**

A quantile-quantile plot (black) of genome-wide association tests. The  $x$ -axis indicates the expected  $-\log_{10} P$ -values under the null hypothesis. The  $y$ -axis shows the observed  $-\log_{10} P$ -values calculated by a linear regression model using PLINK [23]. The line represents  $y=x$ , which corresponds to the null hypothesis. The gray shaded area shows the 95% confidence interval of the null hypothesis. The inflation factor ( $\lambda$ ) is the median of the observed test statistics divided by the median of the expected test statistics. An R package for creating the Q-Q plot, GWAS Tools, was used [32].



**Figure 2.**

Genome-wide association signals. The  $x$ -axis represents chromosomal positions and the  $y$ -axis represents  $-\log_{10}P$ -values calculated by a linear model association analysis. The software, qqman, was used [33].



**Figure 3.**

A quantile-quantile plot (black) of genome-wide association tests. The  $x$ -axis indicates the expected  $-\log_{10} P$ -values under the null hypothesis. The  $y$ -axis shows the observed  $-\log_{10} P$ -values calculated by a linear regression model using PLINK [23]. The line represents  $y=x$ , which corresponds to the null hypothesis. The gray shaded area shows the 95% confidence interval of the null hypothesis. The inflation factor ( $\lambda$ ) is the median of the observed test statistics divided by the median of the expected test statistics. An R package for creating the Q-Q plot, GWAS Tools, was used [32].

