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Individual differences in taste and their association with genes, dietary behaviour, and brain structure

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Abstract

Taste perception plays a key role influencing human dietary behaviour and further health consequences. It has been shown that genetic and neurological factors contribute to variation in taste, but their underlying mechanisms remain largely underexplored. For example, it is unclear how much of the variance in sweet taste is due to genetics, whether the association between sweet and bitter tastes is due to genetic covariance, and whether variation in brain structure is associated with taste. The goal of this work is to extend current knowledge in individual differences in human taste perception of sweetness and bitterness by showing their relationships with genes, dietary behaviour, and brain morphology. We perform quantitative and statistical genetic analyses using an extensively phenotyped and genotyped twin sample of Australian adolescents (n = 1999), with replication and extension making use of two publically available datasets from the Human Connectome Project (HCP; n = 1101) and the UK Biobank (N = 438,870).

In **Chapter 1**, we employed structural equation modelling (variance components analysis) to provide the first evidence that approximately 30% of variation in the perceived intensity of sweet compounds, including sugars (i.e. glucose and fructose) and high-potency sweeteners (i.e. aspartame and neohesperidin dihydrochalcone) is due to genetics. Furthermore, we identified a common genetic factor accounting for more than 75% of the genetic variance in the perception for each of these sweet compounds, suggesting that the perception of both sugars and high-potency sweeteners was regulated by a common set of genes.

In **Chapter 2**, we demonstrated that a quarter of the genetic variance in perceived sweetness (i.e. a weighted mean score of the four sweet tastes from **Chapter 1**) is shared with at least half of the genetic variance in the perceived bitterness of quinine, sucrose octaacetate (SOA), and caffeine. The genetic association between sweetness and the bitterness of propylthiouracil (PROP) becomes evident after adjusting for the *TAS2R38* genotype. These results reveal shared genetic pathways for the human perception of sweetness and bitterness.

To pinpoint the source of genetic variation in bitter taste, in **Chapter 3**, we performed a genome-wide association analysis (GWAS). As previous work was underpowered to detect variants with small effects, we used a bivariate approach to boost power. We identified two putative novel variants with small effects (< 2%) on

chromosomes 7 and 12 for the perceived intensity of denatonium benzoate (DB) and SOA, respectively. We provided the first independent replication for the caffeine bitterness on chromosome 12 and confirmed the previously identified variants on chromosomes 7 and 12 for PROP and quinine, respectively. Building on the common source of genetic variances identified in **Chapter 2**, we showed evidence for pleiotropy that each of the three variants (for quinine, caffeine, and SOA) on chromosome 12 is associated with more than one of the bitter tastes (quinine, SOA, caffeine, and DB). These findings offer a useful starting point for determining the biological pathways linking perception of bitter substances.

We investigated the effect of taste perception on diet-related outcomes. Although previous findings of the association between bitter taste perception and bitter beverage intake were inconsistent, we used two-sample Mendelian randomization (**Chapter 4**) to demonstrate a causal relationship between the perceived bitterness of PROP, quinine, and caffeine and the consumption of coffee, tea, and alcohol among UK Biobank participants. In **Chapter 5**, our longitudinal analyses showed that the perceived sweetness in adolescence is a predictor for body mass index (BMI) in early adulthood. We also showed that this association is partly due to genetics using structural equation modelling and polygenic risk prediction approaches.

In **Chapter 6**, we conducted an exploratory analysis to examine the associations between the volumes of 84 brain regions of interest (ROI) and the perceived sweetness and bitterness (PROP, quinine, caffeine). The volumes of 5 ROIs (right cuneus gyrus, right inferior temporal gyrus, left transverse temporal gyrus, and left and right caudates) were nominally associated with both sweet and bitter tastes. Additionally, we replicated an association between quinine bitterness and the volume of left entorhinal gyrus using data from HCP. This study provides the first evidence for an association between brain morphology and taste intensity ratings.

In conclusion, we used structural equation modelling to show that sweet taste is heritable and the association between sweet and bitter tastes is largely due to genetic covariance. Additionally, bivariate GWAS identified variants with small effects on bitter tastes and revealed their pleiotropy. Furthermore, results from Mendelian randomization and longitudinal analyses evidence the potential causal impact of taste on dietary behaviour and BMI. These findings enhance our understanding in the genetic architecture of taste and shed light on the personalized

nutrition and medicine. Lastly, we showed that the volume of specific brain regions is associated with taste perception, which provides new insights into the gustatory network and suggests a potential role of brain structure in taste.

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Chapter 3: Paul Breslin, Danielle Reed, Nicholas Martin, and Margaret Wright conceived and designed the study. Scott Gordon provided the imputed genetic data. Puya Gharahkani and Zhu Gu assisted in statistical analyses. Danielle Reed, Paul Breslin, and Margaret Wright wrote part of the manuscript. Puya Gharahkani, Zhu Gu, Danielle Reed, Paul Breslin, Margaret Wright, and Nicholas Martin provided critical feedback on the manuscript.

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List of Abbreviations

AIC: Akaike's Information Criterion

BMI: Body Mass Index

CI: Confidence Interval

CNS: Central Nervous System

DB: Denatonium Benzoate

DF: Degrees of Freedom

DZ: Dizygotic

EEG: Electroencephalogram

ENIGMA: Enhancing Neuro Imaging Genetics through Meta-Analysis

eQTL: Expression Quantitative Trait Loci

FFI: Five-Factor Inventory

fMRI: Functional Magnetic Resonance Imaging

GIANT: Genetic Investigation of Anthropometric Traits

gLMS: General Labelled Magnitude Scale

GPCR: G protein-coupled receptor

gSweet: General Sweet factor score

GTEx: Genotype-Tissue Expression project

GWAS: Genome-Wide Association Studies

h^2: Heritability

HCP: Human Connectome Project

LD: Linkage Disequilibrium

LL: Log-Likelihood

MAF: Minor Allele Frequency

mQTL: Methylation Quantitative Trait Loci

MR: Mendelian Randomization

MRI: Magnetic Resonance Imaging

MSG: Monosodium Glutamate

MZ: Monozygotic

NHDC: Neohesperidine Dihydrochalcone

PC: Principal Component

PET: Positron emission tomography

PGRS: Polygenic Risk Scores

PROP: 6-n-propylthiouracil

PTC: Phenylthiocarbamide

QTIM: Queensland Twin Imaging Study

$\mathbf{r_e}$: Environmental Correlation

$\mathbf{r_g}$: Genetic Correlation

ROI: Region of Interest

$\mathbf{r_p}$: Phenotypic Correlation

SD: Standard Deviation

SNP: Single Nucleotide Polymorphism

SOA: Sucrose Octaacetate

UKB: UK Biobank

Introduction

Taste perception has a significant impact on our daily lives. It contributes to our enjoyment by stimulating a desire to eat [1], and hence plays an essential role in nutrition and food selection [2]. Taste is also a natural defence that prevents food poisoning by warning against spoiled foods and potential toxic compounds [3]. However, not everyone has the same ability to taste [4] and such differences have various impacts on dietary behaviour and health consequences. For example, people with lower taste sensitivity are more susceptible to certain diseases, including obesity [5], diabetes [6], and hypertension [7], presumably because they need to consume more salt, sugar, or fat to achieve the same level of taste sensation and satisfaction. In contrast, people with a stronger taste sensitivity can find certain vegetables, such as cabbage and Brussels sprouts, more bitter and less preferable [8-10], and this in turn decreases their vegetable intake [11, 12].

There have been many studies on individual differences in taste from various perspectives, including age, gender, and cultural background. Taste perception changes overtime as we age. In general, taste sensitivity peaks at late childhood [13], and then it declines gradually and the slope becomes flatter when reaching late adulthood [14]. Taste perception also differs by sex. For example, girls are more sensitive to sweetness, bitterness, sourness and saltiness than boys [13, 15] because females enter puberty at earlier ages and a more developed hormonal system better shapes their sensory network [16]. Women are more sensitive to certain bitter compounds than men [17] because women have denser taste buds and papillae on the tongue [18], which directly reflect on the ability to taste [19]. In addition, taste perception and preference shift dramatically during pregnancy due to changes in the production of sex hormone [20]. Whereas how we taste can influence what we eat, a reverse relationship has also been observed. Low-sugar diet for 2-3 months increases the perceived sweetness of the same food [21], and low-salt diet also increases the perceived intensity of salt [22]. Different ethnicities and cultural backgrounds also contribute to differences in taste perception and preference, such that Taiwanese people have stronger preferences for the taste of sucrose and monosodium glutamate (MSG) than Japanese and Australian people [23], and non-Hispanic whites generally have a stronger perceived intensity than Hispanics and African-Americans [24].

Ability to taste can be largely changed by disease status and medical treatments. For example, middle ear infection (otitis media) and radiotherapy on head and neck damage the taste nerves (the chorda tympani nerve [CN VII] and the glossopharyngeal nerve [CN IX]) that convey taste signals from the tongue and oral cavity to the brain and hence modify the taste sensations [25]. Alterations in taste perception also result from brain damages [26, 27] and neurological disorders, such as Alzheimer's disease [28], Parkinson's disease [29], or multiple sclerosis [30].

Rise of taste genetics

Studies on the taste genetics arose from a lab accident in early 1930 when Arthur L. Fox accidentally spilled the powder of a chemical phenylthiocarbamide (PTC) into the air. Some lab members complained about its bitterness, but Fox himself could not taste it [31]. Follow-up studies showed that the ability to perceive PTC was a Mendelian recessive trait, with approximately one-third of individuals finding it tasteless [32, 33]. This finding spurred hundreds of studies around the world to investigate the genetics of PTC perception and how it related to dietary behaviour [34] and diseases [17]. In early 2000, association [35] and linkage [36] analyses mapped the ability to perceive PTC to a locus on chromosome 7 and showed that the haplotype of three single nucleotide polymorphisms (SNP) within a specific bitter taste receptor gene *TAS2R38* accounts for 55% to 85% of the variance, subsequently replicated by others [37, 38]. However, even with a successful story of this discovery [39], the genetic pathways and molecular mechanisms underlying human taste perception remain largely underexplored.

Twin and family studies

Genetic effects on taste have been investigated using twin and family study design, taking the advantage of the genetic relationship between family members. Sensitivity to PTC and its structurally related chemical propylthiouracil (PROP) is largely genetically predisposed (heritability [h^2] = 0.64 – 0.72) [40, 41] and the perception of other bitter compounds, such quinine, caffeine and sucrose octaacetate (SOA), are estimated to be less heritable (h^2 = 0.28 – 0.34) using the same sample [40]. As for other basic taste qualities, sourness is moderately heritable (h^2 = 0.53) [42] and saltiness is largely due to shared environmental effects (e.g. parenting effects on siblings) [42, 43]. No attempt on estimating the heritability of the umami taste (e.g. savoury or meaty taste) has been made, but preferences for meat

and fish are moderately heritable (h^2 = 0.39 – 0.44) [44, 45]. Regarding sweetness, genetic influences on preferences for the sucrose solution and sweet foods range from low [41, 44] to moderate [43] (h^2 = 0.23 – 0.40); however, the degree of genetic influences on the perceived intensity of sweet taste remains unclear [41, 43], which is likely due to lack of statistical power in these studies (n < 700) and sweet intensity ratings being a less stable phenotype compared with sweet preference [41].

In **Chapter 1**, using the largest-to-date twin sample with multiple taste phenotypes (n ~ 2000), we perform structural equation modelling (variance components analysis) to estimate the heritability of the perceived intensity of two sugars (i.e. glucose and fructose) and two high-potency sweeteners (i.e. aspartame [200 times sweeter than sucrose] and neoherperidine dyhydrochalcone [NHDC; 1500-1800 times sweeter than sucrose]), which are commonly used in sugar-free/low-calorie food and beverages nowadays. As the perception of both sweetness and bitterness replies on G protein-coupled receptors (GPCR), in **Chapter 2**, we further investigate whether the association between the perceived sweetness and bitterness is due to genetic covariances, which would provide a hint of whether genetic effects on sweet and bitter taste is at the receptor (peripheral) level.

Genome-wide association study

Genome-wide association studies (GWAS) have been a common approach for the identification of genetic variants associated with a trait [46], which helps in understanding molecular mechanisms and constructing biological pathways. However, since the discovery of the locus within *TAS2R38* for PROP perception, only two successful GWAS on taste (i.e. the identification of variants within the cluster of bitter taste receptor genes on chromosome 12 for quinine bitterness [37] and caffeine bitterness [38]) have been published.

As taste perception, except for PROP bitterness, can be highly polygenic (i.e. the total genetic variance is attributed to multiple independent variants with small effects rather than one variant with a large effect), it requires a large sample with great power to detect associated variants. In **Chapter 3**, we perform a GWAS on the perception of 5 bitter substances (PROP, quinine, SOA, caffeine, and denatonium benzoate [DB]) using the same cohort for the discovery of quinine locus [37] with a 40% increase in sample size. With the exclusion of PROP, these bitter taste phenotypes are highly correlated at both the phenotypic and genetic level (**Chapter**

2). This enables us to use a series of bivariate analyses to further boost power [47] and reveal previously unidentified variants with small effects (< 5%).

Influence of taste perception on diet and health

Taste perception is believed have an impact on health because of its influence on food preference [2], which affects what we eat and further reflects on health [48, 49]. However, the relationship among taste perception, preference, dietary behaviour, and health is complex and inconclusive. For example, findings of the association between perceived bitterness and the intake of bitter beverages, such as coffee, tea, and alcohol [50-57], are limited and inconsistent. Similarly, whether perceived sweetness is associated with BMI has not reach an agreement across studies [5, 13, 58-60]. Possible reasons include that the effect of taste is accumulative so it takes time to reflect on dietary behaviour and health, and hence a relationship cannot be seen in cross-sectional studies. Moreover, these investigations were likely limited by small sample sizes. To overcome these obstacles, in **Chapter 4**, we use Mendelian randomization to assess the causal association between the perceived bitterness of PROP, quinine, and caffeine and the consumption of coffee, tea, and alcohol in the large UK Biobank cohort (N = 438,870). In **Chapter 5**, we conduct a longitudinal analysis to investigate the relationship between perceived sweetness and BMI measured at the same time and BMI measure 9 years later. We further examine their genetic associations using variance components analysis and polygenic risk prediction, which could reveal underlying molecular mechanisms.

Gustatory areas in the brain

Studies on peripheral receptors have been a focus of taste research, but the brain also plays a significant role. When we eat, food chemicals are detected by taste receptors in the oral cavity and signals are sent via gustatory nerves to the brain where taste sensation is generated so we know what we eat and whether we like it or not [61]. Positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) studies have identified areas in the human brain that both respond to taste and are homologous to those found in other primates [62]. However, activation patterns of these taste-responsive regions have been inconsistent across studies [63, 64], and such variation may be due to different taste stimuli used or

tasks performed, as the brain response can be valence-specific [65] or intensity-specific [66], or, more importantly, small sample sizes (n < 100).

Structural variations of specific brain regions are related to human senses, such as olfaction [67] and vision [68], and recent evidence suggests that volumetric differences also associate with taste perception [69, 70]. People with eating disorders, e.g. anorexia nervosa, had larger left gyrus rectus grey matter, and there was an association between its size and the sucrose pleasantness among both patients and healthy individuals [69, 70]. Furthermore, structural alterations (e.g. removing parts of the brain) can modify perceived intensity of taste [71]. In **Chapter 6**, we conduct an exploratory analysis to examine the association between the volume of brain regions and the perceived intensities of sweet and bitter tastes. The findings would provide a clue to whether structural variation of specific brain regions modifies taste and help delineate the human gustatory circuit.

Datasets and statistical analyses

This work employed a dataset of approximately 2000 Australian adolescent twins and siblings with taste phenotypes and genotypes. Participants were recruited as a part of the Brisbane Adolescent Twin Study (BATS) [72], also known as the Brisbane Longitudinal Twin Study (BLTS). Taste tests were conducted between 2003 and 2014, in which participants tasted 4 sweet (glucose, fructose, aspartame, and NHDC) and 5 (PROP, quinine, caffeine, SOA, and DB) bitter substances and rated their perceived intensities on a general Labelled Magnitude Scale (gLMS) [73]. Approximately a third of the sample later participated in the Queensland Twin IMaging (QTIM) study and were MRI scanned.

The twin characteristics (monozygotic [MZ] twins shared 100% of their genetic profiles and dizygotic [DZ] twins on average shared 50%) allow the use of structural equation modelling (variance components modelling [74]). This method partitions the total phenotypic variance of a trait or the phenotypic covariance between traits into genetic (i.e. heritability of a trait or genetic covariance between traits) and environmental components (**Chapters 1, 2, and 5**).

Genotype data allow the use of GWAS to identify genetic variants associated with individual differences in taste perception (**Chapter 3**). We use the software tool GEMMA [75, 76] for GWAS because it controls genetic relationship between

individuals and runs, in addition to the conventional univariate analysis, multivariate analyses to boost statistical power.

Genetic variants identified in GWAS are used as instrumental variables (i.e. genetic proxies of associated phenotypes) in Mendelian randomization [77] to assess causal relationships between taste perception and beverage intake (**Chapter 4**). The beverage intake data on coffee, tea, and alcohol were obtained from a large genetically informative cohort comprising approximately 500,000 volunteers from the United Kingdom (UK Biobank) [78].

We take the publically available GWAS summary of BMI [79] to calculate polygenic risk score of BMI in our twin sample to examine its genetic relationship with taste (**Chapter 5**). This approach borrows the power from the large cohort of the GWAS on BMI (N = 322,154) and the outcomes can compensate the results from variance component modelling.

We conduct mixed-effect linear analyses to examine the association between taste and brain phenotypes (**Chapter 6**). This method controls individual relatedness between family members and is used as an alternative of twin modelling (structural equation modelling). For replication, we use data from the Human Connectome Project (HCP), which includes 1101 participants with taste data on quinine perception and brain MRI images [80].

1

A Common Genetic Influence on Human Intensity Ratings of Sugars and High-Potency Sweeteners

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Chapter 1. A common genetic influence on human intensity ratings of sugars and high-potency sweeteners

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Abstract

The perception of sweetness varies among individuals but the sources of this variation are not fully understood. Here, in a sample of 1901 adolescent and young adults (53.8% female; 243 MZ and 452 DZ twin pairs, 511 unpaired individuals; mean age 16.2 ± 2.8, range 12 – 26 years), we studied the variation in the perception of sweetness intensity of two monosaccharides and two high-potency sweeteners: glucose, fructose, neohesperidine dihydrochalcone, and aspartame. Perceived intensity for all sweeteners decreased with age (2% – 5% per year) and increased with the history of otitis media (6% – 9%). Males rated aspartame slightly stronger than females (7%). We found similar heritabilities for sugars (glucose: h^2 = 0.31, fructose: h^2 = 0.34) and high-potency sweeteners (neoherperidine dyhydrochalcone: h^2 = 0.31, aspartame: h^2 = 0.30); all were in the modest range. Multivariate modelling showed that a common genetic factor accounted for > 75% of the genetic variance in the four sweeteners, suggesting that individual differences in perceived sweet intensity, which are partly due to genetic factors, may be attributed to a single set of genes. This study provided evidence of the shared genetic pathways between the perception of sugars and high-potency sweeteners.

Introduction

The perception of sweet taste varies among individuals [4]. Discovery of genetic variants in the sweet taste genes *TAS1R3* and *GNAT3* and their relationship with sucrose perception establish a role of inborn variation on sweet taste [82-86]. Yet, a firm understanding of the molecular-genetic basis of human sweet perception remains undetermined.

New data from model organisms (i.e. mice) indicate there may be a second system to sense sweetness [87], in addition to T1R3 and gustducin (protein products of *TAS1R3* and *GNAT3*). This second system is sensitive to caloric sugars but not high-potency sweeteners [87]. In humans, genetic studies of sucrose perception, including perceived intensity, pleasantness, and preferred concentration, have suggested a heritability ranging from 0.14 to 0.55 [41, 82, 88-90]. However, there are no heritability estimates for high-potency sweeteners in humans and the degree to which sugars and high-potency sweeteners share molecular mechanisms of sweet taste transduction is not fully understood.

Here, in a large adolescent and young adult twin sample (695 complete twin pairs), we estimated the heritability of perceived intensity for four sweeteners. These included two commonly occurring natural saccharides, glucose and fructose (found in fruits and vegetables), and two high-potency sweeteners, aspartame and neohesperidine dihydrochalcone (NHDC). Using multivariate genetic modelling we examined the extent that any association between the four sweeteners was due to common environmental or genetic factors. Further, this modelling allowed us to investigate whether any of the genetic variance in the high-potency sweeteners could be attributed to a distinct set of genetic factors, separate to that for natural sugars.

Materials and Methods

Participants

Participants were adolescent and young adult twins and their singleton siblings from the Brisbane Adolescent twin study [72] who have participated in previous studies of the genetics of melanoma risk factors [91, 92], and cognition [93, 94]. Taste data reported here were collected between August 2002 and July 2014. The sample comprised 1175 females and 1013 males (mean age 16.2 ± 2.8 years, range 12 to 26 years) from 1052 families, including 316 MZ and 586 DZ complete twin pairs, and 384 singletons (non-twin siblings and unpaired twins) (Table 1-1). This includes all participants from a previous study of bitter perception [40], with a 150% increase in sample size. Zygosity for 92% of the same-sex twins was determined from genotyping (Illumina 610K SNP array) and the remainder by self-report confirmed by study nurses. Participants (or their parents if under 18 years of age) gave written consent to participate in the study which was approved by the Queensland Institute of Medical Research Human Research Ethics Committee.

Table 1-1. Number of families before and after data screening.

Family Type	Initial	After screening [a]
MZ twin pairs	234	189
MZ twin pairs + sibling(s) [b]	82	54
DZ twin pairs	491	380
DZ twin pairs + sibling(s) [b]	95	72
Non-twin singletons/unpaired twins	150	320 [c]

[a] Participants were excluded if they scored water as moderate or higher taste (> 20mm on LMS), had large differences between presentation one and two and had overly high or low total average scores [40].
[b] Families with a twin pair and one or two siblings.
[c] The number of non-twin singletons/unpaired twins increases after cleaning as some twin pair families lose one twin during the screening procedure.

Taste Test

As described previously, the taste test included both bitter and sweet stimuli [40]. Briefly, it included duplicate presentations of 10 different solutions, of which five were bitter (propylthiouracil (PROP), sucrose octaacetate (SOA), quinine HCl, caffeine, and denatonium benzoate), four were sweet (described below) and one was neutral (i.e. water). The four sweet solutions included the two natural sugars, 0.60 M glucose, 0.30 M fructose, and two high-potency sweeteners, 8.0×10^{-5} M neohesperidine dihydrochalcone (NHDC) and 1.4×10^{-3} M aspartame. Each solution and the water control were presented twice (i.e. a total of 20 solutions) in colour coded 2mL polypropylene microcentrifuge tubes with flip tops. The first ten tubes contained one presentation of each compound plus the water control and the next ten contained the same solutions in a different order, but the order of all twenty tubes was the same for all participants (SOA, water, caffeine, glucose, quinine HCl, fructose, NHDC, PROP, aspartame, denatonium benzoate/ fructose, glucose, PROP, aspartame, quinine HCl, NHDC, caffeine, water, SOA, and denatonium benzoate). Participants were instructed to: 1) open the tube, swish the solution around in the mouth for five seconds and spit out, 2) rate the perceived intensity of the solution, 3) rate the quality of the taste, 4) rinse the mouth out four times with tap water and, 5) repeat steps 1 to 4 for each tube. Perceived intensity was rated on a general labelled magnitude scale (LMS) [73] with labels of no sensation (0mm), barely detectable (2mm), weak (7mm), moderate (20mm), strong (40mm), very strong (61mm) and strongest imaginable (114mm). This scale minimises ceiling effects and provides a continuous measure that is desirable for quantitative analysis.

In addition, participants and/or a parent answered questions relating to a previous head injury and otitis media (middle ear infection), which are two common

factors relating to the change in taste perception as they damage the signal transduction from the mouth to the brain [60, 95, 96]. History of head injury was coded as: 1) Never suffered from a head injury; 2) Yes, but not serious; 3) Yes, had either a concussion or loss of consciousness; 4) Yes, both concussion and loss of consciousness. History of otitis media was coded as: 0) Never suffered from middle ear infection; 1) Yes, had suffered from middle ear infection. 27% and 46% of participants had suffered from head injury and otitis media respectively. The taste test took 30 – 45 minutes, while total testing time for all components of the study was approximately two and a half hours.

Before March 2004, the taste test was self-administered as part of a mail and phone study. Test tubes were embedded in an inch-wide polyethylene sheet, rolled up into a padded post bag, and mailed to participants via regular post. The mail pack included both detailed written instructions and a summary sheet of the key points that participants could refer to while completing the test. Participants were instructed not to complete the taste test if suffering from a cold or flu until they had completely recovered, and not to eat or smoke, and drink only water for at least one hour before the test. Approximately 40% of the data were collected via mail and the rest were collected during participants' visits at QIMR Berghofer Medical Research Institute.

Data Screening

Prior to analysis, the data were screened for outliers [40]. Briefly, participants were removed if they 1) rated water stronger than moderate (mean score of first and second presentation > 20mm), 2) had a low (< 200mm) or high (> 1800mm) total score across all 18 taste stimuli or 3) if there was a large discrepancy (> 80mm) between ratings for the first and second presentations. This excluded 13% of the sample, with the final sample comprising 243 MZ and 452 DZ twin pairs and their siblings, and 320 families of unpaired individuals (mean age of 16.2 ± 2.8 years; 1023 females, 878 males) (Table 1-1).

Statistical Analyses

Since intensity scores for all sweeteners were positively skewed, a square root transformation was performed to approximate normal distributions. To establish regularity in sampling and measurement, and to check assumptions of the twin design, homogeneity of means and variances for birth order and zygosity were tested using the statistical package Mx which utilises maximum likelihood (ML)

estimation procedures [74]. Outlying families were detected by using the %p option in Mx that uses the Mahalanobis distance to identify families having excessive similarities or differences relative to other families in the sample and model expectation. As removal of outlying families (1 to 3 families for fructose, NHDC, and aspartame) did not change any of the estimates, these families were included in all analyses. Covariates (sex, age, history of otitis media and head injury) were tested for significance in fixed effects mean models (regressions and deviations from the mean). Models were assessed by comparing double the negative log-likelihood between nested models, as this difference is distributed asymptotically as a χ^2, which is used to decide whether a model is a significant worse fit than its predecessor.

Variance components modelling partitions the variation of a trait into genetic and environmental sources by taking advantage of the differences in genetic relatedness between MZ (share all genes) and DZ (share half of genes) pairs. These known differences allow the estimation of additive genetic (A), non-additive genetic (D), common environment (C) and unique environment (E, includes experimental error) parameters in a variance components model. Because twins in this sample were reared together, the C and D parameters are negatively confounded and as such, cannot be simultaneously estimated [97]. However, if the MZ twin correlation is more than double the DZ twin correlation it is indicative that non-additive genetic influences (including dominance and epistasis) are most important, whereas if the MZ twin correlation is less than double the DZ twin correlation, common environment is likely to be more important.

As there were two measurements for each compound, their differences were tested in univariate models and the phenotypic variation was further partitioned into a test unreliability (U) component using a repeated measures model in which two presentations were treated as observations of one underlying score by constraining the two presentations to be equal. All variance components (A, C, and E) were then constrained to affect each presentation variable equally as both are imperfect measures of the true underlying phenotype. Test unreliability (U) was then estimated from the variance not contributed by A, C, and E.

As perceived taste intensities of the sweeteners were moderately correlated, we specified a multivariate model including all four compounds, utilising the additional information gained from the cross-trait correlations to estimate common

sources of variation between these traits. Three out of four MZ twin correlations were lower than double the DZ twin correlations so a Cholesky decomposition of A, C, and E factors was used as a starting point, with covariates modelled as regressions or deviation effects on the mean. Alternative models of independent pathways and common pathways were also assessed to examine whether there were specific genetic components for all four variables. The best model fit was determined by Akaike's Information Criterion (AIC) that penalizes models for increasing complexity and can be used with non-nested models [98].

Results

The mean perceived intensities of all four sweeteners were between moderate and strong (Table 1-2). There is 5% to 15% difference between first and second presentations for each sweetener. Head injury had no significant effect on any phenotype while age, sex, and otitis media significantly ($p < 0.05$) influenced some measures (Table 1-2). Between the ages of 12 and 26, perceived intensities of all four sweeteners decreased with age (2% to 5% per year). For example, a 14-year-old participant would rate the glucose solution 6.4 higher than a 24-year-old participant. Males rated aspartame 7% more intense than females (27.6 versus 25.7, $p = 0.015$). History of otitis media had a small but consistent positive effect on all sweeteners, with participants who had suffered from middle ear infections rating sweeteners as 6% to 9% more intense than those who had never had a middle ear infection. Phenotypic correlations among the four sweeteners (0.4 – 0.64) were similar to those between duplicate presentations of the same compound (0.48 – 0.58) (Table 1-3).

Table 1-2. Sweet intensity characteristics of the twin sample

	Glucose	Fructose	NHDC [d]	Aspartame
Descriptive statistics				
Pre1 (N=1876-1888) [a]	33.6 ± 18.2	32.6 ± 20	33.2 ± 20.4	27.3 ± 18.6
Pre2 (N=1868-1873) [a]	29.2 ± 18.5	30.9 ± 19.2	35.8 ± 21.7	25.8 ± 18
Mean (N=1882-1890) [a]	31.4 ± 16	31.8 ± 17.7	34.5 ± 18.9	26.6 ± 16.4
Covariate effects				
Sex (95%CI)	0.01 (-0.12, 0.13)	0.08 (-0.06, 0.22)	0.05 (-0.09, 0.20)	**0.18 (0.05, 0.32)**
Age (95%CI)	**-0.06 (-0.08, -0.03)**	**-0.11 (-0.13, -0.08)**	**-0.11 (-0.14, -0.08)**	**-0.08 (-0.11, -0.06)**
Otitis media (95%CI)	**0.19 (0.07, 0.31)**	**0.19 (0.06, 0.33)**	**0.22 (0.09, 0.36)**	**0.15 (0.02, 0.29)**
Twin correlations				
r_{MZ} (95%CI) (238-240 pairs) [ab]	0.31 (0.19, 0.42)	0.33 (0.21, 0.44)	0.32 (0.2, 0.43)	0.27 (0.15, 0.39)
r_{DZ} (95%CI) (446-449 pairs) [ab]	0.22 (0.12, 0.3)	0.19 (0.09, 0.28)	0.12 (0.03, 0.21)	0.17 (0.08, 0.26)
Univariate AEU modelling				
A (95%CIs) [c]	25% (18, 32)	26% (19, 33)	25% (17, 32)	23% (15, 30)
E (95%CIs) [c]	25% (18, 33)	34% (26, 41)	32% (25, 40)	35% (27, 43)
U (95%CIs) [c]	50% (46, 53)	40% (37, 43)	43% (40, 46)	42% (39, 46)

Means, standard deviations, and sample sizes for the perceived intensity (millimetre on a labelled magnitude scale) of sweeteners from presentation 1 and 2 and their means. Estimates of phenotypic variation accounted by unreliability (U). Age, sex, and otitis media covariate effect estimates, and twin correlations. The regression and deviations for covariate effects were applied to the means of the square root transformed variables. Significant covariate effects are shown in bold.

[a] The number of participants (N) changes between presentations, as not all participants completed the entire test.

[b] Twin correlations calculated using means.

[c] Estimates of A (additive genetic component), E (non-shared environmental component), and U (unreliability component) add up to 100% variance for each sweetener.

[d] Neohesperidine dihydrochalcone.

Table 1-3. Phenotypic correlation coefficients among the perceived intensity of four sweeteners

		Glucose		Fructose		NHDC [a]		Aspartame	
		1	2	1	2	1	2	1	2
Glucose	1	-							
	2	**0.48**	-						
Fructose	1	0.62	0.49	-					
	2	0.51	0.63	**0.58**	-				
NHDC [a]	1	0.51	0.50	0.64	0.54	-			
	2	0.43	0.54	0.48	0.53	**0.56**	-		
Aspartame	1	0.48	0.52	0.55	0.54	0.54	0.47	-	
	2	0.40	0.53	0.47	0.49	0.43	0.48	**0.56**	-

Two presentations for each sweetener. Coefficients between duplicate presentations of the same compounds shown in bold.

[a] Neohesperidine dihydrochalcone.

Variance Components Modelling

For all four sweeteners, the MZ twin correlations (0.27 – 0.33) were higher than the DZ correlations (0.12 – 0.22), though both correlations were low-modest (Table 1-2). Univariate ACE models showed no significant worsening of fit after removal of the shared environmental factor for each of the four sweeteners (Δ-2LL ranged from 0 to 1.55). NHDC was further tested in an ADE model as the MZ correlation was more than double the DZ correlation. Removal of the dominant genetic factor from the ADE model did not result in a worse fit for NHDC (Δ-2LL = 0). As we found test unreliability (U) accounted for 40% to 50% of the variance for each sweetener (AEU model; Table 1-2), in all further modelling we used mean intensity, which is a more stable measure.

Model fit of the multivariate models, which included mean intensity ratings for each of the four sweeteners, are shown in Table 1-4. Dropping the shared environmental factors from the full ACE Cholesky did not worsen the model fit (Δ-2LL = 3.50, Δdf = 10, AIC = 1566.288) and revealed one common genetic factor and a specific genetic factor for fructose, NHDC, and aspartame. While the CE model (dropping genetic factors) also provided a better fit than the full (ACE) Cholesky, the AE model was a better fit according to the AIC. A common pathway model (AIC = 1559.356), shown in Figure 1-1, provided the best fit among the models tested (Table 1-4). From the independent pathway model, heritabilities were estimated to be 0.31, 0.34, 0.31, and 0.30 for glucose, fructose, NHDC, and aspartame, respectively. A common genetic factor explained the majority of the genetic variance for glucose (88%), fructose (87%), NHDC (79%), and aspartame (76%) ratings, with specific factors accounting for only a small amount of the genetic variance (12% to 24%). Genetic correlations ranged between 0.78 and 0.89 with the lowest between fructose and aspartame, and the highest between glucose and fructose. Environmental factors accounted for 66% to 70% of the variance.

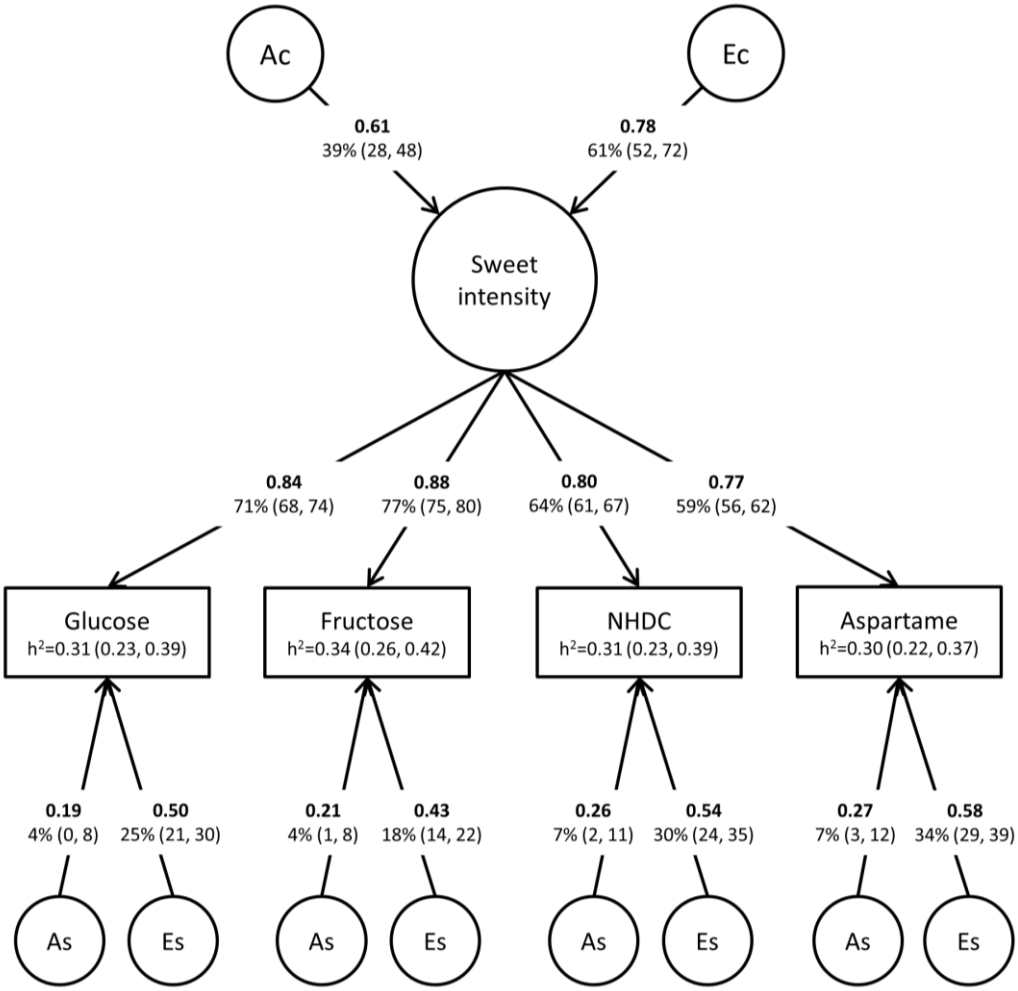

Figure 1-1. Common pathway model for perceived intensity of four sweeteners (standardized path coefficients and percentage of variance with 95% CIs). Ac and Ec are common additive genetic and environmental effects on the intensity measurement of four sweeteners. As and Es are specific genetic and environmental effects for each sweetener. Heritability estimates (h^2) for each sweetener are sums of loadings from the Ac and As. For example, h^2 for glucose is 0.39*0.71 + 0.04. Adding loadings from Ac, Ec, As and Es gives a total variance of 100% for each sweetener.

Table 1-4. Model Fit of the Multivariate Models for Perceived Intensity of Sweeteners

Model		df	-2LL	Δdf	Δ-2LL	AIC
Cholesky	ACE	7495	16572.79	-	-	1582.792
	AE [a]	7505	16576.29	10	3.50	1566.288
	CE [a]	7505	16583.39	10	10.60	1573.389
	E [a]	7515	16661.22	20	88.43	1631.222
Common pathway	**AE**	**7512**	**16583.36**			**1559.356**
Independent pathway	AE	7509	16582.10			1564.096

Abbreviations: degrees of freedom (df); -2 times the log-likelihood (-2LL); Akaike's information criterion (AIC). Best model shown in bold.

[a] Model fitting versus Cholesky full ACE model.

Discussion

This is the first study to estimate the heritability and genetic covariance for perceived intensity of four structurally diverse sweet compounds: glucose, fructose, NHDC, and aspartame. Moderate heritabilities were estimated at 0.31, 0.34, 0.31, and 0.30 for glucose, fructose, NHDC, and aspartame, respectively. These estimates are similar to that previously reported for the sucrose intensity [90]. Our finding that a common genetic factor accounts for most of the genetic variance suggests that a single set of genes influences the perceived intensity of all the sweeteners. This factor explained 23% to 30% of the phenotypic (total) variance, whereas specific additive genetic components accounted for 4% to 7% of the variance. Although there is no evidence for a specific genetic factor influencing the perception of caloric sugars, the common genetic factor could include parallel pathways that may account for a different amount of the genetic variance in the perception of sugars compared with high-potency sweeteners.

We found little evidence for a shared environmental factor for sweet perception, which is also consistent with prior work examining sucrose intensity [90]. Although there is a belief that diets high in sugar can change sweet preference, and sweet foods are often withheld from children early in life to modify this, our findings and those of Keskitalo et al. [90] suggest that common environmental influences on sweet intensity ratings are very small. Similar data from animal models show that mice and rats, either exposed or not exposed to sugar early in life, do not differ as adults in sweet-related behaviour [99, 100].

Univariate AEU modelling showed that test unreliability accounted for nearly 50% of the trait variation. This places an upper ceiling on the heritability estimate by removing variance that can be in part explained by genotype and thus results in an underestimation of the heritability. Unreliability of sweet intensity is commonly found among researchers because sweet responses are subject to influences of circulating insulin, glucagon, leptin, and cannabinoids, which vary from meal to meal, time of day, and from day to day [101, 102]. However, as all sweeteners were tested within a short time frame in this study, the unreliability was more likely to be measurement specific. Therefore, a more stable measure of mean intensity was used and the heritability estimates were similar to those previously reported [90].

We found that the effect of age on sweet perception was similar to that for bitter perception [40], suggesting that as adolescents move from childhood to adulthood, the perceived intensity of both sweet and bitter tastes decreases. Despite the cross-sectional design of this study, the age effect suggests that the taste systems are changing from child-like to adult-like; the results of this study suggest that during this period, the perception of intensity from sweet compounds decreases [85]. Likewise, longitudinal studies of sweet preference in both humans and mice revealed a negative age effect [103, 104].

Similarly, history of otitis media positively affected the perceived intensity of all sweet measures. It has been proposed previously that the damage of otitis media infection to the chorda tympani, a taste nerve from the tongue to the brain that passes behind the tympanum, results in an increase in the number of taste buds per fungiform papilla [95]. More taste buds correspond to more taste receptor cells, and consequently may influence sweetness perception. As a positive effect of otitis media was also observed for bitter compounds [40], it may be that otitis media infection causes a global increase in the intensity of both sweet and bitter tastes.

In contrast to the global effect of age and otitis media, we found a sex effect only for aspartame, with males rating aspartame slightly more intense than females. Previous studies of smaller sample size reported no sex influences on the perceived intensity of aspartame, but only on other perception measures such as sweetness and pleasantness [105, 106]; this suggests the complexity in human perception of sweetness.

In conclusion, this study has examined the perceived intensity of four structurally diverse sweet compounds: two naturally-occurring saccharides (glucose and fructose) and two high-potency sweeteners commonly used as additives to human foods (NHDC and aspartame). We have established that a moderate amount of the variation in the perceived intensity of sweeteners is due to genetic factors, and that there appears to be a single set of genes responsible for most of the variation in perception of these four compounds. This suggests that genetic factor scores for sweet intensity may be optimal for genome-wide association analyses.

2

Is the Association between Sweet and Bitter Perception due to Genetic Variation?

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Chapter 2. Is the association between sweet and bitter perception due to genetic variation?

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Abstract

Perceived intensities of sweetness and bitterness are correlated with one another and each is influenced by genetics. The extent to which these correlations share common genetic variation, however, remains unclear. In a mainly adolescent sample (n = 1901, mean age 16.2 years), including 243 MZ and 452 DZ twin pairs, we estimated the covariance among the perceived intensities of four bitter compounds (6-n-propylthiouracil [PROP], sucrose octaacetate, quinine, caffeine) and four sweeteners (the weighted mean ratings of glucose, fructose, neohesperidine dihydrochalcone, aspartame) with multivariate genetic modelling. The sweetness factor was moderately correlated with sucrose octaacetate, quinine, and caffeine (r_p = 0.35 – 0.40). This was mainly due to a shared genetic factor (r_g = 0.46 – 0.51) that accounted for 17% – 37% of the variance in the three bitter compounds' ratings and 8% of the variance in general sweetness ratings. In contrast, an association between sweetness and PROP only became evident after adjusting for the *TAS2R38* diplotype (r_p increased from 0.18 to 0.32) with the PROP genetic factor accounting for 6% of variance in sweetness. These genetic associations were not inflated by scale use bias, as the cross-trait correlations for both MZ and DZ twins were weak. There was also little evidence for mediation by cognition or behavioural factors. This suggests an overlap of genetic variance between perceptions of sweetness and bitterness from a variety of stimuli, which includes PROP when considering the *TAS2R38* diplotype. The most likely sources of shared variation are within genes encoding post-receptor transduction mechanisms common to the various taste G protein-coupled receptors.

Introduction

Taste perception varies greatly among individuals. For over a decade, intensity ratings of the bitter compound 6-n-propylthiouracil (PROP) have been used to distinguish an individual's "bitter taster status" [108], with those rating it as extremely bitter sometimes described as "supertasters" [18]. Many studies suggested these individuals are also more sensitive to other taste stimuli [51, 109-113], whereas many others have failed to find such associations [114-118]. Therefore, whether individual differences in ratings of a single compound can generalize to other taste stimuli has been questioned and, furthermore, whether there are pan-quality overarching individual differences remains unclear [108, 113, 118].

Most of the perceptual variability in PROP is due to genetic variation within the bitter taste receptor *TAS2R38* [36, 37, 119]. Genetic variation in *TAS2R38* does not appear to be associated with perceived intensity of other taste stimuli (e.g. quinine and sucrose) [120], but some evidence shows that the *TAS2R38* diplotype may modify the association between PROP and other tastes [113]. In addition, the PROP response has been shown to be less predictive of overall perceived taste intensity than are collective ratings of sucrose, sodium chloride, citric acid, and quinine [118], suggesting that PROP ratings are not a sole predictor for overall taste perception. Rather, a more complex association across multiple taste classes, such as general differences in the "gain" of the taste system, appears to be at play.

A parallel body of work reveals that genetics plays a significant role in the perception of different taste qualities, accounting for over 30% of the variance in sweetness, sourness and bitterness [40-42, 81]. Our previous studies identified a shared genetic pathway for taste perception across different bitter compounds, excluding PROP [40], and more recently a common genetic factor for the perception of both sugars and non-caloric sweeteners [81]. Although the perception of sweetness has been weakly-to-moderately correlated with bitterness [41, 113, 118], whether this association is due to shared genes has not been determined in humans. In addition, there is evidence that non-receptor based factors may contribute to the correlation between sweet and bitter taste perceptions. For example, prosocial (e.g. agreeableness) and antisocial (e.g. psychopathy) personalities are associated with elevated sweet and bitter taste preferences, respectively [121, 122]. Further, other

concerns, such as psychometric properties of scale use (e.g. a tendency to rate at one side of the scale [123]) and even intelligence (e.g. higher IQ is associated with less extreme rating styles [124]) need to be raised when studying weak sensory associations.

The present study investigated the sources of association between multiple taste qualities using a large adolescent and young adult twin sample. Genetic covariances between perceived intensity of four sweet (glucose, fructose, neohesperidine dihydrochalcone [NHDC], and aspartame) and four bitter solutions (PROP, SOA, quinine HCl [quinine], and caffeine) were estimated using multivariate genetic modelling. In addition, this study examined the impact of the *TAS2R38* diplotype on the genetic covariances, and potential confounding effects of scale use bias, general cognitive ability, and behavioural factors.

Materials and Methods

Participants

Participants were adolescent and young adult Caucasian twins and their singleton siblings from the Brisbane Adolescent Twin Study [72], also referred to as the Brisbane Longitudinal Twin Study (BLTS). They were originally recruited for a study on melanoma [125], a common form of cancer among light-skinned people. The taste data reported here were collected between August 2002 and July 2014. The sample comprised 243 MZ and 452 DZ twin pairs, including 126 pairs with one to two singleton siblings, and 320 unpaired individuals (mean age of 16.2 ± 2.8 years; 1023 females, 878 males) (Supplementary Table 2-1). This is the same sample as used previously [81]. Zygosity was determined from genotyping (92% of same sex twin pairs) or from self-report. The Queensland Institute of Medical Research Human Research Ethics Committee approved the study. Written consent was obtained from both the participants and their parents (the latter not required for those 18 years and over).

Taste Test

The taste test battery has been described in Chapter 1. Briefly, participants rated the intensity of five bitter (6.0×10^{-4} M PROP, 2.0×10^{-4} M SOA, 1.81×10^{-4} M quinine, 0.05 M caffeine, and 4.99×10^{-6} M denatonium benzoate) and four sweet (0.60 M glucose, 0.30 M fructose, 8.0×10^{-5} M NHDC, and 1.4×10^{-3} M aspartame) solutions using a general Labelled Magnitude Scale (gLMS) [73]. Mean intensity

ratings from duplicate presentations for each of PROP, SOA, quinine and caffeine were used in all analyses. For the four sweet compounds, a weighted mean general sweet (gSweet) factor was used, as perceived intensity of the sweeteners is highly correlated at the genetic level (r_g = 0.78 – 0.89) and most of the variance (71% for glucose, 77% for fructose, 64% for NHDC, and 59% for aspartame) is accounted for by a common genetic factor (Chapter 1). Denatonium benzoate was not included due to the violation of the normality assumption, a criterion for twin modelling [126] (see Statistical Analyses). Statistical transformation failed to overcome this problem because of its distinct bimodal distribution (Supplementary Figure 2-1). In addition, the mean intensity rating for denatonium benzoate was double that of other stimuli, with the most common rating being the strongest imaginable on the scale, suggesting that the concentration may have been too high to detect variation (also known as a "ceiling effect"). The intensity rating characteristics for denatonium benzoate are summarized in Supplementary Table 2-2.

TAS2R38 diplotype

The genotypes for the three *TAS2R38* SNPs (rs713598, rs1726866, and rs10246939), resulting in three amino acid substitutions (A49P, A262V, and V296I), were available for 92% of the sample. Genotyping was done using the Illumina 610-Quad BeadChip (n = 1254) [37] or HumanCoreExome-12 v1.0 BeadChip. The frequencies of the three diplotypes (PAV/PAV = 17%, PAV/AVI = 52% [including 34 participants with rare diplotypes of AAV/AVI or PAV/AAV, which were shown to have similar effects on PROP perception as the PAV/AVI diplotype [119]], and AVI/AVI = 31%) were similar to the frequencies reported previously for PROP sensitivity (i.e. a distribution of 20% high: 50% medium: 30% low-sensitivity tasters) [18, 127].

Statistical Analyses

The heritability and phenotypic correlations among the intensity scores of gSweet and the four bitter substances were estimated using univariate and bivariate variance components modelling. This twin method partitions the phenotypic variance or covariance into additive genetic (A), common environment (C) and unique environment (E, includes experimental error) sources by taking advantage of the differences in the genetic relatedness between MZ twins who share 100% of their genes and DZ pairs who share, on average, 50% of their genes. This method can detect genetic effects by comparing the correlations of MZ and DZ twins and without

requiring the investigation of specific genes. Variance components modelling was performed using the structural equation modelling software package Mx, which utilises maximum likelihood estimation procedures [74]. Prior to modelling, a square root transformation was applied to each of the five intensity scores to obtain a more normal distribution (Supplementary Figure 2-1 and Supplementary Table 2-3). Covariates of age, sex, and otitis media were modelled as regressions or deviation effects on the mean for all models. Damage to the chorda tympani nerve resulting from an otitis media infection can result in an increase in the number of taste buds [95], and consequently may influence taste perception. Our previous studies showed that the history of otitis media was associated with increased perceived intensity (4% – 9%) of the same four sweet and bitter solutions [40, 81]. No outlying families were identified using the %p option in Mx, which uses the Mahalanobis distance to compute a z-score for each family, with values outside the -3.5 to +3.5 range indicating excessive similarities or differences relative to other families in the sample and model expectation.

To estimate the covariance structure between the five traits, a multivariate Cholesky decomposition model [126] was used as a starting point. A series of models, including dropping A and C components, were tested to determine which pattern of covariance best fitted the data. For nested models, we assessed the comparative fit by calculating the difference in double the negative log-likelihood, which is distributed asymptotically as a χ^2. For non-nested models, the Akaike's Information Criterion (AIC), which penalizes models for increasing complexity [98], was used. The effect of the *TAS2R38* diplotype on the covariance structure was tested in a partial dominant model with one covariate for the heterozygous effect (i.e. PAV/AVI = 1 and PAV/PAV = AVI/AVI = 0) and a second covariate for the PAV homozygous effect (i.e. PAV/PAV = 1 and AVI/AVI = PAV/AVI = 0). Consequently, the genetic variance in PROP dropped from 0.72 to 0.20 (compared with an additive or dominant model where PROP genetic variance reduced to 0.25 and 0.24 respectively). This model was used to examine the covariance structure when the *TAS2R38* genetic effect on intensity ratings was removed. A second model in which the low-sensitivity tasters for PROP (30% of the genotyped sample) were excluded from the analyses was also tested.

In addition, this study investigated whether intensity ratings were associated with general cognitive ability (Verbal IQ), assessed with the Multidimensional Aptitude Battery [128], and personality (neuroticism, extraversion, openness to experience, agreeableness, and conscientiousness) assessed with the NEO Five-Factor Inventory (FFI) [129]. Both cognition and personality were assessed at age 16, which for the majority of twins were two years after assessment for sweet and bitter taste. Verbal IQ and personality were available for 1282 and 1277 participants respectively [130]. Further, to assure that the associations were estimated from taste perception and were not inflated by scale use bias, an emphasis score: 0 = neutral, 1 = somewhat, 2 = strongly was calculated by folding the 5-point Likert scale of the sixty responses from the NEO-FFI: strongly agree = 5, agree = 4, neutral = 3, disagree = 2, strongly disagree = 1. We tested these scale bias scores for relation with the taste ratings. Where an association was indicated, the measure was included as a covariate in the multivariate model to examine whether the genetic architecture was changed. Lastly, cross-correlations were calculated between the intensity ratings of PROP for the first born twin with those of their co-twin's ratings for the other four traits, for both MZ and DZ pairs, to examine any genetic effect on scale use rather than taste perception.

Results

Mean ratings, standard deviations, twin correlations, and heritability estimates for the perceived intensity of PROP, SOA, quinine, caffeine and gSweet are shown in Table 2-1. The mean rating was lower for PROP, and the variance was slightly larger, compared with the other bitter compounds. This was due to the distinct differences in PROP response between the three *TAS2R38* diplotype groups and 31% of the participants being low-intensity tasters who could barely detect the bitterness in PROP (Figure 2-1). In contrast, there was no effect of the *TAS2R38* diplotype on SOA, quinine, caffeine or gSweet. Heritability for PROP ($h^2 = 0.73$) was significantly higher than that for SOA, quinine and caffeine ($h^2 = 0.35 – 0.4$), in line with prior work [40]. The mean and heritability ($h^2 = 0.36$) estimates for the gSweet factor are the same as those reported previously [81].

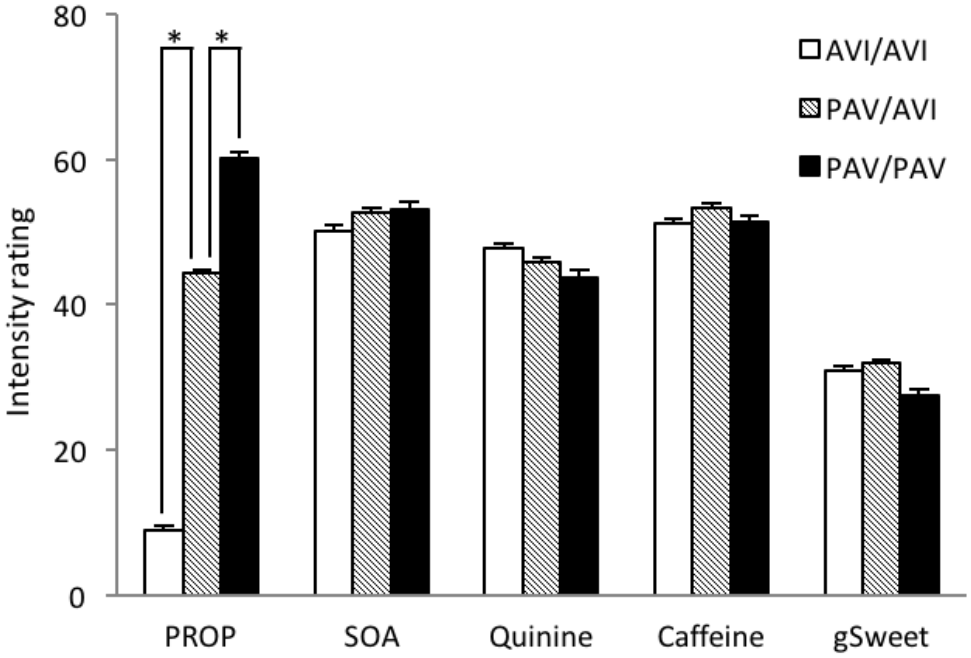

Figure 2-1. Perceived intensity ratings (mean + standard error) for four bitter solutions and the general sweetness factor (a weighted mean rating of glucose, fructose, NHDC and aspartame). Participants grouped by *TAS2R38* diplotypes (n = 527 for AVI/AVI, n = 916 for PAV/AVI, n = 313 for PAV/PAV). * indicates significant differences (Student's t-test, p < 0.001).

Table 2-1. Taste intensity characteristics

	PROP	SOA	Quinine	Caffeine	gSweet
Mean ± SD [a]	36.7 ± 29.6	51.6 ± 22.6	45.6 ± 22.9	52.2 ± 23.3	31.2 ± 15.2
Twin Correlations [b]					
r_{MZ} (95% CI)	0.72 (0.65, 0.78)	0.34 (0.23, 0.45)	0.40 (0.29, 0.50)	0.29 (0.17, 0.41)	0.36 (0.24, 0.46)
r_{DZ} (95% CI)	0.34 (0.26, 0.42)	0.25 (0.10, 0.34)	0.18 (0.09, 0.27)	0.21 (0.12, 0.30)	0.20 (0.17, 0.34)
Heritability (95% CI)	0.73 (0.67, 0.77)	0.40 (0.31, 0.49)	0.40 (0.31, 0.49)	0.34 (0.24, 0.43)	0.36 (0.27, 0.45)
Phenotypic Correlations (95% CI)					
Full Sample					
SOA	0.29 (0.25, 0.34)	-	-	-	-
Quinine	0.25 (0.20, 0.29)	0.59 (0.56, 0.62)	-	-	-
Caffeine	0.31 (0.26, 0.35)	0.64 (0.62, 0.67)	0.63 (0.60, 0.65)	-	-
gSweet	0.22 (0.18, 0.27)	0.35 (0.30, 0.39)	0.40 (0.37, 0.44)	0.40 (0.36, 0.43)	-
TAS2R38 adjusted [c]					
SOA	0.37 (0.33, 0.41)	-	-	-	-
Quinine	0.44 (0.40, 0.47)	0.60 (0.57, 0.63)	-	-	-
Caffeine	0.42 (0.38, 0.46)	0.64 (0.61, 0.67)	0.63 (0.61, 0.66)	-	-
gSweet	0.32 (0.27, 0.36)	0.35 (0.30, 0.39)	0.41 (0.36, 0.45)	0.39 (0.35, 0.43)	-
AVI/AVI excluded [d]					
SOA	0.44 (0.39, 0.48)	-	-	-	-
Quinine	0.48 (0.43, 0.52)	0.60 (0.56, 0.63)	-	-	-
Caffeine	0.46 (0.41, 0.51)	0.63 (0.60, 0.66)	0.62 (0.59, 0.66)	-	-
gSweet	0.31 (0.26, 0.36)	0.33 (0.28, 0.38)	0.37 (0.32, 0.42)	0.37 (0.32, 0.42)	-

Means and standard deviations, MZ and DZ twin correlations, heritability estimates and phenotypic correlations for perceived intensity ratings (millimeters on a labeled magnitude scale) of PROP, SOA, quinine, caffeine and a general sweetness factor (gSweet).

[a] n = 1881 - 1892. The sample size (N) varies as not all participants completed the entire test.

[b] 238-240 MZ and 446-449 DZ twin pairs; all estimates are from univariate AE models.

[c] *TAS2R38* diplotype, available for n = 1756, was tested in a partial dominant model.

[d] N reduced to 1229 when *TAS2R38* AVI/AVI diplotype excluded

Perceived intensity for gSweet was moderately correlated with those for SOA, quinine, and caffeine (r_p = 0.35 – 0.40) and more weakly associated with PROP (r_p = 0.22) (Table 2-1). Multivariate model-fitting showed that the common environmental components (C) could be dropped without loss of fit (Supplementary Table 2-4). Multivariate AE modelling identified a genetic factor (A2 factor in Figure 2-2) accounting for 8% of the variance in gSweet and 17% – 37% of the variance in SOA, quinine, and caffeine. Only 1% of the variance in gSweet was genetically shared with PROP (A1 factor in Figure 2-2). There was also little shared genetic variance between PROP and the other three bitter compounds (i.e. 1% – 3%, A1 in Figure 2-2). Further, the association between gSweet and PROP was largely due to an environmental "PROP" factor (E1), accounting for 27% of the variance in PROP and 6% in gSweet. This environmental factor also accounted for a small amount of the variance (9% – 12%) in SOA, quinine, and caffeine.

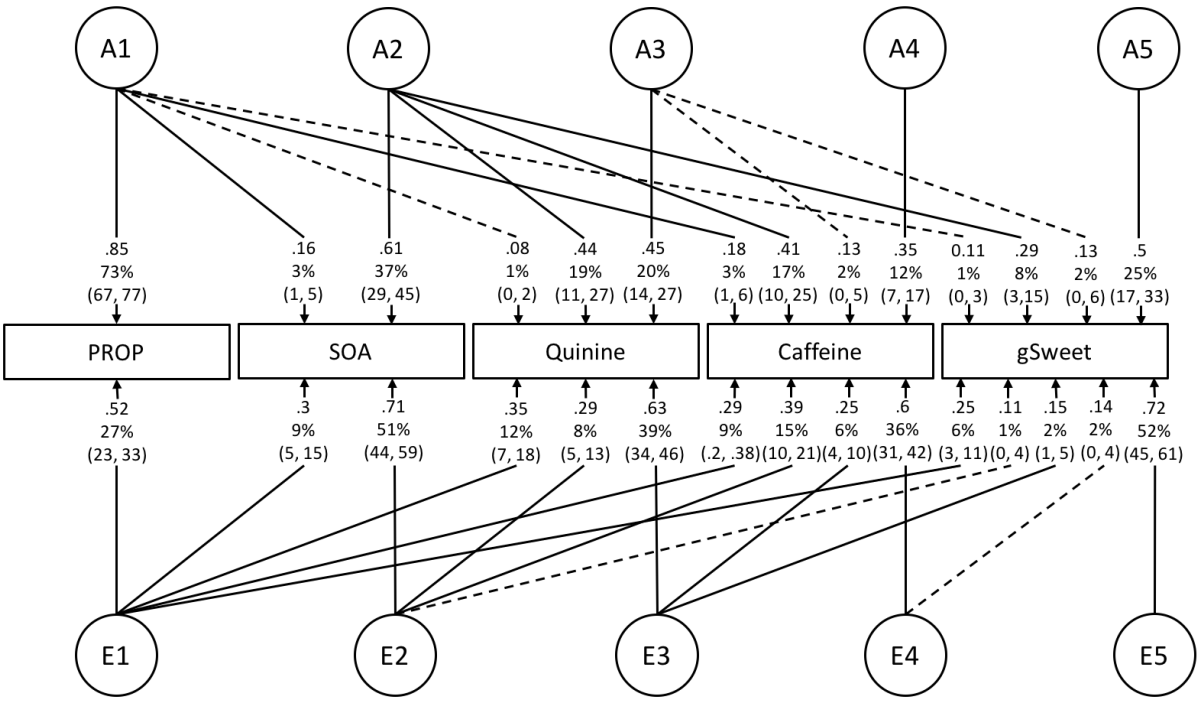

Figure 2-2. The Cholesky AE model showing estimates of standardized path coefficients (can be squared to get the variance) and percentage of variance with 95% CIs and covariation between perceived intensity of PROP, SOA, quinine, caffeine, and gSweet. The boxes and the circles represent observed variables (phenotypes) and latent variables, respectively. A and E are the addictive genetic and non-shared environmental factors. Dash lines are insignificant estimates. See Supplementary Table 2-5 for absolute variances.

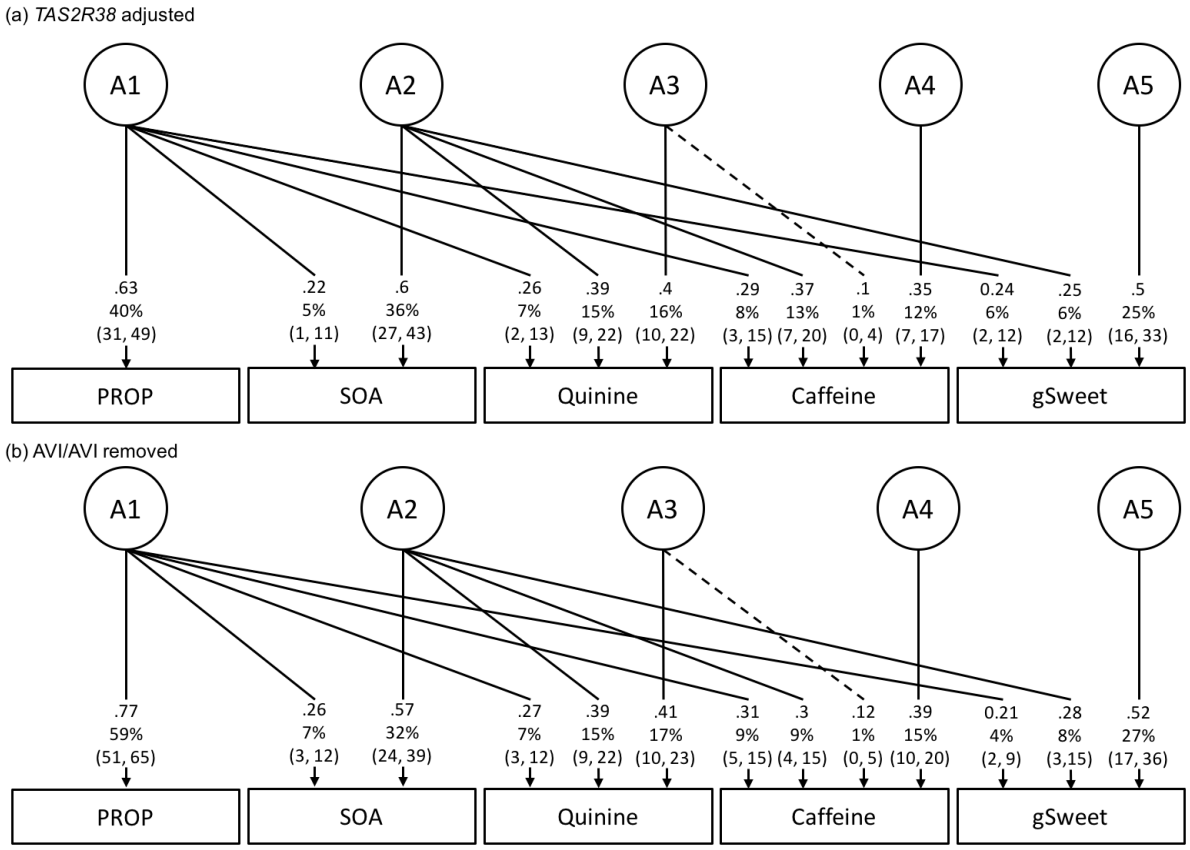

Figure 2-3. The conditioned Cholesky AE models showing estimates of standardized path coefficients (can be squared to get the variance) and percentage of variance with 95% CIs and covariation between perceived intensity of PROP, SOA, quinine, caffeine, and gSweet. The boxes and the circles represent observed variables (phenotypes) and latent variables, respectively. Only additive genetic factors (A) are shown here because estimates of environmental factors (E) are not different from those estimated from the full sample (n = 1901). Dashed lines are insignificant estimates. (a) Adjusting for *TAS2R38* diplotype (n = 1756). * Modelling results with gSweet replaced by glucose and fructose are shown in Supplementary Table 2-7. (b) Participants with *TAS2R38* AVI/AVI diplotype removed (n = 1229). (See Supplementary Table 2-5 for absolute variance.)

When the model was adjusted for the *TAS2R38* diplotype (i.e. the *TAS2R38* genetic effect was removed), the correlation between gSweet and PROP increased (r_p = 0.32), as did the correlation between PROP and the other bitter compounds (r_p increased from 0.25 - 0.31 to 0.37 - 0.44) (Table 2-1). The stronger association was due to an increase in shared genetic variance with 6% of variance in gSweet now overlapping with the genetic variance for PROP (A1 in Figure 2-3a; h^2 of PROP decreased to 0.40 after adjustment). Similarly, after adjusting for *TAS2R38*, there was an increase in shared genetic variance for SOA, quinine and caffeine with PROP. Notably, no increase in the environmental variance (E1) shared with PROP was found for gSweet, SOA, Caffeine or Quinine. Adjusting for *TAS2R38* reduces the absolute genetic variance in PROP, with environmental variance for PROP and the genetic and environmental variances of other tastes remaining the same (Supplementary Table 2-5). In addition, removing PROP low-sensitivity tasters, rather than adjusting for *TAS2R38*, produced a similar covariance structure, even though the sample size was reduced (Figure 2-3b; Supplementary Table 2-5c). This increase in the genetic correlations after adjustment for *TAS2R38* or removal of PROP low-sensitivity tasters (Table 2-2) contrasts with the environmental correlations that remained the same. In terms of genetic variance as a proportion of the heritability (Supplementary Table 2-6), 23% of the genetic variance in gSweet (8% of the variance divided by the heritability of 0.36) overlapped with 46% of genetic variance in quinine, 49% in caffeine, and 94% in SOA, whereas only 3% of the genetic variance in gSweet overlapped with PROP, which increased to 15% after adjusting for the *TAS2R38* diplotype.

Since a commonly based definition of sweet taste is the oral perception of natural sugars, we used the intensity rating for glucose and fructose instead of gSweet to remove any possible bias of a weighted mean intensity rating of both the sugars and high-potency sweeteners; the results were similar (Supplementary Table 2-7).

Table 2-2. Genetic (lower-triangle) and environmental (upper-triangle) correlations (95% confidence intervals) between perceived intensities of four bitter compounds and the general sweet intensity estimated from bivariate AE models.

a. Full sample

		Environmental correlations				
		PROP	SOA	Quinine	Caffeine	gSweet
Genetic correlations	PROP	-	0.38 (0.28, 0.48)	0.45 (0.35, 0.54)	0.36 (0.26, 0.45)	0.32 (0.21, 0.41)
	SOA	0.26 (0.15, 0.37)	-	0.52 (0.44, 0.59)	0.58 (0.51, 0.64)	0.24 (0.14, 0.33)
	Quinine	0.12 (0, 0.23)	0.70 (0.58, 0.80)	-	0.60 (0.53, 0.66)	0.35 (0.25, 0.44)
	Caffeine	0.31 (0.19, 0.42)	0.76 (0.65, 0.86)	0.68 (0.55, 0.79)	-	0.36 (0.27, 0.45)
	gSweet	0.18 (0.05, 0.29)	0.51 (0.35, 0.67)	0.50 (0.33, 0.65)	0.46 (0.27, 0.62)	-

b. *TAS2R38* adjusted

		Environmental correlations				
		PROP	SOA	Quinine	Caffeine	gSweet
Genetic correlations	PROP	-	0.37 (0.27, 0.47)	0.44 (0.34, 0.52)	0.38 (0.28, 0.46)	0.27 (0.16, 0.37)
	SOA	0.36 (0.19, 0.52)	-	0.51 (0.42, 0.58)	0.58 (0.50, 0.64)	0.24 (0.14, 0.33)
	Quinine	0.43 (0.26, 0.58)	0.74 (0.62, 0.85)	-	0.60 (0.52, 0.66)	0.37 (0.27, 0.46)
	Caffeine	0.50 (0.32, 0.65)	0.76 (0.64, 0.85)	0.70 (0.57, 0.81)	-	0.36 (0.27, 0.45)
	gSweet	0.40 (0.22, 0.56)	0.52 (0.36, 0.67)	0.47 (0.29, 0.62)	0.45 (0.26, 0.61)	-

n = 1756. *TAS2R38* diplotype was tested in a partial dominant model.

c. *TAS2R38* AVI/AVI excluded

		Environmental correlations				
		PROP	SOA	Quinine	Caffeine	gSweet
Genetic correlations	PROP	-	0.46 (0.34, 0.56)	0.53 (0.42, 0.62)	0.43 (0.32, 0.54)	0.29 (0.16, 0.41)
	SOA	0.44 (0.27, 0.57)	-	0.50 (0.40, 0.59)	0.60 (0.52, 0.68)	0.19 (0.07, 0.31)
	Quinine	0.44 (0.27, 0.57)	0.75 (0.60, 0.88)	-	0.60 (0.51, 0.68)	0.36 (0.24, 0.47)
	Caffeine	0.52 (0.36, 0.66)	0.68 (0.51, 0.80)	0.66 (0.48, 0.79)	-	0.34 (0.22, 0.45)
	gSweet	0.34 (0.17, 0.50)	0.55 (0.36, 0.74)	0.39 (0.17, 0.58)	0.42 (0.19, 0.62)	-

n = 1229.

Further, there was little evidence of the effect of scale use bias, IQ, or personality on intensity ratings. While some small associations were observed (Supplementary Table 2-8), there was no change in the covariance structure when the emphasis score, IQ, and personality were included as covariates in the multivariate model (Supplementary Table 2-9). In addition, scale use had no effect on the genetic estimates; the cross-trait (e.g. PROP for twin 1 with gSweet for twin 2) correlations for both MZ and DZ twins were low and of similar magnitude (r_{MZ} = 0 – 0.10, r_{DZ} = 0.03 – 0.11; Supplementary Table 2-10).

Discussion

This study examined whether there is heritable genetic overlap between the perception of sweetness and bitterness of eight different compounds. Using multivariate genetic modelling, we showed that up to a quarter of the genetic variance in sweet perception is shared with at least half, or more, of the genetic variance in SOA, quinine and caffeine. Further, after adjustment of the *TAS2R38* diplotype, 15% of genetic variance in sweetness is also in common with PROP perception. These results suggest that human perceptions of sweetness and bitterness are linked through shared genes and the extent of overlap depends on the specific taste stimuli.

Confirming prior work, intensity ratings for sweetness were weakly-to-moderately associated with bitterness ratings [41, 113, 118]. Although Lim *et al*. [118] only found an association between sucrose and quinine and not between sucrose and PROP, their sample size was small (n = 83) compared to Fischer's and ours (n = 1670 and 1901 respectively). This suggests that a bigger sample size is required to detect weak associations with PROP. Our finding that the associations between gSweet and SOA, quinine and caffeine were mainly due to a shared genetic factor supports the current understanding that, in humans, the perception of both sweetness and bitterness is mediated via G protein-coupled receptors (GPCR) and other shared transduction proteins in the oral cavity [4, 131]. Genetic variation in these taste genes has been shown to link to individual differences in the perception of both sweetness [82, 83] and bitterness [37, 38]. Evidence from animal models also shows that knocking out common genes in their downstream signalling pathways, including genes that encode the G-protein alpha-gustducin, Ggamma13, the lipid enzyme phospholipase C beta2, and transient receptor potential ion channel *TRPM5*, leads to a reduced response to both sweet and bitter tastes [132-134].

Our prior work showed that the perception of PROP was weakly associated with the perception of other bitter compounds at the genetic level [40]. Here, using a greatly expanded sample, this study confirmed this lack of strong association, but also identified, after adjusting for the *TAS2R38* diplotype, a shared genetic factor accounting for some of the genetic variance in the perception of PROP as along with other bitter and sweet tastes. This finding supports the two-locus model [135] of the perception of *TAS2R38* associated bitter compounds (i.e. PROP and its structurally

related chemical propylthiocarboamide [119]), with one locus controlling compound-specific tasting and the other locus controlling general taste responsiveness. This shared genetic factor (A1 factor in Figure 2-3) may correspond to a shared pathway at the peripheral level because the perception of PROP is believed to go through the same GPCR-based signalling elements as other bitter and sweet tastes [136, 137]. Alternatively, shared genetic variation could also involve a shared pathway at the central neural level, supporting the hypothesis [138] that a central nervous system (CNS) mechanism influences general responsiveness to tastes. In that study, the ability to perceive thermally induced taste predicted higher taste responses to sweet and bitter stimuli, including PROP, as well as salty, sour and umami taste stimuli. They eliminated the potential confounders of papillae density and gustatory afferents used in signal transduction and concluded that the overall control of gain in the orosensory system is likely centrally determined.

In addition to the perception of sweetness and bitterness, the shared genetic factors identified here could also link to the perception of umami taste, especially at the peripheral level, because a glutamate taste receptor is also a member of the Class 1 Taste GPCR family [131, 139]. Animal studies have shown that these taste qualities (sweetness, bitterness and umami) are encoded by common downstream transduction components that are not believed to be used by ionic taste stimuli (sourness and saltiness) [132, 134]. Future taste genetic studies involving glutamate, salts, and acids could test this hypothesis in humans and would help tease apart which common genes are involved.

To assure that the genetic overlap between sweetness and bitterness was not due to confounding factors, we tested a series of alternative hypotheses. First, we found only subtle associations between personality traits and taste ratings, in contrast to other studies [121, 122]. This difference between our weak personality influence and those previously observed may be due to differences in measures of taste preference rather than taste intensity in the present study. Second, individuals with higher IQ rated taste solutions as less intense. There is some evidence that people with higher IQ are less likely to give extreme ratings [124] and we also observed a negative correlation between IQ and emphasis scores (r_p = -0.24) in this study. Therefore, these people could be more conservative in rating the intensity of taste solutions. Alternatively, the association may be due to pleiotropic effects with shared genes influencing both taste perception and the development of intelligence.

Nevertheless, neither personality traits nor IQ modifies the genetic architecture between sweetness and bitterness. Third, the emphasis scores were not associated with any taste ratings, so an individual's response style is not likely a concern. Lastly, scale use style is not influenced by genetics and did not inflate or deflate the estimates of heritability or genetic covariances. The last test supports that the gLMS can be a valid instrument to study taste genetics. These four tests of alternative hypotheses allow us to conclude more strongly that the genetic correlation between sweetness and bitterness results from true correlates of taste perception and that these estimates of genetic covariances are valid rather than an artefact of scale use.

There are some limitations of this study. First, partitioning trait variance using Cholesky decomposition is restricted by the trait order in the model, with only the last trait in the model including a trait specific factor. For all other traits, the trait-specific variance is pushed to a group factor(s), which can, therefore, elevate covariances between traits. We tested these models in different orders, however, and obtained similar results. Second, although adjusting for the *TAS2R38* diplotype increased the shared genetic variance between PROP and other taste stimuli, the increase was small and the confidence intervals overlapped (e.g. gSweet increased from 3% [confidence intervals: 0 and 9%] to 15% [confidence intervals: 5 and 30%]). Future studies with larger samples could provide more distinct results. Lastly, whether there is a common genetic pathway for the overall taste perception cannot be answered from this study. Although we showed a genetic factor for the perception of both sweet and bitter tastes, even including PROP, it might simply be a specific taste transduction factor that does not also account for the perception of umami, sourness and saltiness.

In conclusion, this study examined the associations among perceived intensities of four bitter compounds – PROP, SOA, quinine, and caffeine – and a weighted sweet factor from four sweeteners, and modelling results identified two latent genetic factors suggesting two shared genetic pathways. We speculate that there are genes responsible for the recognition of a GPCR-taste signal or a general taste signal. Genetic covariation of downstream signalling elements could involve peripheral and/or central mechanisms, including genes encoding molecules that transduce information from the taste receptors to the nerves and neural elements in the gustatory brain circuits. Future genome-wide association analysis in humans could identify common genes across different taste modalities, which have

implications for understanding the molecular mechanism of GPCR-transduced taste perception as well as the taste-based metabolic signals from the gastrointestinal tract, pancreas, liver, thyroid, and elsewhere.

3

Joint Analysis Strengthens the Role of Bitter Receptor Clusters on Chromosomes 7 and 12 in Human Bitter Taste

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Chapter 3. Joint analysis strengthens the role of bitter receptor clusters on chromosomes 7 and 12 in human bitter taste

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Abstract

Human perception of bitter substances is partially genetically determined. A previous genome-wide association study (GWAS) discovered a single nucleotide polymorphism (SNP) within the bitter taste receptor gene *TAS2R19* on chromosome 12 that accounts for 5.8% of the variance in the perceived intensity rating of quinine, and strengthened the classic association between *TAS2R38* genotype and the bitterness of propylthiouracil (PROP). Here we performed a GWAS using the same sample with a 40% increase in sample size (n = 1999) together with a bivariate approach to detect previously unidentified common variants with small effects on bitter perception. We identified two signals, both with small effects (< 2%), within the bitter taste receptor clusters on chromosomes 7 and 12, which influence the perceived bitterness of denatonium benzoate and sucrose octaacetate respectively. We also provided the first independent replication for an association of caffeine bitterness on chromosome 12. Furthermore, we provided evidence for pleiotropic effects on quinine, caffeine, sucrose octaacetate and denatonium benzoate for the three SNPs on chromosome 12 and the functional importance of the SNPs for denatonium benzoate bitterness. These findings provide new insights into the genetic architecture of bitter taste and offer a useful starting point for determining the biological pathways linking perception of bitter substances.

Introduction

Bitterness is a taste sensation that arises when particular chemicals come into contact with receptors in specialized cells on the human tongue [141-143]. But not everyone perceives the same bitterness for a given stimulus; this individual variation is partially genetically determined and can affect food perception, preferences and intake [3, 4, 144]. Genetic effects for bitter taste perception, which are estimated by twin studies, range from 36 to 73% [40, 41, 107], with most of the known variation arising from inborn variation in the bitter receptor gene family (*TAS2R*) [37, 38, 145]. These bitter receptors are in tissues beyond the tongue and oral cavity, including the airways, gut, thyroid, and brain [146] where they may function as toxin detectors or early-stage sentinel systems. Bitter taste responses may reflect how well the receptors detect ligands in other tissues [147]. Historically, the ability to taste one well-studied bitter compound, phenylthiocarbamide (PTC), has been related to many diseases [17]; more recently and more specifically, variation in the PTC taste

receptor is shown to be involved in the immune system [148] and to predict surgical outcome for severe rhinosinusitis [149]. Thus, together with the better-known effects on food intake and nutrition, bitter taste perception is of increasing importance to the medical field.

Given the rising importance of taste genetics, studies have focused on determining the underlying genetic variation that leads to individual differences in bitter perception. An earlier genome-wide association study (GWAS) [37], which included 1457 adolescents from 626 twin families, revealed a single nucleotide polymorphism (SNP) within the bitter taste receptor gene *TAS2R19,* accounting for 5.8% of the variance in the perception of quinine, and replicated the classic association between the bitter taste receptor gene *TAS2R38* and the perception of propylthiouracil (PROP; a chemical relative of PTC). The study, however, could neither detect loci for the other compounds tested, such as caffeine and sucrose octaacetate (SOA), that are likely to be affected by a large number of small-effect alleles nor the previously proposed but yet to be identified second locus for thiourea-containing compounds like PTC and PROP [135].

Drawing on studies of complex traits such as body mass index (BMI) [79] and schizophrenia [150], here we increased the overall sample size by 40% and used multivariate association analysis [76] to identify common genetic variants (minor allele frequency [MAF] $\geq$ 5%) with small effects. Multivariate GWAS has been used to detect SNP associations that did not reach genome-wide significance in univariate analyses, such as autism spectrum disorders [151] and bone mineral density [152]. This method can detect not only pleiotropic genetic variants but also variants associated with only one of the correlated phenotypes [47]. As shown by Stephens [47], bivariate analysis increases power when there is greater separation of genotype groups (0, 1 or 2 copies of the minor allele) in two- versus one-dimensional space. In Figures 3-1a and b, we provide two illustrations of when a joint analysis of two correlated traits can provide greater separation of genotypes associated with the primary trait (trait 1). The first example (a) shows the case where only one trait (trait 1 on the y-axis) is associated with the variant (non-pleiotropic), with bivariate analysis providing better separation of the genotype groups in 2-dimensional space compared with the y-axis alone. A similar boost in signal would be found in a conditional analysis, where the non-associated trait is included as a covariate, as this

removes the non-associated part of the variance in the associated trait (i.e. covariance between two traits) and, therefore, enhances the association. The second example (b) shows that maximum separation can be achieved when both traits (trait 1 on the y-axis, trait 2 on the x-axis) are associated with the variant and the effect of the minor allele on the two is in opposite direction. In the case where a variant has the same effect on both correlated traits (Figure 3-1c), bivariate analysis provides minimum/no increase in power. The bivariate approach is especially well-justified for bitter taste traits because, with the exception of PROP, perception of these bitter substances are highly correlated (r_p = ~0.6) [153] and their genetic variances largely overlap (r_g = ~0.7) [40, 107].

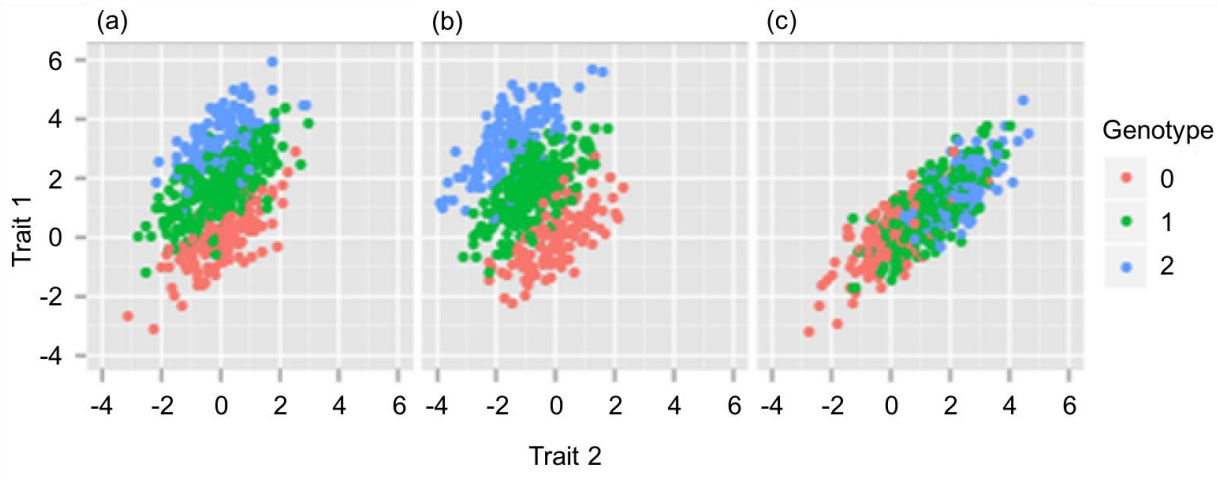

Figure 3-1. Illustration of three scenarios in a bivariate analysis. Each dot represents an individual, coloured according to their genotype (0, 1 or 2 copies of the minor allele). In (a) trait 1 and 2 are correlated but the variant is only associated with trait 1. When considering traits 1 and 2 jointly in testing for association, there is greater separation of the genotype groups for trait 1 in the two-dimensional space compared with the y-axis alone. For example, the blue and green dots would largely overlap in the one-dimensional space along the y-axis. In (b) the minor allele has opposite effects on traits 1 and 2 - increasing trait 1 and decreasing trait 2. The three genotype groups are better separated in the two-dimensional space than for either trait individually. In (c) the minor allele has a similar effect on traits 1 and 2 - increasing both traits. Separation of the three genotype groups in two-dimensional space is no greater than along the y-axis alone. The figures and text are adapted from Figure 1 in Stephens (2013) [47].

Here we aimed to identify common genetic variants with small effects (i.e. 1% – 5%) on the perception of bitterness, building on our previous GWAS [37], which

was too underpowered to detect common genetic variants with small effects. We performed univariate GWAS for the perceived intensity of 5 bitter substances (PROP, quinine, caffeine, SOA, and denatonium benzoate [DB]) using our expanded sample, including 1999 individuals from 929 twin families. As these phenotypes were collected from the same individuals, to boost power we ran a series bivariate GWAS (6 in total) for the correlated phenotypes of quinine, caffeine, SOA and DB [107]. We looked for evidence of pleiotropy for each identified variant. When there was little evidence for pleiotropy, we tested the SNP association with the primary trait conditional on the second. For variants in linkage disequilibrium, we used bidirectional conditional analysis (i.e. including the genotype of one SNP as a covariate at a time to test the association with the other SNP) and plotted the SNP associations for one trait against the other. Finally, to help interpret the genotype-phenotype associations, we examined the potential function of the identified SNPs with bioinformatics tools.

Materials and Methods

Sample

Participants were 1999 adolescent and young adult Caucasian twins and their siblings from 929 families from the Brisbane Adolescent Twin Study [72], also referred to as the Brisbane Longitudinal Twin Study (BLTS), with data collected between August 2002 and July 2014. This sample consisted of 275 MZ and 544 DZ twin pairs, including 155 pairs with one to two singleton siblings, and 184 unpaired individuals (mean age of 16.0 ± 2.8 years [medium 14.5 years, range 11-25 years]; 1075 females, 924 males). It included all participants from a previous GWAS [37], plus a 40% increase in sample size.

Taste Test

The taste test battery has been described in previous chapters. Briefly, it included duplicated presentations of five bitter (6.0×10^{-4} M PROP, 2.0×10^{-4} M SOA, 1.81×10^{-4} M quinine, 0.05 M caffeine, and 4.99×10^{-6} M DB) solutions as well as a paper strip rinsed in a saturated PROP solution (0.059M). Participants were instructed to rate their perceived intensity for each solution and the PROP paper using a general Labelled Magnitude Scale (gLMS) [73] with labels of no sensation (0 mm), barely detectable (2 mm), weak (7 mm), moderate (20 mm), strong (40 mm), very strong (61 mm), and strongest imaginable (114 mm). Mean intensity ratings

from duplicate presentations for each stimulus were used in all analyses. A total of 1757 participants completed the full test battery (solutions and PROP paper) with a further 242 providing an intensity rating for the PROP paper only.

Genotyping, Genetic Imputation and Quality Control

Genotyping was performed with the Illumina 610-Quad BeadChip (n = 1457 individuals) and the HumanCoreExome-12 v1.0 BeadChip (n = 542 individuals), with approximately 700k SNPs passing standard quality control filters, as outlined previously [37]. These SNPs were then phased using ShapeIT [154] and imputed using Minimac3 [155] and the Haplotype Reference Consortium of Caucasian European ancestry (Release 1) [156]. Individuals who were > 6 SDs from the PC1/PC2 centroid were excluded, so our sample was of exclusively European ancestry. To ensure SNPs were imputed with high data quality, SNPs with a call rate < 90%, MAF < 0.05, imputation score < 0.3, and Hardy–Weinberg equilibrium score of $P < 10^{-6}$ were excluded. Approximately 4.4 million SNPs met these criteria and were used in the analyses.

Genome-wide Association Analysis

Univariate and bivariate GWAS were conducted using GEMMA [76], which fits a linear mixed model for each SNP and uses the genetic relatedness matrix to account for the family structure. Covariates included age, sex, a history of ear infection, all of which were shown to be associated with taste intensity ratings [107], and the first five PCs calculated from the genotypes. Bivariate analysis essentially provides a complement to univariate analysis. It can enhance the strength of a SNP association, but the estimated effect on each of the two traits remains. For non-pleiotropic SNPs identified in bivariate analysis, we tested for their associations using conditional analysis of the associated trait conditional on the non-associated trait. When two identified SNPs were correlated, to test whether they were independent signals for the corresponding traits, we performed conditional analyses, by fitting each of the SNPs as an extra covariate. Prior to analyses, intensity ratings for each stimulus were square root transformed to obtain a more normal distribution [107] and then converted to Z-scores. A genome-wide significance threshold was defined as P < 5.0e-8. As four of the phenotypes were correlated (r_p between quinine, caffeine, SOA and DB = 0.58 – 0.64; Chapter 2) the number of independent tests was estimated using a matrix spectral decomposition algorithm [157] at 4.96 and

accordingly a Bonferroni-corrected threshold was defined as P < 1.0e-8. The genomic inflation factor (λ) ranged between 0.99 and 1.02 (Supplementary Figures 3-4 and 3-5), which indicates that potential technical or population stratification artefacts had a negligible impact on the results. As all association analyses were performed under an additive model and all phenotypes were converted to Z-scores, variance explained by a SNP was calculated as $2 \times MAF \times (1 - MAF) \times \beta^2$. Manhattan and Q-Q plots were created using the "fastman" package [158] in R. Regional association plots were created using Locuszoom [159].

Functional annotation of the identified SNPs

To examine the potential role of the identified SNPs, we used Haploreg v4.1 [160] for functional annotation. Briefly, it annotates all index SNPs and their correlated SNPs (r^2 was set to be ≥ 0.8 for this study) by their associated chromatin states (e.g., conserved regions and DNAse hypersensitivity sites) from the Roadmap epigenomics project [161] and Encode project [162] and their effects on regulatory motifs. It also reports the effect of SNPs on gene expression in multiple tissues from eQTL (expression quantitative trait loci) studies, including results from the GTEx [163] project portal. Use of functional annotation provides more information about the putative role of a specific gene as well as developing mechanistic hypotheses of the impact of the SNP on phenotypes (e.g. variation in taste perception). More details are provided in Supplementary Table 3-3.

Ethical Statement

The Queensland Institute of Medical Research Human Research Ethics Committee approved the study. Written informed consent was obtained from both the participants and their parents (the latter not required for those 18 years and over) before participation. All methods were performed in accordance with the relevant guidelines and regulations.

Results

We confirmed two previously identified associations with large effects on PROP and quinine, provided the first independent replication of an association for caffeine, and revealed two new associations with small effects (< 2%) on SOA and DB (Table 3-1). In addition, we found evidence for pleiotropic effects on quinine, caffeine, SOA and DB.

Table 3-1. Genetic variants associated with human bitter taste perception.

Trait 1	SNP	Chr:Position	A1/A2	MAF	β	SE	r^2	P	Trait 2			
									Quinine	Caffeine	SOA	DB
									P_bivariate			
Quinine	rs10772420	12:11174276	G/A	0.469	-0.337	0.034	5.67%	**7.8e-23***	-	**4.8e-65***	**1.8e-24***	**6.4e-26***
Caffeine	rs2597979†	12:11189966	G/C	0.163	0.264	0.048	1.91%	**4.2e-8**	**8.4e-24***	-	**2.8e-10***	**4.5e-11***
SOA	rs67487380	12:11194384	A/G	0.275	-0.202	0.040	1.63%	3.8e-7	**5.4e-13***	**4.5e-8**	-	2.4e-6
DB	rs10261515	7:141398707	G/A	0.491	-0.136	0.037	0.93%	2.5e-4	**3.1e-8**	4.0e-6	5.6e-4	-
PROP solution	rs10246939	7:141672604	C/T	0.443	0.968	0.028	46.20%	**2.8e-199***				
PROP paper	rs10246939	7:141672604	C/T	0.441	0.534	0.032	14.08%[a]	**5.4e-59***				
PROP paper	rs6761655‡	2:218218646	G/A	0.186	-0.246	0.044	1.83%	**2.7e-8**				

We report the top SNP from the peak association. SNPs that were not identified in our previous GWAS are underlined. Allele frequency and effect sizes are reported with reference to allele A1. Base-pair position is based on GRCh37; A1/A2, minor/major allele; MAF, minor allele frequency; β, the effect size; SE, standard error of the β; r^2, percent variance of the trait accounted for by the SNP; P, P-value from the univariate association analysis of trait 1; P_bivariate, P-value from the bivariate association analysis of traits 1 and 2; SOA, sucrose octaacetate; DB, denatonium benzoate; **bold**, P < genome-wide significance threshold of 5.0e-8; *, P < corrected significance threshold of 1.0e-8; †, an independent replication; ‡, no evidence of replication. See Supplementary Tables 3-1 – 3-7 for the full list of SNPs.

[a] rs10246939 accounted only a third of the variance in PROP paper compared to PROP solution. This was partly due to the lower heritability of PROP paper (h^2 = 0.40) compared to PROP solution (h^2 = 0.71, Supplementary Table 3-8).

Table 3-2. Conditional analyses of correlated SNPs on chromosome 12 associated with the perception of quinine, caffeine and sucrose octaacetate (SOA).

Trait	SNP	Association (P-value)	Association conditional on correlated SNP (P-value)		
			rs10772420	rs2597979	rs67487380
Quinine	**rs10772420**	7.8e-23	-	3.0e-19	1.5e-10
	rs2597979	4.3e-3	4.4e-2	-	-
	rs67487380	1.5e-13	0.12	-	-
Caffeine	rs10772420	2.5e-3	-	0.38	-
	rs2597979	4.2e-8	4.4e-6	-	9.7e-8
	rs67487380	0.11	-	0.47	-
SOA	rs10772420	1.0e-4	-	-	0.47
	rs2597979	0.38	-	-	0.63
	rs67487380	3.8e-7	7.6e-4	5.3e-7	-

r^2 = 0.24 between rs10772420 and rs2597979; r^2 = 0.43 between rs10772420 and rs67487380; r^2 = 0.08 between rs2597979 and rs67487380. SNP in bold in the second column are index SNPs for the corresponding traits from Table 3-1.

Confirmation of the locus on chromosome 12 influencing quinine and pleiotropic effects

The peak association for quinine was a missense variant within the bitter taste receptor gene *TAS2R19* on chromosome 12 (rs10772420, Figure 3-2a). As expected, with the increase in sample size the association was stronger (P = 7.8e-23) than that found in the initial GWAS (P = 1.8-e15) [37], and the peak SNP explained almost the same amount of variance (5.67%). In the bivariate analysis, which included caffeine, there was a further boost in signal (P = 4.8e-65, Table 3-1). This was due to the nominal association of caffeine with rs10772420 (P = 2.5e-3; Figure 3-2b) and the effect of the minor allele being in the opposite direction to quinine (i.e. decrease in caffeine versus increase in quinine perception), which provided greater separation of the rs10772420 genotypes in two-dimensional space (as illustrated in Figure 3-1b). A much smaller increase in the quinine signal was found in the bivariate analysis with SOA (P = 1.8e-24) and DB (P = 6.4e-26). Both compounds (SOA: P = 1.0e-4; DB: P = 2.8e-3) were nominally associated with rs10772420 (Figure 3-2c and d), but the effect of the minor allele was in the same direction as that for quinine, resulting in little/no further separation of the genotypes in two-dimensional space (as illustrated in Figure 3-1c). Notably the size and direction of the effect of rs10772420 on the four bitter substances varied (Supplementary Figure 3-1; Supplementary Table 3-9): the strongest effect was on quinine (β= -0.337; 5.67% of the variance or 12.32% of the

genetic variance), with a smaller fraction of the variance being explained for caffeine (β = 0.107; 0.57/1.24% of the total/genetic variance), SOA (β = -0.137; 0.94/2.04% of the total/genetic variance) and DB (β = -0.106; 0.56/1.22% of the total/genetic variance). In Figure 3-3 we show that variants with the largest effect on quinine - a cluster of 263 SNPs - were also associated with SOA, caffeine and DB, and that this cluster was separate to the top SNPs for SOA (a cluster of 167 SNPs) and caffeine (a cluster of 116 SNPs).

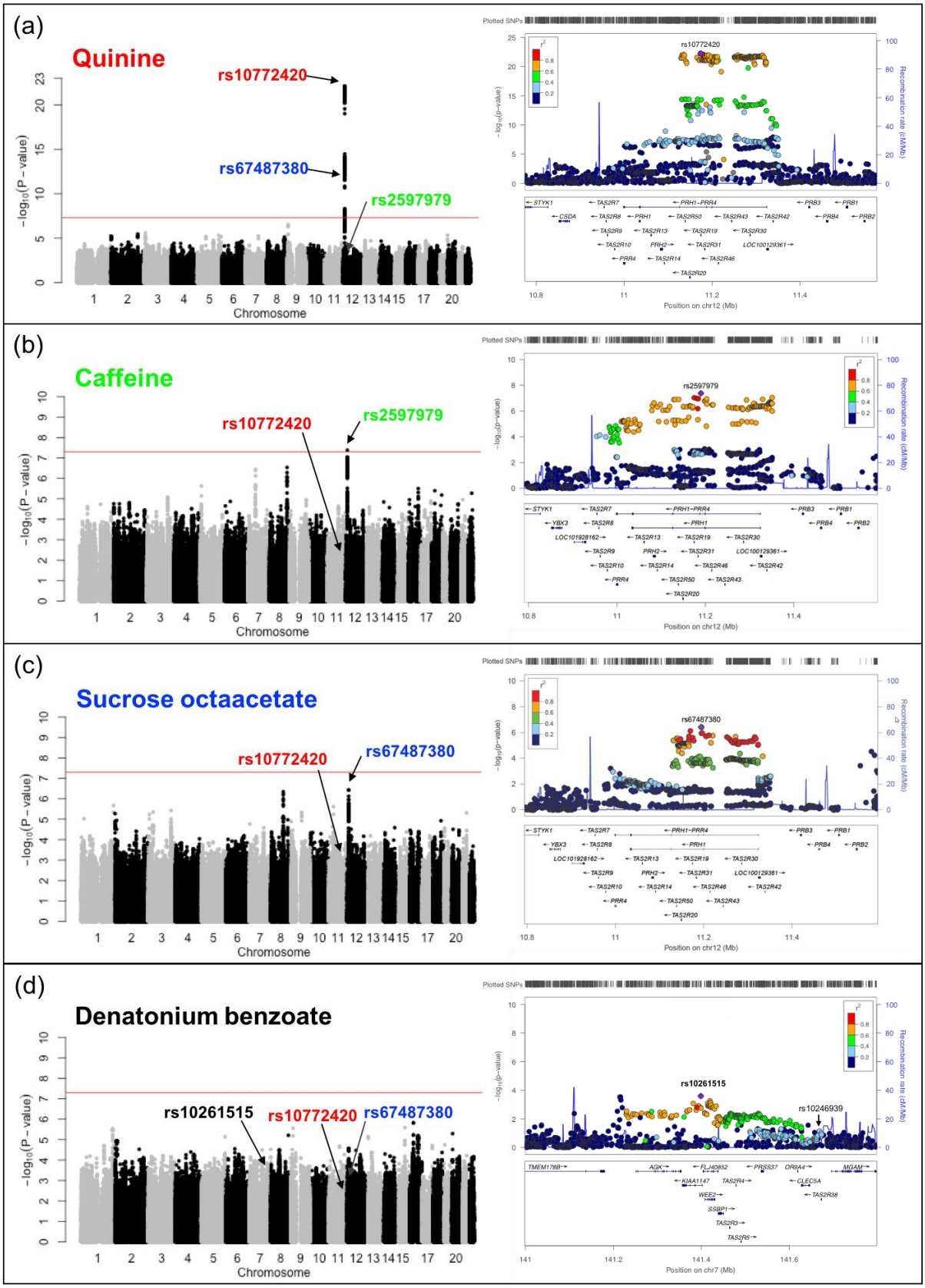

Figure 3-2. Common variants associated with the perception of bitter taste: (a) quinine, (b) caffeine, (c) sucrose octaacetate, and (d) denatonium benzoate (n = 1757). The left half of the figure shows the Manhattan plots, displaying the association P-value for each SNP in the genome (displayed as –log10 of the P-

value). The red line indicates the genome-wide significance threshold of P = 5.0e-8. rs10772420 (labelled in red), rs2597979 (labelled in green), and rs67487380 (labelled in blue) are the most significant SNP within a putative or associated locus for quinine, caffeine, and sucrose octaacetate, respectively. rs10261515 is labelled in (d) because it reaches genome-wide significance in the bivariate analysis (Table 3-1 and Figure 3-4). The right half of the figure shows regional plots ± 400kb for the top SNPs on chromosomes 12 (a, b, and c) and 7 (d) with gene model below. Plots are zoomed to highlight the genomic region that likely harbors the causal variant. The top SNP for PROP (rs10246939) is also labelled in the regional plot in (d).

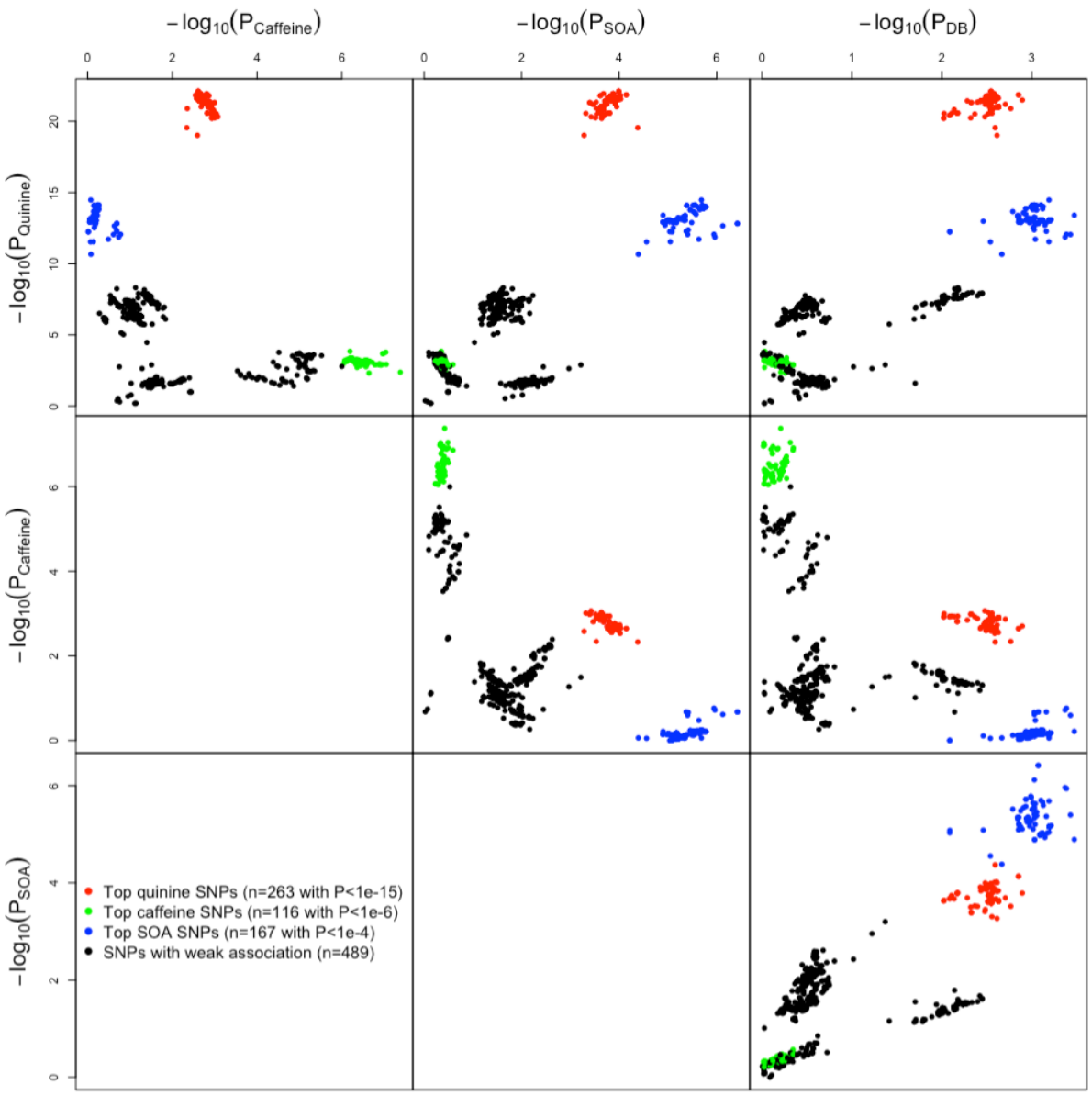

Figure 3-3. Top SNP associations on chromosome 12 for perceived intensity of quinine, sucrose octaacetate (SOA), caffeine and denatonium benzoate (DB). The red, blue and green clusters represent the top SNP associations with quinine, SOA and caffeine respectively. The top SNPs for these three bitter compounds are clustered separately from one another, even though the lead SNPs (rs10772420 for quinine; rs2597979 for caffeine; rs67487380 for SOA) of each cluster are correlated ($r^2_{rs10772420\text{-}rs2597979}$ = 0.24; $r^2_{rs10772420\text{-}rs67487380}$ = 0.43; $r^2_{rs2597979\text{-}rs67487380}$ = 0.08). The top SNPs for DB in this genomic region overlap with the tops SNPs for SOA, but the strengths of the associations with DB are weaker. In addition, there is evidence of pleiotropy. The red cluster is strongly associated with quinine, and more weakly associated with caffeine, SOA and DB; the blue cluster is associated with quinine, SOA and DB; the green cluster is associated with quinine and caffeine. A total of 1035 SNPs on chromosome 12 between 10950000 and 113550000 base pairs are plotted here.

Independent replication of a SNP association on chromosome 12 for caffeine

For caffeine perception, we identified a peak association on chromosome 12 (rs2597979, P = 4.2e-8; Figure 3-2b), which accounted for a maximum trait variance of 1.91%. This SNP was in high linkage disequilibrium with that identified in a previous GWAS for caffeine detection threshold [38] (r^2 = 0.84 with rs2708377), and therefore we provided the first independent replication for this association. Further support was provided by our bivariate caffeine-quinine analysis (P = 8.4e-24). The enhancement in signal due to quinine also being associated with rs2597979 (P = 4.3e-3), with the effect in the opposite direction to caffeine (Supplementary Figure 3-1). Since the lead SNPs for caffeine (rs2597979) and quinine (rs10772420) were weakly correlated (r^2 = 0.24), we tested whether the associations could be driven by the same SNP using conditional analysis, where each of the genotypes are included as a covariate. The caffeine-rs2597979 association remained (P = 4.4e-6; Table 3-2) after conditioning on the lead SNP for quinine, whereas the caffeine-rs10772420 association disappeared (P = 0.38) after conditioning on rs2597979, indicating that the caffeine-rs2597979 association was not driven by rs10772420. For quinine, the results of the conditional analysis were less clear. While the quinine-rs10772420 association remained highly significant after conditioning on the lead SNP for caffeine (P = 3.0e-19), a weak quinine-rs2597979 association remained after conditioning on rs10772420 (P = 0.044). Figure 3-3 shows that the top caffeine SNPs are weakly associated with quinine and largely independent from the top quinine SNPs.

In contrast to quinine, we found little evidence for an association of either SOA or DB with rs2597979 (SOA: P = 0.38; DB: P = 0.62). The small boost in the caffeine-rs2597979 association found in the bivariate analysis (caffeine and SOA: P = 2.8e-10; caffeine and DB: P = 4.5e-11) was likely due to the correlation between the traits, which was supported by the boost in the caffeine-rs2597979 association when the intensity ratings for SOA (P = 5.9e-11) or DB (P = 7.9e-12) were included as a covariate. Figure 3-3 shows that the caffeine-associated SNPs are largely independent from SOA/DB-associated SNPs in this genomic region of chromosome 12.

Putative novel associations identified in bivariate analyses influencing SOA and DB

The strongest association for SOA was found on chromosome 12 (rs67487380, P = 3.8e-7; Figure 3-2c). This SNP was also associated with quinine (P = 1.5e-13; Table 3-2, Figure 3-2a) and DB (P = 8.5e-4), with the size and direction of the effect being similar to that for SOA (Supplementary Figure 3-1), so that the boost in signal found in the bivariate SOA-quinine analysis (P = 5.4e-13; Table 3-1) was likely due to quinine. Even so, we found that the SOA-rs67487380 association remained when we conditioned on the lead SNP for quinine (P = 7.6e-4, Table 3-2), which is moderately correlated with rs67487380 (r^2 = 0.43), whereas the SOA-rs10772420 association was lost (P = 0.47) when rs67487380 was included as a covariate. Similarly, for quinine, the rs10772420 association remained after conditioning on the lead SOA SNP (P = 1.5e-10), but the quinine-rs67487380 association disappeared (P = 0.12, Table 3-2), after conditioning on the lead quinine SNP. These conditional analysis results indicated that each of lead SNPs for SOA and quinine represents the main signal for its corresponding taste. Figure 3-3 clearly shows that the top SNPs for SOA and quinine are clustered separately from each other, whereas the top SNPs for DB in the genomic region on chromosome 12 largely overlap with the top SNPs for SOA.

In contrast to quinine and DB, caffeine was not associated with the lead SOA SNP (P = 0.11; Table 3-2). A small boost in signal in the bivariate SOA-caffeine analysis (P = 4.5e-8) was largely due to the correlation between SOA and caffeine. Further, the SOA-rs67487380 association remained after conditioning on the intensity rating for caffeine (P = 1.0e-8), indicating that the covariance between SOA and caffeine was not due to this SNP. Figure 3-3 shows that the top SNPs for SOA and caffeine are largely separated and this is because their lead SNPs are only subtly correlated (r^2 = 0.08 between rs67487380 and rs2597979).

For DB, while all SNP associations had a P-value > 1.0e-6 (Figure 3-2d), one association on chromosome 7 (P = 2.5e-4) was boosted in the bivariate DB-quinine analysis (rs10261515, P = 3.1e-8, Table 3-1, Figure 3-4). The bivariate signal was mainly driven by the SNP association with DB, as there was no evidence for an association between quinine and rs10261515 (P = 0.15), and the DB signal was boosted after conditioning on the intensity score for quinine (P-value changed from

2.5e-4 to 1.9e-8). There was no evidence that this DB-associated SNP was associated with caffeine (P = 0.81) or SOA (P = 0.15), and little evidence of a signal boost in either the DB-caffeine (P = 4.0e-6) or DB-SOA (P = 5.6e-4) bivariate analyses (Table 3-1).

The SNP rs10261515 is located within *KIAA1147* on chromosome 7, nearby three bitter taste receptor genes *TAS2R3*, *TAS2R4* and *TAS2R5* (Figure 3-2d), and is 274 kb upstream of the PROP-associated SNP rs10246939, with which it is weakly correlated (r^2 = 0.23; Figure 3-2d). When we conditioned on the lead SNP for PROP, the DB-rs10261515 association remained (P = 9.0e-4), including after the additional adjustment for the quinine score (P = 1.7e-5).

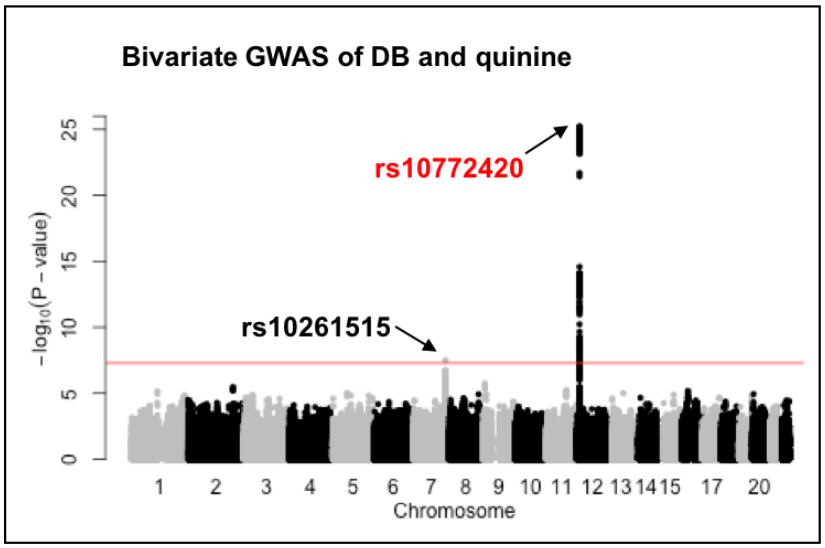

Figure 3-4. Manhattan plot showing a common variant (rs10261515) on chromosome 7 associated with the perception of denatonium benzoate (DB) based on the Bivariate GWAS of DB and quinine (n = 1757). The signal on chromosome 7 is driven by DB (P = 2.5e-4 in the univariate analysis) not quinine (P = 0.15). The signal on chromosome 12 is mainly due to the association of rs10772420 with quinine rather than DB as shown in Figures 3-2a and d. The red line indicates the genome-wide significance threshold of P = 5.0e-8.

Confirmation of previously identified locus on chromosome 7 influencing PROP

The peak association for PROP was the well-known missense variant rs10246939 within the bitter taste receptor gene *TAS2R38* on chromosome 7 (Table 3-1, Supplementary Figure 3-2), confirming our previous findings [37]. For PROP

paper, we identified a secondary locus within the *DIRC3* gene on chromosome 2 (rs6761655 and its completely correlated SNP rs6736242 [r^2 = 1.0], P = 2.7e-8, Supplementary Figure 3-2b). This SNP accounted for a maximum trait variance of 1.83% in PROP paper and showed a weaker but nominally significant association with the perception of PROP solution (P = 7.4e-4). We note that this signal was present in the previous GWAS [37] (Supplementary Figure 3-3), but was less obvious (i.e. not a solid peak as there were fewer [2.3 million] SNPs used in the earlier GWAS) and therefore was not reported. However, we found no evidence for this association with PROP perception in one previously reported GWAS of 225 Brazilians [145], as well as two unpublished GWAS, one of ~500 individuals from the Silk Road population and one of ~2500 Italians (Supplementary Table 3-10). We further searched for this association in an earlier linkage study [164], which prepared PROP paper in the exact same way as the present study, but the closest marker was ~500kb away from rs6761655 and it was not associated.

Functional annotation of the identified SNPs

We performed functional analysis (i.e. the SNP effect on gene expression and DNA methylation) for five of the six SNPs in Table 3-1 using the bioinformatics tool Haploreg [160]. We did not include rs6761655 here due to lack of replication in the independent datasets. We also searched for bitter taste receptors that have been shown to respond to these bitter substances in human cell-based functional studies [165, 166]. The key results are presented in Table 3-3 and a summary of the functional analysis can be found in Supplementary Table 3-11.

The SNPs for quinine (rs10772420) and PROP (rs10246939) are missense variants within *TAS2R19* and *TAS2R38* respectively. In addition, the caffeine-associated SNP (rs2597979) is highly correlated with a missense variant rs10743938 (r^2 = 0.92) within *TAS2R31*. This SNP has two possible allele changes of T>A and T>G, leading to residue changes of Leu162Met and Leu162Val respectively. In the present study, only rs10743938:T>A passed quality control and its association with caffeine had a P-value of 1.1e-7 (Supplementary Table 3-2).

Further, the SNPs for quinine, caffeine, and SOA are common expression quantitative loci (eQTL) for five bitter taste receptor genes (*TAS2R14*, *TAS2R20*, *TAS2R31*, *TAS2R43*, *TAS2R64P*) on chromosome 12, and the expression of other bitter taste receptors in the same region is regulated by one or two of these three

SNPs, e.g. the expression of *TAS2R46* is only regulated by the SOA and quinine associated SNP rs67487380. The DB-associated SNP rs10261515 influences the expression of the bitter taste receptor genes, *TAS2R4* and *TAS2R5*, on chromosome 7. T2R4 is more likely to a receptor for DB because the allele (rs10261515 G allele) for weaker DB intensity rating is associated with a lower expression level of *TAS2R4* and the opposite (higher) for *TAS2R5*. In addition, DB can activate T2R4 but T2R5 in cell-based functional analysis. Results from the cell-based functional analysis do not necessarily agree with the results from the bioinformatics functional analysis. For example, the quinine-associated SNP rs10772420 is a missense variant within *TAS2R19* and it regulates both gene expression and DNA methylation of *TAS2R19*, but T2R19 does not respond to quinine. We note that neither of these bioinformatics and cell-based functional analyses were based on human taste tissues.

Table 3-3. Bioinformatics and cell-based functional studies of the genetic variants associated with bitter taste perception.

Trait	Index SNP	GENCODE genes	Non-synonymous SNPs in LD ($r^2 \geq 0.8$) with index SNP	eQTL[a]	mQTL[b]	Cell-based functional analysis[c]
Quinine	rs10772420	*TAS2R19*	rs10772420 in *TAS2R19*	*TAS2R10*, *TAS2R14*, *TAS2R19*, *TAS2R20*, *TAS2R31*, *TAS2R43*, *TAS2R50*, *TAS2R64P*	*TAS2R19*	T2R4, T2R7, T2R10, T2R14, T2R31, T2R39, T2R40, T2R43, T2R46
Caffeine	rs2597979	*PRR4*, *TAS2R31*	rs10743938 in *TAS2R31*	*TAS2R14*, *TAS2R15*, *TAS2R20*, *TAS2R31*, *TAS2R43*, *TAS2R45*, *TAS2R64P*	*TAS2R19*, *TAS2R50*	T2R7, T2R10, T2R14, T2R43, T2R46
SOA	rs67487380	*PRR4*		*TAS2R10*, *TAS2R12*, *TAS2R14*, *TAS2R15*, *TAS2R19*, *TAS2R20*, *TAS2R31*, *TAS2R43*, *TAS2R46*, *TAS2R64P*		T2R46
DB	rs10261515	*KIAA1147*		*TAS2R4*, *TAS2R5*		T2R4, T2R8, T2R10, T2R13, T2R30, T2R39, T2R43, T2R46
PROP	rs10246939	*MGAM*, *TAS2R38*	rs713598, rs1726866 and rs10246939 in *TAS2R38*	*TAS2R5*, *TAS2R38*		T2R4, T2R38

[a] The genotype of the index SNP and/or correlated SNPs ($r^2 \geq 0.8$) is associated with the expression of these bitter taste receptor genes.
[b] The genotype of the index SNP and/or correlated SNPs ($r^2 \geq 0.8$) is associated with the methylation of DNA fragments within these bitter taste receptor genes.
[c] Bitter taste receptors shown to respond to bitter substances in cell-based functional analysis using human embryotic kidney cells [165, 166].

Discussion

In this study of bivariate GWAS on human taste perception, we identify two putative novel associations, including rs67487380 on chromosome 12 for SOA-elicited bitter taste and rs10261515 on chromosome 7 for DB-elicited bitter taste. In addition, we provide the first independent replication of an association on chromosome 12 for caffeine bitterness (rs2597979) and confirm the previously reported associations for quinine bitterness (rs10772420 on chromosome 12) and PROP bitterness (rs10246939 on chromosome 7). All variants are located within the bitter taste receptor clusters on chromosomes 7 and 12, highlighting the importance of these two regions in the genetics of bitter taste. Further, we show evidence of pleiotropy for those variants on chromosome 12 and the functional importance of the DB-associated SNP.

This is the first GWAS study to identify a SNP (rs67487380 on chromosome 12) association with human perception of SOA. In mice, a major locus for SOA perception (*soa*) was reported in the early 1990s [167]. Interestingly, the mouse *soa* locus also affects the perception of other bitter substances, including quinine, DB, PROP, but not caffeine [168, 169]. Here we show that rs67487380 is also associated with the perception of quinine and DB, but not caffeine or PROP ($P > 0.05$). SOA activates human T2R46 but no other T2Rs in heterologous expression assays [166]. It is possible that rs67487380 regulates the perception of SOA through its effect on mRNA expression because the G allele for weaker SOA intensity is also associated with a lower expression level of *TAS2R46*. Nevertheless, rs67487380 could still be a proxy for true causal variants.

The finding of the novel association between DB and the SNP rs10261515 suggests that there may be a second locus on chromosome 7 that affects human bitter taste perception (the first is the locus within *TAS2R38* for PROP). Heterologous expression studies using human embryotic kidney (HEK) cells transfected with *TAS2Rs* have shown that DB activates T2R4 but no other bitter taste receptors in this region (e.g. T2R3, T2R5, and T2R38) [165]. In addition, the human T2R4 is the ortholog of mouse T2R8, which also responds to DB [141]. Our functional annotation results provide further support for T2R4 as a DB bitter taste receptor, since the allele (rs10261515 G allele) for a lower perceived intensity of DB is associated with lower expression level of *TAS2R4* mRNA.

The SNP association for caffeine perception replicated a previous GWAS of 608 Brazilian adults [38]. In that study the lead SNP accounted for 8.9% of the variance in caffeine sensitivity, compared with our estimate of 1.9%. Similarly, the quinine-associated SNP accounted for 23.2% of the variance in quinine sensitivity in the Brazilian study, which is four times of the effect estimated here. This difference in effect sizes is likely due to two main factors. First, the taste scores in the Brazilian sample were corrected for overall-taste-sensitivity (an average score of the perception of sweet, umami, sour, salty and bitter compounds), which removed ~30% of the variance in the perception of caffeine and quinine. Without correction, rs10772420 accounted for 13.2% of the variance in quinine, and the caffeine association was not detected due to low power. Second, the Brazilian study used a detection threshold approach, which measures overall oral sensitivity, compared with our measure of bitter taste intensity. Regardless, both studies identified the same variants for caffeine, quinine as well as PROP, indicating that these are likely to be valid associations among human bitter taste perception and these T2R-rich regions of chromosomes 7 and 12.

The functional annotation of the caffeine-associated SNP showed that the highly correlated SNP (rs10743938) is a missense mutation that could affect the function of T2R31. Although this is the first evidence linking this bitter taste receptor to the perception of caffeine, genetic variants in *TAS2R31* have been shown to affect the perception of quinine [170] and acesulfame potassium [171] (a non-nutritive sweetener with bitter aftertaste). Prior cell-based functional studies [165] reported that caffeine does not activate T2R31 in heterologous expression assays; rather, it activates T2R7, -10, -14, -43, and -46, and that the summed expression level of these activated T2Rs increases with the perceived intensity of caffeine [50]. However, comparing results from bioinformatics and cell-based analyses can be limited by two major factors. Here, we report associations for the index (lead) SNP with the lowest P-value, but since this SNP is in a linkage disequilibrium block, the association could be driven by any variant within the block. Second, these cell-based functional assays [165] were conducted in heterologous systems (i.e. HEK cells transfected with *TAS2Rs*), which may not always recapitulate human sensory experience faithfully [172]. We observed a similar difference for quinine, with the lead quinine-associated SNP rs10772420 constituting a missense mutation in *TAS2R19*. Yet T2R19 does not respond to quinine in functional expression assays [165]. Therefore, a better method

to identify causal SNPs for the foreseeable future is to tightly integrate genetic-perceptual association results with those of taste receptor cell-based assays using human taste tissues, such as taste buds or cultured human taste cells [173].

This study provides the first evidence for antagonistic genetic pleiotropy in bitter taste. The two SNPs rs10772420 and rs2597979 have opposite effects on the perceived intensity of quinine and caffeine (Supplementary Figure 3-1; Supplementary Table 3-3) and this largely enhances the strengths of their associations (P-value) in the bivariate analysis (Figure 3-1b). As bitter-tasting substances (e.g. caffeine [174]) can have both beneficial and detrimental effects, the antagonistic pleiotropy may be an evolutionary consequence that avoids over and under consumption.

The top SNPs for quinine, caffeine, and SOA were correlated (r^2 = 0.08 – 0.43) and each could have various effects on one another. These correlations are due to the linkage disequilibrium between polymorphisms within bitter taste receptor genes on chromosome 12, which results in common haplotypes for nearby genes and long-range haplotypes for more distant ones [175, 176]. Previous studies have revealed a complex bitter substance – receptor relationship, with one bitter compound activating multiple T2Rs and one T2R responding to multiple bitter substances [165, 166, 177]. Taken together, it is likely that the perception of a bitter taste can be mediated by multiple T2Rs, and SNPs identified in the present study could represent haplotypes that regulate several T2Rs together. We have attempted to illustrate this in Figure 3-5 by taking the perception of quinine and caffeine as an example. The lead SNP for quinine (rs10772420) is correlated with several SNPs (the regional association plot in Figure 3-2a) that regulate the T2Rs for quinine (cell-based functional analysis results in Table 3-3). Also, the lead SNP for caffeine (rs2597979) is correlated with SNPs (Figure 3-2b) that regulate T2Rs for caffeine (Table 3-3). In addition, the common T2Rs for the two tastes are regulated by SNPs that are in linkage disequilibrium with the two lead SNPs. We note that the real regulatory network can be more complex than this, such that one T2R can be regulated by multiple SNPs. Whereas we used conditional analysis (Table 3-2) and plotted the SNP associations against the three tastes (Figure 3-3) to show that each of the lead SNPs represents the main signal in the linkage disequilibrium block, the clusters of nearby bitter receptors and many variants in high linkage disequilibrium create challenges in separating causal from non-causal variants.

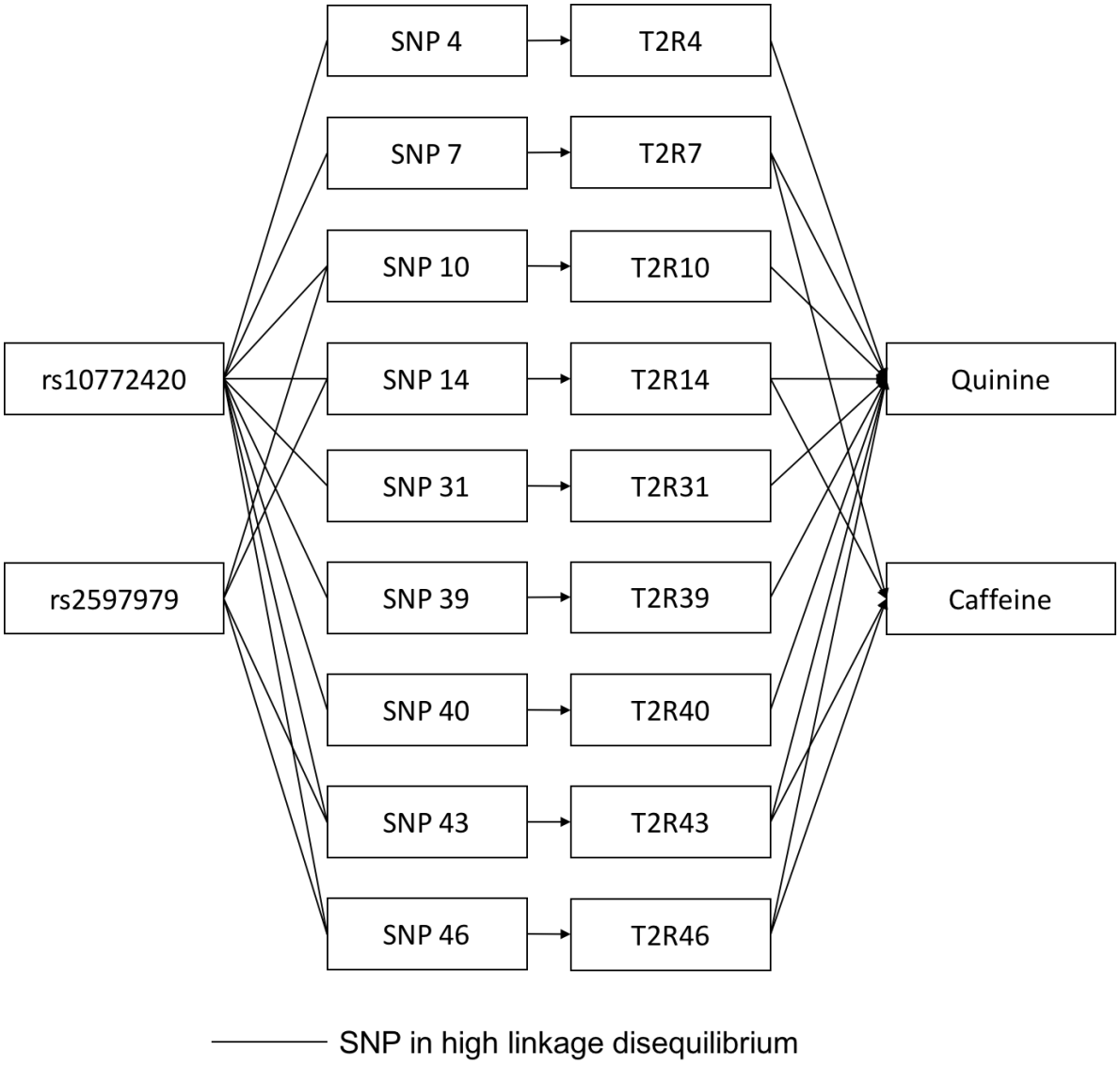

Figure 3-5. Potential model of the SNP regulation of human bitter taste perception. Quinine can be detected by bitter taste receptors T2R4, -7, -10, -14, -31, -39, -40, -43, and -46 on chromosome 12, and caffeine can be detected by the T2R7, -14, -43, and -46 (as summarized in Table 3-3), which overlap the T2Rs for quinine. Here we assume that each T2R is regulated by a major SNP with the corresponding number. rs10772420 is associated with the perception of quinine via its correlated SNPs; rs2597979 is associated with the perception of caffeine via its correlated SNPs.

Perceptual studies of bitter taste also have reported that individual differences in perceived bitterness from multiple compounds show positive correlations. Most relevant to the present work, past studies demonstrated a strong correlation of perceived bitter taste intensities among DB, SOA, and quinine [153]. This observation harkens to that reported in the present study for rs67487380 on

chromosome 12. Furthermore, individual differences in bitterness from SOA, caffeine and quinine were also observed, suggesting a linkage between SOA receptor variants and caffeine receptor variants [153]. This too reflects associations observed in the present data set. Perhaps, a linkage disequilibrium block accounts both for the genetic architecture as well as the bitterness perception associations.

Prior work using pedigree segregation analysis has proposed that the perception of PTC (a structurally related chemical to PROP) is modulated by a 2-locus model [135], but the location of a second locus has been unclear for nearly 30 years. Here we found no support for an association with *TAS2R1*, which was suggested by a prior family-based linkage study [164], but identified a putative secondary locus within the *DIRC3* gene on chromosome 2, which accounted for an additional 1.83% of the variance (4.58% of the genetic variance) in the perception of PROP paper. While we found no evidence for replication using three independent datasets – from one published study (i.e. the Brazilian sample) and two unpublished (the Silk Road and the Italian samples), we note that there are considerable differences across studies (e.g., sample age [all other studies used adults], ethnicity, and delivery method [the Brazilian study used PROP solution]), which may have influenced our ability to replicate their findings. We did a further search for this association using available data from an early linkage study [164], which used the same PROP paper, but were severely limited by the sparsity of markers, as none were close to the SNP identified in the present study. Ideally, we need to test for this association using the same methods and materials (i.e. the perceived intensity of saturated PROP paper measured from adolescents with European ancestry), but at this stage the signal does not appear to be sufficiently robust to be detected with alternative methods.

The strengths of the present study include the use of the largest-to-date sample with multiple taste phenotypes from the same individuals, which increases the statistical power via bivariate association analysis. We show that the association signals (P-value) for quinine and caffeine (rs10772420 and rs2597979 respectively) were stronger in the bivariate compared with the univariate analysis, but the estimated effect size remains the same. The signal boosts in these already established associations serve as a proof of principle for using bivariate GWAS. We also show that, through the discovery of the association of DB, a signal can be enhanced when only one of two correlated traits is associated. This is useful for

identifying non-pleiotropic SNPs for correlated phenotypes. We used multiple levels of analysis (conditional on genotype and phenotype) as well as cluster plots to disentangle the pleiotropic nature of these SNPs with bitter tastes and provide additional support for the signals identified in the bivariate analyses. We attempted to obtain data to replicate every novel association. However, we were unable to test the association for SOA and DB, due to no other datasets being available. Given the enhancement in the known signals for both quinine and caffeine in the bivariate analyses, together with the post-hoc bioinformatics analyses, as well as prior functional analyses, we believe the SOA and DB hits are unlikely to be false positives. Further, findings from multivariate GWAS of other phenotypes, e.g. levels of plasma lipids [178], have been replicated in independent studies. The variants for SOA and DB account for less than 10% of the genetic variance (< 2% of trait variance) of their associated traits, suggesting that there are more variants with smaller effects. The remaining genetic variance could be partly due to rare variants because SNPs with an MAF smaller than 5% were excluded here and rare variants can have a large effect on complex traits [179].

In conclusion, this study reveals the influence of multiple variants on bitter taste and demonstrates the benefits of multivariate analysis over the conventional univariate GWAS. Recent advancement in the methodology of multivariate GWAS (i.e. MTAG [180]) could make multivariate analysis easier to apply because it uses individual summary level results from different studies and does not require correlated phenotypes to be collected from the same sample. Whereas our previous twin analysis (Chapter 2) provided strong evidence of pleiotropy for the perception of several bitter compounds (except for PROP), there are numerous causal models that could underlie this shared genetic aetiology. Identification of specific SNPs/genes involved offers a useful starting point for determining the biological pathways linking perception of bitter substances and for delineating of the mechanisms involved. Future studies integrating bioinformatics and functional analyses using human taste tissues will provide stronger evidence in identifying true causal variants, which could assist personalized nutrition and precision medicine.

4

Understanding the Role of Bitter Taste Perception in Coffee, Tea and Alcohol Consumption through Mendelian Randomization

Chapter modified from a manuscript submitted to Scientific Reports (June 2018). I presented the preliminary results at the Brisbane Life Scientist Symposium in November 2017 (see Supplementary Document for the abstract).

Chapter 4. Understanding the role of bitter taste perception in coffee, tea and alcohol consumption through Mendelian randomization

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Abstract

Coffee, tea and alcohol are widely consumed bitter beverages of high public health interest because they are implicated in various health conditions. Consumption behaviour of these beverages can be shaped by individual differences in bitter taste perception but findings from observational studies are unclear and provide no insight to causality. This study aimed to examine the causal relationship between perception of three bitter substances, propylthiouracil (PROP), quinine, and caffeine, and the consumption of coffee, tea, and alcohol. We applied a two-sample Mendelian randomization analyses. Genetic instruments for the perception of PROP (rs1726866), quinine (rs10772420) and caffeine (rs2597979) were obtained from a genome-wide association study of Australian Twins of European ancestry (sample 1; Chapter 3). These association of these variants with consumption of coffee, tea and alcohol were obtained from a population-based cohort study including up to 438,870 UK Biobank participants of white-British ancestry (sample 2). The results showed that, with every 2-standard deviation change in the predicted taste perception, a higher perceived intensity of caffeine increased coffee consumption by 0.076 (P = 1.6e-9) cups per day, whereas higher perceived intensities of PROP and quinine decreased coffee consumption by 0.034 (P = 7.2e-4) and 0.052 (P = 1.9e-7) cups per day. For tea consumption, an inverse relationship with each bitter taste was observed due to the negative correlation between coffee and tea intake. Higher perceived intensities of PROP and quinine increased tea consumption by 0.067 (P = 3.7e-7) and 0.059 (P = 2.1e-8) cups per day, and a higher perceived intensity of caffeine decreased coffee consumption by 0.094 (P = 2.1e-8) cups per day. For alcohol, only the perception of PROP was associated, with every 2-standard deviation increase in the predicted perceived intensity leading to a lower alcohol intake (P = 5.9e-10). We present strong evidence that bitter taste perception is causally associated with intake of coffee, tea and alcohol, with the patterns of causal relationships varying by bitter stimulus. These results provide insights into the development and prevention of their addictive consumption behaviour and consequential health outcomes.

Introduction

Coffee, tea and alcohol are widely consumed beverages with bitter taste that most humans naturally dislike at first tasting. They have been implicated in both

beneficial and adverse health effects [174, 181]. Individual differences in metabolizing caffeine [182, 183] and ethanol [184-188] present in these beverages may shape their consumption, whereas the influence of taste factors remain unclear.

The relationship between the perception of bitter compounds, such as propylthiouracil (PROP), quinine, and caffeine, and the consumption behaviour of these bitter beverages had been inconsistent across various studies [50-57, 189-191]. As performing taste tests is a time-consuming process, these investigations likely lacked sufficient power to convincingly rule out moderate effects due to sample size limitations, which complicates causal inferences. However, these issues can be overcome by recent advances in taste genetics and statistical genetic methods.

Heritabilities for the perceived intensity of PROP, quinine, and caffeine have been estimated to be 0.73, 0.40, and 0.36 in classical twin studies [41, 107]. Furthermore, candidate gene and genome-wide association studies (GWAS) have pinpointed precise genetic factors [37, 38], including SNPs in/near bitter taste receptor genes *TAS2R38* for PROP, *TAS2R19* for quinine, and *TAS2R31* for caffeine. Identification of these genetic variants enables Mendelian randomization analysis (MR), a technique commonly used in disease epidemiology, to make causal inferences of an exposure on outcomes of interest using genetic variants (i.e. SNPs) as instrumental variables. Its fundamental principle is based on Mendel's Law of Independent Assortment whereby genetic variants are shuffled at meiosis, conceptually mimicking a "natural" randomized trial [77, 192, 193]. Since genetic variants are randomized at birth at conception, they can be used as risk factor instruments that are generally free from confounding and reverse causality to evaluate the relationship of a risk factor on a specific outcome of interest [194, 195].

In this study, we investigate the causal relationship between bitter taste perception and the consumption of coffee, tea and alcohol through a MR framework. We use confirmed genetic markers for the perception of PROP (rs1726866), quinine (rs10772420) and caffeine (rs2597979) separately as genetic proxies for bitter taste perception and test their associations with the consumption of coffee, tea and alcohol among more than 400,000 participants in the UK Biobank cohort [78].

Methods

Study group description

The UK Biobank recruited 502,650 participants aged 37-73 years at 21 centres across England, Wales and Scotland in 2006-2010 [78]. This study was under generic approval from the UK National Health Service National Research Ethics Service. Written informed consent was obtained from participants.

Genetic data from UK Biobank

All UK Biobank participants have been genotyped using the Affymetrix UK BiLEVE Axiom array or Affymetrix UK Biobank Axiom® array comprising 805,426 markers in the official release. Imputations were performed using IMPUTE2 and UK10K haplotype and Haplotype Reference Consortium (HRC) reference panels, as described elsewhere [78]. For the current analysis, all SNPs of interest were genotyped or imputed with high quality (minimum INFO score of 0.95) based on the HRC reference panel samples. Our present analyses focused on the population of white-British ancestry, as determined by similarity of ancestral principal component values (PC1, PC2) to those who self-reported and were classified as white-British [78]. Using this criterion, we identified 438,870 individuals of white-British ancestry.

Genetic instruments for bitter taste

Genetic proxies for perceived intensity of PROP, quinine and caffeine were identified from our GWAS (Chapter 3), which used a subset of the Brisbane Adolescent Twin study (n = 1757; aged 12-25; 54% females; all European ancestry) [72]. Taste phenotypes were collected between 2003 and 2014. Participants were asked to rate the perceived intensity of 6.0×10^{-4} M PROP, 1.81×10^{-4} M quinine, and 0.05 M caffeine on a general labelled magnitude scale (gLMS). All three SNP-taste phenotype associations have also been confirmed in independent studies [35, 36, 38].

Each of the three taste-associated SNPs was genome-wide significant and explained an appreciable amount of trait variation (rs1726866 accounted for 46% variance in PROP, P = 5.6e-198; rs10772420 accounted for 6% of the variance in quinine, P = 7.8e-23; rs2597979 accounted for 2% of the variance in caffeine, P = 4.2e-8), rendering them suitable instruments for our MR analyses. All the SNPs satisfy the F-statistic > 10 criterion for being a strong instrument (Supplementary Table 4-1). In the GWAS, taste intensity ratings were square root transformed and then converted into Z-scores. Here, we assigned the effect allele (EA) to represent the allele associated with higher intensity ratings. The EA for rs1726866 (G allele),

rs10772420 (A allele), and rs2597979 (G allele) increased the intensity rating of PROP, quinine, and caffeine by 0.965, 0.337, and 0.264 standard deviations (SD), respectively.

Beverage consumption phenotypes

For bitter beverages consumption, we collated the following traits based on the touch-screen questionnaire completed by UK Biobank participants at the assessment centre. Amounts of coffee and tea consumption were quantified separately based on self-reported daily cups per day consumption, e.g. “How many cups of coffee do you drink each DAY? (including decaffeinated coffee)” and “How many cups of tea do you drink each DAY? (including black and green tea)”. For alcohol, participants were asked “About how often do you drink alcohol?” and to report on their drinking behaviour on a 6-point frequency scale (Never, Special occasions only, 1-3 times a month, 1-2 times a week, 3-4 times a week, Daily or almost daily). Participants who preferred not to answer were excluded from the analyses. For participants completing the assessment at multiple times (n > 1), we computed their average consumption.

To assess risks of heavy-consumption, we separated light/non-drinkers from heavy drinkers according to the lower quartile and upper quartile of the consumption distribution. For coffee, light/non-drinkers were classified as participants with < 2 cups per day and heavy drinkers as > 4 cups per day of coffee intake. The cut-offs were < 2 cups/day and > 5 cups/day for tea-drinking. For alcohol, non-drinkers were defined as individuals reporting no consumption of alcohol, while heavy-drinkers were individuals who consume more frequently than 3-4 times weekly.

Statistical Analysis for beverage consumption in UK Biobank

Associations between each bitter-taste SNP and bitter beverage consumption were estimated through linear and logistic additive models using BOLT-LMM [196] (linear) and *plink2* [197] (logistic). Covariates included age, sex and the first 10 principal components (PC). Using genetic PCs, we identified 438,870 Europeans of white-British ancestry, described elsewhere [198]. The use of BOLT-LMM allows the use of a linear mixed model to adjust for cryptic relatedness, hence maximizing power as related individuals can be retained in the analyses. However, BOLT-LMM was only formulated for quantitative traits; for binary traits, such as drinker status, the software *plink2* [197] was used after excluding related individuals.

Mendelian randomization analyses

We applied a two-sample MR approach [193]. The SNP-taste perception estimates were derived from our GWAS of taste perception (Chapter 3) while the SNP-beverage consumption association was derived from an independent sample, the UK Biobank. The MR estimate of the effect of bitter taste perception on consumption traits can be calculated using the wald-type ratio estimator [77, 199]. The causal magnitude of association can then be estimated through $\beta_{IVW} = \beta_{bev}/\beta_{bitter}$, where β_{bev} refers to the magnitude of association on quantity of bitter beverages for each perception-increasing allele and β_{bitter} refers to the magnitude of association for per-SD change in square-rooted perception score for each perception-decreasing allele. For the analyses on logistic traits (e.g. non- vs heavy-drinkers), the equivalent causal odds ratio can be estimated using log $OR_{IVW} = log\ OR_{drink\ status}/\beta_{bitter}$ where $log\ OR_{drink\ status}$ refers to the (log) increase in drinker risk per perception-decreasing allele. The standard errors of our MR estimates were derived using the delta method. Estimates were then scaled (multiplied by 2) to reflect a 2-SD change in perception score. All statistical analyses were performed using in-house scripts written in the statistical package R. As we investigated 3 different bitter stimuli against 3 different beverages of interest in our analyses, we conservatively set our Bonferroni-corrected P-value to be 0.00556 (0.05/9). We further assessed SNP associations with potential confounders through a look-up effort on publicly available GWAS summary results [200].

Results

Descriptive

Table 4-1 below summarizes the baseline characteristics including self-reported measurements of bitter beverage consumption among 438,870 individuals that were included in our analyses. The genotype frequencies for each of the bitter SNPs are shown in Supplementary Table 4-2.

Table 4-1. Baseline characteristics of 438,870 individuals from the UK Biobank cohort included in the analyses.

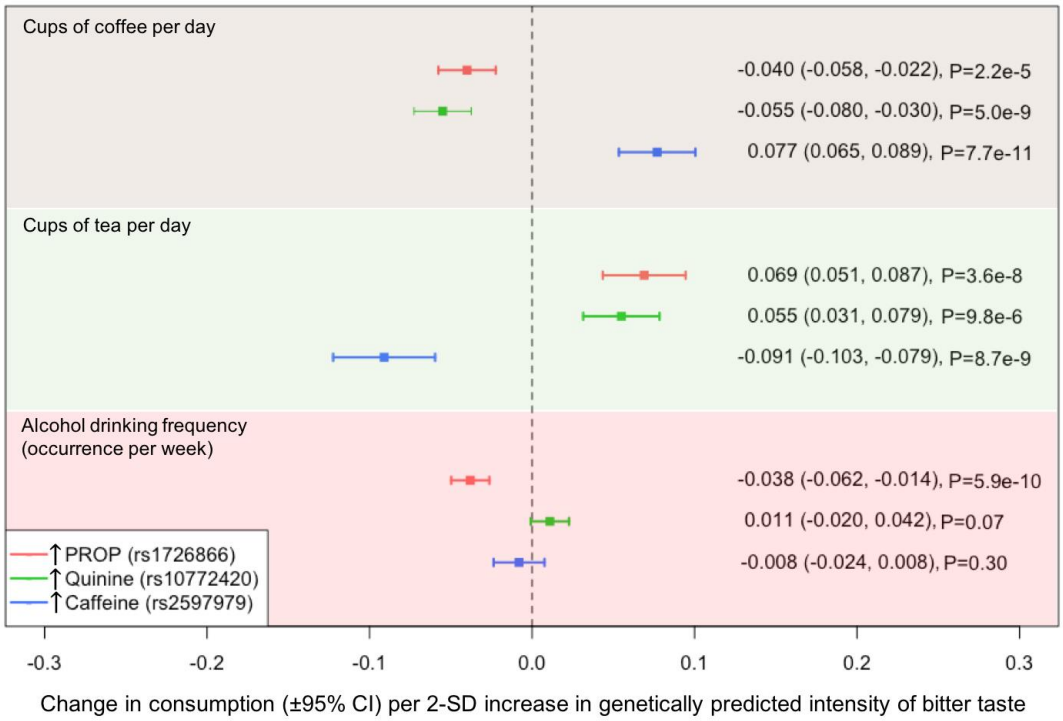
Variable	Statistics	Value based on 438,870 participants
Age	Mean (SD)	56.5 (8.09)
Sex	N of males (%)	223,040 (45.8%)
Height (cm)	Mean (SD)	168.5 (9.28)
BMI	Mean (SD)	27.42 (4.78)
Smoking	N (%)	
Ever smoked		194,764 (40.16%)
Self-reported alcohol drinking frequency	N (%)	
Never		39,434 (8.1%)
Special occasions only		55,197 (11.35%)
1-3 times/month		56,299 (11.58%)
1-2 times/ week		123,195 (25.33%)
3-4 times/week		115,327 (23.71%)
Daily or almost daily		96,870 (19.91%)
Coffee consumption	Mean (SD)	
Coffee (cups per day)		2.13 (2.11)
Tea consumption	Mean (SD)	
Tea (cups per day)		3.51 (2.87)

Mendelian randomization estimates of bitter perception on coffee and tea consumption

Associations between bitter SNPs and the consumption of bitter beverage are summarized in Supplementary Tables 4-3 (linear) and 4-4 (logistic). Instrumental variable estimates for per 2-SD unit increase in perceived bitterness on consumption quantity are shown in Figure 4-1a. We identified strong associations between genetically predicted higher perception for each bitter taste and coffee consumption ($P < 1e-4$). The magnitude of association for higher caffeine perception (rs2597979) is consistently in the opposite direction compared to perception of PROP (rs1726866) and quinine (rs10772420), providing evidence that higher perception of caffeine is causally associated with increased risk of being a heavy coffee drinker ($P = 7e-4$;Figure 4-1b), with similar evidence for association with higher cups per day ($P = 5.2e-4$), but higher perception of PROP and quinine results in lower coffee consumption.

Genetically predicted higher PROP and quinine intensity was associated with increased tea consumption, with a change of 0.069 ($P = 3.6e-8$) and 0.055 ($P = 9.8e-6$) cups per day per 2-SD increase in perception score. In contrast, higher caffeine perception leads to a decrease in tea consumption (beta = -0.091, $P = 8.7e-9$). Similar patterns of association were observed when comparing non-tea drinkers against heavy tea drinkers (see Figure 4-1b). All associations remained statistically significant upon Bonferroni correction.

(a)



(b)

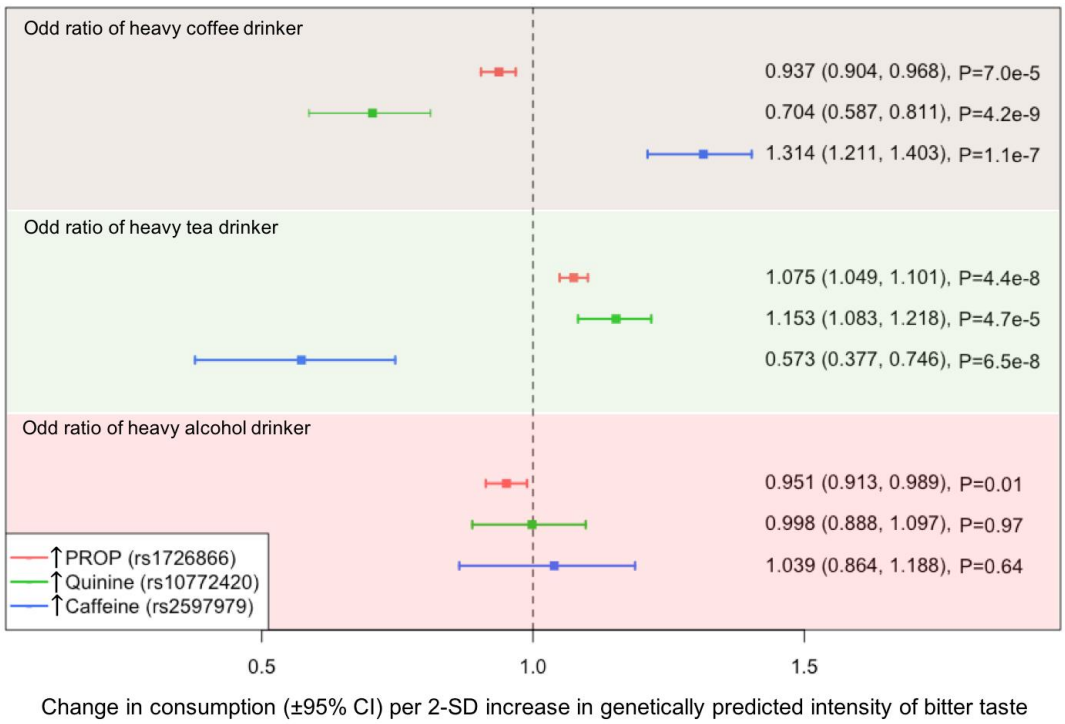


Figure 4-1. Causal effects of perceived bitterness of PROP, quinine, and caffeine on (A) the intake of coffee, tea, and alcohol and (B) the odd ratio of heavy coffee, tea, and alcohol drinker.

Conditional Mendelian randomization analyses to address inverse correlation between coffee and tea

As each of the three bitter tastes had opposite effects on the intake of coffee and tea, we investigated whether this was driven by the negative correlation between coffee and tea intake in the UK Biobank (r = -0.3). To remove the covariance between coffee and tea intake, we performed conditional analyses by adjusting for cups per day of tea consumption when estimating the MR causal estimates on bitter perception of coffee, and vice versa. Our conditional analyses (Supplementary Table 4-5) revealed that the association between predicted quinine and caffeine perception and coffee intake remained after conditioning for tea intake, and the association between predicted PROP and caffeine perception and tea intake remained after adjusting for coffee intake.

When stratified by non-drinker status on tea (or coffee) for the association between bitter taste and coffee (or tea) (Supplementary Tables 4-6 and 4-7), the direction of effect was largely consistent with the unstratified model - with larger standard errors due to a loss of effective sample size. However, the magnitude of positive (negative) association for caffeine perception on coffee (tea) among non-tea (coffee) drinkers was larger than the unstratified model (e.g. beta_coffee among non-tea drinkers = 0.248; as compared to 0.077 from the original model), suggesting a potential beverage preference based on caffeine perception. Complete results for the conditional and stratified analyses can be found in Supplementary Tables 4-5 to 4-7.

Mendelian randomization estimates for alcohol intake

The MR analysis of bitter taste perception on alcohol varied by stimuli (Figure 4-1). For PROP, increased predicted intensity was associated with lower alcohol consumption (for a 2-SD increase in PROP intensity, beta = -0.038 frequency score, P = 5.9e-10). Our MR analyses reveal weak evidence that genetically predicted increased quinine perception is associated with higher alcohol consumption as the confidence interval overlaps zero. Genetically predicted caffeine perception was not associated with alcohol consumption (beta = -0.008, P = 0.3). For drinker status, direction of association was largely consistent (Figure 4-1b), but none of the associations between predicted bitter taste and drinking status were statistically significant after correcting for multiple testing.

Investigating sex differences on the causal association between bitter taste and bitter beverage consumption

In light of previously suggested confounding effect of sex on the association between bitter perception and these beverages [54, 57, 189], we attempted to evaluate whether these differences were observed in our data, by performing our MR analyses separately for each sex (Supplementary table 4-8). We found no consistent evidence to support sex-difference, except for the association between caffeine perception and tea intake which appeared to be much stronger in females (for a 2-SD increase in caffeine perception score, beta = -0.523 cups per day, P = 1.4e-10 in females and beta = -0.181 cups per day, P = 0.06 in males).

Discussion

We investigated the perception of different bitter compounds on beverage consumption in a large population-based cohort (UK Biobank) using a MR instrumental variable approach. To summarize, our MR analyses indicated that genetic prediction of higher perceived intensity of PROP and quinine decreased coffee consumption and that prediction of higher perceived intensity of caffeine increased coffee consumption; opposite relationships were observed for tea consumption. For alcohol, higher predicted intensity of PROP resulted in lower consumption but the perception of quinine and caffeine had no clear influence. These findings highlight a potential role of taste perception in consumption behaviour and that the underlying mechanisms are specific to each of the bitter tastes.

Previous studies investigating the effect of PROP taster status on coffee consumption had conflicting findings. Many reported no association [51, 52, 55, 56], but one study of Polish elderly women showed that tasters consume coffee more frequently [54]. These data may be confounded by the use of coffee condiments as PROP tasters are more likely to drink coffee with milk, cream, and sweetener than PROP non-tasters [51]. Nevertheless, not only were these studies plagued by the caveats of observational study designs, they were also statistically underpowered due to low sample sizes (n < 500). Here we showed that the *TAS2R38* genotype was associated with coffee intake among the large UK Biobank cohort, with increased predicted perceived intensity leading to a lower intake. The direction of association could be attributed to the inborn aversion towards bitterness, which is a defensive mechanism that prevents ingestion of poisonous food [3]. Here the effect size is

notably small, so the association could only be observed when the sample is large enough.

Quinine is a commonly used stimulus to examine bitter taste response and it is also a source of bitterness in coffee [201]. However, previous work on the effect of quinine perception on coffee drinking is limited, with one study showing that coffee drinkers tend to be less sensitive to quinine [53]. Our results provide the first evidence that increased predicted perceived intensity of quinine leads to a lower coffee intake and a lower risk of being a heavy coffee drinker. The association is in the same direction as that of PROP but the effect of quinine is stronger, presumably because the perception of quinine could better reflect an individual's sensation to general bitterness.

Unexpectedly, for caffeine, the direction of effect was opposite to the other two bitter substances PROP and quinine. Caffeine intake has been related to caffeine perception. For example, a higher intake of caffeine is associated with lower sensitivity to caffeine [53], whereas caffeine daily users tend to rate caffeine, at a perceivable concentration, more bitter than non-daily users [50]. The present study suggests that an increased predicted perceived intensity of caffeine leads to a higher intake of coffee and a higher risk of being a heavy coffee drinker. Additionally, caffeine demonstrated the largest magnitude of association among the three bitter tastes. Caffeine is a non-volatile component of coffee and it contributes to the perceived strength, body and bitterness of coffee [202]. It is possible that caffeine adds an extra flavour to coffee for individuals with a stronger ability to perceive it, which further modifies their drinking behaviour. Nevertheless, caffeine metabolism still plays a major role in coffee consumption behaviour [182].

For the relationship between bitter taste perception and tea, conflicting findings have been found between PROP perception and the sensory acceptance of green tea [190, 191]. Here we showed that a higher predicted perceived intensity of PROP and quinine increased tea intake and a higher predicted perceived intensity of caffeine decreased the intake. These effects of bitter taste on tea consumption were in the opposite direction compared with those on coffee consumption, consistent with the negative correlation between coffee and tea intake in the UK Biobank cohort ($r = -0.3$). A negative relationship of same magnitude has also been reported in an Australian population sample ($N = 3908$) [203]. Our sensitivity analyses, which used the instrumental variable estimate on tea conditional on coffee (and vice versa),

provided more conservative results that tea intake was associated with PROP and caffeine and coffee intake was associated with quinine and caffeine. Similar results were observed in post-hoc analyses examining the effect of bitter taste on tea intake among non-tea drinkers and the effects on coffee among non-coffee drinkers, though the standard errors were larger due to a loss of effective sample size. We note that the tea drinking behaviour largely varies in the United Kingdom due to its mixed cultural backgrounds. Our findings were based on the intake of black and green tea, and the effects may differ for other tea types with different taste profiles.

Apart from coffee and tea, earlier studies have also suggested alcohol elicits bitterness in humans [204, 205] and such bitter sensation can mediate consumption behaviour [206]. PROP taster status and the *TAS2R38* genotype have been suggested to mediate alcohol consumption [57, 189, 206, 207], with inconsistencies being found between sexes. Our MR findings indicate that increased predicted perceived intensity of PROP leads to a lower intake but presented no evidence indicative of a difference between males and females. Through the stratified MR analyses by types of alcohol, we further showed that such association is mainly driven by red wine (Supplementary Table 4-9). We found no effect of quinine and caffeine perception on alcohol intake. Since quinine perception can be considered as taste response to general bitterness, the null association indicates that other taste, such as sweet taste perception [205, 206], may be a more important factor influencing alcohol consumption.

The strengths of our study include the use of a two-sample approach in MR to avoid biased estimates [199]. Since the relationship between SNP instruments and the exposure of interest (bitter taste) is independent from the instrument with the outcome, this approach potentially avoids winner's curse and weak instrument bias [193, 199]. Since taste perception can change overtime, our MR design of utilizing the SNP estimates on bitter taste perception during adolescence/early adulthood as an instrument allows us to evaluate the impact of taste factors on bitter beverage consumption in later life. Additionally, the use of a 2-SD change in taste perception allow us to assess the effect of changing from one taster status to another (equivalent to an interquartile change), without worrying about within-individual variation in taste perception as observed in earlier test-retest validations [40].

A major limitation for our study is the relatively small sample size (n = 1757) of the discovery sample used to estimate the SNP-bitter taste associations. However,

this limitation is unlikely to have substantially biased our finding as all of the SNPs used in this study have been replicated in other independent GWAS [35, 36, 38]. Despite our inability to perform sensitivity analyses that explore the validity of instrumental variables, particularly in relation to bias due to potential horizontal pleiotropy [199] due to having just one genetic instruments per trait, all of the SNPs (or their highly correlated SNPs) are missense variants in bitter taste receptors genes (Chapter 3). The biological evidence greatly reduces the chance for residual pleiotropy biasing our findings.

The response amongst those invited to UK Biobank was ~5% and participants differ considerably from the underlying population with respect to socioeconomic position, health and survival [208]. This could introduce selection bias, including with genetic associations and MR analyses [209]. For example, non-response is related to genetic predictors of a number of mental health and lifestyle/behavioural outcomes [210]. This will not have affected the association of genetic variants with taste perception which was done in a cohort with a > 72% response rate [72], but if consumption of tea, coffee or alcohol are related to socioeconomic position the associations in UKB may be biased. For example, there is evidence of a positive association between socioeconomic position and alcohol consumption (i.e. higher consumption in those with higher education and socioeconomic position [211]). However, sensitivity analyses (Supplementary Table 4-10) evaluating the association between these bitter taste SNPs and proxies of socio-economic status (i.e. UK Biobank townsend deprivation index and number of vehicle in household) found no evidence of association. Hence, it is unlikely for our inference to have been affected by these selection biases.

Given that the SNPs used as instrument variables explain appreciable amounts of variation in bitter taste perception as compared to bitter beverage intake, it is highly unlikely to manifest a bi-directional causal effect. However, we looked up the SNP associations with a list of behavioural and disease traits correlated to coffee consumption (e.g. diet behaviour, smoking, anorexia, and insomnia; Supplementary table 4-10) from on publicly available GWAS summary database [200] and found no association reaching statistical significance after adjusting for multiple testing.

The use of the wald-type estimator to evaluate the causal effect has an intrinsic assumption that the exposure-outcome relationship is linear. For behavioural traits like coffee consumption, this assumption can be violated when we assess the

effect of bitter taste on individuals at the extreme end of the trait distribution (i.e. non-tasters, or extremely sensitive tasters). Here, our stratified approach of making a separate hypothesis for i) amount of coffee consumed using cups per day and ii) non-drinker vs extreme drinker (or drinker for the case of alcohol traits) allow us to complement this investigation. It was shown that in most cases, the inference drawn for the cups per day (quantitative) phenotype was similar to those estimated on drinker status. Both results provide evidence against a quadratic relationship between bitter taste and bitter beverage consumptions. However, individual level data on both taste perception and beverage intake will be required to evaluate causal effects stratified at different consumption categories.

Overall, our findings demonstrate that differences in bitter taste perception is causally associated with bitter beverage consumption behaviour. This delivers an important message that, in addition to inborn variations, factors altering taste perception, such as disease status (e.g. middle ear infection) and medical treatment (radiotherapy on brain and neck) [212], may also modify our dietary behaviour. Given the popularity of these bitter beverages, future studies are necessary to investigate the underlying biology of how altered perception can potentially contribute to their addiction, which may shed light on the prevention and have public health implications.

5

Sweet Taste Perception is Associated with Body Mass Index at the Phenotypic and Genetic level

Chapter published in Twin Research and Human Genetics [213]

Chapter 5. Sweet taste perception is associated with body mass index at the phenotypic and genotypic level

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Abstract

Investigations on the relationship between sweet taste perception and body mass index (BMI) have been inconclusive. Here we report a longitudinal analysis using a genetically informative sample of 1,576 Australian adolescent twins to explore the relationship between BMI and sweet taste. First, we estimated the phenotypic correlations between perception scores for four different sweet compounds (glucose, fructose, neohesperidine dihydrochalcone [NHDC], and aspartame) and BMI. Then, we computed the association between adolescent taste perception and BMI in early adulthood (reported 9 years later). Finally, we used twin modelling and polygenic risk prediction analysis to investigate the genetic overlap between BMI and sweet taste perception. Our findings revealed that BMI in early adulthood was significantly associated with each of the sweet perception scores, with the strongest correlation observed for aspartame with r = 0.09 (P = 0.007). However, only limited evidence of association was observed between sweet taste perception and BMI that was measured at the same time (in adolescence), with the strongest evidence of association observed for glucose with a correlation coefficient of r = 0.06 (P = 0.029) and for aspartame with r = 0.06 (P = 0.035). We found a significant (P < 0.05) genetic correlation between glucose and NHDC perception and BMI. Our analyses suggest that sweet taste perception in adolescence can be a potential indicator of BMI in early adulthood. This association is further supported by evidence of genetic overlap between the traits, suggesting that some BMI genes may be acting through biological pathways of taste perception.

Introduction

A high body mass index (BMI) is associated with increased risks for metabolic, cardiovascular and respiratory diseases and several types of cancer, such as breast and colorectal cancers [214]. Obesity can arise as a result of genetic predisposition [215], metabolic problems [216], hormonal changes [217], physical activity [218], and/or eating behaviour [219]. The latter is heavily influenced by cognitive factors that allow individuals to assign a subjective reward value to specific kinds of foods [220]. One of these (cognitive) factors is taste perception, which is the sensory impression of food on the tongue.

Among the five basic human taste qualities (i.e. sweet, bitter, sour, salty, and umami), sweetness is generally considered pleasant at moderate intensities and

favoured by most individuals [221]. Sweetness is detected by sweet taste receptors in the oral cavity, which send signals to the brain where taste sensation is elicited [222]. Sweet taste receptors are also expressed along the digestive system, including the pancreas, bladder, gastrointestinal, and adipose tissues [223], where they do not evoke sweet sensation but are involved in many physiological functions, including glucose homeostasis [223], insulin secretion [224], and adipogenesis [225].

Earlier studies investigating the association between BMI and sweet taste perception have presented inconsistent findings. Some studies reported that obese individuals perceive the same candy or sucrose solution as less sweet than their non-obese counterparts [5, 60]. However, a handful of other studies did not find a direct association between BMI and sweet taste perception or sensitivity [13, 58, 59].

Twin studies have reported that individual differences in both sweet taste perception and BMI are partially attributable to genetic variation (h^2 = 0.30 – 0.34 for sweet taste perception [Chapter 1]; h^2 = 0.47 – 0.90 for BMI [226]) . Recent genome-wide association studies (GWAS) of BMI identified an enrichment of associated genetic variants involved in the central nervous system (CNS) that might be linked to sweet taste perception. These include genetic variants in loci associated with insulin, glucose and adipogenesis regulation [79], as well as energy balance, regulation of appetite, and food intake preferences [215]. However, it is still unclear whether some genes are jointly responsible for both the regulation of sweet taste perception and BMI.

In the present study, we used longitudinal and genetic data from a sample of 1,576 young Australian twins to investigate the relationship between sweet taste and BMI. We first examined the association between sweet taste perception and BMI both measured during adolescence. Next, we examined whether adolescent taste perception predicted BMI later in life (9 years later). Finally, we assessed the genetic overlap between BMI and sweet perception using twin modelling and polygenetic risk scores approaches to determine whether the association between the two traits was due to shared genetic components.

Materials and Methods

Data

Participants were adolescent and young adult twins and their singleton siblings from the Brisbane Adolescent Twin Study (BATS) [72], also referred to as

the Brisbane Longitudinal Twin Study (BLTS). They completed a taste test at around 15.8 ± 2.6 years old and had their BMI measured at the clinic (two thirds) or self-reported (one third). They reported their BMI again approximately 9 years later as part of a follow-up study. Descriptive statistics for the participants are displayed in Table 5-1. The study was conducted in accordance with the Declaration of Helsinki and informed consent was obtained from all adult participants and from parents for participants under age of 18. Approval for this study was obtained from the Human Research Ethics Committee of QIMR Berghofer Medical Research Institute.

Table 5-1. Descriptive statistics of participants and their taste perception.

	Early Age (12-26)	Later Age (18-38)
N of participants	1576	998
MZ pairs	205	101
DZ pairs	395	197
Siblings	376	402
Age (years)	15.8 ± 2.6	25.2 ± 4
Female	53.5%	61.1%
Height (cm)	162.7 ± 8.2	171.8 ± 9.7
Weight (kg)	54.9 ±11.6	70.9 ± 15.1
BMI (kg/m^2)	20.6 ± 3.5	23.8 ± 4.0
Intensity rating		
Glucose	31.2 ± 15.7	
Fructose	31.9 ± 17.6	
NHDC	34.4 ± 18.6	
Aspartame	26.3 ± 16.0	
gSweet	31.1 ± 15.0	

Data are presented as mean ± standard deviation. Intensity ratings are millimetre on a labelled magnitude scale. NHDC: neohesperidine dihydrochalcone. gSweet: general sweet intensity.

The taste test included 10 different solutions, of which five were bitter, four were sweet (described below) and one was neutral (i.e. water, as control) [81]. The four sweet solutions included two sugars (0.60 M glucose and 0.30 M fructose) and two high-potency (low/non-caloric) sweeteners (8.0×10^{-5} M neohesperidine dihydrochalcone [NHDC] and 1.4×10^{-3} M aspartame). Each solution and the water control were presented twice (i.e. a total of 20 solutions) in colour-coded 2mL polypropylene microcentrifuge tubes with flip tops. The first ten tubes contained one presentation of each compound plus the water control and the next ten contained the same solutions in a different order. The order of all twenty tubes was the same for all

participants. Participants were instructed to: 1) open the tube, swish the solution in the mouth for five seconds and spit out, 2) rate the perceived intensity of the solution, 3) rinse the mouth out four times with tap water and, 4) repeat steps 1 to 4 for each tube. Perceived intensity was rated on a general labelled magnitude scale (gLMS) [73] with labels of no sensation (0mm), barely detectable (2mm), weak (7mm), moderate (20mm), strong (40mm), very strong (61mm), and strongest imaginable (114mm). Participants marked a line on the scale where they thought the sensation fitted. The mean intensity ratings from duplicate presentations were used in this study. As Chapter 1 showed that a common genetic component accounted for most of the variance in intensity scores of each sweetener (71% for glucose, 77% for fructose, 64% for NHDC, and 59% for aspartame), here we calculated a general sweet intensity rating (gSweet) using the weighted mean of intensity ratings of the four sweeteners (Chapter 2). Intensity scores were square root transformed to approximate the normal distribution.

Genotyping Quality Control

Participants were genotyped using the IlluminaHuman610W-Quad bead chip. Standard quality control was performed on genotyped variants: single nucleotide polymorphism (SNP) with call rate < 0.95, deviation from Hardy-Weinberg equilibrium (HWE) p-value < 10^{-6} or minor allele frequency (MAF) < 0.01 were excluded [227]. To control for population stratification, we excluded individuals which lay beyond 6 standard deviations from the first 2 genotypic principal components centroid of the 1000 Genome European descent populations. Imputation was carried out based on the August 4, 2010 version of the publicly released 1000 Genomes Project European genotypes using MACH [228]. We implemented a filtering threshold of 0.3 on the r^2 metric for each of the SNPs in-line with recommended practice in MACH, and a MAF > 0.01.

Twin modelling

Phenotypic and genetic correlations between measures of taste intensity and BMI were estimated using bivariate variance components modelling in the structural equation software package Mx, which utilises maximum likelihood estimation procedures [74]. Variance components modelling partitions the variation of a trait into genetic and environmental sources by leveraging the degree of genetic differences between monozygotic twins (MZ; share all genes) and dizygotic twins (DZ; share half

of genes) pairs. These known differences allow the estimation of additive genetic (A), common environment (C), and unique environment (E, which also includes experimental error and random noise) parameters in a variance components model (ACE). The comparative fit of models was assessed by calculating the Log-likelihood Ratio Test (LRT) statistics [229]. All the models were adjusted for sex and the participants' corresponding age.

Polygenic risk scores

To complement the genetic correlation estimates, we also carried out a polygenic risk prediction analysis by computing polygenic risk scores (PGRS) of our trait of interest (in this case, BMI) and then using this genetic profile to predict another trait (i.e. taste perception) [230]. In short, the PGRS describes predicted phenotypic values that are genetically derived. It is computed by aggregating the estimated effects of many variants multiplied by the number of observed effect allele into a single score for each individual, to mimic a genetic proxy (profile scoring) for the underlying phenotypic trait. In our analysis, we used the association estimates from the latest GWAS meta-analysis summary statistics of BMI from the Genetic Investigation of Anthropometric Traits (GIANT) consortium including up to 339,225 participants [79]. We extracted effect estimates of the SNPs that were computed based on participants (n = 322,154) of European ancestry only. This approach could yield increased power to detect genetic correlations due to the large sample size of the GIANT analysis. Given that the samples in this study were also part of the GIANT BMI GWAS, these GWAS estimates were recomputed after removing the overlapping samples. Next, we selected variants to compute the PGRS based on 10 different P-value thresholds (< 0.00001, 0.001, 0.01, 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 0.75). Linkage disequilibrium (LD) clumping was performed to remove redundant SNPs ($r^2 > 0.2$) within a 500kb window for each of the variants in the PGRS. The computation of PGRS and LD clumping were carried out using PLINK [197] by multiplying the number copies of each effect allele with the reported magnitude of association and summing over all the relevant SNPs. Ambiguous SNPs with complementary strands (A/T, G/C) were removed. Specifically, the number of independent genetic variants for each of the thresholds were 2071, 8403, 27638, 79871, 136195, 237876, 333747, 424237 and 511295 SNPs. Finally, we converted each of the PGRS to standard Z-scores ($\mu = 0$ and $\sigma = 1$) and tested their

association with the different sweet taste perception scores using a linear mixed model: fitting the first three principal components derived from the genotypes, age and sex as covariates, and the family ID as random effect.

The polygenic risk prediction approach carries a high multiple testing burden given that each of the PGRS is tested for their association with sweet taste perception. However, given that the different sweet taste perception are correlated between each other and that each of the PGRS are highly redundant, we estimated the number of independent tests using a matrix spectral decomposition algorithm [157]. This algorithm estimates the equivalent number of independent variables from a correlation matrix, by examining the ratio of observed eigenvalue variance to its theoretical maximum. The estimated number of independent test was 7 and thus a Bonferroni-corrected significance threshold was P = 0.05/7 = 0.007.

Results

We investigated whether sweet taste perception in adolescence was predictive of BMI in adolescence (15.8 ± 2.6 years old) and early adulthood (25.2 ± 4.0 years old). In our regression analyses, we found an association between the perceived intensity of sweetness and BMI at the same age in adolescence, with glucose and aspartame showing significant correlations of r = 0.056 (P = 0.035) and r = 0.058 (P = 0.029) respectively (Table 5-2). When investigating whether sweet taste perception was predictive of early adulthood BMI, we found stronger associations with all sweet perception scores ranging from r = 0.07 for fructose to r = 0.09 for aspartame (Table 5-2). Intensity ratings of water were used as a negative control, and, as expected, they were not associated with BMI.

Table 5-2. Summary of phenotypic and genetic correlations between taste intensity ratings and BMI.

	BMI same age		BMI later age	
Phenotypic correlations	r_p ± s.e.	P	r_p ± s.e.	P
gSweet	0.05 ± 0.03	0.059	**0.08 ± 0.03**	**0.012**
Glucose	**0.06 ± 0.03**	**0.035**	**0.08 ± 0.03**	**0.020**
Fructose	0.04 ± 0.03	0.196	**0.07 ± 0.03**	**0.034**
NHDC	0.04 ± 0.03	0.122	**0.07 ± 0.03**	**0.025**
Aspartame	**0.06 ± 0.03**	**0.029**	**0.09 ± 0.03**	**0.007**
H2O	-0.02 ± 0.03	0.579	-0.03 ± 0.03	0.406
Genetic correlations	r_g ± s.e.	P	r_g ± s.e.	P
gSweet	0.10 ± 0.06	0.092	0.16 ± 0.08	0.053
Glucose	**0.13 ± 0.06**	**0.030**	**0.21 ± 0.08**	**0.017**
Fructose	0.08 ± 0.06	0.173	0.14 ± 0.08	0.150
NHDC	0.07 ± 0.06	0.260	0.10 ± 0.08	0.267
Aspartame	0.12 ± 0.06	0.077	0.18 ± 0.08	0.090
H2O	0.00 ± 0.06	0.955	-0.12 ± 0.08	0.172

All values are calculated using bivariate variance components modelling in the structural equation software package Mx. Genetic correlations are calculated from bivariate AE model as C components can be dropped without worsening the model fit (P = 0.28 - 0.87 for each trait). gSweet: general sweet intensity. NHDC: neohesperidine dihydrochalcone. s.e.: standard error. Estimates in bold are statistically different from 0.

Using twin modelling, we found a positive genetic correlation between perceived intensity of all the tastes and BMI at same and later age. However, only the perceived intensity of glucose showed a significant association (same age: r_g = 0.13, P = 0.03; later age: r_g = 0.21, P = 0.017) (Table 5-2). The environmental correlations were not significantly different from zero (data not shown).

To further assess the genetic overlap between BMI and sweet taste perception, we carried out a polygenic risk prediction analysis. Based on the GWAS of BMI from the GIANT consortium, we computed individual BMI PGRS including a different number of variants based on P-value thresholds and tested their association with sweet taste perception and BMI itself. As anticipated, each of the PGRS were strongly associated with BMI (e.g. the PGRS based on SNPs with a P-value < 0.2 in the GIANT GWAS had an r = 0.2; $P < 1.0 \times 10^{-15}$) (Supplementary Figure 5-1). Through this approach, we found a significant genetic correlation between BMI and NHDC (Figure 5-1) but not with glucose as with the twin modelling analysis.

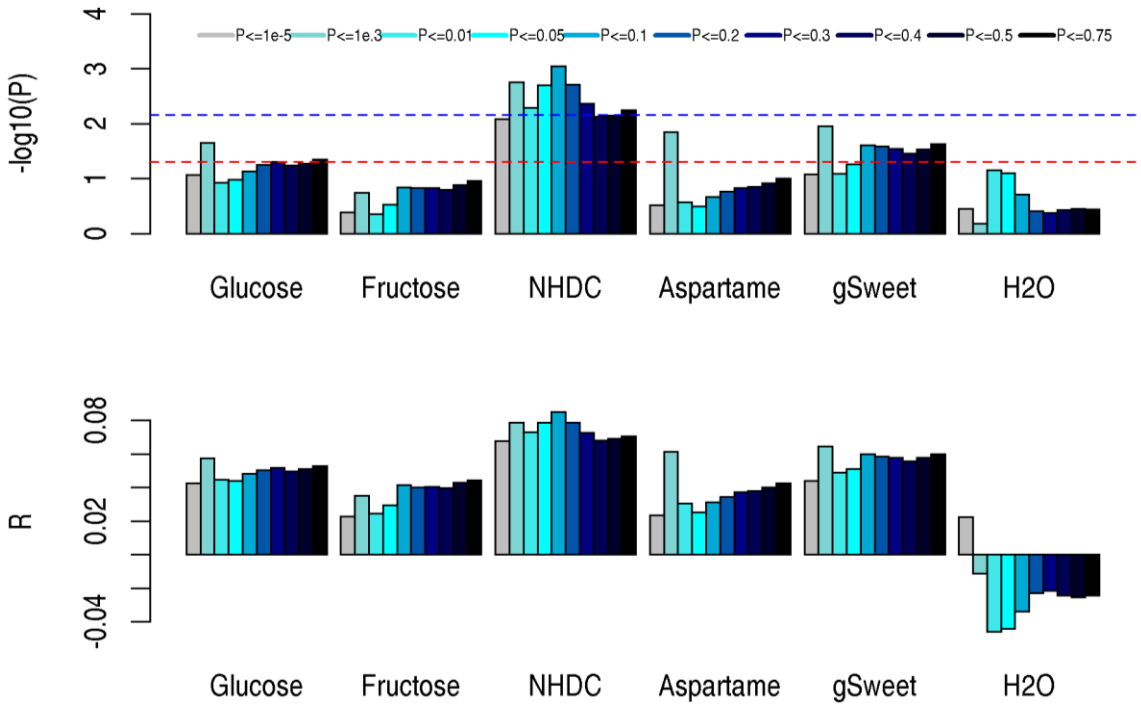

Figure 5-1. Results of the polygenic risk prediction. The bars correspond to the association between each of the polygenic risk scores (PGRS) of BMI computed based on the specified P-value thresholds (< 0.00001, 0.001, 0.01, 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 0.75) and the different sweet taste intensity scores. P-values of the association are shown in the logarithmic scale in the upper part of the figure while spearman correlations (r) are shown in the bottom part. Red dotted line shows the nominal P-value (0.05) on –log10 scale. Blue dotted line shows the significance threshold after accounting for multiple testing. As an example, the first bar should be interpreted as the correlation between a genetically-predicted BMI value based on SNPs with a P-value < 1.0 x 10^{-5} in the GIANT BMI GWAS and glucose. gSweet: general sweet intensity. NHDC: neohesperidine dihydrochalcone.

Discussion

We found that the perceived intensity of sweet solutions measured during adolescence was positively associated with BMI measured at the same age, and that the association was stronger with BMI measured 9 years later. We also found evidence of a positive genetic correlation of glucose and NHDC with BMI.

In contrast to findings from previous cross-sectional studies, which showed negative associations [5, 60] or no associations between sweet taste perception and BMI [13, 58, 59], we found a subtle but positive association with BMI measured at the same age. The differences may result from variation in study designs and sample demographics. Bartoshuk *et al*. [60] reported a negative association between

perceived sweetness of a candy and BMI in a large sample of college students (n = 3700) whereas the taste measures in our study were adolescents' intensity ratings of sweet solutions. Overberg *et al*. [5] collected data from adolescents as well as children (age ranged from 6 to 18) and they reported a negative association by dichotomising participants into either obese (BMI > 97th percentile) or normal weight (BMI < 90th percentile). In our study, BMI was defined as a continuous variable, which provided us more robust estimates with respect to the magnitude and direction of the correlation. In addition, studies [13, 58, 59] that showed no association typically have relatively small sample sizes (n < 100), which suggests that larger samples are necessary to detect a significant association.

Our finding that sweet taste perception has a higher association with BMI at a later age (as compared to BMI obtained at the same time of taste perception measurement) yields several possible explanations. The first could be the cumulative effect of sweet preferences. Individual differences in sweet perception may gradually contribute to a bigger variation in preferences for sweetness, which directly influence sweet food consumption, and further lead to a bigger variation in BMI later in life. Secondly, during adolescence, diet preferences are strongly influenced by cultural practise and familial influences [231], rather than the true taste preferences. Also, BMI at a young age is susceptible to body and hormonal changes rather than sweet food consumption, compared to that in a later age when body mass change becomes more stable.

Our two genetic approaches independently identified significant correlations between BMI and sweet taste perception; one with glucose and the other with NHDC. Nevertheless, these correlations were all positive regardless of taste stimuli and the underlying modelling approaches. In the context of our study, the twin modelling and PGRS analyses complement each other in the sense that the PGRS models the cross-trait genetic overlap based on common genetic variants, while conventional twin models incorporate a larger spectrum of information including undiscovered causal SNPs and rare variants in determining co-heritability.

The genetic overlap we observed tends to agree with the literature. Simple carbohydrates (sugars) as well as complex carbohydrates (oligosaccharides and polysaccharides) are the main source of energy for the human body, and over-consumption of these sweet compounds can result in weight gain as excess energy intake is transformed and stored as body fat [232]. While the GIANT consortium

identified several BMI-associated genes with putative functions in the brain [79], some of those genes could be responsible for sweet taste perception by influencing dietary behaviour. Similarly, there is evidence that individual differences in taste responses are due to differences in signal processing in the CNS [138]. Other pathways may also contribute to the genetic overlap between sweet perception and BMI. For example, the neurobiology of food reward might help explain why the use of non-caloric sweeteners can result in weight gain without affecting calorie intake [233]. Lastly, apart from the neurological and possible mechanisms explained above, sweet taste receptors in the mouth and guts can also regulate energy metabolisms through various mechanisms, such as their effects on insulin secretion [223, 224]. Sweet taste perception appears to play an important role in regulating BMI; however, in this study we were not able to prove the directionality of this association.

The strengths of our study included that the twin data allowed us to estimate the genetic and environmental contributions to the correlation between sweet taste and BMI. Also, the polygenic risk score of BMI derived from common variants provided further support for the genetic correlation between the two and made our findings more robust. Lastly, the longitudinal data for BMI measured 9 years apart allowed us to test the long-term effects of sweet taste perception on BMI. We must also acknowledge some limitations. For example, the effective sample size ($N_{\text{same age}}$ = 1576 and $N_{\text{later age}}$ = 998) could be considered small for a twin study, and the use of self-reported BMI might potentially introduce some modest bias, as it has been pointed out that participants tend to slightly overestimate their height and underestimate their weight [234].

In conclusion, we showed that adolescent sweet taste perception is associated with BMI at both adolescence and early adulthood, and that this association is partly explained through their genetic overlap. Identification of the shared genetic architecture could improve our understanding of the genetic pathways underlying both sweet taste perception and weight gain. As we obtain more study samples in the future, we can use the SNPs that strongly associate with sweet taste perception as genetic instruments to make causal inference (i.e. Mendelian randomization studies [Chapter 4]) about the effect of sweet taste perception on weight gain and diet-related disorders, which may provide insights into the prevention and treatment.

6

Is there an Association between Brain Structure and Perceived Intensity of sweet and Bitter Tastes?

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Chapter 6. Is there an association between brain structure and perceived intensity of sweet and bitter tastes?

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Abstract

Functional neuroimaging studies have identified brain regions associated with human taste perception, but only a few studies investigated the associations with brain structure. Here, in this exploratory study, we examined the association between the volumes of 82 brain regions of interest (ROI) and the perceived intensities of sweet (glucose, fructose, aspartame, and neohesperidin dihydrochalcone) and bitter (propythiouracil [PROP], quinine, and caffeine) substances in a large Australian healthy cohort (n = 559). We showed that the volumes of 3 cortical (right cuneus gyrus, left transverse temporal gyrus, right inferior temporal gyrus) and one subcortical structure (both left and right caudate) were associated with more than one tastes ($P < 0.05$) and tended to be associated with both sweet and bitter tastes in the same direction, suggesting that these brain regions could be broadly tuned for taste sensation. A further 11 ROIs were associated with a specific taste, suggesting that these brain regions may be more

narrowly turned ($P < 0.05$; *sweetness*: left pars triangularis, left banks of the superior temporal sulcus, left caudal anterior cingulate cortex, posterior cingulate cortex; *PROP*: right isthmus cingulate cortex, right thalamus, right pars orbital cortex [only after adjusting for *TAS2R38* genotype]; *caffeine*: right superior fontal gyrus, right hippocampus; *quinine*: left entorhinal cortex, left amygdala). Using brain imaging and quinine ratings available from the Human Connectome Project ($n = 1101$), we replicated the association between the left entorhinal cortex volume and quinine bitterness ($r = -0.06$, $P = 2.0 \times 10^{-2}$). This study provides the first evidence that, even in healthy people, variation in brain structure is associated with taste intensity ratings, and provides new insights into the role of the brain structure in taste perception.

Introduction

Our brain plays a significant role in taste perception. When we eat, food chemicals are detected by taste receptors in the oral cavity and signals are sent via gustatory nerves to the brain where taste sensation is generated so we know what we eat and whether we like it or not [61]. Positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) studies show that there are areas in the human brain that both respond to taste and are homologous to those found in other primates [62]. However, identification of these taste-responsive brain regions has been inconsistent across studies [64], and this may be due to the use of different taste stimuli and tasks performed, as the brain response can be valence-specific [65] or intensity-specific [66], or, more importantly, the small sample size ($n < 100$). Therefore, more knowledge is required to construct the gustatory pathway in the human brain.

Structural variations of specific brain regions relate to human senses, such as olfaction [67] and vision [68], and recent evidence suggests that volumetric differences also associate with taste. People with eating disorders, e.g. anorexia nervosa, had larger left gyrus rectus grey matter [69, 70], and there was an association between its size and the sucrose pleasantness among both patients and healthy controls. Furthermore, structural alterations (e.g. removing parts of the brain) can modify perceived intensity of taste [71]. These findings suggest a new direction to investigate the linkage between brain regions and taste. Here, using a large population sample ($n = 559$), we conducted an exploratory study to investigate whether there was an association between brain structure and taste perception.

Methods and Materials

Sample

Participants were a subset of the Brisbane Adolescent Twin Study (BATS) [72], consisting of 59 complete monozygotic and 107 complete dizygotic twin pairs and 285 unpaired twins or singleton siblings, from 361 families. The sample included 351 females and 208 males and all of them were right-handed and healthy. Participants completed taste test at the mean age of 16.7 ($\pm$ 2.7 standard deviations [SDs]) years, and were MRI scanned approximately 5.0 ($\pm$ 1.5 SDs) years later (mean age at scan = 21.7 $\pm$ 3.2 SDs years), as part of the Queensland Twin IMagining (QTIM) study [235]. Prior to scanning, participants were screened for neurological and psychiatric conditions, including loss of consciousness for more than 5 minutes, and general MRI contraindications. Zygosity of same-sex twin pairs was determined using a commercial kit (AmpFISTR Profiler Plus Amplification Kit, ABI) and later confirmed by genome-wide single nucleotide polymorphism genotyping (Illumina Human610-Quad BeadChip). This study was approved by Human Research Ethics Committees at the University of Queensland, QIMR Berghofer Medical Research Institute, and UnitingCare Health. Written consent was obtained from both the participants and their parents (the latter not required for those 18 years and over).

Taste Test

The taste test has been described in previous chapters. Briefly, participants were instructed to taste five bitter (6.0×10^{-4} M propylthiouracil [PROP], 2.0×10^{-4} M sucrose octaacetate [SOA], 1.81×10^{-4} M quinine, 0.05 M caffeine, and 4.99×10^{-6} M denatonium benzoate [DB]) and four sweet (0.60 M glucose, 0.30 M fructose, 8.0×10^{-5} M neoherperidine dihydrochalcone [NHDC], and 1.4×10^{-3} M aspartame) solutions and to rate their perceived intensities using a general Labelled Magnitude Scale (gLMS). Here we used a general sweet factor score, which is a weighted mean accounting for most of the variance in the perceived intensity of the four sweet tastes (71% for glucose, 77% for fructose, 64% for NHDC, and 59% for aspartame; Chapter 2), and ratings for three bitter solutions: PROP, a widely studied bitterness phenotype that approximately half of its variance is due to the genetic variation within the bitter taste receptor gene *TAS2R38* [107]; quinine, a commonly used bitter agent to test perceived bitterness and aversiveness; caffeine, a basic ingredient of the

most popular bitter drink, coffee, and can have various impacts on brain [236]. Ratings for SOA and DB were not included because they were less common taste phenotypes, but their results are provided in the supplementary documents.

Brain Imaging

Structural T1-weighted 3D brain images were acquired using a 4T Bruker Medspec (Bruker, Germany) whole-body MRI system paired with a transverse electromagnetic (TEM) head coil (TR = 1500 ms, TE = 3.35 ms, TI = 700 ms, 240 mm FOV, 0.9 mm slice thickness, 256 or 240 slices depending on acquisition orientation (86% coronal [256 slices], 14% sagittal [240 slices]) and corrected for intensity inhomogeneity with SPM12 before analysis (Wellcome Trust Centre for Neuroimaging, London, UK; http://www.fil.ion.ucl.ac.uk/spm). Volumes of the 82 regions of interest (ROIs), including 34 gyral-based regions from the Desikan-Killiany atlas [237] plus 7 subcortical volumes from each hemisphere, were extracted using FreeSurfer (v5.3; http://surfer.nmr.mgh.harvard.edu/) as previously reported [238]. Cortical reconstructions and ROI labelling were checked using the procedures of the Enhancing Neuro Imaging Genetics through Meta-Analysis (ENIGMA) consortium (enigma.ini.usc.edu), with incorrectly delineated cortical structures excluded from the analysis. Descriptive statistics of these brain and taste phenotypes are provided in Supplementary Table 6-1.

Statistical Analysis

Statistical analyses were performed using a linear mixed model implemented in the R package 'hglm' [239]. Prior to analysis taste intensity ratings were regressed by age at taste test, sex, and history of otitis media, which were shown to associate with taste ratings [107]. Brain phenotypes were regressed by age at brain scan, sex, and total brain volume ('BrainSeg' in FreeSurfer). Taste and brain phenotypes greater or smaller than mean $\pm$ 3 SDs were considered as outliers and removed from the analysis (up to 40 outliers; see Supplementary Tables for details). Covariates included the fixed effect of the time interval between the taste test and brain scan and the random effect of the family relationship matrix with values of 1, 0.5, and 0 assigned to monozygotic twins, dizygotic twins/siblings, and unrelated individuals. Analyses of PROP were further adjusted for the *TAS2R38* genotype. Given that the 4 taste phenotypes are correlated (Chapter 2) and so are the 82 brain phenotypes [240], we used a matrix spectral decomposition algorithm [157] to

estimate the number of independent phenotypes to be 3 taste and 55 brain phenotypes. A Bonferroni-corrected significance threshold was set at P = 0.05/(3 x 55) = 3.2 x 10^{-4}.

Results

While no association reached the corrected threshold (presented as a heatmap in Figure 6-1 [See Supplementary Tables 6-2 to 6-5 for details]), we found several patterns for these associations. ROIs from both hemispheres tended to associate with a taste in the same direction. Using the conventional threshold of P < 0.05, the sweetness factor was associated with 6 ROIs and bitter tastes were associated with 4 to 7 ROIs. The volumes of 3 cortical regions (right cuneus gyrus, left transverse temporal gyrus, right inferior temporal gyrus) and 2 subcortical regions (left and right caudate) were associated with more than one taste and tended to be associated with both sweet and bitter tastes. The cortical associations were all positive – larger volumes being associated with increased taste intensity ratings, whereas large left and right caudate volumes were associated with decreased taste intensity ratings.

Eleven regions were associated with only one taste (P < 0.05). These included 4 cortical regions in the left hemisphere being associated with sweet perception (3 positive associations with pars triangularis, caudal anterior cingulate cortex, and posterior cingulate cortex volumes and a negative association with banks of the superior temporal sulcus), 3 ROIs in the right hemisphere being associated with PROP perception (2 positive associations with pars orbital cortex [only after adjusting for the *TAS2R38* genotype] and thalamus volumes and one negative association with isthmus cingulate cortex), 2 ROIs in the right hemisphere being association with caffeine perception (a positive association with hippocampus volume and a negative association with superior frontal gyrus volume), and 2 ROIs in the left hemisphere being associated with quinine perception (a positive association with amygdala volume and a negative association with entorhinal cortex volume). We note that the *TAS2R38* genotype was not associated with any ROIs (P > 0.05), and while the number of ROIs associated with PROP perception increased from 2 to 3 after adjusting for the *TAS2R38* genotype (Supplementary Tables 6-5 and 6-6), the overall association pattern did not change much.

We further compared the associations with quinine perception with those from the Human Connectome Project (HCP) S1200 release [80], which included the same brain phenotypes and perceived intensity ratings of only one taste solution (i.e. 1.0 x 10^{-3} M quinine solution, which was 10 times more concentrated than ours) from 1101 adults with mainly European ancestry (Figure 6-1 and Supplementary Table 6-7). We found five associations ($P < 0.05$; all negative) with cortical regions in HCP, two of which were also associated in QTIM. The negative association between the left entorhinal cortex volume and quinine perception in QTIM ($r = -0.12$, $P = 3.7 \times 10^{-3}$) was replicated in HCP ($r = -0.06$, $P = 2.0 \times 10^{-2}$) (Figure 6-2). Analyzing the two datasets together gave a stronger association ($r = -0.08$, $P = 1.2 \times 10^{-3}$). In contrast, an inverse association between the left transverse temporal gyrus and quinine was observed in HCP (QTIM: $r = 0.11$, $P = 6.9 \times 10^{-3}$; HCP: $r = -0.07$, $P = 3.8 \times 10^{-3}$). Although the other 3 associated ROIs in HCP (right lateral occipital gyrus and bilateral orbitofrontal cortex) showed no association in QTIM, their direction of association tended to be the same.

Discussion

In this exploratory study, we showed that the volumes of 5 ROIs (right cuneus gyrus, left transverse temporal gyrus, right inferior temporal gyrus, and caudate from each hemisphere) were associated with both sweet and bitter tastes, suggesting that these brain structures could be more broadly tuned for taste sensation. In particular for cuneus and caudate, responses to a more general taste (i.e. not only for sweetness and bitterness but also for sourness and saltiness) have been reported in fMRI [64] and electroencephalogram (EEG) [241] studies, respectively. Transverse temporal gyrus, also known as Heschl's gyrus, is the primary auditory cortex. A larger transverse temporal gyrus volume, bilaterally, has been associated with increased perceived intensity of hearing [242]. Here we provide evidence that the volume of transverse temporal gyrus also relates to the perceived intensity of taste.

Among the 11 associations for more narrowly tuned ROIs found in QTIM, the association between left entorhinal cortex and quinine was robustly replicated in the HCP dataset. The entorhinal cortex is involved in learning of food avoidance, though lesions of entorhinal cortex in rats affect the learning of odour aversion [243] rather than taste [244]. Further investigation of this brain region in humans will help interpret our finding. We could not find a perfect explanation for the inverse

association between quinine and transverse temporal gyrus in HCP, but this could be due to differences between the two studies, such as the concentration of quinine solution (QTIM: 1.81×10^{-4} M vs HCP: 1.0×10^{-3} M).

Regarding the volume of the primary (anterior insula and frontal operculum) and the secondary (orbitofrontal cortex) gustatory cortices, we found no association for insula in both QTIM and HCP, but there was evidence of associations for regions overlapping the frontal operculum (i.e. pars orbitalis and pars triangularis) in QTIM and for lateral orbitofrontal cortex in HCP. We note that, in QTIM, the test-retest reliability for insula ($r = 0.57$ and 0.32 for left and right) was low compared to other ROIs [240]. In the present study, we did not observe an association between sweetness and left gyrus rectus (medial orbitofrontal cortex), whose volume was previously linked to sucrose pleasantness [69, 70]. This is consistent with functional studies that, when tasting a sucrose solution, neural responses in medial orbitofrontal cortex appears to correlate with pleasantness rather than intensity ratings [66].

A limitation of our study is that the taste and brain phenotypes were collected at different times in QTIM. We included the time interval as a covariate to control for this, and found no significant effects ($P > 0.05$) for any of the associated regions, except for the right inferior temporal gyrus ($P \sim 3e\text{-}3$) where a longer time interval leads to a larger volume. Our findings may also be limited by the use of brain ROI measures, because an ROI covers a pre-defined brain region, which may contain areas that are not related to taste. Future vertexwise analyses could help pinpoint associated brain regions. Further, except for Quinine, which was collected as part of the HCP, there is no other dataset available that has both brain imaging and taste phenotypes, so it was not possible to replicate associations for any of the other taste phenotypes.

This is the first large-scale study showing that taste perception is associated with the volumes of specific brain regions among healthy individuals. Whether there is a causal relationship between taste and brain structure can be tested using a two-sample Mendelian randomization. This is similar to what we have done in Chapter 4 with the outcome phenotypes of beverage intake replaced by brain volumes. The associations between taste-associated SNPs (from Chapter 3) and brain volumes can be obtained from the well-powered cohorts from the ENIGMA Consortium [245] and UK Biobank Imaging Study [246], each of which collected brain imaging data

from more than 30,000 and 20,000 participants, respectively. The results could not only be used to validated our findings but also elucidate the effect of brain structure on shaping taste perception and vice versa.

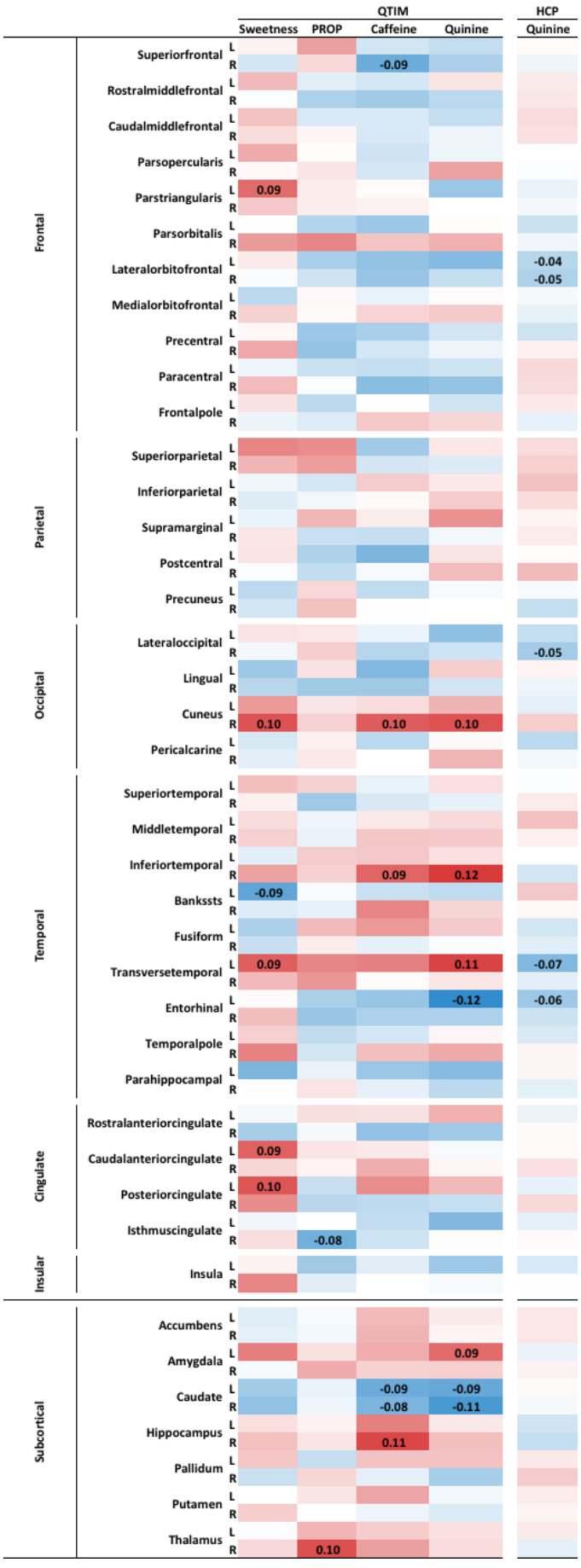

Figure 6-1. Heatmap showing correlations between taste perception and brain structure volumes in QTIM (n = 559) and HCP (n = 1101). In QTIM, Sweetness scores are associated with 6 brain regions. PROP scores are associated with 2 regions (increases to 3 regions after adjusting for the *TAS2R38* genotype; Supplementary Table 6-6). Caffeine and quinine scores are associated with 6 and 7 regions respectively. The volumes of right cuneus and left transverse temporal gyri are positively associated with both sweetness and two of the bitter tastes. The volumes of right inferior temporal gyrus and caudate in each hemisphere are associated with quinine and caffeine. In HCP, quinine scores are associated with 5 brain regions and the association with left entorhinal cortex is in the same direction with that in QTIM, whereas the association with left transverse temporal gyrus is in the opposite direction. L and R indicate the volume of left and right hemisphere of the brain respectively. Only correlation coefficients with P-value < 0.05 are shown.

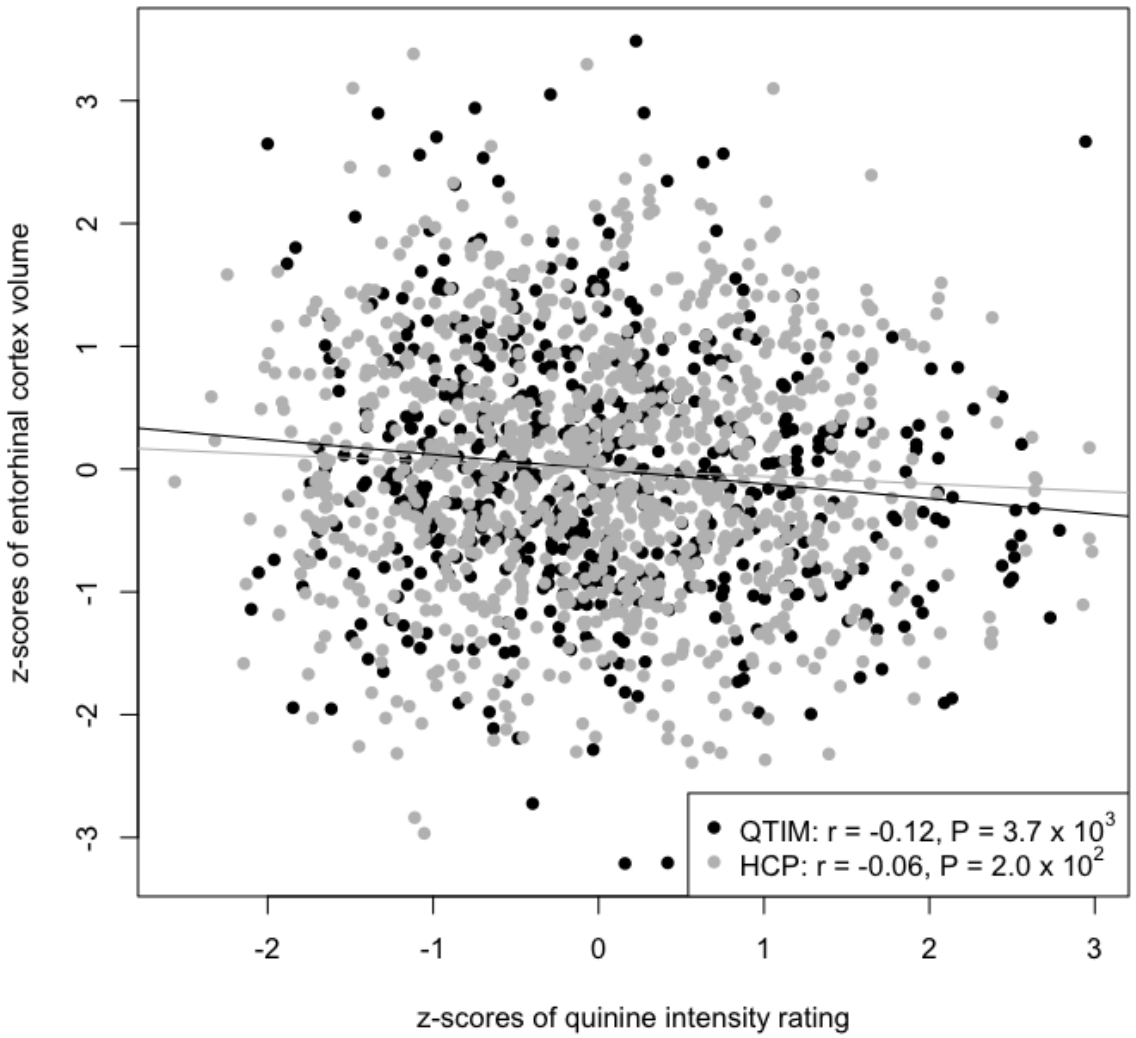

Figure 6-2. Scatter plots showing the association between quinine intensity rating and entorhinal cortex volume in QTIM (n = 558) and HCP (n = 1101). Every SD increase in quinine intensity rating is associated with 0.12 and 0.06 SD decrease in entorhinal cortex volume in QTIM and HCP, respectively. No point is covered by the legend.

7

General Discussion

Chapter 7. General Discussion

This work extends our understanding of individual differences in human taste perception of sweetness and bitterness by showing their relationships with genes, dietary behaviour, and brain morphology. We applied quantitative and statistical genetic methods (including variance component modelling, GWAS, Mendelian randomization, polygenic risk score prediction, and mixed-effect linear regression) on the largest-to-date genetically informative twin sample with multiple taste and brain phenotypes (n = 1999 Australian twins and siblings) and data from the UK biobank cohort (N = 438,870) and HCP (n = 1101). We estimated the heritability of sweet taste perception and the genetic association between sweet and bitter tastes. We identified novel loci associated with bitter tastes and revealed their pleiotropy. We demonstrated the effect of taste perception on beverage intake and BMI. Lastly, we showed the association between taste and the volume of specific brain regions. Key findings from each chapter are summarized in Table 7-1.

Table 7-1. Summary of key findings from each chapter.

Chapter	Aims	Findings
1. A common genetic influence on human intensity ratings of sugars and high-potency sweeteners.	- Estimate heritability of perceived intensity ratings of sugars and high-potency sweeteners. - Investigate their genetic overlap.	• h^2 = 0.31 for glucose. • h^2 = 0.34 for fructose. • h^2 = 0.31 for NHDC. • h^2 = 0.30 for aspartame. • A common genetic factor accounts for more than 75% of the genetic variance in each sweet taste.
2. Is the association between sweet and bitter perception due to genetic variation?	- Investigate the source of association between the perceived intensity of sweetness (a factor score of glucose, fructose, NHDC, and aspartame) and bitterness (PROP, SOA, quinine, and caffeine).	• The sweetness is moderately correlated with SOA, quinine, and caffeine (r_p = 0.35 – 0.40). • A shared genetic factor accounts for 8% of the variance in sweetness and 17% – 37% of the variance in SOA, quinine, and caffeine (r_g = 0.46 – 0.51). • The association between sweetness and PROP becomes evident after adjusting for the *TAS2R38* diplotype (r_p increases from 0.22 to 0.32 and r_g increases from 0.18 to 0.40).
3. Joint analysis strengthens the role of bitter receptor clusters on chromosomes 7 and 12 in human bitter taste	- Identify variants with small effects (< 5%) on bitter taste using bivariate GWAS.	• Two putative novel associations within clusters of bitter taster receptor genes on chromosomes 7 and 12 for DB (rs10261515, r^2 = 0.93%, univariate P_{DB} = 2.5e-4, bivariate $P_{DB\text{-}Quinine}$ = 3.1e-8) and SOA (rs67487380; r^2 = 1.63%, univariate P_{SOA} = 3.8e-7, bivariate $P_{SOA\text{-}Quinine}$ = 5.4e-13, bivariate $P_{SOA\text{-}caffeine}$ = 4.5e-8)

		respectively. • Independent replication for an association for caffeine on chromosome 12 (rs2597979, r^2 = 1.91%, P = 4.2e-8). • Top SNPs for quinine, SOA, and caffeine on chromosome 12 are pleiotropic to the perception of quinine, caffeine, SOA, and DB.
4. Understanding the role of bitter taste perception in coffee, tea and alcohol consumption through Mendelian randomization	- Investigate the causal relationship between perceived bitterness of PROP, quinine, and caffeine and the intake of bitter beverages coffee, tea, and alcohol.	• With every 2-SD change in the predicted bitterness, a higher perceived intensity of caffeine increases coffee consumption by 0.076 (P = 1.6e-9) cups per day, whereas higher perceived intensities of PROP and quinine decrease coffee consumption by 0.034 (P = 7.2e-4) and 0.052 (P = 1.9e-7) cups per day. • For tea consumption, a higher perceived intensity of caffeine decreases coffee consumption by 0.094 (P = 2.1e-8) cups per day, and higher perceived intensities of PROP and quinine increase tea consumption by 0.067 (P = 3.7e-7) and 0.059 (P = 2.1e-8) cups per day. The opposite effects of bitter tastes are due to the negative correlation between coffee and tea intake. • For alcohol, only the perception of PROP is associated, with every 2-SD decrease in the predicted perceived intensity leading to a higher frequency of alcohol intake (beta = 0.038, P = 5.9e-10).
5. Sweet taste perception is associated with body mass index at the phenotypic and genetic level	- Investigate the associations between sweet tastes (glucose, fructose, NHDC, and aspartame) and BMI measured (1) at the same time (in adolescence) and (2) 9 years later (young adulthood). - Estimate their genetic associations using variance components analysis and polygenic risk score prediction.	• Suggestive associations between sweet tastes and BMI measured at the same time: Glucose, r_p = 0.06, P = 0.035. Fructose, r_p = 0.04, P = 0.196. NHDC: r_p = 0.04, P = 0.122. Aspartame, r_p = 0.06, P = 0.029. • Stronger associations between sweet tastes and BMI measured 9 years later: Glucose, r_p = 0.08, P = 0.020. Fructose, r_p = 0.07, P = 0.034. NHDC: r_p = 0.07, P = 0.025. Aspartame, r_p = 0.09, P = 0.007. • The associations with glucose and NHDC are partly due to genetics.
6. Is there an association between brain structure and perceived intensity of sweet and bitter tastes?	- Explore the associations between the volume of 82 brain regions of interest and perceived intensity of sweetness and bitterness of PROP, quinine, and caffeine in QTIM (n = 559) and QTIM (n = 1101).	• Four brain regions (right cuneus gyrus, right inferior temporal gyrus, left transverse temporal gyrus, and both left and right caudate volumes) are nominally associated with the perceived intensities of both sweet and bitter tastes in QTIM. • The negative association between quinine perception and the entorhinal gyrus volume is found in both QTIM and HCP.

Heritability of sweet taste perception

We showed that the heritabilities of the perceived intensity for both sugars and artificial sweeteners are approximately 0.3 (**Chapter 1**), which is smaller than that for sourness ($h^2 = 0.5$) [42] and bitterness ($h^2 \sim 0.7$ for PROP/PTC and ~ 0.4 for other bitter tastes [40, 41]) and larger than saltiness ($h^2 = 0$) [42, 43]. Our large twin cohort overcame the power issue present in other studies [41, 43] and allowed us to use variance components modelling to provide this first solid estimate of the degree of genetic effect on sweet taste perception.

Previous studies sequencing sweet taste receptor genes and genes involved in the sweet perception pathway suggested that variants within/nearby *TAS1R3* [82] and *GNAT3* [83] respectively account for 16% and 13% of the variance of sucrose sensitivity, but such large amounts of variance explained were likely overestimated. If these variants accounted for a total of 29% of the variance, which is over 85% of the total genetic variance of sweet taste, they should be easily replicated and widely reported, which is not the case. Besides, those associations were found using a small sample (n ≥ 160) with mixed ethnicities (Caucasians, Asians, and African-Americans) without replication. We looked up these associations in our Australian Caucasian twin sample (n = 1757) and a U.S. Caucasian twin sample (n = 686) and found no replications, except for a nominal association between rs307355 and sucrose intensity in the U.S. sample (P = 0.03; Table 7-2). This suggests that the effects of these genes could be specific to sensitivity ratings rather the intensity ratings, or, their effects are simply too small so a large sample is required for replication. Additionally, genes apart from the sweet taste receptor system may also play a significant role, such as genes with putative functions in the brain that regulate BMI could also be responsible for sweet taste perception as suggested in **Chapter 5**. Nevertheless, we note that variants within *TAS1R3* are found to be associated with sweet preference [84, 85] and the risk of dental caries [247] in adults, *rather than with sweet perception*.

Table 7-2. Associations (P-values) between variants within TAS1R3 and GNAT3 and sweet taste phenotypes in the QIMR Berghofer and the Monell samples.

	QIMR (n = 1757 Australian twins and siblings)					Monell (n = 686 U.S. twins)		
SNP	**Glucose Intensity**	**Fructose Intensity**	**Aspartame Intensity**	**NHDC Intensity**	**gSweet**	**Sucrose Intensity**	**Sucrose Sweetness**	**Sucrose Liking**
TAS1R3								
rs307355	0.64	0.21	0.69	0.56	0.37	0.03*	0.14	0.24
rs35744813	0.61	0.20	0.39	0.57	0.36	0.38	0.20	0.40
GNAT3								
rs7792845	0.99	0.37	0.80	0.81	0.70	0.28	0.31	0.32
rs940541	0.52	0.67	0.36	0.83	0.70	0.47	0.42	0.76
rs1107660	0.69	0.89	0.14	0.97	0.61	0.40	0.35	0.82
rs1107657	0.71	0.93	0.15	0.92	0.64	0.39	0.39	0.83
rs1524600	0.45	0.88	0.68	0.91	0.70	0.81	0.91	0.22
rs6467217	0.45	0.84	0.64	0.91	0.68	0.81	0.91	0.22
rs6970109	0.45	0.84	0.64	0.91	0.68	0.79	0.85	0.32
rs6975345	0.53	0.89	0.59	0.99	0.68	0.63	0.86	0.49
rs10242727	0.49	0.86	0.51	0.96	0.63	0.63	0.86	0.49
rs6467192	0.30	0.91	0.38	0.98	0.55	0.74	0.93	0.42
rs6961082	0.73	0.15	0.99	0.15	0.65	0.84	0.98	0.38

Only one association has P < 0.05, followed by *. Associations are calculated using GEMMA, as used in Chapter 3.

Genetic architecture of sweet and bitter taste

In **Chapter 1** we found a common genetic factor accounting for most of the genetic variance for each of the four sweet taste phenotypes, with specific sweet taste genetic factors accounting for < 25% of the total genetic variance. A different genetic architecture was found for bitter taste, with the unique/specific genetic factors for quinine and caffeine accounting for 50% and 35% of the total genetic variance, respectively (**Chapter 2**). These results suggest that humans have more specialized genetic pathways for the perception of different bitter substances compared with sweet substances, but more supporting evidence at the molecular level is required.

In **Chapter 3** we showed that bitter taste receptor genes played a key role for the genetic covariances between bitter tastes as they appeared in the top associations for each bitter taste and that the top SNPs for quinine, caffeine, and SOA had pleiotropic influences on the perception of quinine, caffeine, SOA, and DB. Interestingly, while we found a strong positive genetic association between the perception of quinine and caffeine (r_g = 0.68; **Chapter 2**), the top SNP for quinine (rs10772420) was inversely associated with the perception of quinine and caffeine (**Chapter 3**), so was the top SNP for caffeine (rs2597979). This indicates that these top SNPs actually reduce the strength of genetic association between the perception of quinine and caffeine. Nevertheless, we note that these top SNPs are within a large linkage disequilibrium block and could be proxies of the true causal SNPs, which might not have such obvious opposite influences on the two tastes.

If the peripheral receptor system accounted for the genetic covariance between bitter tastes, it might also partly account for the genetic association between sweet and bitter tastes (**Chapter 2**) because both sweet and bitter receptors are from the GPCR family, which share downstream signalling molecules [136, 137]. In addition, genetic variants in the bitter taste receptor genes could also influence sweet taste perception, such as the effect of *TAS2R31* on the perception of a non-nutritive sweetener acesulfame potassium [171].

Taste, diet, and health

Previous findings of the relationship between taste and other phenotypes were mostly reported in cross-sectional studies, which cannot make causal inferences. Using Mendelian randomization (**Chapter 4**), we demonstrated that bitter taste perception is causally associated with the intake of bitter beverages. This work could not be done without the identification genetic variants for PROP, quinine, and caffeine (**Chapter 3**). Further in a longitudinal study (**Chapter 5**) we showed that adolescent sweet perception is a predictor of early adult BMI. These findings together highlight the importance of taste perception in dietary behaviour. The causal relationship indicates that factors modifying taste perception can have further impact on diet and health and could support the previous findings that the primary cause of morbidity in 20% of cancer patients is malnutrition rather than malignancy [248, 249], as cancer treatments (e.g. chemo- and radiotherapies) can modify sensory perception [250] that further leads to a decrease in dietary intake and the development of food aversion [251]. Our efforts to explore the mechanisms underlying individual differences in taste could provide strategies to recover altered taste and prevent the unexpected negative impact on quality of life.

A peep into the human gustatory circuit from a different angle

We used the largest-to-date cohorts with both brain and taste phenotypes (n = 559 and 1101 in QTIM and HCP, respectively) to show that taste perception is associated with the volume of specific brain regions (**Chapter 6**). However, the association pattern we found varies from what has been reported in functional imaging studies [63, 64]. For example, the entorhinal cortex, which was associated with quinine perception in both QTIM and HCP, was not reported in the two largest meta-analyses of functional imaging studies [63, 64]. Also, the repeatedly reported gustatory region – insula cortex – was not associated in our work. These

inconsistencies suggest that the associated brain regions we found may play a different role in taste perception compared to those identified in functional imaging studies. For example, neurons in these regions could function consistently (e.g. connecting taste cortices) regardless of tasting status so there is no obvious change in the neuronal activity. Additionally, the associations we found were weak (r_p ~ 0.1) and were not significant when using a more stringent (Bonferroni-corrected) threshold, indicating that the power of our sample remains too small. Alternatively, this could suggest that other brain phenotypes, e.g. tissue density [252], should be investigated when exploring brain gustatory circuit.

Limitations and future directions

In addition to the limitations discussed in each chapter, there are general limitations to this work. The primary sample we used included only healthy Caucasians living in Australia and most taste data were collected at age 14. Although this implies that our findings may only apply to this specific population, it indicates that they are less likely to be influenced by potential confounding factors such as age [14], race [24], ethnicity [23], and health status [25]. Additionally, there was no significant sex effect on taste perception (except for aspartame [**Chapter 1**] and SOA [40]) in our adolescent sample, whereas previous studies reported sex differences in younger [13, 15] and older groups [17]. Nevertheless, this work can serve as a starting point for future studies with different sample characteristics to investigate taste genetics.

Our sample contained multiple sweet and bitter taste phenotypes, but no data was available for other taste modalities (e.g. sourness, saltiness, and umami taste). Therefore, we could not test whether the common genetic factor for sweet and bitter tastes (**Chapter 2**) is also for the perception of umami taste, whose receptors are also members of the GPCR family [131, 139], and not for sour and salty tastes, whose receptors are believed to be ion channels [132, 134]. A more complete picture of the genetic covariances across taste modalities can be examined using the twin sensory data collected by the Monell Chemical Senses Center (n > 1000 and increases every year) [41]. The dataset includes taste ratings on sweet (sucrose), bitter (PTC and quinine), sour (citric acid), salty (sodium chloride and potassium chloride) solutions, and more (e.g. vegetable juice). Additionally, each taste stimulus was rated for not only perceived intensity but also sweetness, bitterness, sourness,

saltiness, burn, and liking. This informative twin dataset will further allow testing the relationship between different qualities of the same taste, e.g. whether the association between sweetness, intensity, and liking of a sucrose solution is due to genetic covariance.

Sample size is a major obstacle hindering the progress of taste genetics because both twin modelling and GWAS require large power to show a significant effect particularly when the effect size is small (e.g. lower heritability and genetic variants with small effects). Genetically informative data on taste are relatively limited compared to disease traits or other sensory traits, such as vision [253-255] or hearing [256-258]. This is presumably due to impaired taste having no immediate threat to life, or to a lesser extent. A foreseeable solution to the power issue is to form a "Taste Genetics" consortium. It has been a trend to bring research groups with similar interests together and there has been many successful genetic discoveries through consortium collaboration, e.g. the Enhancing Neuro Imaging Genetics through Meta-Analysis (ENIGMA) Consortium [245] and the Psychiatric Genomics Consortium (PGC) [259]. There are a few genetically informative cohorts on taste perception, including the American [41], the Brazilian [38], the Australian (ours), and the Silk Road and the Italian samples (see **Chapter 3**). Bringing these data together for meta-analysis or mega-analysis would provide enhanced power to identify loci that could not be found in each sub-sample. We note that taste phenotypes vary between studies so it will be easier to accumulate data on more common phenotypes (e.g. perception of PROP, quinine, caffeine, sucrose), whereas others will rely on new data collection, such as the perception of SOA and DB.

Integrating genomic data with metabolomics data will provide a deeper understanding of the biological pathways of human taste perception. GWAS points out top signals, which are not necessarily the causal SNPs or within the responsible genes. Additional information of the identified SNPs can be obtained from analyses of metabolomic data such as eQTL or mQTL results. For example, GTEx [163] and Haploreg [160] are web-based tools for functional annotating the SNPs of interest, which we used to link the novel association for DB to the bitter taste receptor T2R4 in **Chapter 3**. Other tools, such as PrediXcan [260] and MetaXcan [261], are developed to use GWAS summaries to impute gene expression levels for prioritizing associated genes. Furthermore, there are tools that employ GWAS and eQTL summaries to perform multi-SNP Mendelian randomization, such as SMR [262] and

gSMR [263], to test causal associations between the gene expression levels and traits of interests. These approaches help target the right genes for follow-up functional validation in cell-based assays or animal models. Besides, it is very important to choose right tissues/cells for functional validation because the same protein can have various functions in different tissues, e.g. T2R38 is responsible for perception in the mouth and immune response in the airway [264, 265]. Streamlining these steps can accelerate research discovery not only for taste but overall medical and biological sciences.

Mendelian randomization is not ready to test the effect of taste other than the perception of PROP/PTC, quinine, and caffeine because this method relies on strong and reliable SNP associations, which are often discovered in GWAS, to be used as genetic instruments/proxies. However, since there has been hundreds of studies showing the relationships between PROP/PTC perception and dietary behaviour [34] and diseases [17] plus we showed the effects of quinine and caffeine on beverage intake in **Chapter 4**, it would be useful to do a phenome-wide Mendelian randomization to investigate the effect of these tastes on diet-related phenotypes, such as the intake of sugar, salt, and the risk of being overweight [266], cardiovascular diseases, and even cancer [267]. The SNP associations for these traits can be acquired from the Gene Atlas [200] and the Global Biobank Engine [http://gbe.stanford.edu], which contain genetic associations for hundreds of traits of UK Biobank participants. The outcomes will not only expand our understanding of the casual effect of taste but also provide hints to direct taste research, with potentially higher success/return rates.

Conclusion

This work contributes to the literature of taste sciences by expanding current knowledge in its genetics and relationships with diet and brain morphology. It also reveals existing obstacles and brings out new directions to pursue. We believe that taste research is promising in terms of personalized nutrition and medicine and the prevention of public health issues, which will benefit human beings and build a healthy world for tomorrow.

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Supplementary Documents

Chapter 1

None.

Chapter 2

Supplementary Table 2-1. Number of families before and after data screening.

Family Type	Initial	After screening [a]
MZ twin pairs	234	189
MZ twin pairs + sibling(s) [b]	82	54
DZ twin pairs	491	380
DZ twin pairs + sibling(s) [b]	95	72
Non-twin singletons/unpaired twins	150	320 [c]

46% of the sample had suffered a middle ear infection and it was included as a covariate in all analyses. 27% of the sample had a history of head injury but it had no effect on all intensity ratings and thus was not included (Chapter 1).
[a] Participants were excluded if they scored water as moderate or higher taste (> 20 mm on gLMS), had large differences between presentation one and two and had overly high or low total average scores (Chapter 1).
[b] Families with a twin pair and one or two siblings.
[c] The number of non-twin singletons/unpaired twins increases after cleaning as some twin pair families lose one twin during the screening procedure.

Supplementary Table 2-2. Taste intensity characteristics of denatonium benzoate.

Mean ± SD [a]	79.5+24.8
Twin Correlations [b]	
r_{MZ} (95% CI)	0.41 (0.3, 0.51)
r_{DZ} (95% CI)	0.19 (0.1, 0.28)
Heritability (95% CI)	0.43 (0.33, 0.52)
Correlations (95% CI)	
Full Sample	
PROP	0.29 (0.25, 0.34)
SOA	0.63 (0.6, 0.66)
Quinine	0.58 (0.55, 0.61)
Caffeine	0.62 (0.59, 0.65)
gSweet	0.43 (0.4, 0.47)
TAS2R38 adjusted [c]	
PROP	0.37 (0.33, 0.41)
SOA	0.63 (0.6, 0.66)
Quinine	0.6 (0.56, 0.63)
Caffeine	0.63 (0.6, 0.65)
gSweet	0.44 (0.4, 0.48)
AVI/AVI excluded [d]	
PROP	0.45 (0.4, 0.49)
SOA	0.62 (0.58, 0.65)
Quinine	0.57 (0.53, 0.61)
Caffeine	0.6 (0.56, 0.64)
gSweet	0.41 (0.36, 0.46)

Mean and standard deviation, MZ and DZ twin correlations, heritability estimate for perceived intensity ratings (millimeters on a labeled magnitude scale) of denatonium benzoate and phenotypic correlations with PROP, SOA, quinine, caffeine and a general sweetness factor (gSweet).
[a] n = 1882.
[b] 238 MZ and 446 DZ twin pairs. Estimates are from univariate AE models.
[c] *TAS2R38* diplotype, available for n = 1756, was tested in a partial dominant model.
[d] N reduced to 1229 when *TAS2R38* AVI/AVI diplotype excluded

Supplementary Table 2-3. Kurtosis and skewness of taste intensity ratings before and after square root transformation.

	Kurtosis		Skewness	
	Original	Sqrt transformed	Original	Sqrt transformed
PROP	2.3963	1.9849	0.6164	-0.0931
SOA	2.5823	2.6556	0.3702	-0.1761
Quinine	2.8460	2.7351	0.5484	-0.1110
Caffeine	2.7097	2.5954	0.5125	-0.0202
Denatonium Benzoate	2.2380	3.2557	-0.4214	-0.8116
gSweet	5.0308	3.2773	1.1864	0.4587

The square root transformation approximates the intensity rating of gSweet to a normal distribution and does not worsen the distributions of those for PROP, SOA, quinine and caffeine.

Supplementary Table 2-4. Model fit of the Cholesky multivariate modelling for perceived intensity ratings of PROP, SOA, quinine, caffeine and gSweet.

	Model	-2LL	df	AIC	Δ-2LL	Δdf	p
Full sample (n = 1901)	ACE	23236.74	9377	4482.743			
	AE	**23242.78**	**9392**	**4458.779**	**6.036**	**15**	**0.98**
	CE	23343.004	9392	4559.004	106.261	15	8.39e-16
	E	23676.959	9407	4862.959	440.216	30	1.96e-74
TAS2R38 adjusted [a] (n = 1756)	ACE	20216.56	8661	2894.561			
	AE	**20225.1**	**8676**	**2873.103**	**8.542**	**15**	**0.9**
	CE	20257.308	8676	2905.308	40.747	15	3.50e-4
	E	20428.84	8691	3046.84	212.279	30	2.44e-29
AVI/AVI excluded (n = 1229)	ACE	14413.51	6047	2319.511			
	AE	**14424.27**	**6062**	**2300.269**	**10.758**	**15**	**0.77**
	CE	14462.502	6062	2905.308	48.991	15	1.76e-5
	E	14632.462	6077	2478.462	218.951	30	1.33e-30

Abbreviations: degrees of freedom (df); -2 times the log-likelihood (-2LL); Akaike's information criterion (AIC).
All models are fitted versus Cholesky full ACE model. Best models are shown in bold.
[a] *TAS2R38* diplotype was tested in a partial dominant model.

Supplementary Table 2-5. Absolute variance (95% confidence intervals) in perceived intensities of PROP, SOA, quinine, caffeine, and the general sweet intensity accounted for by each genetic (A) and environmental (E) factor in Cholesky AE model.

a. Full sample

	A1	**A2**	A3	A4	A5
PROP	0.72 (0.64, 0.81)				
SOA	0.02 (0.01, 0.05)	**0.36 (0.27, 0.45)**			
Quinine	0.01 (0, 0.02)	**0.19 (0.11, 0.27)**	0.20 (0.13, 0.27)		
Caffeine	0.03 (0.01, 0.06)	**0.17 (0.10, 0.25)**	0.02 (0, 0.05)	0.12 (0.07, 0.17)	
gSweet	0.01 (0, 0.03)	**0.08 (0.03, 0.15)**	0.02 (0, 0.06)	0 (0, 0.02)	0.24 (0.16, 0.31)
	E1	E2	E3	E4	E5
PROP	0.27 (0.23, 0.32)				
SOA	0.09 (0.05, 0.15)	0.49 (0.42, 0.58)			
Quinine	0.12 (0.07, 0.18)	0.08 (0.05, 0.13)	0.38 (0.33, 0.45)		
Caffeine	0.08 (0.04, 0.14)	0.15 (0.1, 0.21)	0.06 (0.04, 0.10)	0.35 (0.30, 0.40)	
gSweet	0.06 (0.02, 0.11)	0.01 (0, 0.03)	0.02 (0.01, 0.05)	0.02 (0, 0.03)	0.49 (0.43, 0.57)

n = 1901. A2, shown in bold, is the only common genetic factor for gSweet and the bitter compounds SOA, quinine, caffeine.

b. Adjusted for *TAS2R38* diplotype.

	A1	**A2**	A3	A4	A5
PROP	**0.20 (0.15, 0.25)**				
SOA	**0.05 (0.01, 0.10)**	**0.34 (0.26, 0.43)**			
Quinine	**0.07 (0.02, 0.13)**	**0.14 (0.08, 0.22)**	0.16 (0.10, 0.22)		
Caffeine	**0.08 (0.03, 0.15)**	**0.13 (0.07, 0.20)**	0.01 (0, 0.04)	0.12 (0.07, 0.17)	
gSweet	**0.05 (0.01, 0.11)**	**0.06 (0.02, 0.12)**	0 (0, 0.03)	0 (0, 0.02)	0.24 (0.15, 0.32)
	E1	E2	E3	E4	E5
PROP	0.30 (0.26, 0.35)				
SOA	0.08 (0.04, 0.13)	0.49 (0.42, 0.57)			
Quinine	0.12 (0.07, 0.18)	0.08 (0.05, 0.13)	0.40 (0.34, 0.46)		
Caffeine	0.09 (0.05, 0.15)	0.14 (0.09, 0.20)	0.06 (0.03, 0.09)	0.34 (0.30, 0.39)	
gSweet	0.04 (0.02, 0.08)	0.01 (0, 0.04)	0.03 (0.01, 0.06)	0.01 (0, 0.04)	0.49 (0.43, 0.57)

n = 1756. The genetic variance in PROP reduces from 0.72 to 0.20 after adjustment whereas its environmental variance remains. The total genetic and total environmental variances in SOA, quinine, caffeine, and gSweet do not change after adjustment. Both A1 and A2, shown in bold, are common genetic factors for intensity ratings of sweet and bitter tastes. *TAS2R38* diplotype was tested in a partial dominant model.

c. *TAS2R38* AVI/AVI excluded.

	A1	**A2**	A3	A4	A5
PROP	**0.37 (0.31, 0.43)**				
SOA	**0.07 (0.03, 0.12)**	**0.31 (0.23, 0.39)**			
Quinine	**0.07 (0.03, 0.12)**	**0.14 (0.08, 0.22)**	0.16 (0.1, 0.23)		
Caffeine	**0.09 (0.05, 0.15)**	**0.09 (0.04, 0.15)**	0.01 (0, 0.05)	0.15 (0.10, 0.21)	
gSweet	**0.04 (0.01, 0.09)**	**0.08 (0.03, 0.15)**	0 (0, 0.03)	0 (0, 0.02)	0.27 (0.17, 0.36)
	E1	E2	E3	E4	E5
PROP	0.26 (0.22, 0.31)				
SOA	0.13 (0.08, 0.19)	0.46 (0.40, 0.53)			
Quinine	0.17 (0.11, 0.24)	0.05 (0.03, 0.09)	0.37 (0.32, 0.43)		
Caffeine	0.12 (0.07, 0.19)	0.14 (0.09, 0.19)	0.06 (0.03, 0.09)	0.33 (0.29, 0.39)	
gSweet	0.05 (0.02, 0.10)	0 (0, 0.02)	0.03 (0.01, 0.06)	0.01 (0, 0.04)	0.49 (0.42, 0.57)

n = 1229. Participants with *TAS2R38* AVI/AVI diplotypes were excluded. The genetic variance in PROP reduces from 0.72 to 0.37 after adjustment whereas its environmental variance remains. The total genetic and total environmental variances in SOA, quinine, caffeine, and gSweet do not change after adjustment. Both A1 and A2, shown in bold, are common genetic factors for intensity ratings of sweet and bitter tastes. Both A1 and A2, shown in bold, are common genetic factors for intensity ratings of sweet and bitter tastes.

Supplementary Table 2-6. Genetic variance accounted for by each genetic factor in the Cholesky AE models.

		A1	A2	A3	A4	A5
Full Sample	PROP	100%				
	SOA	6.2% (1.9, 12.5)	93.8% (87.5, 98.2)			
	Quinine	1.4% (0, 5.2)	45.8% (31.3, 61.2)	52.8% (37.3, 67.7)		
	Caffeine	9.3% (3.4, 17.5)	49.0% (34.0, 64.5)	5.9% (0.5, 15.8)	35.8% (22.7, 49.8)	
	gSweet	3.2% (0.3, 8.7)	23.4% (10.3, 41.5)	4.4% (0, 15.8)	0.1% (0, 6.2)	68.9% (51.5, 83.3)
TAS2R38 Adjusted [a]	PROP	100%				
	SOA	12.3% (3.4, 24.5)	87.7% (75.5, 96.6)			
	Quinine	17.8% (6.7, 31.5)	39.3% (25.1, 55.6)	42.9% (27.7, 58.1)		
	Caffeine	23.5% (10.2, 39.9)	38.2% (23.6, 54)	3.1% (0, 11.6)	35.2% (22.4, 49.1)	
	gSweet	15.1% (4.7, 29.9)	16.4% (5.8, 32.2)	0.8% (0, 8.5)	0% (0, 0)	67.7% (49.9, 82.5)
AVI/AVI excluded	PROP	100%				
	SOA	17.7% (8.8, 28.1)	82.3% (71.9, 91.2)			
	Quinine	18.6% (9.6, 28.8)	38.1% (24.5, 54.1)	43.3% (27.8, 58.3)		
	Caffeine	26.7% (15.5, 39.6)	25.2% (13.1, 38.6)	4.2% (0, 13.9)	43.9% (31.5, 57.6)	
	gSweet	11.2% (4.1, 20.9)	20% (8.3, 37.3)	0.2% (0, 8.7)	0.3% (0, 6.2)	68.4% (47.0, 83.2)

[a] *TAS2R38* diplotype was tested in a partial dominant model.

Supplementary Table 2-7. Standardized variance (95% confidence intervals) in perceived intensities of PROP, SOA, quinine, caffeine, and glucose or fructose accounted for by each genetic (A) and environmental (E) factor in Cholesky AE model adjusted for the TAS2R38 diplotype.

a. Glucose

	A1	A2	A3	A4	A5
PROP	40% (31, 49)				
SOA	5% (1, 11)	36% (27, 43)			
Quinine	7% (2, 13)	15% (9, 22)	16% (10, 22)		
Caffeine	8% (3, 15)	13% (7, 20)	1% (0, 4)	12% (7, 17)	
Glucose	4% (1, 9)	3% (1, 8)	0% (0, 3)	1% (0, 5)	26% (17, 34)
	E1	E2	E3	E4	E5
PROP	60% (51, 69)				
SOA	8% (4, 14)	51% (44, 59)			
Quinine	12% (7, 18)	9% (5, 13)	41% (35, 48)		
Caffeine	9% (5, 15)	14% (10, 21)	6% (3, 10)	35% (30, 40)	
gSweet	3% (1, 7)	2% (0, 5)	3% (1, 6)	1% (0, 3)	58% (50, 66)

n = 1756.

b. Fructose

	A1	A2	A3	A4	A5
PROP	40% (31, 49)				
SOA	5% (1, 11)	36% (27, 43)			
Quinine	7% (2, 13)	15% (9, 22)	16% (10, 22)		
Caffeine	8% (3, 15)	13% (7, 20)	1% (0, 4)	12% (7, 17)	
Fructose	4% (1, 10)	5% (1, 10)	1% (0, 5)	0% (0, 4)	25% (15, 33)
	E1	E2	E3	E4	E5
PROP	60% (51, 69)				
SOA	8% (5, 14)	51% (44, 59)			
Quinine	12% (7, 18)	9% (5, 13)	41% (35, 48)		
Caffeine	9% (5, 15)	15% (10, 21)	6% (3, 9)	35% (30, 40)	
Fructose	2% (1, 6)	1% (0, 2)	2% (0, 5)	1% (0, 4)	59% (51, 68)

n = 1756.

Supplementary Table 2-8. Phenotypic correlations between taste intensities and IQ, personality and emphasis scores estimated from bivariate ACE models.

	IQ	Neuroticism	Extraversion	Openness	Agreeableness	Conscientiousness	Emphasis
PROP	-0.11*	0.04	0.02	-0.05	-0.07*	-0.03	-0.02
SOA	-0.15*	0.07*	0.03	-0.07*+	-0.06*+	-0.04	-0.02
Quinine	-0.14*	0.07*	0.05	-0.05	-0.04	-0.05	0
Caffeine	-0.13*	0.07*	0.02	-0.04	-0.06*+	-0.04	-0.02
gSweet	-0.07*	0.05	0.02	0.00	-0.03	-0.05	0

n = 1244~1256. *p < 0.05 before correction for multiple testing. +Insignificant after adjusting for IQ.

Supplementary Table 2-9. Standardized variance in five taste traits in Choleskly AE models adjusted for the TAS2R38 diplotype and further adjusted for IQ, neuroticism, openness and agreeableness.

a. IQ

	A1	A2	A3	A4	A5
PROP	38% (28, 46)				
SOA	8% (3, 15)	31% (22, 38)			
Quinine	3% (0, 8)	15% (8, 23)	16% (9, 23)		
Caffeine	9% (4, 17)	12% (6, 19)	2% (0, 6)	14% (9, 19)	
gSweet	11% (5, 19)	4% (1, 9)	2% (0, 9)	0% (0, 2)	21% (11, 29)
	E1	E2	E3	E4	E5
PROP	62% (54, 72)				
SOA	8% (4, 14)	53% (46, 62)			
Quinine	16% (11, 23)	8% (4, 12)	42% (36, 48)		
Caffeine	9% (5, 14)	14% (9, 19)	5% (3, 8)	35% (30, 41)	
gSweet	3% (1, 7)	2% (0, 5)	2% (0, 4)	2% (0, 5)	54% (46, 62)

n = 1282.

b. Neuroticism

	A1	A2	A3	A4	A5
PROP	37% (27, 46)				
SOA	7% (2, 13)	30% (22, 38)			
Quinine	4% (0, 9)	14% (8, 22)	16% (9, 23)		
Caffeine	9% (3, 16)	11% (5, 18)	1% (0, 5)	13% (8, 19)	
gSweet	9% (4, 17)	3% (0, 8)	2% (0, 8)	0% (0, 3)	22% (12, 30)
	E1	E2	E3	E4	E5
PROP	63% (54, 73)				
SOA	10% (5, 15)	54% (46, 62)			
Quinine	16% (10, 23)	8% (4, 12)	43% (37, 49)		
Caffeine	9% (5, 15)	15% (10, 21)	6% (3, 9)	36% (31, 42)	
gSweet	4% (1, 8)	3% (1, 7)	2% (0, 4)	3% (1, 6)	52% (45, 60)

n = 1277.

c. Agreeableness

	A1	A2	A3	A4	A5
PROP	38% (29, 47)				
SOA	7% (2, 13)	30% (21, 37)			
Quinine	4% (1, 9)	14% (7, 22)	16% (9, 23)		
Caffeine	9% (3, 16)	11% (5, 18)	1% (0, 5)	13% (8, 19)	
gSweet	9% (4, 17)	3% (0, 9)	2% (0, 8)	0% (0, 3)	22% (12, 30)
	E1	E2	E3	E4	E5
PROP	62% (53, 71)				
SOA	10% (5, 15)	54% (46, 62)			
Quinine	16% (10, 23)	8% (4, 12)	43% (37, 49)		
Caffeine	9% (5, 14)	15% (10, 21)	6% (3, 9)	36% (31, 42)	
gSweet	4% (1, 8)	3% (1, 7)	1% (0, 4)	3% (1, 6)	52% (45, 60)

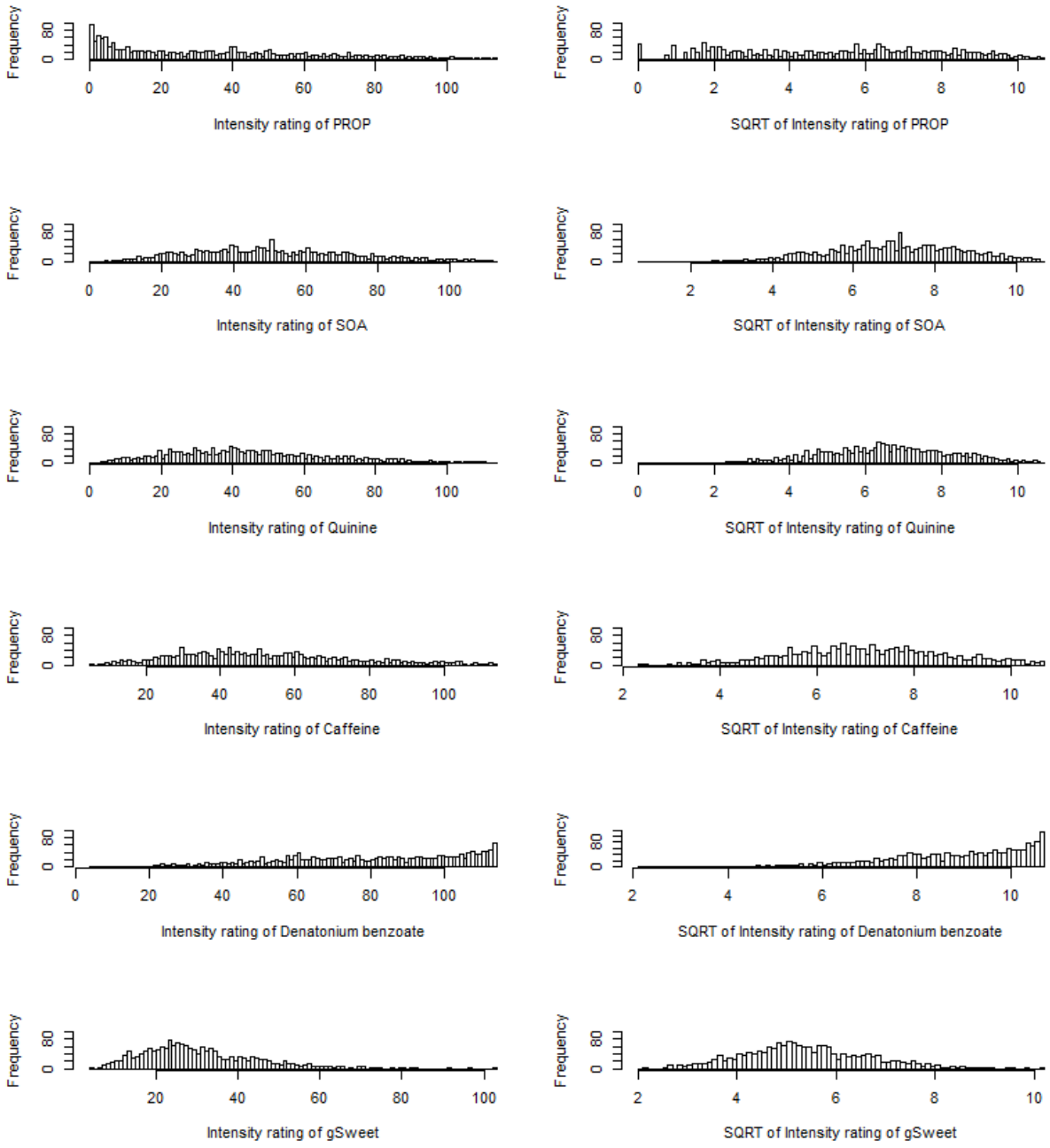
n = 1277.

The multivariate model adjusted for *TAS2R38* was used for comparison because it provided a better fit (AIC = 2873.103) than the model without adjustment (AIC = 4127.487) using the same sample (n = 1756).

Supplementary Table 2-10. Phenotypic correlations between PROP rating from one twin and ratings of SOA, quinine, caffeine, and gSweet from co-twin for MZ and DZ twins.

	MZ	DZ
SOA	0.06 (-0.07, 0.18)	0.08 (-0.01, 0.17)
Quinine	0 (-0.12, 0.13)	0.03 (-0.07, 0.12)
Caffeine	0.09 (-0.04, 0.21)	0.11 (0.02, 0.20)
gSweet	0.10 (-0.03, 0.22)	0.10 (0, 0.19)

n = 1244~1256.



Supplementary Figure 2-1. Distribution of intensity ratings before and after square root transformation.

Chapter 3

Supplementary Table 3-1. Top 100 SNPs on chromosome 12 associated with the perceived intensity of quinine.

Chr:Position	SNP	A1/A2	MAF	Beta	SE	P
12:11173455	rs10743937	C/T	0.469	-0.337	0.034	7.84e-23
12:11173490	rs10772419	C/A	0.469	-0.337	0.034	7.84e-23
12:11174276	rs10772420	G/A	0.469	-0.337	0.034	7.84e-23
12:11134701	rs2900554	T/G	0.468	-0.337	0.034	1.15e-22
12:11177223	rs11054173	A/T	0.469	-0.336	0.034	1.16e-22
12:11178100	rs9651854	C/T	0.469	-0.336	0.034	1.16e-22
12:11180204	rs2900578	T/C	0.469	-0.336	0.034	1.16e-22
12:11180340	rs2010481	A/G	0.469	-0.336	0.034	1.16e-22
12:11189176	rs2060705	G/A	0.475	-0.338	0.034	1.39e-22
12:11192290	rs2597981	G/A	0.475	-0.338	0.034	1.39e-22
12:11258446	rs2443739	C/G	0.467	-0.337	0.034	1.46e-22
12:11131570	rs2218820	T/C	0.468	-0.336	0.034	1.58e-22
12:11133472	rs7136588	T/C	0.468	-0.336	0.034	1.59e-22
12:11137325	rs10772396	T/C	0.468	-0.336	0.034	1.61e-22
12:11314285	rs34241192	C/A	0.467	-0.336	0.034	1.68e-22
12:11315043	rs35021653	T/C	0.467	-0.336	0.034	1.68e-22
12:11182874	rs34763234	G/A	0.47	-0.335	0.034	1.75e-22
12:11183255	rs10845293	G/A	0.47	-0.335	0.034	1.80e-22
12:11285300	rs2708371	C/G	0.467	-0.335	0.034	2.37e-22
12:11285075	rs977473	T/A	0.467	-0.335	0.034	2.38e-22
12:11285130	rs1960613	G/T	0.467	-0.335	0.034	2.38e-22
12:11308428	rs61928597	C/T	0.467	-0.335	0.034	2.42e-22
12:11308774	rs35340812	G/A	0.467	-0.335	0.034	2.42e-22
12:11309593	rs35699328	C/T	0.467	-0.335	0.034	2.42e-22
12:11310004	rs61928602	C/T	0.467	-0.335	0.034	2.44e-22
12:11310295	rs3906996	C/T	0.467	-0.335	0.034	2.44e-22
12:11311520	rs7310047	G/A	0.467	-0.335	0.034	2.44e-22
12:11312860	rs6488355	C/T	0.467	-0.335	0.034	2.44e-22
12:11312877	rs6488356	A/G	0.467	-0.335	0.034	2.44e-22
12:11312948	rs7486717	A/C	0.467	-0.335	0.034	2.44e-22
12:11313673	rs7955495	C/T	0.467	-0.335	0.034	2.47e-22
12:11266222	rs2264229	C/G	0.466	-0.335	0.034	2.47e-22
12:11253373	rs2597972	A/G	0.467	-0.335	0.034	2.49e-22
12:11307312	rs34082341	T/C	0.467	-0.334	0.034	2.54e-22
12:11307693	rs7302010	A/G	0.467	-0.334	0.034	2.54e-22
12:11306777	rs7976211	G/T	0.467	-0.334	0.034	2.54e-22
12:11307147	rs35124606	G/C	0.467	-0.334	0.034	2.58e-22
12:11306346	rs34536990	T/C	0.467	-0.334	0.034	2.60e-22
12:11263015	rs2708354	A/C	0.467	-0.335	0.034	2.74e-22
12:11293821	rs35017789	A/G	0.468	-0.333	0.034	2.83e-22
12:11294026	rs7312327	G/A	0.468	-0.333	0.034	2.83e-22
12:11294191	rs7298544	A/C	0.468	-0.333	0.034	2.83e-22
12:11296917	rs61931270	G/A	0.468	-0.333	0.034	2.83e-22
12:11296975	rs36115011	C/G	0.468	-0.333	0.034	2.83e-22
12:11297165	rs34685506	C/A	0.468	-0.333	0.034	2.83e-22
12:11297752	rs6488350	G/A	0.468	-0.333	0.034	2.83e-22
12:11297854	rs6488351	A/G	0.468	-0.333	0.034	2.83e-22
12:11299449	rs35856529	A/G	0.468	-0.333	0.034	2.83e-22
12:11299605	rs35893804	C/T	0.468	-0.333	0.034	2.83e-22
12:11299685	rs34288418	T/C	0.468	-0.333	0.034	2.83e-22
12:11299687	rs34843817	A/G	0.468	-0.333	0.034	2.83e-22

12:11299851	rs34927715	A/G	0.468	-0.333	0.034	2.83e-22
12:11299977	rs61931278	G/C	0.468	-0.333	0.034	2.83e-22
12:11300006	rs61931279	C/T	0.468	-0.333	0.034	2.83e-22
12:11300369	rs7973730	C/T	0.468	-0.333	0.034	2.83e-22
12:11304086	rs61928564	T/A	0.468	-0.333	0.034	2.83e-22
12:11304159	rs61928565	G/T	0.468	-0.333	0.034	2.83e-22
12:11304327	rs61928566	T/C	0.468	-0.333	0.034	2.83e-22
12:11305373	rs34548551	G/C	0.468	-0.333	0.034	2.83e-22
12:11305514	rs34769150	G/T	0.468	-0.333	0.034	2.83e-22
12:11296329	rs35280352	C/T	0.468	-0.333	0.034	2.84e-22
12:11305724	rs7959320	A/G	0.468	-0.333	0.034	2.88e-22
12:11306064	rs7965506	T/C	0.468	-0.333	0.034	2.88e-22
12:11198678	rs2708322	T/C	0.468	-0.334	0.034	2.91e-22
12:11204944	rs2597992	T/A	0.468	-0.333	0.034	2.96e-22
12:11206217	rs2597994	A/G	0.468	-0.333	0.034	2.96e-22
12:11207864	rs2708386	A/C	0.468	-0.333	0.034	2.96e-22
12:11208989	rs2597998	C/T	0.468	-0.333	0.034	2.96e-22
12:11209938	rs2598000	T/C	0.468	-0.333	0.034	2.96e-22
12:11211904	rs2708383	C/T	0.468	-0.333	0.034	2.96e-22
12:11250938	rs2443094	A/G	0.468	-0.333	0.034	2.96e-22
12:11256413	rs2597966	C/T	0.467	-0.334	0.034	3.01e-22
12:11256437	rs2600332	T/C	0.467	-0.334	0.034	3.01e-22
12:11256660	rs2708361	C/T	0.467	-0.334	0.034	3.01e-22
12:11257001	rs2597965	A/G	0.467	-0.334	0.034	3.01e-22
12:11257149	rs2600341	T/C	0.467	-0.334	0.034	3.01e-22
12:11257235	rs2597964	A/G	0.467	-0.334	0.034	3.01e-22
12:11257876	rs2708359	T/A	0.467	-0.334	0.034	3.01e-22
12:11258011	rs2597963	C/T	0.467	-0.334	0.034	3.01e-22
12:11258149	rs2600349	C/T	0.467	-0.334	0.034	3.01e-22
12:11258253	rs2708358	C/T	0.467	-0.334	0.034	3.01e-22
12:11259871	rs2599405	C/G	0.467	-0.334	0.034	3.01e-22
12:11259894	rs2597960	A/G	0.467	-0.334	0.034	3.01e-22
12:11260410	rs2597959	A/C	0.467	-0.334	0.034	3.01e-22
12:11260644	rs2600338	G/A	0.467	-0.334	0.034	3.01e-22
12:11260761	rs2600339	G/C	0.467	-0.334	0.034	3.01e-22
12:11261123	rs2708356	G/A	0.467	-0.334	0.034	3.01e-22
12:11261967	rs2600342	T/G	0.467	-0.334	0.034	3.01e-22
12:11262086	rs2597952	T/C	0.467	-0.334	0.034	3.01e-22
12:11262265	rs2599411	A/G	0.467	-0.334	0.034	3.01e-22
12:11262724	rs2597951	G/C	0.467	-0.334	0.034	3.01e-22
12:11262955	rs2597950	T/C	0.467	-0.334	0.034	3.01e-22
12:11263089	rs2597949	G/A	0.467	-0.334	0.034	3.01e-22
12:11263112	rs2708353	A/G	0.467	-0.334	0.034	3.01e-22
12:11263318	rs2597947	T/C	0.467	-0.334	0.034	3.01e-22
12:11263744	rs75814885	T/G	0.467	-0.334	0.034	3.01e-22
12:11264798	rs2708352	T/C	0.467	-0.334	0.034	3.01e-22
12:11264980	rs2264190	G/A	0.467	-0.334	0.034	3.01e-22
12:11265851	rs2600344	C/T	0.467	-0.334	0.034	3.01e-22
12:11266342	rs2264192	C/T	0.467	-0.334	0.034	3.01e-22

Supplementary Table 3-2. Top 100 SNPs on chromosome 12 associated with the perceived intensity of caffeine.

Chr:Position	SNP	A1/A2	MAF	Beta	SE	P
12:11189966	rs2597979	G/C	0.163	0.26	0.05	4.17e-8
12:11351229	rs1669436	C/G	0.139	0.28	0.05	8.98e-8
12:11174753	rs1868769	G/A	0.194	0.23	0.04	9.18e-8
12:11180299	rs2084648	A/T	0.193	0.23	0.04	1.04e-7
12:11183451	rs10743938	A/T	0.193	0.23	0.04	1.09e-7
12:11184140	rs10845296	G/A	0.193	0.23	0.04	1.09e-7
12:11203065	rs2597988	T/C	0.145	0.26	0.05	1.12e-7
12:11329548	rs73053413	T/C	0.14	0.27	0.05	1.21e-7
12:11139589	rs10772398	C/T	0.147	0.26	0.05	1.24e-7
12:11128666	rs10772395	C/T	0.147	0.26	0.05	1.28e-7
12:11131462	rs6488333	C/T	0.147	0.26	0.05	1.28e-7
12:11202522	rs2257110	G/C	0.145	0.26	0.05	1.30e-7
12:11293130	rs2600337	G/C	0.14	0.27	0.05	1.37e-7
12:11198433	rs2708323	A/G	0.145	0.26	0.05	1.64e-7
12:11199734	rs2597984	T/C	0.145	0.26	0.05	1.64e-7
12:11345936	rs1650024	A/G	0.141	0.27	0.05	1.91e-7
12:11345005	rs1669421	T/C	0.141	0.26	0.05	2.21e-7
12:11345136	rs1650022	T/G	0.141	0.26	0.05	2.21e-7
12:11346562	rs1669424	T/C	0.141	0.26	0.05	2.21e-7
12:11083677	rs10772391	T/C	0.144	0.26	0.05	2.29e-7
12:11347219	rs1669425	A/G	0.141	0.26	0.05	2.33e-7
12:11347223	rs1650025	G/C	0.141	0.26	0.05	2.33e-7
12:11309537	rs34692077	T/C	0.143	0.26	0.05	2.51e-7
12:11347649	rs1669426	T/C	0.141	0.26	0.05	2.53e-7
12:11347716	rs1650026	T/C	0.141	0.26	0.05	2.53e-7
12:11347751	rs1650027	A/G	0.141	0.26	0.05	2.53e-7
12:11347798	rs1650028	A/G	0.141	0.26	0.05	2.53e-7
12:11348296	rs1427754	C/T	0.141	0.26	0.05	2.53e-7
12:11348862	rs1669430	T/C	0.141	0.26	0.05	2.53e-7
12:11348886	rs1669431	G/T	0.141	0.26	0.05	2.53e-7
12:11349622	rs1650032	A/G	0.141	0.26	0.05	2.53e-7
12:11349671	rs2600373	A/G	0.141	0.26	0.05	2.53e-7
12:11349938	rs1669434	T/G	0.141	0.26	0.05	2.53e-7
12:11350263	rs1669435	T/C	0.141	0.26	0.05	2.53e-7
12:11350661	rs1650033	T/C	0.141	0.26	0.05	2.53e-7
12:11350951	rs1650034	T/G	0.141	0.26	0.05	2.53e-7
12:11350963	rs1650035	T/G	0.141	0.26	0.05	2.53e-7
12:11345572	rs1650023	A/G	0.141	0.26	0.05	2.54e-7
12:11342401	rs1669415	C/T	0.141	0.26	0.05	2.63e-7
12:11342415	rs1669416	G/C	0.141	0.26	0.05	2.63e-7
12:11342525	rs1669417	G/A	0.141	0.26	0.05	2.63e-7
12:11343422	rs1669419	G/A	0.141	0.26	0.05	2.63e-7
12:11349605	rs1669432	G/T	0.14	0.26	0.05	2.64e-7
12:11349732	rs1669433	A/G	0.14	0.26	0.05	2.64e-7
12:11311787	rs61928603	C/T	0.148	0.26	0.05	2.70e-7
12:11343964	rs1650021	T/A	0.141	0.26	0.05	2.79e-7
12:11203459	rs2597990	G/A	0.144	0.26	0.05	2.86e-7
12:11098139	rs2418223	T/A	0.147	0.25	0.05	3.05e-7
12:11341878	rs61928650	T/C	0.141	0.26	0.05	3.16e-7
12:11215852	rs1817043	G/A	0.144	0.26	0.05	3.31e-7
12:11216315	rs2708377	C/T	0.144	0.26	0.05	3.31e-7
12:11216972	rs2255418	C/T	0.144	0.26	0.05	3.31e-7
12:11217237	rs2599415	G/A	0.144	0.26	0.05	3.31e-7

12:11338781	rs1669413	C/A	0.142	0.26	0.05	3.59e-7
12:11079998	rs10743936	C/T	0.147	0.25	0.05	3.68e-7
12:11307615	rs4763634	T/C	0.142	0.26	0.05	3.88e-7
12:11164751	rs7315843	G/A	0.148	0.25	0.05	3.93e-7
12:11165233	rs1376249	A/T	0.148	0.25	0.05	3.93e-7
12:11324559	rs8181	C/G	0.141	0.26	0.05	4.07e-7
12:11326071	rs2900127	G/A	0.141	0.26	0.05	4.07e-7
12:11326315	rs4763637	T/C	0.141	0.26	0.05	4.07e-7
12:11328768	rs1650051	G/A	0.141	0.26	0.05	4.07e-7
12:11329053	rs1669406	G/A	0.141	0.26	0.05	4.07e-7
12:11329249	rs1669407	C/T	0.141	0.26	0.05	4.07e-7
12:11331479	rs187328	T/C	0.141	0.26	0.05	4.07e-7
12:11331726	rs319266	G/A	0.141	0.26	0.05	4.07e-7
12:11332584	rs319269	C/A	0.142	0.26	0.05	4.09e-7
12:11333542	rs319270	C/A	0.142	0.26	0.05	4.09e-7
12:11337442	rs319277	G/A	0.142	0.26	0.05	4.09e-7
12:11338983	rs1650019	T/C	0.141	0.26	0.05	4.15e-7
12:11316437	rs61928609	A/C	0.142	0.26	0.05	4.18e-7
12:11165540	rs7306087	A/C	0.149	0.25	0.05	4.19e-7
12:11167674	rs2900577	G/C	0.149	0.25	0.05	4.19e-7
12:11307278	rs4763632	A/G	0.142	0.26	0.05	4.24e-7
12:11315112	rs61928606	G/A	0.141	0.26	0.05	4.29e-7
12:11150579	rs1463237	C/T	0.148	0.25	0.05	4.34e-7
12:11305844	rs7962445	T/C	0.142	0.26	0.05	4.38e-7
12:11119119	rs6488331	T/C	0.159	0.24	0.05	4.39e-7
12:11320130	rs4763636	A/G	0.142	0.26	0.05	4.40e-7
12:11320643	rs61928615	A/G	0.142	0.26	0.05	4.40e-7
12:11324401	rs1047713	C/G	0.142	0.26	0.05	4.53e-7
12:11320297	rs61928614	A/G	0.142	0.26	0.05	4.58e-7
12:11311159	rs7298947	T/C	0.142	0.26	0.05	4.59e-7
12:11311590	rs7296270	A/T	0.142	0.26	0.05	4.59e-7
12:11311947	rs61928604	C/T	0.142	0.26	0.05	4.59e-7
12:11313886	rs7973298	T/C	0.142	0.26	0.05	4.59e-7
12:11150551	rs4388985	G/A	0.149	0.25	0.05	4.61e-7
12:11158728	rs10772414	C/G	0.149	0.25	0.05	4.61e-7
12:11171846	rs10734843	A/G	0.149	0.25	0.05	4.61e-7
12:11309750	rs35318883	T/C	0.141	0.26	0.05	4.62e-7
12:11091432	rs3741843	C/T	0.147	0.25	0.05	4.72e-7
12:11252797	rs2597975	C/T	0.143	0.25	0.05	5.06e-7
12:11252845	rs2597974	T/C	0.143	0.25	0.05	5.06e-7
12:11338555	rs1669409	A/T	0.142	0.26	0.05	5.06e-7
12:11267880	rs2600347	G/A	0.142	0.25	0.05	5.41e-7
12:11299218	rs7487324	C/T	0.142	0.25	0.05	5.42e-7
12:11300255	rs61931280	C/T	0.142	0.25	0.05	5.42e-7
12:11304413	rs61928567	T/C	0.142	0.25	0.05	5.42e-7
12:11170837	rs10732561	T/G	0.148	0.24	0.05	5.68e-7
12:11261589	rs2600340	T/C	0.143	0.25	0.05	5.71e-7

Supplementary Table 3-3. Top 100 SNPs on chromosome 12 associated with the perceived intensity of sucrose octaacetate (SOA).

Chr:Position	SNP	A1/A2	MAF	Beta	SE	P
12:11194384	rs67487380	A/G	0.275	-0.202	0.040	3.78e-7
12:11195162	rs1901188	T/C	0.275	-0.202	0.040	3.78e-7
12:11170152	rs10845291	T/C	0.274	-0.197	0.040	7.52e-7
12:11196583	rs7310224	G/A	0.264	-0.198	0.040	1.09e-6
12:11195322	rs35969800	A/G	0.265	-0.197	0.040	1.14e-6
12:11211734	rs35846189	C/A	0.321	-0.183	0.038	1.66e-6
12:11211781	rs35413384	C/G	0.321	-0.183	0.038	1.66e-6
12:11220455	rs11526041	A/C	0.321	-0.183	0.038	1.75e-6
12:11205292	rs2900581	G/A	0.321	-0.182	0.038	1.89e-6
12:11205343	rs2900582	A/G	0.321	-0.182	0.038	1.89e-6
12:11166968	rs7310849	G/A	0.32	-0.181	0.038	1.99e-6
12:11272192	rs7313683	G/T	0.348	-0.185	0.039	2.06e-6
12:11252729	rs35097305	C/A	0.32	-0.182	0.038	2.16e-6
12:11315644	rs1349553	G/A	0.315	-0.185	0.039	2.23e-6
12:11167763	rs10845290	A/G	0.284	-0.187	0.039	2.32e-6
12:11175414	rs4763235	G/C	0.321	-0.179	0.038	2.52e-6
12:11256031	rs28419178	T/C	0.319	-0.181	0.038	2.65e-6
12:11138852	rs1376251	T/C	0.321	-0.179	0.038	2.80e-6
12:11141752	rs2418302	T/C	0.321	-0.179	0.038	2.80e-6
12:11314022	rs35746980	T/C	0.315	-0.183	0.039	2.82e-6
12:11318574	rs1551193	A/C	0.314	-0.183	0.039	3.00e-6
12:11147660	rs11054140	T/C	0.321	-0.178	0.038	3.05e-6
12:11259611	rs66840927	T/G	0.319	-0.180	0.039	3.26e-6
12:11166536	rs11054164	T/C	0.32	-0.177	0.038	3.47e-6
12:11166578	rs1901190	C/T	0.32	-0.177	0.038	3.47e-6
12:11143223	rs12296784	A/C	0.266	-0.186	0.040	3.83e-6
12:11272738	rs3851590	C/G	0.261	-0.194	0.042	3.95e-6
12:11131212	rs2900553	T/G	0.32	-0.176	0.038	4.11e-6
12:11131791	rs7138953	A/G	0.32	-0.176	0.038	4.11e-6
12:11177580	rs10772421	A/C	0.266	-0.185	0.040	4.25e-6
12:11262180	rs4763627	A/G	0.318	-0.178	0.039	4.38e-6
12:11311958	rs2290318	C/G	0.315	-0.179	0.039	4.42e-6
12:11312026	rs2290319	A/C	0.315	-0.179	0.039	4.43e-6
12:11263238	rs4763628	C/A	0.319	-0.178	0.039	4.59e-6
12:11304132	rs34373518	G/A	0.317	-0.178	0.039	4.60e-6
12:11263799	rs112665659	C/A	0.319	-0.178	0.039	4.61e-6
12:11263373	rs4763629	G/A	0.319	-0.178	0.039	4.68e-6
12:11309606	rs34274000	A/G	0.315	-0.179	0.039	4.78e-6
12:11291030	rs7316032	G/A	0.317	-0.177	0.039	5.32e-6
12:11293408	rs34708147	G/A	0.317	-0.177	0.039	5.32e-6
12:11299571	rs34666803	C/T	0.317	-0.177	0.039	5.32e-6
12:11281517	rs67961444	C/A	0.317	-0.177	0.039	5.46e-6
12:11285233	rs7980677	C/T	0.317	-0.177	0.039	5.48e-6
12:11289324	rs6488346	C/T	0.317	-0.177	0.039	5.48e-6
12:11264628	rs35376087	T/A	0.318	-0.175	0.039	6.12e-6
12:11266214	rs145696441	C/G	0.318	-0.175	0.039	6.12e-6
12:11266828	rs77096743	C/A	0.318	-0.175	0.039	6.12e-6
12:11271711	rs68186227	C/T	0.318	-0.175	0.039	6.12e-6
12:11216751	rs67861347	G/A	0.329	-0.172	0.038	6.18e-6
12:11165446	rs4763613	T/C	0.331	-0.170	0.038	6.53e-6
12:11154236	rs10772412	C/T	0.327	-0.171	0.038	6.69e-6
12:11149532	rs1450839	G/A	0.331	-0.169	0.038	6.82e-6
12:11149711	rs10845279	A/C	0.331	-0.169	0.038	6.82e-6

12:11149720	rs10845280	G/A	0.331	-0.169	0.038	6.82e-6
12:11149769	rs10845281	C/T	0.331	-0.169	0.038	6.82e-6
12:11150033	rs12226919	T/G	0.331	-0.169	0.038	6.82e-6
12:11150046	rs12226920	T/G	0.331	-0.169	0.038	6.82e-6
12:11150214	rs11054142	A/G	0.331	-0.169	0.038	6.82e-6
12:11150319	rs11054143	C/T	0.331	-0.169	0.038	6.82e-6
12:11150884	rs7301234	A/G	0.331	-0.169	0.038	6.82e-6
12:11150969	rs7135941	C/T	0.331	-0.169	0.038	6.82e-6
12:11151003	rs7301364	A/G	0.331	-0.169	0.038	6.82e-6
12:11151213	rs7301713	A/T	0.331	-0.169	0.038	6.82e-6
12:11151826	rs10845282	A/G	0.331	-0.169	0.038	6.82e-6
12:11152029	rs11054144	T/C	0.331	-0.169	0.038	6.82e-6
12:11152200	rs11054145	T/C	0.331	-0.169	0.038	6.82e-6
12:11152350	rs11054146	A/G	0.331	-0.169	0.038	6.82e-6
12:11152775	rs2060702	T/C	0.331	-0.169	0.038	6.82e-6
12:11153206	rs1450840	C/A	0.331	-0.169	0.038	6.82e-6
12:11153547	rs11054147	T/C	0.331	-0.169	0.038	6.82e-6
12:11154906	rs12321023	A/G	0.331	-0.169	0.038	6.82e-6
12:11156123	rs10772413	T/C	0.331	-0.169	0.038	6.82e-6
12:11157120	rs7138834	G/A	0.331	-0.169	0.038	6.82e-6
12:11158299	rs10845284	T/C	0.331	-0.169	0.038	6.82e-6
12:11158390	rs10845285	A/G	0.331	-0.169	0.038	6.82e-6
12:11159050	rs10845286	T/C	0.331	-0.169	0.038	6.82e-6
12:11159135	rs10845287	G/C	0.331	-0.169	0.038	6.82e-6
12:11159512	rs11054152	G/C	0.331	-0.169	0.038	6.82e-6
12:11159693	rs11054154	T/C	0.331	-0.169	0.038	6.82e-6
12:11160459	rs10772415	T/A	0.331	-0.169	0.038	6.82e-6
12:11160740	rs4298989	T/C	0.331	-0.169	0.038	6.82e-6
12:11160840	rs1450841	A/G	0.331	-0.169	0.038	6.82e-6
12:11161343	rs7133669	G/A	0.331	-0.169	0.038	6.82e-6
12:11161448	rs36104587	A/G	0.331	-0.169	0.038	6.82e-6
12:11161496	rs35653945	G/T	0.331	-0.169	0.038	6.82e-6
12:11161838	rs28569398	A/G	0.331	-0.169	0.038	6.82e-6
12:11161936	rs28654530	C/T	0.331	-0.169	0.038	6.82e-6
12:11161976	rs28498385	C/T	0.331	-0.169	0.038	6.82e-6
12:11162014	rs28630880	C/T	0.331	-0.169	0.038	6.82e-6
12:11162131	rs11054156	G/A	0.331	-0.169	0.038	6.82e-6
12:11162140	rs11054157	A/G	0.331	-0.169	0.038	6.82e-6
12:11162287	rs11054158	T/C	0.331	-0.169	0.038	6.82e-6
12:11162442	rs11054159	A/C	0.331	-0.169	0.038	6.82e-6
12:11162533	rs11054160	C/T	0.331	-0.169	0.038	6.82e-6
12:11162679	rs11054161	A/C	0.331	-0.169	0.038	6.82e-6
12:11162785	rs11054162	A/G	0.331	-0.169	0.038	6.82e-6
12:11162790	rs11054163	A/G	0.331	-0.169	0.038	6.82e-6
12:11162991	rs4763606	G/A	0.331	-0.169	0.038	6.82e-6
12:11163012	rs4763607	C/T	0.331	-0.169	0.038	6.82e-6
12:11163058	rs4763608	C/T	0.331	-0.169	0.038	6.82e-6

Supplementary Table 3-4. Top 100 SNPs on chromosome 7 associated with the perceived intensity of denatonium benzoate (DB) from the bivariate analysis of DB and quinine. P_univaraite_DB is the P-value from the univariate analysis of DB. P_univariate DB_adjQ is the P-value from the univariate analysis of DB adjusted for the quinine score. P_bivariate DB_Q is the P-value from the bivariate analysis of DB and quinine.

Chr:Position	SNP	A1/A2	MAF	Beta Univariate DB	SE Univariate DB	P univariate DB	P univariate DB_adjQ	P bivariate DB_Q
7:141398707	rs10261515	G/A	0.491	-0.136	0.037	2.54e-4	1.94e-8	3.15e-8
7:141428042	rs2366501	T/C	0.498	0.118	0.037	1.49e-3	2.00e-7	1.90e-7
7:141416846	rs6964456	T/C	0.499	-0.120	0.037	1.18e-3	1.74e-7	1.92e-7
7:141419499	rs12672225	G/T	0.496	0.127	0.037	5.63e-4	1.17e-7	1.96e-7
7:141421245	rs12113603	G/A	0.5	0.118	0.037	1.38e-3	2.10e-7	2.28e-7
7:141421842	rs4726471	G/A	0.5	-0.118	0.037	1.38e-3	2.10e-7	2.28e-7
7:141421101	rs6948307	T/C	0.5	-0.118	0.037	1.38e-3	2.10e-7	2.28e-7
7:141425690	rs34184650	T/C	0.499	0.117	0.037	1.58e-3	2.48e-7	2.50e-7
7:141421031	rs6947065	T/G	0.5	-0.119	0.037	1.26e-3	2.13e-7	2.52e-7
7:141420264	rs10257653	T/C	0.499	0.119	0.037	1.25e-3	2.26e-7	2.74e-7
7:141418573	rs10262864	G/T	0.499	0.119	0.037	1.24e-3	2.35e-7	2.85e-7
7:141415102	rs9942597	T/C	0.5	-0.119	0.037	1.25e-3	2.45e-7	2.89e-7
7:141415514	rs9942694	G/A	0.5	0.119	0.037	1.25e-3	2.45e-7	2.89e-7
7:141421623	rs17162425	A/C	0.499	0.116	0.037	1.62e-3	2.82e-7	2.94e-7
7:141420390	rs6966981	T/C	0.496	0.124	0.037	7.32e-4	1.83e-7	3.06e-7
7:141420707	rs6971275	T/C	0.496	0.124	0.037	7.43e-4	1.92e-7	3.25e-7
7:141420768	rs6967301	A/G	0.496	0.124	0.037	7.45e-4	1.93e-7	3.26e-7
7:141389640	rs7791469	A/G	0.487	-0.115	0.036	1.60e-3	2.71e-7	3.26e-7
7:141414317	rs2072180	A/G	0.496	0.123	0.037	8.11e-4	2.11e-7	3.30e-7
7:141422153	rs4726472	C/T	0.496	0.120	0.037	1.10e-3	2.53e-7	3.62e-7
7:141417484	rs4726470	T/A	0.496	0.121	0.037	9.96e-4	2.74e-7	4.27e-7
7:141433634	rs1008318	T/G	0.495	0.119	0.037	1.26e-3	3.30e-7	4.52e-7
7:141601523	rs1285900	C/A	0.471	0.092	0.037	1.32e-2	1.92e-6	5.17e-7
7:141429767	rs6464452	A/G	0.433	0.115	0.037	2.05e-3	4.52e-7	5.29e-7
7:141543711	rs12670179	C/T	0.469	0.099	0.038	8.57e-3	1.67e-6	6.21e-7
7:141601043	rs1285899	T/A	0.471	0.085	0.037	2.09e-2	3.51e-6	6.95e-7
7:141435436	rs12533304	C/T	0.496	0.100	0.037	7.95e-3	1.63e-6	7.00e-7
7:141501943	rs17524275	C/G	0.486	0.096	0.037	9.41e-3	1.94e-6	7.17e-7
7:141530918	rs6969093	A/G	0.478	0.104	0.037	5.29e-3	1.27e-6	7.33e-7
7:141529611	rs4530955	T/A	0.478	0.107	0.037	4.21e-3	1.11e-6	7.38e-7
7:141530176	rs7780596	T/A	0.478	0.104	0.037	5.34e-3	1.29e-6	7.41e-7
7:141532934	rs12703410	A/G	0.477	0.102	0.037	5.83e-3	1.45e-6	7.92e-7
7:141483590	rs12530637	A/G	0.397	0.086	0.039	2.64e-2	4.97e-6	8.25e-7
7:141505318	rs62477743	G/T	0.486	0.094	0.037	1.10e-2	2.42e-6	8.41e-7
7:141504196	rs11766522	G/A	0.486	0.094	0.037	1.11e-2	2.45e-6	8.53e-7
7:141532989	rs12703411	A/C	0.477	0.104	0.037	5.29e-3	1.47e-6	8.65e-7
7:141519959	rs1074968	G/C	0.478	0.100	0.037	7.44e-3	1.81e-6	8.97e-7
7:141592840	rs1285954	A/G	0.471	0.085	0.037	2.19e-2	4.28e-6	9.24e-7
7:141381467	rs12703403	G/C	0.45	-0.124	0.037	8.37e-4	3.92e-7	9.29e-7
7:141517831	rs11770781	C/T	0.478	0.101	0.037	6.69e-3	1.78e-6	9.57e-7
7:141517511	rs2214839	C/T	0.478	0.101	0.037	6.69e-3	1.78e-6	9.57e-7
7:141465363	rs6962760	C/T	0.491	0.103	0.037	5.10e-3	1.47e-6	1.01e-6
7:141589691	rs1285956	G/A	0.471	0.086	0.037	1.98e-2	4.20e-6	1.05e-6
7:141495604	rs1859646	A/G	0.486	0.101	0.037	6.07e-3	1.76e-6	1.05e-6
7:141398942	rs13231650	T/C	0.433	0.114	0.037	1.81e-3	6.46e-7	1.08e-6
7:141497070	rs34378880	A/G	0.485	0.098	0.037	8.12e-3	2.20e-6	1.10e-6
7:141412310	rs1476640	T/C	0.434	0.104	0.037	4.88e-3	1.37e-6	1.12e-6

7:141501747	rs12703408	G/C	0.483	0.100	0.037	6.96e-3	2.06e-6	1.17e-6
7:141501672	rs17464668	A/G	0.483	0.100	0.037	6.96e-3	2.06e-6	1.17e-6
7:141525153	rs7791243	T/A	0.478	0.099	0.037	7.78e-3	2.26e-6	1.19e-6
7:141626494	rs1285931	C/G	0.451	0.079	0.037	3.42e-2	8.14e-6	1.24e-6
7:141591345	rs1285955	A/G	0.47	0.084	0.037	2.42e-2	5.66e-6	1.26e-6
7:141544787	rs892355	C/T	0.476	0.095	0.037	1.02e-2	3.03e-6	1.28e-6
7:141524776	rs6464454	A/G	0.478	0.100	0.037	7.06e-3	2.28e-6	1.30e-6
7:141498467	rs11762219	C/T	0.483	0.102	0.037	5.89e-3	1.97e-6	1.31e-6
7:141530057	rs4636113	A/T	0.474	0.106	0.037	4.44e-3	1.61e-6	1.33e-6
7:141475161	rs35046848	A/G	0.399	0.089	0.038	2.03e-2	4.87e-6	1.37e-6
7:141504604	rs11763806	T/C	0.483	0.098	0.037	8.21e-3	2.58e-6	1.38e-6
7:141503999	rs58726075	A/G	0.483	0.098	0.037	8.21e-3	2.58e-6	1.38e-6
7:141502792	rs11766169	C/T	0.483	0.098	0.037	8.11e-3	2.57e-6	1.39e-6
7:141578863	rs1830211	C/T	0.47	0.093	0.037	1.20e-2	3.44e-6	1.44e-6
7:141607214	rs1799658	G/A	0.471	0.082	0.037	2.69e-2	6.86e-6	1.46e-6
7:141504298	rs11769672	A/G	0.483	0.097	0.037	8.55e-3	2.79e-6	1.49e-6
7:141496256	rs9648785	C/T	0.483	0.103	0.037	5.25e-3	1.99e-6	1.56e-6
7:141540548	rs12533399	C/T	0.476	0.096	0.037	9.38e-3	3.28e-6	1.63e-6
7:141464765	rs2270009	T/C	0.486	0.104	0.037	4.45e-3	1.80e-6	1.69e-6
7:141439907	rs6464453	C/G	0.494	0.098	0.037	8.47e-3	2.79e-6	1.72e-6
7:141468253	rs12667295	T/C	0.49	0.100	0.037	6.32e-3	2.43e-6	1.74e-6
7:141467838	rs974008	G/A	0.49	0.100	0.037	6.32e-3	2.43e-6	1.74e-6
7:141486321	rs11773137	G/A	0.49	0.093	0.037	1.13e-2	3.83e-6	1.78e-6
7:141540492	rs12532841	G/A	0.476	0.097	0.037	8.54e-3	3.31e-6	1.79e-6
7:141537735	rs12669721	G/T	0.476	0.097	0.037	8.54e-3	3.31e-6	1.79e-6
7:141538865	rs12703412	G/A	0.476	0.097	0.037	8.54e-3	3.31e-6	1.79e-6
7:141442213	rs2301924	A/G	0.495	0.094	0.037	1.21e-2	3.77e-6	1.81e-6
7:141407716	rs6963959	A/C	0.431	0.106	0.037	4.19e-3	1.64e-6	1.82e-6
7:141518505	rs34706333	C/A	0.474	0.101	0.037	6.60e-3	2.55e-6	1.83e-6
7:141512088	rs13223389	A/C	0.474	0.101	0.037	6.76e-3	2.59e-6	1.83e-6
7:141518836	rs12534862	G/A	0.474	0.101	0.037	6.67e-3	2.58e-6	1.84e-6
7:141514009	rs34285424	C/T	0.474	0.101	0.037	6.74e-3	2.60e-6	1.84e-6
7:141514595	rs7779209	A/G	0.474	0.101	0.037	6.71e-3	2.60e-6	1.85e-6
7:141565938	rs1918301	C/T	0.47	0.091	0.037	1.42e-2	4.79e-6	1.85e-6
7:141407424	rs4726468	T/C	0.43	0.107	0.037	3.84e-3	1.59e-6	1.94e-6
7:141442652	rs2013816	G/A	0.495	0.095	0.037	1.11e-2	3.82e-6	2.01e-6
7:141614005	rs1285912	G/A	0.471	0.078	0.037	3.56e-2	1.11e-5	2.14e-6
7:141561184	rs1655265	C/T	0.469	0.090	0.037	1.52e-2	5.63e-6	2.16e-6
7:141558725	rs2436718	G/A	0.469	0.090	0.037	1.52e-2	5.63e-6	2.16e-6
7:141561751	rs2695133	G/A	0.469	0.090	0.037	1.52e-2	5.63e-6	2.16e-6
7:141466179	rs62476658	A/G	0.487	0.105	0.037	4.26e-3	2.14e-6	2.20e-6
7:141564780	rs1285896	T/C	0.469	0.088	0.037	1.75e-2	6.43e-6	2.28e-6
7:141524822	rs7806962	A/G	0.475	0.102	0.037	5.91e-3	2.82e-6	2.32e-6
7:141480936	rs17464086	A/G	0.489	0.097	0.036	7.66e-3	3.53e-6	2.35e-6
7:141478800	rs2234002	A/G	0.489	0.097	0.036	7.66e-3	3.53e-6	2.35e-6
7:141444414	rs17462840	C/G	0.494	0.092	0.037	1.36e-2	4.96e-6	2.35e-6
7:141526913	rs7804754	C/T	0.479	0.098	0.037	8.24e-3	3.71e-6	2.35e-6
7:141469679	rs2023998	A/G	0.487	0.103	0.037	5.01e-3	2.52e-6	2.40e-6
7:141572858	rs1285895	A/G	0.47	0.088	0.037	1.77e-2	6.90e-6	2.43e-6
7:141489911	rs2234007	A/G	0.489	0.089	0.037	1.50e-2	5.86e-6	2.44e-6
7:141488985	rs10952507	A/G	0.489	0.089	0.037	1.50e-2	5.86e-6	2.44e-6
7:141489866	rs2234006	T/C	0.489	0.089	0.037	1.50e-2	5.86e-6	2.44e-6
7:141475054	rs4535645	C/T	0.49	0.098	0.037	7.60e-3	3.58e-6	2.46e-6

Supplementary Table 3-5. Top 100 SNPs on chromosome 7 associated with the perceived intensity of PROP solution.

Chr:Position	SNP	A1/A2	MAF	Beta	SE	P
7:141672604	rs10246939	C/T	0.443	0.968	0.028	2.80e-199
7:141672705	rs1726866	G/A	0.443	0.965	0.028	5.62e-198
7:141662394	rs2695135	T/C	0.282	0.756	0.040	1.80e-73
7:141658390	rs2436717	T/C	0.278	0.741	0.039	2.24e-73
7:141661450	rs6962383	C/A	0.28	0.749	0.040	1.82e-72
7:141660700	rs1726867	A/G	0.281	0.740	0.039	7.28e-72
7:141653637	rs7794708	T/C	0.275	0.696	0.039	1.81e-66
7:141639975	rs1285944	C/T	0.282	0.687	0.039	8.13e-65
7:141636563	rs1285950	C/A	0.282	0.687	0.039	1.38e-64
7:141647022	rs1285968	A/G	0.279	0.683	0.039	1.17e-63
7:141613205	rs13240104	G/A	0.282	0.677	0.039	4.09e-63
7:141613248	rs34588922	C/T	0.282	0.677	0.039	4.09e-63
7:141612717	rs6976028	T/C	0.282	0.676	0.039	5.09e-63
7:141546847	rs12703413	A/G	0.278	0.676	0.039	5.28e-62
7:141544200	rs9640205	G/C	0.273	0.684	0.040	5.41e-62
7:141551958	rs34894166	C/T	0.279	0.674	0.039	1.12e-61
7:141564646	rs62475469	A/G	0.279	0.674	0.039	1.70e-61
7:141560990	rs60165685	A/G	0.278	0.674	0.039	2.22e-61
7:141586441	rs6955562	G/C	0.276	0.676	0.039	2.43e-61
7:141556519	rs58093678	G/C	0.278	0.673	0.039	3.38e-61
7:141560655	rs17133534	T/G	0.279	0.673	0.039	3.60e-61
7:141562424	rs13232651	T/C	0.279	0.673	0.039	3.60e-61
7:141533757	rs35647444	T/G	0.279	0.672	0.039	4.94e-61
7:141633062	rs1527309	T/C	0.28	0.671	0.039	5.11e-61
7:141531140	rs6969430	A/G	0.28	0.667	0.039	6.91e-60
7:141590705	rs10808016	G/T	0.281	0.663	0.039	9.97e-60
7:141532187	rs10464444	A/G	0.28	0.666	0.039	1.01e-59
7:141588426	rs35634557	C/T	0.283	0.661	0.039	1.31e-59
7:141565357	rs6957037	G/A	0.282	0.660	0.039	3.35e-59
7:141588055	rs873818	T/G	0.281	0.662	0.039	3.77e-59
7:141543098	rs9640357	T/C	0.282	0.659	0.039	3.94e-59
7:141543810	rs35836873	G/A	0.282	0.659	0.039	3.94e-59
7:141543882	rs9640358	A/G	0.282	0.659	0.039	3.94e-59
7:141544100	rs9640204	G/A	0.28	0.660	0.039	8.05e-59
7:141537968	rs11765575	G/A	0.282	0.657	0.039	8.48e-59
7:141544199	rs9640359	A/C	0.275	0.666	0.040	1.04e-58
7:141584184	rs13235900	G/A	0.279	0.659	0.039	2.58e-58
7:141585166	rs1980369	G/T	0.279	0.659	0.039	2.58e-58
7:141574911	rs12668089	T/C	0.281	0.655	0.039	2.78e-58
7:141575800	rs12668693	T/C	0.281	0.655	0.039	2.78e-58
7:141577186	rs2082551	G/A	0.281	0.655	0.039	2.78e-58
7:141569606	rs6959360	A/C	0.281	0.654	0.039	3.40e-58
7:141573055	rs12534927	C/T	0.281	0.654	0.039	3.77e-58
7:141567569	rs2163953	C/T	0.281	0.653	0.039	4.89e-58
7:141661585	rs10435196	T/A	0.248	0.686	0.042	6.28e-57
7:141658886	rs67596995	G/A	0.25	0.678	0.041	7.06e-57
7:141662547	rs12531134	C/T	0.249	0.687	0.042	1.06e-56
7:141656487	rs11762634	A/G	0.25	0.661	0.041	1.16e-55
7:141657465	rs13235385	T/C	0.246	0.666	0.041	3.47e-55
7:141654892	rs2570407	C/A	0.251	0.658	0.041	3.97e-55
7:141637810	rs1594777	G/A	0.253	0.642	0.041	1.04e-52
7:141638297	rs12531781	T/C	0.253	0.642	0.041	1.04e-52
7:141638429	rs13227402	T/C	0.253	0.642	0.041	1.04e-52

7:141642285	rs1594776	T/C	0.248	0.636	0.041	3.02e-51
7:141639215	rs13237944	A/C	0.249	0.635	0.041	4.63e-51
7:141646430	rs2293460	T/C	0.246	0.632	0.041	4.96e-50
7:141646434	rs2293461	G/A	0.246	0.632	0.041	4.96e-50
7:141630267	rs12539499	C/T	0.251	0.626	0.041	2.83e-49
7:141593434	rs12538701	T/C	0.252	0.622	0.041	8.30e-49
7:141544734	rs892354	T/C	0.251	0.622	0.041	8.72e-49
7:141544095	rs9640203	G/A	0.25	0.624	0.041	1.07e-48
7:141628704	rs11770855	C/T	0.25	0.622	0.041	1.32e-48
7:141550780	rs13236432	C/G	0.251	0.621	0.041	1.47e-48
7:141590684	rs10952509	C/A	0.25	0.622	0.041	1.51e-48
7:141627899	rs13222726	G/A	0.25	0.619	0.041	2.20e-48
7:141602476	rs11765106	G/A	0.252	0.616	0.041	4.46e-48
7:141609424	rs7802271	A/T	0.254	0.610	0.041	7.34e-48
7:141609758	rs7782886	A/G	0.254	0.610	0.041	7.34e-48
7:141610572	rs35412929	C/T	0.254	0.610	0.041	7.34e-48
7:141610891	rs994808	C/A	0.254	0.610	0.041	7.34e-48
7:141611285	rs994809	C/T	0.254	0.610	0.041	7.34e-48
7:141611392	rs7808421	G/A	0.254	0.610	0.041	7.34e-48
7:141611499	rs7789123	C/G	0.254	0.610	0.041	7.34e-48
7:141612116	rs11767119	A/G	0.254	0.610	0.041	7.34e-48
7:141612621	rs11767947	A/G	0.254	0.610	0.041	7.34e-48
7:141614110	rs11769089	A/G	0.253	0.611	0.041	8.40e-48
7:141614190	rs11765974	G/A	0.253	0.611	0.041	8.40e-48
7:141605899	rs7786202	C/T	0.253	0.613	0.041	1.11e-47
7:141531917	rs34726057	C/T	0.252	0.611	0.041	1.39e-46
7:141579215	rs10952508	G/T	0.25	0.607	0.041	1.99e-46
7:141611955	rs7785954	G/A	0.258	0.592	0.041	2.32e-45
7:141526020	rs6967189	C/T	0.255	0.591	0.042	1.42e-43
7:141511858	rs12703409	C/T	0.253	0.593	0.042	3.63e-42
7:141510353	rs35010424	T/C	0.25	0.587	0.043	1.62e-40
7:141627149	rs1285933	G/A	0.469	0.479	0.036	1.80e-38
7:141614005	rs1285912	G/A	0.471	0.479	0.036	3.24e-38
7:141607214	rs1799658	G/A	0.47	0.480	0.036	3.46e-38
7:141616506	rs745162	G/A	0.471	0.478	0.036	3.92e-38
7:141615875	rs1285914	G/A	0.471	0.478	0.036	5.03e-38
7:141475161	rs35046848	A/G	0.399	0.497	0.038	5.08e-38
7:141615867	rs1285913	C/G	0.471	0.478	0.036	5.33e-38
7:141591345	rs1285955	A/G	0.47	0.477	0.036	1.20e-37
7:141589691	rs1285956	G/A	0.471	0.477	0.036	1.26e-37
7:141429767	rs6464452	A/G	0.432	0.482	0.037	1.64e-37
7:141592840	rs1285954	A/G	0.47	0.476	0.036	1.65e-37
7:141601043	rs1285899	T/A	0.471	0.476	0.036	1.74e-37
7:141601523	rs1285900	C/A	0.471	0.475	0.036	1.99e-37
7:141563970	rs1433594	A/G	0.466	0.476	0.036	2.04e-37
7:141368300	rs12154227	T/C	0.424	-0.483	0.037	2.50e-37
7:141549635	rs34708913	A/T	0.465	0.475	0.036	3.14e-37

Supplementary Table 3-6. Top 100 SNPs on chromosome 7 associated with the perceived intensity of PROP paper.

Chr:Position	SNP	A1/A2	MAF	Beta	SE	P
7:141672705	rs1726866	G/A	0.441	0.535	0.032	3.38e-59
7:141672604	rs10246939	C/T	0.441	0.534	0.032	5.40e-59
7:141658390	rs2436717	T/C	0.278	0.461	0.038	4.37e-33
7:141660700	rs1726867	A/G	0.281	0.457	0.038	8.77e-32
7:141661450	rs6962383	C/A	0.28	0.458	0.039	1.73e-31
7:141662394	rs2695135	T/C	0.282	0.459	0.039	2.41e-31
7:141653637	rs7794708	T/C	0.276	0.433	0.037	4.68e-30
7:141639975	rs1285944	C/T	0.283	0.427	0.037	2.87e-29
7:141636563	rs1285950	C/A	0.283	0.427	0.037	3.32e-29
7:141647022	rs1285968	A/G	0.279	0.424	0.037	9.69e-29
7:141613205	rs13240104	G/A	0.282	0.415	0.037	5.34e-28
7:141613248	rs34588922	C/T	0.282	0.415	0.037	5.34e-28
7:141612717	rs6976028	T/C	0.283	0.413	0.037	9.20e-28
7:141633062	rs1527309	T/C	0.282	0.413	0.038	2.61e-27
7:141586441	rs6955562	G/C	0.276	0.409	0.038	1.68e-26
7:141546847	rs12703413	A/G	0.279	0.407	0.038	1.96e-26
7:141551958	rs34894166	C/T	0.279	0.406	0.038	2.32e-26
7:141533757	rs35647444	T/G	0.279	0.405	0.038	2.89e-26
7:141590705	rs10808016	G/T	0.282	0.404	0.038	3.00e-26
7:141531140	rs6969430	A/G	0.28	0.406	0.038	3.11e-26
7:141532187	rs10464444	A/G	0.28	0.406	0.038	3.40e-26
7:141588426	rs35634557	C/T	0.283	0.403	0.038	3.44e-26
7:141564646	rs62475469	A/G	0.28	0.405	0.038	3.46e-26
7:141556519	rs58093678	G/C	0.279	0.405	0.038	3.53e-26
7:141560655	rs17133534	T/G	0.279	0.405	0.038	3.63e-26
7:141562424	rs13232651	T/C	0.279	0.405	0.038	3.63e-26
7:141560990	rs60165685	A/G	0.279	0.405	0.038	4.13e-26
7:141544200	rs9640205	G/C	0.273	0.408	0.038	6.69e-26
7:141544100	rs9640204	G/A	0.279	0.401	0.038	1.22e-25
7:141588055	rs873818	T/G	0.281	0.400	0.038	1.31e-25
7:141537968	rs11765575	G/A	0.282	0.397	0.038	2.00e-25
7:141584184	rs13235900	G/A	0.279	0.400	0.038	2.06e-25
7:141585166	rs1980369	G/T	0.279	0.400	0.038	2.06e-25
7:141543098	rs9640357	T/C	0.282	0.396	0.038	2.78e-25
7:141543810	rs35836873	G/A	0.282	0.396	0.038	2.78e-25
7:141543882	rs9640358	A/G	0.282	0.395	0.038	3.62e-25
7:141565357	rs6957037	G/A	0.282	0.395	0.038	4.15e-25
7:141567569	rs2163953	C/T	0.281	0.394	0.038	6.24e-25
7:141574911	rs12668089	T/C	0.281	0.393	0.038	6.97e-25
7:141575800	rs12668693	T/C	0.281	0.393	0.038	6.97e-25
7:141577186	rs2082551	G/A	0.281	0.393	0.038	6.97e-25
7:141569606	rs6959360	A/C	0.281	0.393	0.038	7.20e-25
7:141658886	rs67596995	G/A	0.25	0.410	0.039	7.70e-25
7:141573055	rs12534927	C/T	0.28	0.392	0.038	8.83e-25
7:141544199	rs9640359	A/C	0.274	0.397	0.038	1.10e-24
7:141661585	rs10435196	T/A	0.247	0.410	0.040	2.72e-24
7:141662547	rs12531134	C/T	0.248	0.410	0.040	4.32e-24
7:141654892	rs2570407	C/A	0.251	0.398	0.039	4.74e-24
7:141657465	rs13235385	T/C	0.245	0.402	0.039	5.92e-24
7:141656487	rs11762634	A/G	0.25	0.397	0.039	6.23e-24
7:141637810	rs1594777	G/A	0.253	0.387	0.039	7.01e-23
7:141638297	rs12531781	T/C	0.253	0.387	0.039	7.01e-23
7:141638429	rs13227402	T/C	0.253	0.387	0.039	7.01e-23

7:141642285	rs1594776	T/C	0.248	0.384	0.039	2.13e-22
7:141639215	rs13237944	A/C	0.249	0.384	0.039	2.71e-22
7:141614110	rs11769089	A/G	0.253	0.378	0.039	5.97e-22
7:141614190	rs11765974	G/A	0.253	0.378	0.039	5.97e-22
7:141630267	rs12539499	C/T	0.251	0.375	0.039	2.86e-21
7:141646430	rs2293460	T/C	0.245	0.377	0.039	3.02e-21
7:141646434	rs2293461	G/A	0.245	0.377	0.039	3.02e-21
7:141628704	rs11770855	C/T	0.25	0.373	0.039	4.30e-21
7:141609424	rs7802271	A/T	0.254	0.367	0.039	8.13e-21
7:141609758	rs7782886	A/G	0.254	0.367	0.039	8.13e-21
7:141610572	rs35412929	C/T	0.254	0.367	0.039	8.13e-21
7:141610891	rs994808	C/A	0.254	0.367	0.039	8.13e-21
7:141611285	rs994809	C/T	0.254	0.367	0.039	8.13e-21
7:141611392	rs7808421	G/A	0.254	0.367	0.039	8.13e-21
7:141611499	rs7789123	C/G	0.254	0.367	0.039	8.13e-21
7:141612116	rs11767119	A/G	0.254	0.367	0.039	8.13e-21
7:141612621	rs11767947	A/G	0.254	0.367	0.039	8.13e-21
7:141627899	rs13222726	G/A	0.25	0.370	0.039	8.62e-21
7:141590684	rs10952509	C/A	0.25	0.370	0.039	1.13e-20
7:141593434	rs12538701	T/C	0.252	0.369	0.039	1.18e-20
7:141602476	rs11765106	G/A	0.252	0.365	0.039	2.54e-20
7:141605899	rs7786202	C/T	0.252	0.362	0.039	4.85e-20
7:141611955	rs7785954	G/A	0.257	0.356	0.039	8.37e-20
7:141544095	rs9640203	G/A	0.249	0.362	0.040	1.32e-19
7:141544734	rs892354	T/C	0.251	0.355	0.039	3.56e-19
7:141550780	rs13236432	C/G	0.251	0.355	0.039	3.84e-19
7:141531917	rs34726057	C/T	0.253	0.354	0.039	5.57e-19
7:141579215	rs10952508	G/T	0.25	0.353	0.039	6.28e-19
7:141526020	rs6967189	C/T	0.256	0.349	0.039	2.23e-18
7:141511858	rs12703409	C/T	0.254	0.343	0.040	3.23e-17
7:141510353	rs35010424	T/C	0.252	0.337	0.041	1.93e-16
7:141616506	rs745162	G/A	0.471	0.265	0.034	1.91e-14
7:141614005	rs1285912	G/A	0.471	0.265	0.034	2.45e-14
7:141615875	rs1285914	G/A	0.47	0.263	0.035	3.83e-14
7:141615867	rs1285913	C/G	0.47	0.263	0.035	3.92e-14
7:141627149	rs1285933	G/A	0.469	0.260	0.034	5.98e-14
7:141592840	rs1285954	A/G	0.472	0.260	0.035	8.25e-14
7:141591345	rs1285955	A/G	0.471	0.259	0.035	1.05e-13
7:141607214	rs1799658	G/A	0.47	0.258	0.035	1.21e-13
7:141589691	rs1285956	G/A	0.472	0.258	0.035	1.31e-13
7:141563970	rs1433594	A/G	0.467	0.257	0.035	1.91e-13
7:141601043	rs1285899	T/A	0.471	0.255	0.035	2.15e-13
7:141549635	rs34708913	A/T	0.467	0.256	0.035	2.21e-13
7:141530057	rs4636113	A/T	0.474	0.257	0.035	2.32e-13
7:141518505	rs34706333	C/A	0.474	0.258	0.035	2.39e-13
7:141518836	rs12534862	G/A	0.474	0.257	0.035	2.43e-13
7:141522086	rs79390963	G/A	0.475	0.256	0.035	2.58e-13

Supplementary Table 3-7. Top 100 SNPs on chromosome 2 associated with the perceived intensity of PROP paper and their associations with PROP solution.

Chr:Position	SNP	A1/A2	MAF	Beta_paper	SE_paper	P_paper	P_solution
2:218218646	rs6761655	G/A	0.186	-0.246	0.044	2.69e-8	7.38e-4
2:218218695	rs6736242	A/G	0.186	-0.246	0.044	2.69e-8	7.38e-4
2:218219311	rs7586502	A/G	0.142	-0.259	0.049	1.31e-7	1.03e-3
2:218220180	rs80312552	G/A	0.14	-0.258	0.049	1.70e-7	9.43e-4
2:218219166	rs6435978	T/C	0.142	-0.256	0.049	1.85e-7	2.48e-3
2:218219074	rs6435976	T/C	0.142	-0.253	0.049	2.15e-7	2.34e-3
2:218219139	rs6435977	A/G	0.143	-0.254	0.049	2.18e-7	2.12e-3
2:218221469	rs4674157	A/G	0.14	-0.255	0.049	2.59e-7	1.07e-3
2:218220849	rs4674155	A/G	0.139	-0.252	0.049	3.60e-7	1.67e-3
2:218220860	rs4674156	T/C	0.139	-0.252	0.049	3.60e-7	1.67e-3
2:218218855	rs6707253	G/A	0.142	-0.248	0.049	3.77e-7	3.04e-3
2:218218774	rs6707229	C/A	0.143	-0.248	0.049	4.00e-7	3.09e-3
2:218188539	rs13023129	C/T	0.232	-0.211	0.042	4.99e-7	6.02e-3
2:218216220	rs1863193	C/T	0.211	-0.215	0.043	5.25e-7	7.78e-4
2:218214057	rs13008830	C/T	0.211	-0.214	0.043	6.69e-7	5.76e-4
2:218175472	rs4141835	A/G	0.231	-0.205	0.041	8.12e-7	5.93e-3
2:218200026	rs13432162	A/G	0.229	-0.206	0.042	1.14e-6	1.75e-3
2:218177694	rs1863183	T/C	0.235	-0.201	0.041	1.18e-6	1.09e-2
2:218197359	rs16857324	C/T	0.231	-0.204	0.042	1.47e-6	2.73e-3
2:218226173	rs13417769	G/A	0.137	-0.240	0.051	2.24e-6	5.42e-3
2:218228349	rs7561131	C/T	0.205	-0.203	0.043	3.31e-6	1.38e-3
2:218220232	rs112802287	G/A	0.208	-0.195	0.042	4.26e-6	3.89e-4
2:218222700	rs4674158	T/A	0.209	-0.193	0.042	5.50e-6	6.82e-4
2:218207688	rs5028238	G/A	0.233	-0.193	0.043	6.09e-6	1.20e-3
2:160387482	rs34251858	C/A	0.094	0.258	0.059	1.31e-5	1.41e-2
2:112444296	rs10186692	G/T	0.451	0.150	0.035	1.58e-5	2.82e-2
2:112443852	rs10175681	T/C	0.449	0.148	0.034	1.83e-5	2.03e-2
2:218219697	rs78832202	G/A	0.102	-0.240	0.056	2.01e-5	8.90e-2
2:218219641	rs79707432	A/G	0.102	-0.239	0.056	2.02e-5	1.02e-1
2:218219226	rs55848226	A/G	0.105	-0.235	0.055	2.28e-5	1.37e-1
2:160340777	rs35745662	C/T	0.102	0.245	0.058	2.49e-5	4.27e-2
2:160362019	rs34081025	A/T	0.102	0.243	0.057	2.54e-5	3.12e-2
2:96777168	rs2312955	T/G	0.34	0.151	0.036	2.70e-5	2.96e-1
2:160340014	rs13017222	A/G	0.102	0.245	0.058	2.71e-5	4.22e-2
2:96780716	rs2229169	T/G	0.34	0.151	0.036	2.74e-5	2.67e-1
2:96751395	rs2140938	C/T	0.353	0.153	0.036	2.78e-5	5.76e-1
2:96774981	rs7561198	C/G	0.34	0.151	0.036	2.81e-5	3.06e-1
2:96781986	rs3111873	G/C	0.34	0.151	0.036	2.82e-5	2.79e-1
2:96784934	rs2692894	T/G	0.34	0.151	0.036	2.82e-5	2.79e-1
2:105548062	rs10496387	T/C	0.273	-0.166	0.040	2.89e-5	2.94e-1
2:218163031	rs74910011	T/G	0.119	-0.223	0.053	3.04e-5	4.38e-1
2:96780122	rs4907299	T/G	0.314	0.160	0.038	3.22e-5	1.68e-1
2:96931846	rs2301707	C/T	0.322	0.155	0.037	3.29e-5	4.40e-1
2:105605488	rs7574780	C/A	0.267	-0.166	0.040	3.49e-5	4.04e-1
2:96831355	rs1724125	A/G	0.34	0.150	0.036	3.53e-5	3.40e-1
2:160352948	rs71423016	T/C	0.097	0.244	0.059	3.70e-5	5.67e-2
2:112445659	rs6708131	C/T	0.448	0.143	0.035	3.78e-5	3.54e-2
2:105602952	rs6543279	G/A	0.267	-0.165	0.040	3.79e-5	4.11e-1
2:96794957	rs2917662	A/G	0.34	0.148	0.036	3.81e-5	2.91e-1
2:96794982	rs2969491	T/C	0.34	0.148	0.036	3.81e-5	2.91e-1
2:96787899	rs1168965	C/G	0.34	0.148	0.036	4.18e-5	2.98e-1
2:105555869	rs7595767	G/T	0.272	-0.162	0.040	4.44e-5	4.06e-1
2:112447888	rs10174353	C/T	0.448	0.142	0.035	4.49e-5	4.81e-2

2:105536116	rs2889336	G/C	0.286	-0.163	0.040	4.54e-5	2.75e-1
2:96855241	rs4907230	A/G	0.323	0.152	0.037	4.55e-5	4.40e-1
2:105557895	rs2033303	T/C	0.273	-0.161	0.039	4.57e-5	3.78e-1
2:218227288	rs78096412	G/T	0.098	-0.237	0.058	4.61e-5	1.17e-1
2:218157502	rs74899380	C/G	0.109	-0.229	0.056	4.65e-5	4.77e-1
2:105550991	rs72832219	C/A	0.271	-0.162	0.040	4.78e-5	3.03e-1
2:112441433	rs6728061	A/G	0.449	0.140	0.034	4.80e-5	1.60e-2
2:218227410	rs73991072	T/C	0.097	-0.237	0.058	4.95e-5	1.17e-1
2:96777340	rs7604842	C/T	0.342	0.147	0.036	5.06e-5	3.90e-1
2:218222372	rs28542381	A/G	0.101	-0.230	0.057	5.06e-5	1.17e-1
2:112447296	rs55937046	G/A	0.449	0.141	0.035	5.07e-5	4.75e-2
2:218157508	rs74631012	G/A	0.109	-0.228	0.056	5.07e-5	4.66e-1
2:218188700	rs10804257	C/T	0.113	-0.222	0.055	5.11e-5	4.43e-1
2:96774786	rs10183151	T/G	0.342	0.146	0.036	5.35e-5	4.13e-1
2:105544888	rs6734108	A/G	0.27	-0.161	0.040	5.52e-5	3.72e-1
2:96822373	rs1030864	G/A	0.341	0.146	0.036	5.76e-5	3.73e-1
2:105559947	rs6705953	C/T	0.273	-0.159	0.039	5.87e-5	4.12e-1
2:160352115	rs13003356	C/T	0.093	0.243	0.060	5.94e-5	2.89e-2
2:218226516:1	rs77820558	C/T	0.099	-0.231	0.058	5.98e-5	1.20e-1
2:218194886	rs56163890	A/G	0.112	-0.222	0.055	6.02e-5	3.28e-1
2:96825363	rs1168968	A/G	0.34	0.145	0.036	6.31e-5	3.50e-1
2:112451387	rs3860380	G/A	0.449	0.140	0.035	6.40e-5	4.55e-2
2:96745729	rs2692936	A/G	0.348	0.144	0.036	6.50e-5	6.59e-1
2:96880147	rs58448550	T/G	0.324	0.148	0.037	6.50e-5	4.70e-1
2:96751871	rs2692937	A/G	0.347	0.144	0.036	6.64e-5	6.52e-1
2:96737083	rs2579552	G/A	0.347	0.143	0.036	7.03e-5	6.37e-1
2:96756547	rs2692893	T/C	0.346	0.144	0.036	7.09e-5	5.54e-1
2:112437140	rs1464095	G/A	0.449	0.135	0.034	7.47e-5	1.20e-2
2:96741944	rs2579550	A/G	0.347	0.143	0.036	7.49e-5	6.82e-1
2:96742833	rs2579549	G/T	0.347	0.143	0.036	7.49e-5	6.82e-1
2:105531516	rs34489771	T/G	0.265	-0.160	0.040	7.61e-5	3.58e-1
2:218177312	rs55884900	G/A	0.121	-0.208	0.052	7.78e-5	5.73e-1
2:218162330	rs78665806	G/A	0.12	-0.210	0.053	7.85e-5	5.56e-1
2:112440772	rs4459742	G/A	0.446	0.137	0.035	7.94e-5	1.12e-2
2:96737860	rs2692934	T/C	0.347	0.142	0.036	8.07e-5	6.59e-1
2:218159244	rs6752033	T/C	0.16	-0.191	0.048	8.12e-5	6.99e-1
2:96813480	rs1168976	A/G	0.341	0.143	0.036	8.12e-5	3.66e-1
2:96814075	rs1168975	A/G	0.341	0.143	0.036	8.12e-5	3.66e-1
2:96814928	rs1168974	A/G	0.341	0.143	0.036	8.12e-5	3.66e-1
2:96815492	rs1168972	A/G	0.341	0.143	0.036	8.12e-5	3.66e-1
2:96816606	rs1168970	T/C	0.341	0.143	0.036	8.12e-5	3.66e-1
2:179039491	rs334128	T/G	0.422	0.141	0.036	8.25e-5	2.66e-2
2:218181721	rs79286679	C/T	0.116	-0.213	0.054	8.35e-5	4.05e-1
2:228136823	rs12619141	T/A	0.148	-0.190	0.048	8.58e-5	5.21e-2
2:228137049	rs12619189	G/A	0.148	-0.190	0.048	8.58e-5	5.21e-2
2:228137357	rs78908239	A/T	0.147	-0.189	0.048	8.82e-5	4.39e-2
2:105563377	rs113534447	A/G	0.271	-0.155	0.040	8.91e-5	4.38e-1

Supplementary Table 3-8. Mean, standard deviation, and heritability estimates for the perceived intensity of bitter tastes.

Trait	Mean	SD	Heritability	SE
PROP	36.54	29.73	0.71	0.03
PROP paper	38.82	28.95	0.40	0.04
Quinine	46.03	22.77	0.38	0.05
Caffeine	52.32	23.28	0.31	0.05
SOA	52.03	22.62	0.40	0.05
DB	79.50	24.77	0.45	0.05

PROP, propylthiouracil. SOA, sucrose octaacetate. DB, denatonium benzoate. SD/SE, standard deviation/error. Perceived intensity were ratings on general Labelled Magnitude Scale (gLMS). Heritabilities were estimated using GEMMA based on genetic relatedness matrix.

Supplementary Table 3-9. Phenotypic and genetic variance in the perceived intensity of quinine, caffeine, sucrose octaacetate (SOA) and denatonium benzoate (DB) explained by rs10772420, rs2597979, rs67487380 and rs10261515.

	Phenotypic variance explained (%)				Genetic variance explained (%)			
SNP	Quinine	Caffeine	SOA	DB	Quinine	Caffeine	SOA	DB
rs10772420	5.67	0.57	0.94	0.56	14.92	1.49	2.46	1.48
rs2597979	0.52	1.91	0.05	0.02	1.69	6.15	0.16	0.05
rs67487380	3.36	0.10	1.63	0.72	8.40	0.25	4.07	1.79
rs10261515	0.14	0.00	0.15	0.93	0.31	0.01	0.32	2.06

Supplementary Table 3-10. Top PROP paper-associated SNPs (P < 1e-6) on chromosome 2 in the current study (n = 1999) and their associations in GWAS of (1) 225 subjects from the general population of the São Paulo metropolitan area of Brazil, (2) 466 subjects from Silk Road and (3) 2588 subjects from three Italian cohorts.

SNP	Chr:Position	A1/A2	MAF	β	SE	r^2	P Current Sample	P Brazilian Sample	P Silk Road Sample	P Italian Sample
rs6761655	2:218218646	G/A	0.186	-0.246	4.41e-2	1.83%	2.69e-8	NA	0.95	0.99
rs6736242	2:218218695	A/G	0.186	-0.246	4.41e-2	1.83%	2.69e-8	NA	-	-
rs7586502	2:218219311	A/G	0.142	-0.259	4.88e-2	1.63%	1.31e-7	NA	-	-
rs80312552	2:218220180	G/A	0.140	-0.258	4.91e-2	1.60%	1.70e-7	NA	-	-
rs6435978	2:218219166	T/C	0.142	-0.256	4.89e-2	1.59%	1.85e-7	0.87	-	-
rs6435976	2:218219074	T/C	0.142	-0.253	4.87e-2	1.56%	2.15e-7	NA	-	-
rs6435977	2:218219139	A/G	0.143	-0.254	4.88e-2	1.58%	2.18e-7	NA	-	-
rs4674157	2:218221469	A/G	0.140	-0.255	4.93e-2	1.56%	2.59e-7	NA	-	-
rs4674155	2:218220849	A/G	0.139	-0.252	4.94e-2	1.52%	3.60e-7	NA	-	-
rs4674156	2:218220860	T/C	0.139	-0.252	4.94e-2	1.52%	3.60e-7	NA	-	-
rs6707253	2:218218855	G/A	0.142	-0.248	4.87e-2	1.50%	3.77e-7	NA	-	-
rs6707229	2:218218774	C/A	0.143	-0.248	4.87e-2	1.50%	4.00e-7	NA	-	-
rs13023129	2:218188539	C/T	0.232	-0.211	4.19e-2	1.59%	4.99e-7	NA	-	-
rs1863193	2:218216220	C/T	0.211	-0.215	4.27e-2	1.54%	5.25e-7	NA	-	-
rs13008830	2:218214057	C/T	0.211	-0.214	4.30e-2	1.53%	6.69e-7	NA	-	-
rs4141835	2:218175472	A/G	0.231	-0.205	4.14e-2	1.49%	8.12e-7	0.95	-	-

We give allele frequency and effect sizes with reference to allele A1. Base-pair position is based on GRCh37; A1/A2, minor/major allele; MAF, minor allele frequency; β, the effect size; SE, standard error of the β; r^2, percent variance of the trait accounted for by the SNP; NA, SNP information was not available. The PMID is 22132133 for the GWAS of the Brazilian sample. For the Silk Road and Italian studies, only the P-values for rs6761655 is shown here because they are obtained through personal communication with Dr. Robino Antonietta from the Italian Ministry of Health.

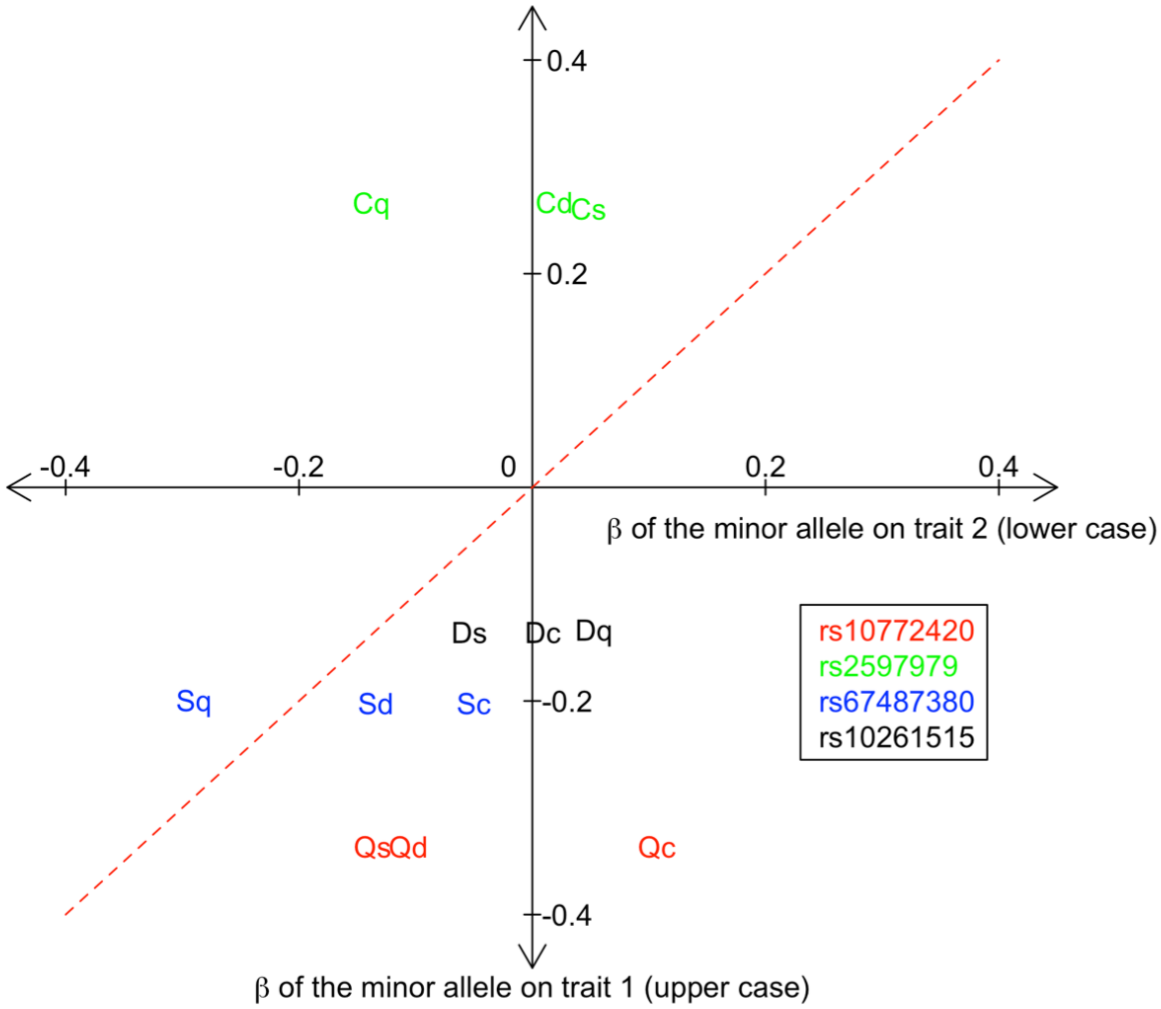
Supplementary Table 3-11. Results of the annotation conducted with Haploreg 4.1.

Chr	pos_hg38	Lead_SNP, Trait[a]	Non-syn SNPs in LD[b]	CR[c]	DNAse[d]	Histone marks[e]	Motifs changed[f]	eQLT[g]	mQTL[h]	GENCODE genes
7	141972804	rs10246939, PROP solution & paper	Ala49Pro [rs713598] and Ile296Val [rs10246939] in *TAS2R38*	✓	Pancreas	Enhancer In skin and blood	DMRT7, Evi-1_4, KAP1, NF-Y, PLZF, Pou2f2, PU.1, Smad3, Sox	CLEC5A, KIAA1147, TAS2R5, TAS2R38, WEE2-AS1, ENSG00000257093.2_141356528_141362651, ENSG00000257093.2_141363972_141364040, ENSG00000257093.2_141364704_141364854, ENSG00000257093.2_141385278_141385436, ENSG00000228775		MGAM, TAS2R38
12	11020856	rs10772420, quinine	Arg299Cys [rs10772420] in *TAS2R19*		Skin		Maf	PRH1, PRR4, TAS2R10, TAS2R14, TAS2R19, TAS2R20, TAS2R31, TAS2R43, TAS2R50, TAS2R64P, ENSG00000212124.2_11174218_11175219	chr12:11067603-11067653 [hg18 coord probe cg25677688] TAS2R19	TAS2R19
12	11037367	rs2597979, caffeine	Leu162Met, Leu162Val [rs10743938] in *TAS2R31*				NRSF, PU.1, SPIB	PRR4, RP11-785H5.1, TAS2R14, TAS2R15, TAS2R20, TAS2R31, TAS2R43, TAS2R45, TAS2R64P, ENSG00000231887.2_11035134_11035297,	chr12:11030521-11030571 [hg18 coord probe cg11032157] TAS2R19, chr12:11067603-11067653 [hg18 coord probe cg25677688] TAS2R50	PRR4, TAS2R31
12	11041785	rs67487380, SOA					BDP1, CTCF, GLI, HNF4, Ifr, LXR, NRSF, Rad21, Pax-4, RXRA, RXRA, SMC3, TATA, Znf143	RP11-434C1.1, RP11-785H5.1, RP11-785H5.2, PRB2, PRR4, TAS2R10, TAS2R12, TAS2R14, TAS2R15, TAS2R19, TAS2R20, TAS2R31, TAS2R43, TAS2R46, TAS2R64P, ENSG00000111215.5_10999644_10999966, ENSG00000212124.2_11174218_11175219, ENSG00000255837.1_11149094_11150474, ENSG00000256436.1_11182986_11184006		PRR4
7	141698907	rs10261515, DB		✓	Blood, immune cell, stem cell, brain, heart, stomach, muscle.	Promoter in lung; enhancer in stem cell, immune cell, liver, brain, stomach, blood, colon, heart, lung.	CDP, Hoxa5	AC004918.1-1|KIAA1147, AGK, CD1B, CLEC5A, FLJ40852, hmm33189, KIAA1147, LCHN, LOC254719, OR9A3P, PRSS37, RP11-744I24.2, RP5-894A10.6, TAS2R4, TAS2R5, WEE2, WEE2-AS1, ENSG00000127366.4_141490017_141491166, ENSG00000257093.2_141356528_141362651, ENSG00000257093.2_141363972_141364040, ENSG00000257093.2_141364704_141364854, ENSG00000257093.2_141364987_141365118, ENSG00000257093.2_141366087_141366225, ENSG00000257093.2_141373867_141374020, ENSG00000257093.2_141385278_141385436, ENSG00000257093.2_141386359_141386458, ENSG00000257093.2_141401686_141401953, ENSG00000228775, ENSG00000257093.2_141356528_141362651		KIAA1147

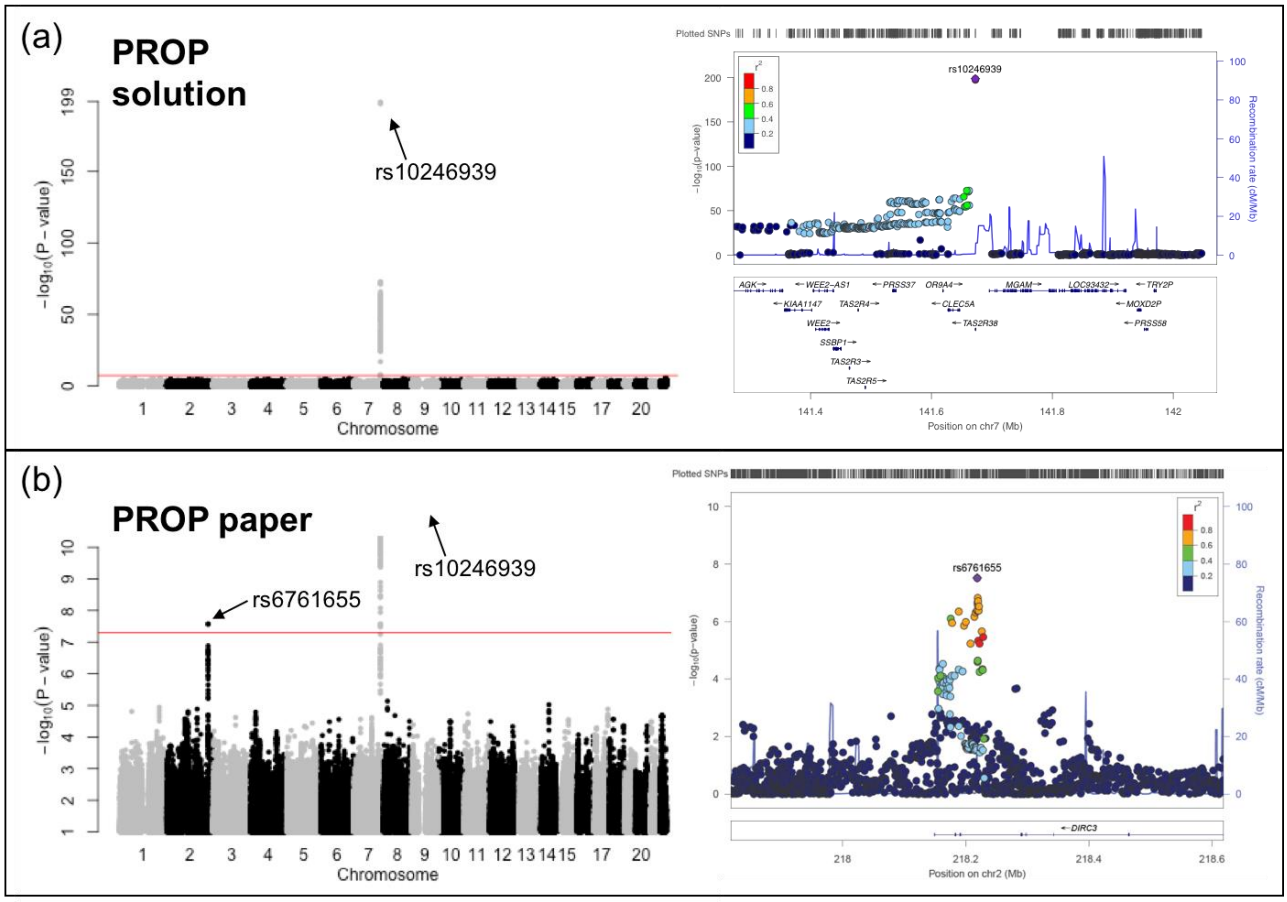
pos_hg38, base-pair position in the Genome Reference Consortium human genome build 38; CR, conserved region; eQTL, expression quantitative trait loci; LD, linkage disequilibrium; mQTL, methylation quantitative trait loci; SNP, single-nucleotide polymorphism. SOA, sucrose octa-acetate; DB, dentonium benzoate. [a]Lead SNP and the associated bitter taste. [b]Non-synonymous SNPs in LD (CEU: $r^2 \geqslant 0.80$) with lead SNP. [c]Check marks (✓) denote the presence of a conserved region (spanning lead SNP and its correlated proxies, CEU: $r^2 \geqslant 0.8$). [d]Tissues with the presence of DNase-I hypersensitivity sites at region spanning lead SNP and its correlated proxies, CEU: $r^2 \geqslant 0.8$. DNAse hypersensitivity sites are related to transcriptional activity because these are chromatin regions that are less condensed and more accessible to transcription factors. [f]Enhancer (H3K4me1) or promoter (H3K4me3) histone marks spanning lead SNP and its correlated proxies, CEU: $r^2 \geqslant 0.8$. Enhancer: a short region of DNA (50-1500 bp) that can be bound by proteins (activators) to increase the transcription of a gene. An enhancer can be 1MB upstream/downstream of its targeted gene. Promoter: a region of DNA (100-1000 bp) that initiates transcription of a gene. A promoter is located near transcription start site (5') of a gene. [f]Regulatory motifs altered by lead SNP and its correlated proxies. Regulatory motifs are short nucleotide sequences typically upstream of genes where transcription factors bind to control the expression of genes. [g]Expression QTL (*cis*-eQTL, variants are within 1 Mb up- and downstream of the transcription start site) for lead SNP and its correlated proxies, CEU: $r^2 \geqslant 0.8$, derived from eQTL studies including results with P < 1e-5 from the GTEx (Genotype-Tissue Expression) and GRASP (Genome-Wide Repository of Associations Between SNPs and Phenotypes). Tissues include esophagus, muscle, salivary gland, lung, heart, ovary, uterus, artery, fallopian tube, vagina, skin, cervix, bladder, thyroid, adipose, colon, breast, testis, nerve, small intestine, pituitary, pancreas, colon, stomach, prostate, adrenal gland, liver, spleen, and kidney. [h]Methylation QTLs for lead SNP derived from temporal cortex, frontal cortex and caudal pons. The results are obtained using GRASP. All associations have P < 1e-8.

Brief summary of Supplementary Table 3-11

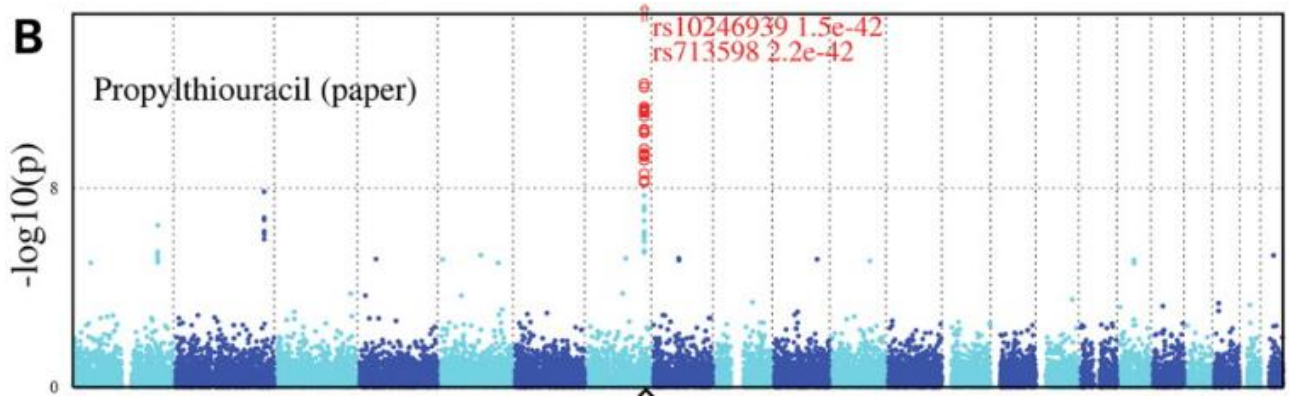
1. PROP, quinine, caffeine, and SOA-associated SNPs include missense variants.
2. PROP, quinine, and DB-associated SNPs are located within conserved region.
3. Except for caffeine-associated SNPs, all SNPs are in high DNAse hypersensitivity sites.
4. PROP, SOA, and DB-associated SNPs are inside promoter and/or enhancer regions.
5. All SNPs may cause regulatory motif change of a protein.
6. All SNPs are eQTLs.
7. Quinine, caffeine, and SOA-associated SNPs are mQTLs.
8. None of these analyses was performed using taste tissues.



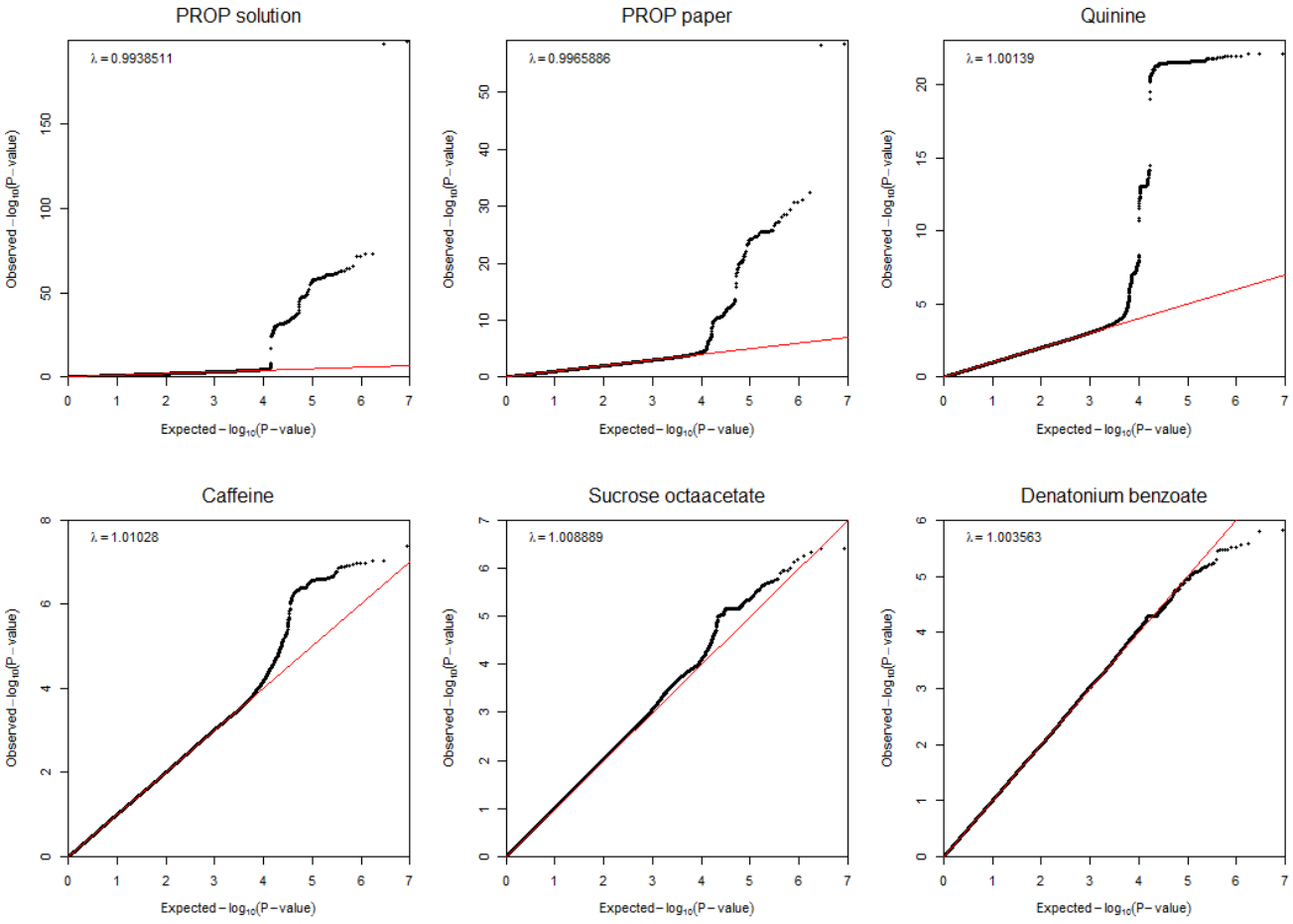
Supplementary Figure 3-1. Direction and size of the effects of SNP associations on the perceived intensities of quinine, caffeine, sucrose octaacetate (SOA) and denatonium benzoate (DB). rs10772420 has the strongest effect on quinine is in the same direction (negative) as its effect on SOA and DB (Qs and Qd) but the opposite (positive) on caffeine (Qc). The effect size (β; see Supplementary Table 9 for variance explained) is the largest for quinine, and it is smaller but at a similar level for the others. The minor allele of rs2597979 has opposite effects on caffeine and quinine (Cq). The direction of effect on SOA and DB (Cs and Cd) is the same as that on caffeine but the size of their effects is subtle. The minor allele of rs67487380 has negative effects on all bitter tastes. The effect size for SOA is similar to those for quinine and DB (Sq and Sd), which positions them close to the diagonal line. The effect on caffeine is the minimum. The minor allele of rs10261515 has the largest and negative effect on DB. Its effects on the others are subtle, but they tend to be negative, null and positive on SOA, caffeine and quinine (Ds, Dc and Dq), respectively. add effect sizes for each SNPs.



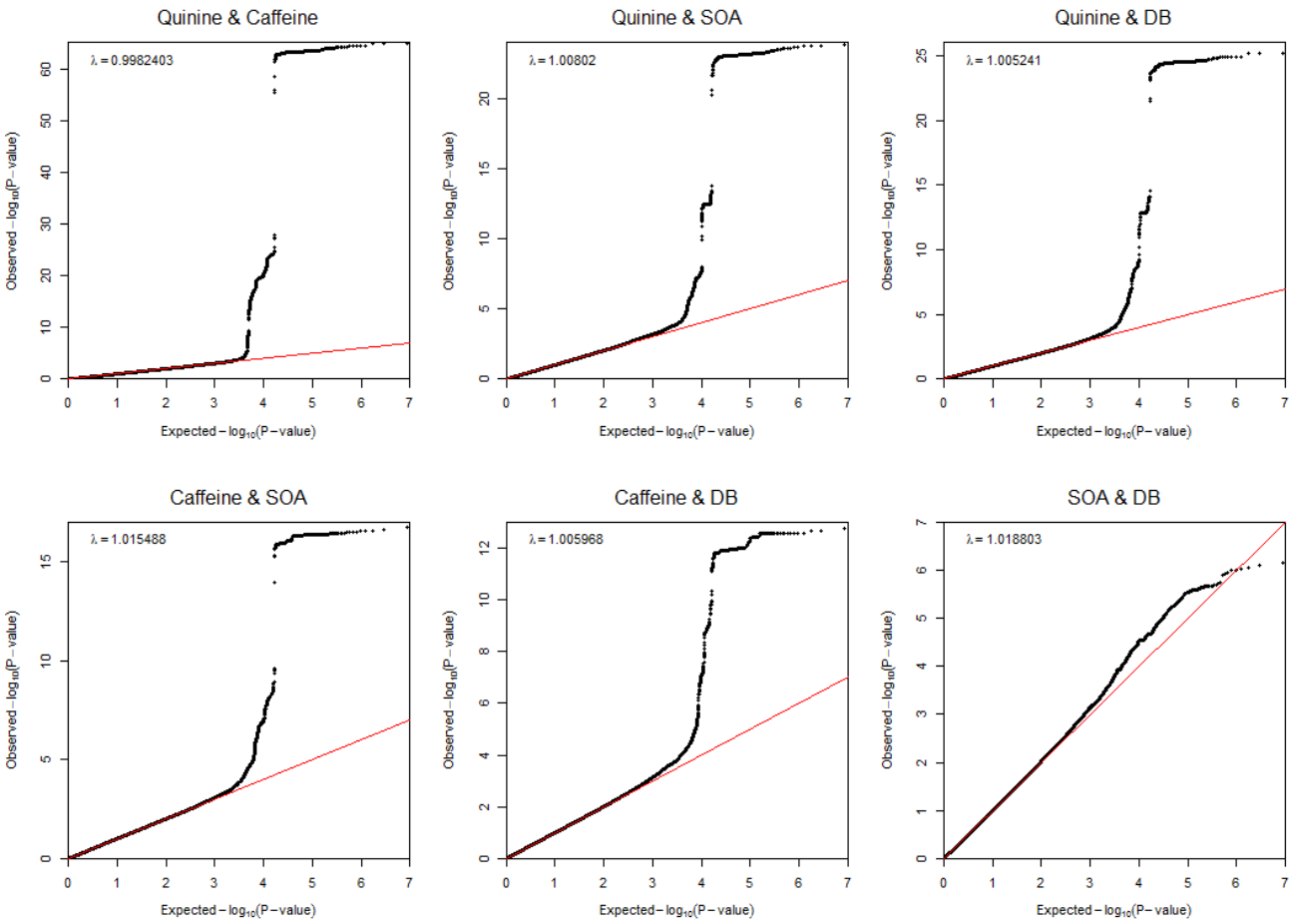
Supplementary Figure 3-2. Univariate GWAS for the perception of (a) PROP solution (n = 1757) and (b) PROP paper (n = 1999). Left part are Manhattan plots displaying the association P-value for each SNP in the genome (displayed as $-\log_{10}$ of the P-value). The red line indicates the genome-wide significance threshold of P = 5.0e-8. Right part are regional plots ± 400kb from the top SNPs on chromosome 7 for PROP solution and chromosome 2 for PROP paper with the gene model below.



Supplementary Figure 3-3. Univariate GWAS for the perception of PROP paper from our previous GWAS of 1756 Australian adolescents. A Manhattan plot displays the association P-value for each SNP in the genome (displayed as $-\log_{10}$ of the P-value). This figure is the Figure 1B from our previous published paper (PMID: 20675712).



Supplementary Figure 3-4. The Q-Q plots for each of the univariate analyses. PROP: propylthiouracil.



Supplementary Figure 3-5. The Q-Q plots for each of the bivariate analyses. SOA: sucrose octaacetate. DB: denatonium benzoate

Chapter 4

Supplementary Table 4-1. Genetic instruments of perceived intensity for PROP, quinine, and caffeine.

Trait	SNP	Chr	EA	Beta	SE	r^2	P-value
PROP	rs1726866	7	G	9.65E-01	2.81E-02	45.94%	5.62E-198
Quinine	rs10772420	12	A	3.37E-01	3.38E-02	5.67%	7.84E-23
Caffeine	rs2597979	12	G	2.64E-01	4.80E-02	1.91%	4.17E-08

EA is the effect allele corresponding to increasing level of perceived intensity. SE is the standard error. r^2 refers to the proportion of phenotypic variance explained by the SNP. Association estimates were extracted from Chapter 3.

Supplementary Table 4-2. Frequencies of bitter perception SNP genotypes in UK Biobank samples (N=438,870)

SNP	Ref. Allele (A1)	Alt. Allele (A2)	Ref. Allele Frequency	Genotype Frequency (A1A1, A1A2, A2A2)
rs1726866	G	A	0.45	0.21,0.49,0.30
rs10772420	G	A	0.48	0.23,0.50,0.27
rs2597979*	G	C	0.21	0.05,0.32,0.63

*The genotypes for rs2597979 were imputed using the HRC reference panel. Genotype values were given in dosages, and rounded to the nearest integer in calculating percentage of allele carriers.

Supplementary Table 4-3. SNP associations with the intake of coffee, tea, and alcohol (linear).

		Coffee				Tea				Alcohol		
SNP	EA	Beta	SE	P-value		Beta	SE	P-value		Beta	SE	P-value
rs1726866	G	-2.00e-2	4.70e-3	2.20e-5		3.45e-2	6.26e-3	3.60e-8		-1.92e-2	3.10e-3	5.90e-10
rs10772420	A	-2.74e-2	4.69e-3	5.00e-9		2.76e-2	6.24e-3	9.80e-6		5.61e-3	3.09e-3	7.00e-2
rs2597979	G	3.86e-2	5.93e-3	7.70e-11		-4.55e-2	7.90e-3	8.70e-9		-4.05e-3	3.91e-3	3.00e-1

EA is the effect allele corresponding to increasing level of perceived intensity. SE is the standard error.

Supplementary Table 4-4. SNP associations with the drinker status of coffee, tea, and alcohol (logistic).

		Coffee				Tea				Alcohol		
SNP	**EA**	**Beta**	**SE**	**P-value**		**Beta**	**SE**	**P-value**		**Beta**	**SE**	**P-value**
rs1726866	G	-2.96e-2	7.45e-3	7.01e-5		3.77e-2	6.88e-3	4.36e-8		0.977	8.95e-3	1.05e-2
rs10772420	A	-4.37e-2	7.44e-3	4.19e-9		2.80e-2	6.87e-3	4.69e-5		1.000	8.94e-3	9.66e-1
rs2597979	G	4.97e-2	9.36e-3	1.13e-7		-4.69e-2	8.68e-3	6.47e-8		1.005	1.13e-2	6.38e-1

EA is the effect allele corresponding to increasing level of perceived intensity. SE is the standard error.

Supplementary Table 4-5. Cross-conditional MR analyses to evaluate association between increased bitter perception on coffee/tea intake.

Taste	**2-SD increase in Bitter against coffee adjusted for tea intake**			**2-SD increase in Bitter against tea adjusted for coffee intake**		
	Beta	**SE**	**P-value**	**Beta**	**SE**	**P-value**
PROP	-0.025	0.01	1.22e-2	0.055	0.013	3.47e-5
Quinine	-0.135	0.028	1.27e-6	0.094	0.038	1.34e-2
Caffeine	0.22	0.045	1.07e-6	-0.248	0.062	5.88e-5

SE is the standard error.

Supplementary Table 4-6. MR association for increased bitter perception with coffee intake among tea non-drinkers.

Taste	**Unstratified* (N = 408,191)**				**Among tea non-drinkers (tea per day <2) (N = 90,706)**				**Among strict tea non-drinkers (tea per day <1) (N = 58,951)**		
	Beta	**SE**	**P-value**		**Beta**	**SE**	**P-value**		**Beta**	**SE**	**P-value**
PROP	-0.040	0.009	2.20e-5		-0.031	0.025	0.211		-0.018	0.034	0.590
Quinine	-0.055	0.009	5.00e-9		-0.231	0.071	0.001		-0.248	0.096	0.010
Caffeine	0.077	0.012	7.70e-11		0.248	0.114	0.030		0.260	0.154	0.090

*Unstratified model were performed using BOLT-LMM to account for genetic relatedness among individuals to maximise power. Stratified analyses were performed after removing related individuals. SE is the standard error.

Supplementary Table 4-7. MR association for increased bitter perception with tea intake among non-coffee drinkers

Taste	Unstratified (n=425,378)				Among coffee non-drinkers (coffee per day <2) (n=165,751)				Among strict coffee non-drinkers (coffee per day <1) (n=85,077)		
	Beta	SE	P-value		Beta	SE	P-value		Beta	SE	P-value
PROP	0.069	0.013	3.60e-8		0.042	0.021	0.052		0.042	0.033	0.208
Quinine	0.055	0.012	9.80e-6		0.085	0.062	0.167		0.065	0.096	0.498
Caffeine	-0.091	0.016	8.70e-9		-0.314	0.100	0.002		-0.317	0.156	0.042

*Unstratified model were performed using BOLT-LMM to account for genetic relatedness among individuals to maximise power. Stratified analyses were performed after removing related individuals. SE is the standard error.

Supplementary Table 4-8. MR association of bitter taste and bitter beverage consumption quantity stratified by sex.

Bitter taste	Beverage	Sex-specific causal estimate of decreased perception on consumption behaviour							Chisq-diff.*	P-value of diff.*
		Males			Females					
		Beta	SE	P-value	Beta	SE	P-value			
PROP	Coffee	-0.042	0.016	8.33e-3	-0.040	0.013	2.15e-3		0.007	0.932
Quinine		-0.214	0.045	2.17e-6	-0.147	0.037	8.48e-5		1.318	0.251
Caffeine		0.297	0.073	4.76e-5	0.308	0.060	3.22e-7		0.012	0.912
PROP	Tea	0.093	0.021	7.97e-6	0.047	0.018	7.48e-3		2.843	0.092
Quinine		0.145	0.060	1.47e-2	0.189	0.050	1.78e-4		0.315	0.574
Caffeine		-0.181	0.096	6.03e-2	-0.523	0.081	1.37e-10		7.334	0.007
PROP	Alcohol	-0.045	0.009	1.82e-6	-0.040	0.010	3.00e-5		0.169	0.681
Quinine		0.005	0.027	0.858	0.058	0.027	3.27e-2		1.931	0.165
Caffeine		0.033	0.044	0.454	-0.098	0.044	2.62e-2		4.429	0.035

SE is the standard error. *Difference between males and females.

Supplementary Table 4-9. Stratified analyses on association of PROP perception on different types of wine

Consumption	Beta	SE	P-value
All alcohol (frequency score)	-0.038	0.006	5.9e-10
White wine (glass/day)	0.076	0.024	1.3e-3
Red wine (glass/day)	-0.171	0.028	1.3e-9

Note: Association estimates are scaled to 2-SD increase in PROP perception.

Supplementary Table 4-10. Association of bitter perception SNP with potential confounders on beverage consumption

SNP	Perception	Ref. Allele	Potential Confounder	Beta	P-value
Diet					
rs1726866	PROP	A	Cheese intake	-5.83e-3	9.35e-3
rs10772420	Quinine	A	Cheese intake	4.72e-3	3.53e-2
rs10845296	Caffeine	A	Cheese intake	8.49e-3	2.37e-3
rs1726866	PROP	A	Variation in diet	7.99e-4	0.535
rs10772420	Quinine	A	Variation in diet	-1.50e-3	0.243
rs10845296	Caffeine	A	Variation in diet	-4.56e-4	0.776
Tobacco use					
rs1726866	PROP	A	Smoking status	6.24e-4	0.658
rs10772420	Quinine	A	Smoking status	1.89e-4	0.893
rs10845296	Caffeine	A	Smoking status	7.83e-4	0.655
rs1726866	PROP	A	Current tobacco smoking	2.69e-4	0.678
rs10772420	Quinine	A	Current tobacco smoking	4.44e-4	0.493
rs10845296	Caffeine	A	Current tobacco smoking	3.89e-4	0.630
Beverage temperature					
rs1726866	PROP	A	Hot drink temperature	5.47e-4	0.652
rs10772420	Quinine	A	Hot drink temperature	1.48e-3	0.221
rs10845296	Caffeine	A	Hot drink temperature	-1.95e-3	0.196
Sleep related traits					
rs1726866	PROP	A	Sleep duration	-3.77e-3	0.107
rs10772420	Quinine	A	Sleep duration	-2.50e-3	0.283
rs10845296	Caffeine	A	Sleep duration	-3.21e-3	0.269
rs1726866	PROP	A	G47 Sleep disorders	-5.17e-5	0.835
rs10772420	Quinine	A	G47 Sleep disorders	-3.88e-6	0.988
rs10845296	Caffeine	A	G47 Sleep disorders	-2.07e-5	0.947
rs1726866	PROP	A	sleep apnoea	-8.58e-5	0.495
rs10772420	Quinine	A	sleep apnoea	1.36e-5	0.914
rs10845296	Caffeine	A	sleep apnoea	-2.31e-5	0.883
Socio-economic					
rs1726866	PROP	A	Townsend deprivation index at recruitment	3.59e-3	0.549
rs10772420	Quinine	A	Townsend deprivation index at recruitment	6.49e-3	0.279
rs10845296	Caffeine	A	Townsend deprivation index at recruitment	-1.51e-4	0.984
rs1726866	PROP	A	Body mass index (BMI)	-5.78e-3	0.535
rs10772420	Quinine	A	Body mass index (BMI)	-4.11e-4	0.965
rs10845296	Caffeine	A	Body mass index (BMI)	-6.50e-3	0.580
rs1726866	PROP	A	Number of vehicles in household	-3.28e-3	0.070
rs10772420	Quinine	A	Number of vehicles in household	-1.92e-3	0.287
rs10845296	Caffeine	A	Number of vehicles in household	1.16e-3	0.607

Estimates were extracted from the GENEATLAS PheWAS database for UK Biobank traits (available at http://geneatlas.roslin.ed.ac.uk/). SNP rs10845296 is used as the best proxy in high-LD (Linkage Disequilibrium) with rs2597979 at an r^2 of 0.83. Ref. allele refers to the allele associated with the magnitude of association on the potential confounder for a given SNP.

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Influence of bitter taste perception on coffee, tea and alcohol consumption

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Coffee, tea and alcohol consumption are associated with various health conditions. Individual differences in taste shape food preferences and dietary behaviour. However, findings from observational studies on bitter taste perception and bitter-tasting beverage consumption are limited and inconclusive. We used Mendelian randomization to examine causal associations between the perception of three bitter substances, caffeine, quinine and propylthiouracil, and the intake of coffee, tea and alcohol among 409,000 UK Biobank participants. The results showed that genetically predicted perception of caffeine (rs2597979) was positively associated with coffee consumption, whereas predicted perception of quinine (rs10772420) and propylthiouracil (rs1726866) was negatively associated with coffee consumption. For tea, the associations are at the opposite direction. Alcohol intake was negatively associated with predicted perception of propylthiouracil. We demonstrated the causal relationships between bitter taste and bitter-tasting beverage consumption, further providing insight into the development and prevention of addiction on these beverages and its health consequences.

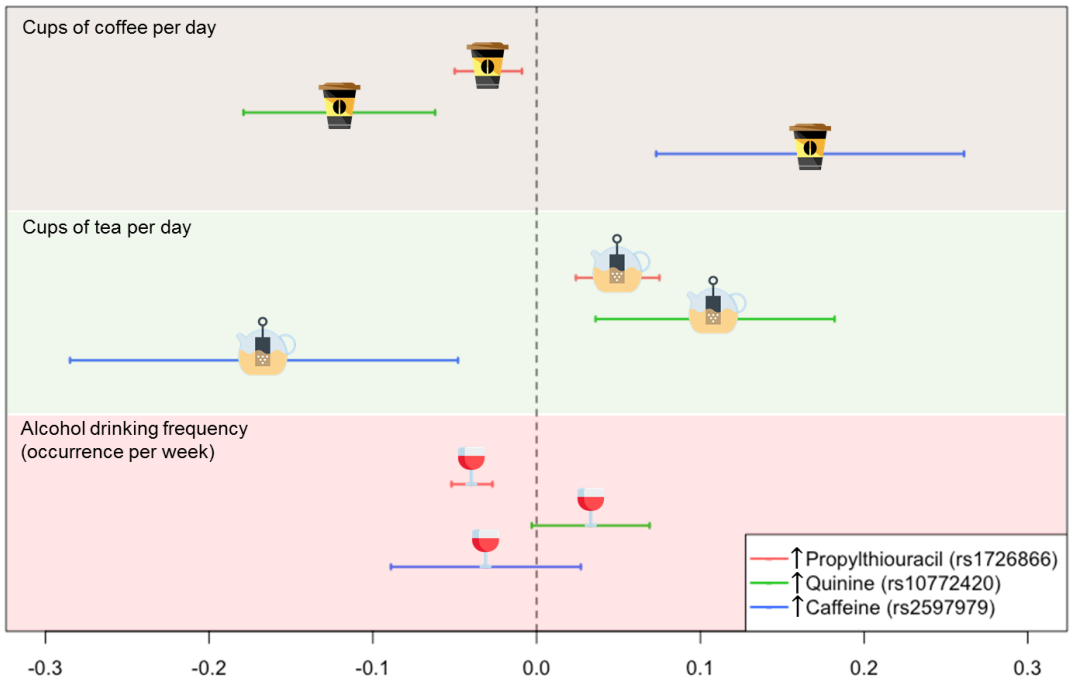
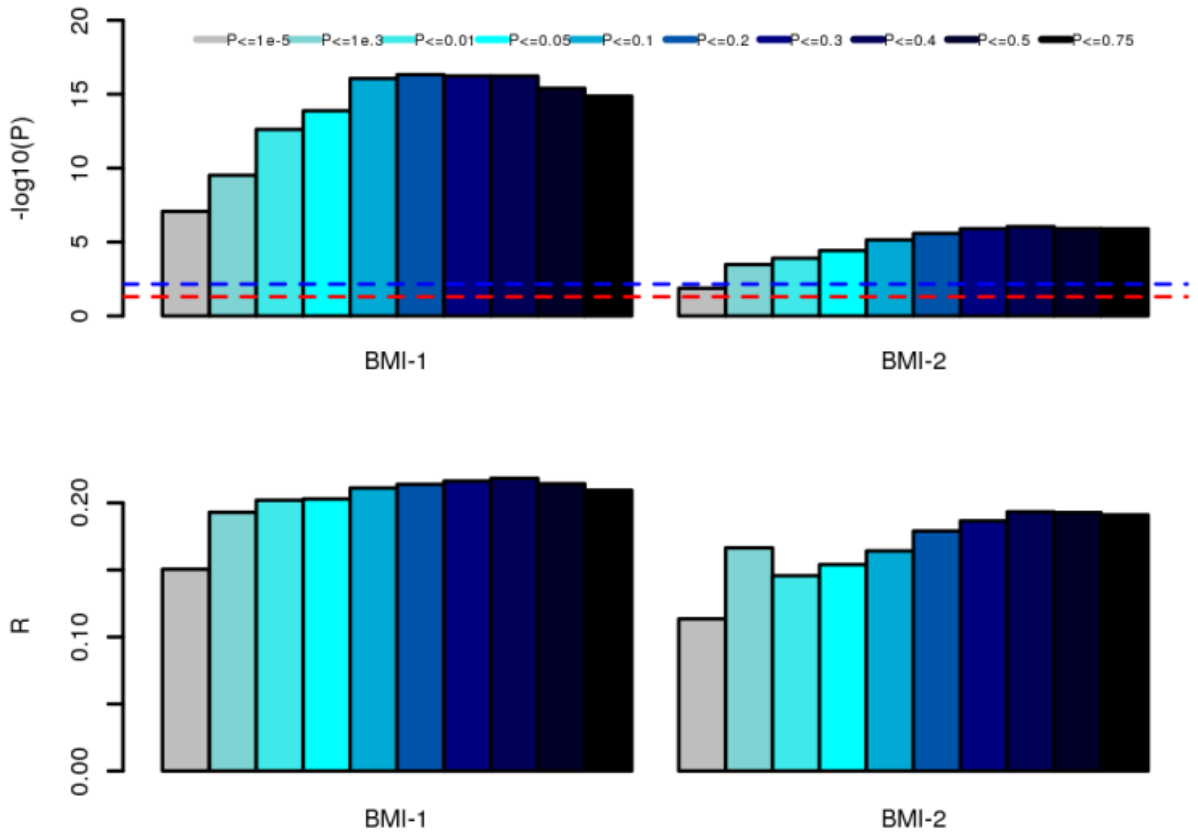


Figure. Effect of genetically predicted perception of bitter tastes on coffee, tea and alcohol consumption. For coffee, every 2-SD increase in predicted ratings (e.g., ratings of Strong vs Moderate on a general Labelled Magnitude Scale) of propylthiouracil and quinine leads to 0.03 and 0.12 fewer cups per day. Every 2-SD increase in predicted ratings of caffeine leads to 0.17 more cups per day. The three bitter tastes have opposite effects on tea. For alcohol, a higher predicted intensity of propylthiouracil leads to lower intake. Icons of coffee, tea and wine are designed by Roundicons, Smashicons and Freepik from www.flaticon.com.



Supplementary Figure 5-1. Correlation Between PGRS of BMI and BMI at the age of taste (BMI-1) and BMI 8 year later (BMI-2).

Chapter 6

Supplementary Table 6-1. Means and standard deviations of the volume of 82 brain regions (mm^3) and intensity ratings of 4 taste solutions. L and R indicate the volume is extracted for left and right hemisphere respectively.

Trait		Mean	SD
Superiorfrontal	L	25231.51	2814.10
Superiorfrontal	R	23728.82	2585.80
Rostralmiddlefrontal	L	17168.97	2526.18
Rostralmiddlefrontal	R	16402.25	2205.08
Caudalmiddlefrontal	L	7295.87	1287.88
Caudalmiddlefrontal	R	6477.83	1301.25
Parsopercularis	L	5636.38	918.53
Parsopercularis	R	4468.55	697.65
Parstriangularis	L	4129.98	670.25
Parstriangularis	R	4611.97	798.76
Parsorbitalis	L	2352.27	360.75
Parsorbitalis	R	2701.37	423.32
Lateralorbitofrontal	L	7333.59	995.59
Lateralorbitofrontal	R	7023.36	887.62
Medialorbitofrontal	L	5065.64	709.43
Medialorbitofrontal	R	5050.21	689.92
Precentral	L	14574.89	1826.91
Precentral	R	14315.77	1812.49
Paracentral	L	3785.63	590.51
Paracentral	R	4207.45	734.25
Frontalpole	L	881.12	179.28
Frontalpole	R	1104.12	211.73
Superiorparietal	L	14293.69	1942.22
Superiorparietal	R	14533.67	1998.66
Inferiorparietal	L	13473.34	2124.75
Inferiorparietal	R	16469.48	2383.42
Supramarginal	L	12212.46	1900.68
Supramarginal	R	11467.19	1820.56
Postcentral	L	10386.17	1442.58
Postcentral	R	9901.54	1549.91
Precuneus	L	10738.76	1405.76
Precuneus	R	11124.21	1503.47
Lateraloccipital	L	12100.25	1567.39
Lateraloccipital	R	12466.07	1723.31
Lingual	L	6877.38	1025.56
Lingual	R	7208.11	980.20
Cuneus	L	3135.17	494.18
Cuneus	R	3388.44	543.67
Pericalcarine	L	2248.07	395.20
Pericalcarine	R	2464.63	421.94
Superiortemporal	L	12569.51	1710.49
Superiortemporal	R	11896.11	1562.96
Middletemporal	L	10008.35	1812.46
Middletemporal	R	10993.63	1818.32
Inferiortemporal	L	8864.62	1585.91
Inferiortemporal	R	8913.35	1656.07
Bankssts	L	2667.12	507.50
Bankssts	R	2568.12	458.83
Fusiform	L	9275.03	1305.85
Fusiform	R	9453.18	1320.68
Transversetemporal	L	1264.65	237.22
Transversetemporal	R	969.51	185.21
Entorhinal	L	1534.59	351.14
Entorhinal	R	1430.68	426.84
Temporalpole	L	2240.49	483.36
Temporalpole	R	2029.40	484.02
Parahippocampal	L	2361.58	336.71
Parahippocampal	R	2199.39	308.60
Rostralanteriorcingulate	L	2938.91	562.33
Rostralanteriorcingulate	R	2338.79	503.31
Caudalanteriorcingulate	L	2052.40	491.53
Caudalanteriorcingulate	R	2340.55	554.80
Posteriorcingulate	L	3514.94	582.15
Posteriorcingulate	R	3520.22	565.09
Isthmuscingulate	L	2851.76	520.06

Isthmuscingulate	R	2624.08	476.65
Insula	L	6884.53	873.66
Insula	R	6926.51	914.00
Accumbens	L	684.67	123.67
Accumbens	R	732.03	112.92
Amygdala	L	1676.85	212.16
Amygdala	R	1695.26	232.55
Caudate	L	3982.09	488.10
Caudate	R	4159.23	533.75
Hippocampus	L	4216.38	449.22
Hippocampus	R	4304.58	439.57
Pallidum	L	1672.22	231.86
Pallidum	R	1550.92	190.54
Putamen	L	6370.69	733.53
Putamen	R	5928.72	705.53
Thalamus	L	8090.54	879.91
Thalamus	R	7492.56	802.62
Sweetness		31.81	16.10
PROP		36.28	30.33
Quinine		45.31	22.79
Caffeine		53.52	23.70

Supplementary Table 6-2. Association between sweet intensity ratings and brain volumes. Significant associations are bold. Interval is the time interval between taste test and brain scan. Taste is the perceived intensity ratings of sweet solutions. r is the correlation coefficient between taste and brain phenotypes. L and R indicate the volume is extracted for left and right hemisphere respectively.

Region of interest		n	Beta Interval	SD Interval	P Interval	Beta Taste	SD Taste	P Taste	r Taste
Superiorfrontal	L	546	-58.78	54.12	2.78e-1	1.10	5.43	8.40e-1	0.01
Superiorfrontal	R	546	-62.96	51.04	2.18e-1	-3.26	5.22	5.32e-1	-0.03
Rostralmiddlefrontal	L	545	-114.98	47.33	1.56e-2	5.03	4.98	3.14e-1	0.04
Rostralmiddlefrontal	R	543	-71.47	47.09	1.30e-1	0.00	4.85	1.00	0.00
Caudalmiddlefrontal	L	546	-6.48	30.98	8.34e-1	2.73	3.19	3.92e-1	0.04
Caudalmiddlefrontal	R	546	-1.64	30.34	9.57e-1	1.61	3.36	6.31e-1	0.02
Parsopercularis	L	548	-28.27	23.07	2.21e-1	2.80	2.42	2.47e-1	0.05
Parsopercularis	R	545	-4.88	16.83	7.72e-1	0.19	1.83	9.18e-1	0.00
Parstriangularis	**L**	**546**	**-4.98**	**16.66**	**7.65e-1**	**3.73**	**1.80**	**3.86e-2**	**0.09**
Parstriangularis	R	547	3.98	20.54	8.47e-1	1.72	2.25	4.43e-1	0.03
Parsorbitalis	L	547	5.24	9.02	5.61e-1	-0.01	0.98	9.90e-1	0.00
Parsorbitalis	R	548	-2.36	11.01	8.30e-1	1.65	1.18	1.60e-1	0.06
Lateralorbitofrontal	L	547	25.59	21.95	2.45e-1	0.67	2.26	7.66e-1	0.01
Lateralorbitofrontal	R	547	-12.58	20.12	5.32e-1	-0.17	2.15	9.36e-1	0.00
Medialorbitofrontal	L	548	-32.72	18.43	7.66e-2	-1.81	1.96	3.57e-1	-0.04
Medialorbitofrontal	R	547	19.94	16.76	2.35e-1	1.17	1.83	5.23e-1	0.03
Precentral	L	543	-27.36	36.96	4.60e-1	0.39	3.68	9.15e-1	0.00
Precentral	R	541	-42.36	36.88	2.52e-1	4.70	3.81	2.19e-1	0.05
Paracentral	L	544	-20.37	14.83	1.70e-1	-0.35	1.60	8.25e-1	-0.01
Paracentral	R	543	-34.92	17.88	5.16e-2	1.86	1.89	3.25e-1	0.04
Frontalpole	L	547	-0.20	5.07	9.69e-1	0.23	0.55	6.81e-1	0.02
Frontalpole	R	547	3.54	5.93	5.51e-1	-0.17	0.65	7.97e-1	-0.01
Superiorparietal	L	545	-20.65	38.15	5.89e-1	6.94	3.83	7.11e-2	0.07
Superiorparietal	R	544	-8.21	39.55	8.36e-1	4.50	4.11	2.75e-1	0.04
Inferiorparietal	L	545	-48.37	42.20	2.53e-1	-0.88	4.30	8.38e-1	-0.01
Inferiorparietal	R	548	-60.03	48.42	2.16e-1	-2.31	5.11	6.52e-1	-0.02
Supramarginal	L	539	1.86	38.09	9.61e-1	-1.19	3.92	7.61e-1	-0.01
Supramarginal	R	537	-3.26	37.71	9.31e-1	1.43	3.97	7.18e-1	0.02
Postcentral	L	543	-27.91	30.15	3.55e-1	1.20	3.15	7.04e-1	0.02
Postcentral	R	541	20.18	32.04	5.29e-1	-0.13	3.40	9.70e-1	0.00
Precuneus	L	544	1.50	25.70	9.53e-1	-2.49	2.62	3.43e-1	-0.04
Precuneus	R	545	-10.04	27.70	7.17e-1	-1.83	2.81	5.17e-1	-0.03
Lateraloccipital	L	546	2.37	34.55	9.45e-1	1.44	3.56	6.87e-1	0.02
Lateraloccipital	R	547	16.26	38.85	6.76e-1	-0.75	4.00	8.52e-1	-0.01
Lingual	L	545	-29.87	25.69	2.46e-1	-3.74	2.62	1.55e-1	-0.06
Lingual	R	546	-14.14	23.97	5.56e-1	-2.52	2.42	3.00e-1	-0.04
Cuneus	L	546	6.75	12.90	6.01e-1	1.94	1.29	1.34e-1	0.06
Cuneus	**R**	**545**	**2.48**	**13.31**	**8.52e-1**	**3.40**	**1.37**	**1.32e-2**	**0.10**
Pericalcarine	L	546	1.86	11.15	8.68e-1	-0.63	1.05	5.49e-1	-0.02
Pericalcarine	R	549	13.09	11.89	2.72e-1	-0.47	1.11	6.75e-1	-0.02
Superiortemporal	L	548	81.29	37.33	3.03e-2	3.54	3.73	3.42e-1	0.04
Superiortemporal	R	546	18.59	33.91	5.84e-1	0.77	3.42	8.23e-1	0.01
Middletemporal	L	548	79.36	43.18	6.74e-2	2.30	4.25	5.89e-1	0.02
Middletemporal	R	547	47.39	40.56	2.44e-1	2.89	4.17	4.90e-1	0.03
Inferiortemporal	L	547	2.24	37.57	9.52e-1	-1.55	3.84	6.86e-1	-0.02
Inferiortemporal	R	547	117.76	38.26	2.28e-3	5.32	3.96	1.80e-1	0.05
Bankssts	**L**	**547**	**-13.22**	**11.55**	**2.53e-1**	**-2.85**	**1.26**	**2.44e-2**	**-0.09**
Bankssts	R	546	-11.80	10.67	2.69e-1	-0.52	1.16	6.53e-1	-0.02
Fusiform	L	541	-11.36	26.39	6.67e-1	-3.57	2.88	2.15e-1	-0.05
Fusiform	R	546	1.96	27.78	9.44e-1	-2.32	2.96	4.33e-1	-0.03
Transversetemporal	**L**	**546**	**7.11**	**5.93**	**2.31e-1**	**1.51**	**0.66**	**2.15e-2**	**0.09**
Transversetemporal	R	546	4.08	4.68	3.84e-1	0.50	0.51	3.22e-1	0.04
Entorhinal	L	545	7.67	8.68	3.77e-1	0.05	0.95	9.54e-1	0.00
Entorhinal	R	543	28.54	10.68	7.92e-3	1.14	1.11	3.05e-1	0.04
Temporalpole	L	548	63.23	13.52	4.26e-6	1.00	1.42	4.79e-1	0.03
Temporalpole	R	545	36.72	13.52	6.92e-3	2.58	1.43	7.19e-2	0.07
Parahippocampal	L	543	-13.41	8.83	1.30e-1	-1.75	0.92	5.73e-2	-0.08
Parahippocampal	R	545	10.32	7.90	1.93e-1	-0.01	0.81	9.93e-1	0.00
Rostralanteriorcingulate	L	548	-17.67	13.49	1.91e-1	-0.20	1.44	8.89e-1	-0.01
Rostralanteriorcingulate	R	546	-14.72	13.01	2.59e-1	-1.72	1.43	2.30e-1	-0.05
Caudalanteriorcingulate	**L**	**544**	**-1.22**	**12.85**	**9.24e-1**	**3.14**	**1.41**	**2.59e-2**	**0.09**
Caudalanteriorcingulate	R	547	-2.42	15.07	8.72e-1	0.93	1.63	5.66e-1	0.02
Posteriorcingulate	**L**	**546**	**1.22**	**13.73**	**9.29e-1**	**3.51**	**1.46**	**1.69e-2**	**0.10**

Posteriorcingulate	R	545	-8.46	13.31	5.25e-1	2.29	1.42	1.07e-1	0.07
Isthmuscingulate	L	545	-5.79	11.09	6.02e-1	-0.25	1.16	8.30e-1	-0.01
Isthmuscingulate	R	546	-6.51	11.73	5.79e-1	0.58	1.20	6.30e-1	0.02
Insula	L	545	34.59	18.09	5.69e-2	0.34	1.84	8.52e-1	0.01
Insula	R	543	43.45	17.87	1.57e-2	3.14	1.81	8.38e-2	0.07
Accumbens	L	546	-7.51	3.31	2.39e-2	-0.16	0.34	6.44e-1	-0.02
Accumbens	R	545	-3.11	2.73	2.56e-1	-0.11	0.28	6.93e-1	-0.02
Amygdala	L	547	-0.36	5.32	9.47e-1	1.00	0.55	7.14e-2	0.08
Amygdala	R	544	-8.66	5.72	1.31e-1	-0.07	0.59	9.02e-1	-0.01
Caudate	L	543	15.96	12.16	1.92e-1	-1.58	0.96	1.04e-1	-0.05
Caudate	R	540	4.92	12.82	7.02e-1	-1.96	1.05	6.57e-2	-0.06
Hippocampus	L	545	18.15	10.37	8.17e-2	0.48	0.99	6.27e-1	0.02
Hippocampus	R	545	9.51	10.15	3.50e-1	0.93	0.91	3.10e-1	0.04
Pallidum	L	545	8.24	5.45	1.32e-1	0.46	0.55	4.07e-1	0.03
Pallidum	R	544	-1.64	3.81	6.68e-1	-0.31	0.39	4.30e-1	-0.03
Putamen	L	546	3.40	16.90	8.41e-1	0.01	1.51	9.93e-1	0.00
Putamen	R	543	-0.86	15.09	9.54e-1	1.11	1.35	4.11e-1	0.03
Thalamus	L	541	-1.88	14.63	8.98e-1	-0.10	1.50	9.46e-1	0.00
Thalamus	R	548	17.94	13.27	1.78e-1	0.73	1.24	5.56e-1	0.02

Supplementary Table 6-3. Association between intensity ratings of quinine and brain volumes. Significant associations are bold. Interval is the time interval between taste test and brain scan. Taste is the perceived intensity ratings of quinine solutions. r is the correlation coefficient between taste and brain phenotypes. L and R indicate the volume is extracted for left and right hemisphere respectively.

Region of interest		n	Beta Interval	SD Interval	P Interval	Beta Taste	SD Taste	P Taste	r Taste
Superiorfrontal	L	555	-59.63	53.53	2.66e-1	-2.74	3.26	4.01e-1	-0.03
Superiorfrontal	R	555	-71.52	50.91	1.61e-1	-3.69	3.16	2.44e-1	-0.05
Rostralmiddlefrontal	L	554	-111.47	46.79	1.77e-2	1.11	2.97	7.09e-1	0.02
Rostralmiddlefrontal	R	552	-73.66	46.89	1.17e-1	-2.95	2.91	3.12e-1	-0.04
Caudalmiddlefrontal	L	555	-8.11	30.60	7.91e-1	-1.57	1.91	4.12e-1	-0.03
Caudalmiddlefrontal	R	555	-3.30	30.41	9.14e-1	-0.46	2.01	8.20e-1	-0.01
Parsopercularis	L	557	-32.16	22.86	1.60e-1	-0.35	1.45	8.09e-1	-0.01
Parsopercularis	R	554	-4.51	16.68	7.87e-1	1.43	1.09	1.90e-1	0.06
Parstriangularis	L	555	-5.24	16.43	7.50e-1	-1.49	1.06	1.62e-1	-0.06
Parstriangularis	R	556	6.05	20.31	7.66e-1	0.01	1.33	9.92e-1	0.00
Parsorbitalis	L	556	6.41	8.91	4.72e-1	0.02	0.58	9.66e-1	0.00
Parsorbitalis	R	557	-3.02	10.99	7.84e-1	0.78	0.71	2.71e-1	0.05
Lateralorbitofrontal	L	555	20.39	21.54	3.45e-1	-2.28	1.34	9.04e-2	-0.07
Lateralorbitofrontal	R	556	-12.13	19.91	5.43e-1	-1.03	1.28	4.21e-1	-0.03
Medialorbitofrontal	L	557	-32.58	18.31	7.59e-2	0.08	1.17	9.48e-1	0.00
Medialorbitofrontal	R	556	19.33	16.61	2.45e-1	0.81	1.09	4.58e-1	0.03
Precentral	L	552	-28.97	36.32	4.26e-1	-1.41	2.21	5.22e-1	-0.03
Precentral	R	550	-44.18	36.19	2.23e-1	-0.54	2.30	8.15e-1	-0.01
Paracentral	L	553	-21.25	14.60	1.46e-1	-0.62	0.95	5.14e-1	-0.03
Paracentral	R	552	-38.03	17.64	3.17e-2	-1.74	1.13	1.23e-1	-0.06
Frontalpole	L	556	0.14	5.05	9.77e-1	-0.21	0.33	5.20e-1	-0.03
Frontalpole	R	556	3.27	5.97	5.84e-1	0.22	0.39	5.65e-1	0.02
Superiorparietal	L	554	-16.95	37.67	6.53e-1	0.83	2.31	7.20e-1	0.01
Superiorparietal	R	553	-6.01	39.03	8.78e-1	-1.22	2.45	6.19e-1	-0.02
Inferiorparietal	L	554	-41.00	42.06	3.30e-1	0.89	2.61	7.34e-1	0.01
Inferiorparietal	R	557	-55.58	48.10	2.49e-1	2.23	3.05	4.65e-1	0.03
Supramarginal	L	548	7.38	37.49	8.44e-1	3.72	2.32	1.10e-1	0.06
Supramarginal	R	547	-0.01	37.44	1.00	-0.39	2.35	8.69e-1	-0.01
Postcentral	L	552	-24.42	29.93	4.15e-1	0.69	1.86	7.10e-1	0.02
Postcentral	R	550	23.89	31.65	4.51e-1	2.01	2.01	3.18e-1	0.04
Precuneus	L	553	10.33	25.85	6.90e-1	-0.16	1.59	9.20e-1	0.00
Precuneus	R	554	-6.55	27.60	8.13e-1	0.01	1.69	9.95e-1	0.00
Lateraloccipital	L	555	-0.54	34.32	9.88e-1	-3.38	2.14	1.15e-1	-0.07
Lateraloccipital	R	556	20.56	38.27	5.92e-1	-1.61	2.37	4.99e-1	-0.03
Lingual	L	554	-28.69	25.42	2.60e-1	1.13	1.56	4.70e-1	0.03
Lingual	R	555	-12.45	23.74	6.00e-1	-0.94	1.44	5.14e-1	-0.03
Cuneus	L	555	6.21	12.87	6.30e-1	0.86	0.78	2.68e-1	0.04
Cuneus	**R**	**554**	**2.88**	**13.11**	**8.26e-1**	**2.02**	**0.81**	**1.38e-2**	**0.10**
Pericalcarine	L	555	2.08	11.00	8.50e-1	0.06	0.63	9.19e-1	0.00
Pericalcarine	R	558	14.34	11.72	2.23e-1	0.75	0.66	2.62e-1	0.04
Superiortemporal	L	557	85.67	37.25	2.23e-2	1.04	2.24	6.44e-1	0.02
Superiortemporal	R	554	19.54	33.82	5.64e-1	-0.73	2.09	7.29e-1	-0.01
Middletemporal	L	557	82.62	42.85	5.51e-2	1.38	2.53	5.87e-1	0.02
Middletemporal	R	556	47.62	40.54	2.41e-1	1.92	2.50	4.42e-1	0.03
Inferiortemporal	L	556	-1.78	37.17	9.62e-1	1.05	2.30	6.48e-1	0.02
Inferiortemporal	**R**	**556**	**113.04**	**38.06**	**3.21e-3**	**6.76**	**2.37**	**4.58e-3**	**0.12**
Bankssts	L	556	-10.43	11.48	3.64e-1	-0.68	0.76	3.70e-1	-0.04
Bankssts	R	555	-12.80	10.60	2.28e-1	0.42	0.70	5.50e-1	0.03
Fusiform	L	550	-12.32	26.52	6.42e-1	1.39	1.74	4.23e-1	0.03
Fusiform	R	555	2.52	27.59	9.27e-1	-0.09	1.77	9.60e-1	0.00
Transversetemporal	**L**	**555**	**6.64**	**6.00**	**2.69e-1**	**1.07**	**0.39**	**6.87e-3**	**0.11**
Transversetemporal	R	555	3.49	4.68	4.56e-1	0.11	0.30	7.26e-1	0.01
Entorhinal	**L**	**554**	**8.04**	**8.56**	**3.48e-1**	**-1.64**	**0.56**	**3.71e-3**	**-0.12**
Entorhinal	R	551	29.24	10.47	5.57e-3	-0.82	0.66	2.11e-1	-0.05
Temporalpole	L	557	60.82	13.51	9.29e-6	0.11	0.85	8.95e-1	0.01
Temporalpole	R	554	34.86	13.41	9.70e-3	1.05	0.85	2.20e-1	0.05
Parahippocampal	L	552	-12.49	8.73	1.53e-1	-0.95	0.55	8.70e-2	-0.07
Parahippocampal	R	554	9.35	7.84	2.34e-1	-0.46	0.49	3.45e-1	-0.04
Rostralanteriorcingulate	L	556	-18.63	13.27	1.61e-1	0.94	0.85	2.68e-1	0.05
Rostralanteriorcingulate	R	555	-13.15	12.97	3.11e-1	-1.09	0.85	1.98e-1	-0.05
Caudalanteriorcingulate	L	553	-1.05	12.84	9.35e-1	-0.10	0.84	9.05e-1	-0.01
Caudalanteriorcingulate	R	556	-3.27	15.04	8.28e-1	0.12	0.98	8.98e-1	0.01
Posteriorcingulate	L	555	0.90	13.67	9.47e-1	0.87	0.88	3.21e-1	0.04

Posteriorcingulate	R	554	-9.18	13.19	4.87e-1	-0.66	0.85	4.33e-1	-0.03
Isthmuscingulate	L	554	-4.88	10.99	6.57e-1	-1.25	0.69	7.22e-2	-0.07
Isthmuscingulate	R	554	-4.67	11.50	6.85e-1	0.04	0.71	9.58e-1	0.00
Insula	L	554	34.01	17.74	5.63e-2	-1.50	1.09	1.69e-1	-0.06
Insula	R	552	42.42	17.75	1.75e-2	-0.12	1.09	9.15e-1	0.00
Accumbens	L	554	-7.30	3.28	2.69e-2	0.07	0.21	7.52e-1	0.01
Accumbens	R	554	-2.85	2.73	2.98e-1	0.03	0.17	8.50e-1	0.01
Amygdala	**L**	**556**	**0.76**	**5.29**	**8.86e-1**	**0.68**	**0.33**	**4.04e-2**	**0.09**
Amygdala	R	553	-9.17	5.66	1.07e-1	0.25	0.35	4.82e-1	0.03
Caudate	**L**	**552**	**15.41**	**12.06**	**2.04e-1**	**-1.55**	**0.56**	**7.00e-3**	**-0.09**
Caudate	**R**	**547**	**3.40**	**12.71**	**7.90e-1**	**-1.93**	**0.64**	**3.03e-3**	**-0.11**
Hippocampus	L	554	19.36	10.31	6.19e-2	0.20	0.59	7.28e-1	0.01
Hippocampus	R	554	10.13	10.09	3.17e-1	0.56	0.56	3.19e-1	0.04
Pallidum	L	554	9.21	5.50	9.53e-2	0.30	0.33	3.69e-1	0.04
Pallidum	R	552	-1.17	3.81	7.59e-1	-0.29	0.23	2.03e-1	-0.05
Putamen	L	555	3.64	16.88	8.29e-1	-0.17	0.91	8.55e-1	-0.01
Putamen	R	552	-2.00	14.97	8.94e-1	-0.47	0.82	5.66e-1	-0.02
Thalamus	L	550	-2.47	14.67	8.67e-1	0.39	0.90	6.68e-1	0.02
Thalamus	R	556	17.32	13.24	1.92e-1	0.40	0.75	5.92e-1	0.02

Supplementary Table 6-4. Association between intensity ratings of caffeine and brain volumes. Significant associations are bold. Interval is the time interval between taste test and brain scan. Taste is the perceived intensity ratings of caffeine solutions. r is the correlation coefficient between taste and brain phenotypes. L and R indicate the volume is extracted for left and right hemisphere respectively.

Region of interest		n	Beta Interval	SD Interval	P Interval	Beta Taste	SD Taste	P Taste	r Taste
Superiorfrontal	L	555	-59.41	53.59	2.69e-1	-2.04	3.15	5.17e-1	-0.03
Superiorfrontal	**R**	**555**	**-73.83**	**50.82**	**1.47e-1**	**-6.34**	**3.04**	**3.78e-2**	**-0.09**
Rostralmiddlefrontal	L	554	-113.33	46.70	1.57e-2	-1.71	2.87	5.53e-1	-0.02
Rostralmiddlefrontal	R	552	-73.97	46.76	1.15e-1	-3.90	2.81	1.66e-1	-0.06
Caudalmiddlefrontal	L	555	-8.60	30.62	7.79e-1	-0.97	1.85	5.99e-1	-0.02
Caudalmiddlefrontal	R	555	-3.87	30.42	8.99e-1	-1.05	1.94	5.90e-1	-0.02
Parsopercularis	L	557	-32.68	22.87	1.54e-1	-0.93	1.40	5.08e-1	-0.03
Parsopercularis	R	554	-6.10	16.65	7.14e-1	-0.61	1.06	5.63e-1	-0.02
Parstriangularis	L	555	-5.14	16.43	7.55e-1	0.07	1.03	9.47e-1	0.00
Parstriangularis	R	556	5.83	20.33	7.75e-1	0.24	1.29	8.53e-1	0.01
Parsorbitalis	L	556	5.78	8.93	5.18e-1	-0.77	0.56	1.71e-1	-0.06
Parsorbitalis	R	557	-3.28	11.05	7.66e-1	0.56	0.69	4.18e-1	0.03
Lateralorbitofrontal	L	556	22.78	21.73	2.95e-1	-2.00	1.30	1.27e-1	-0.06
Lateralorbitofrontal	R	556	-12.54	19.94	5.30e-1	-1.76	1.23	1.55e-1	-0.06
Medialorbitofrontal	L	557	-33.31	18.22	6.82e-2	-0.33	1.12	7.67e-1	-0.01
Medialorbitofrontal	R	556	18.89	16.57	2.55e-1	0.65	1.05	5.37e-1	0.03
Precentral	L	552	-31.14	36.32	3.92e-1	-2.57	2.14	2.31e-1	-0.05
Precentral	R	550	-45.08	36.21	2.14e-1	-1.31	2.23	5.57e-1	-0.02
Paracentral	L	553	-21.76	14.62	1.38e-1	-0.75	0.92	4.13e-1	-0.03
Paracentral	R	552	-38.09	17.64	3.15e-2	-1.84	1.08	9.07e-2	-0.07
Frontalpole	L	556	0.31	5.06	9.52e-1	0.01	0.32	9.86e-1	0.00
Frontalpole	R	556	3.58	5.94	5.47e-1	0.29	0.37	4.45e-1	0.03
Superiorparietal	L	554	-18.65	37.52	6.20e-1	-2.98	2.23	1.82e-1	-0.05
Superiorparietal	R	553	-6.95	39.02	8.59e-1	-1.48	2.37	5.34e-1	-0.03
Inferiorparietal	L	554	-39.75	41.97	3.44e-1	1.89	2.51	4.54e-1	0.03
Inferiorparietal	R	557	-55.64	48.02	2.47e-1	0.21	2.94	9.42e-1	0.00
Supramarginal	L	548	6.40	37.62	8.65e-1	0.61	2.25	7.87e-1	0.01
Supramarginal	R	546	-0.23	37.42	9.95e-1	-1.81	2.26	4.24e-1	-0.03
Postcentral	L	552	-27.01	29.79	3.65e-1	-3.31	1.80	6.77e-2	-0.08
Postcentral	R	550	22.86	31.58	4.70e-1	-0.21	1.94	9.15e-1	0.00
Precuneus	L	553	9.26	25.81	7.20e-1	-1.37	1.54	3.75e-1	-0.04
Precuneus	R	554	-7.49	27.47	7.85e-1	0.03	1.63	9.84e-1	0.00
Lateraloccipital	L	555	0.36	34.54	9.92e-1	-0.57	2.07	7.84e-1	-0.01
Lateraloccipital	R	556	19.15	38.32	6.18e-1	-2.35	2.30	3.09e-1	-0.04
Lingual	L	554	-30.29	25.38	2.34e-1	-2.71	1.51	7.38e-2	-0.07
Lingual	R	555	-12.75	23.66	5.90e-1	-1.94	1.40	1.68e-1	-0.06
Cuneus	L	555	6.36	12.87	6.21e-1	0.39	0.76	6.09e-1	0.02
Cuneus	**R**	**554**	**2.75**	**13.18**	**8.35e-1**	**1.84**	**0.79**	**2.05e-2**	**0.10**
Pericalcarine	L	555	1.85	10.97	8.66e-1	-0.62	0.61	3.16e-1	-0.04
Pericalcarine	R	558	13.95	11.76	2.37e-1	0.00	0.65	9.98e-1	0.00
Superiortemporal	L	557	84.08	37.31	2.51e-2	-0.72	2.17	7.41e-1	-0.01
Superiortemporal	R	554	18.07	33.86	5.94e-1	-1.10	2.01	5.83e-1	-0.02
Middletemporal	L	557	81.03	42.89	6.01e-2	0.81	2.47	7.43e-1	0.01
Middletemporal	R	556	45.85	40.53	2.59e-1	1.96	2.42	4.18e-1	0.03
Inferiortemporal	L	556	-2.19	37.18	9.53e-1	1.73	2.23	4.37e-1	0.03
Inferiortemporal	**R**	**556**	**113.51**	**37.99**	**3.04e-3**	**5.06**	**2.29**	**2.79e-2**	**0.09**
Bankssts	L	556	-10.53	11.46	3.59e-1	-0.55	0.73	4.46e-1	-0.03
Bankssts	R	555	-12.39	10.49	2.38e-1	1.14	0.67	8.69e-2	0.07
Fusiform	L	550	-11.69	26.47	6.59e-1	2.61	1.69	1.23e-1	0.06
Fusiform	R	555	2.56	27.56	9.26e-1	-0.61	1.71	7.23e-1	-0.01
Transversetemporal	L	555	6.51	6.02	2.80e-1	0.70	0.38	6.81e-2	0.08
Transversetemporal	R	555	3.42	4.67	4.65e-1	0.02	0.29	9.53e-1	0.00
Entorhinal	L	554	8.02	8.66	3.55e-1	-0.80	0.55	1.44e-1	-0.06
Entorhinal	R	551	28.70	10.44	6.32e-3	-0.78	0.64	2.21e-1	-0.05
Temporalpole	L	557	60.76	13.48	9.02e-6	-0.50	0.82	5.46e-1	-0.02
Temporalpole	R	554	34.45	13.45	1.08e-2	0.78	0.83	3.49e-1	0.04
Parahippocampal	L	552	-12.49	8.75	1.54e-1	-0.76	0.54	1.57e-1	-0.06
Parahippocampal	R	554	9.40	7.81	2.30e-1	-0.17	0.47	7.25e-1	-0.01
Rostralanteriorcingulate	L	556	-18.52	13.23	1.63e-1	0.35	0.82	6.69e-1	0.02
Rostralanteriorcingulate	R	555	-13.27	12.99	3.07e-1	-1.23	0.81	1.31e-1	-0.06
Caudalanteriorcingulate	L	553	-1.09	12.83	9.32e-1	0.26	0.81	7.52e-1	0.01
Caudalanteriorcingulate	R	556	-2.97	15.01	8.43e-1	1.07	0.94	2.55e-1	0.05
Posteriorcingulate	L	555	1.18	13.64	9.31e-1	1.37	0.85	1.08e-1	0.07

Posteriorcingulate	R	554	-8.95	13.18	4.97e-1	-0.74	0.82	3.69e-1	-0.04
Isthmuscingulate	L	554	-4.56	11.04	6.80e-1	-0.58	0.67	3.92e-1	-0.04
Isthmuscingulate	R	554	-5.07	11.48	6.59e-1	-0.48	0.69	4.87e-1	-0.03
Insula	L	554	33.64	17.83	6.02e-2	-0.44	1.06	6.77e-1	-0.02
Insula	R	552	41.69	17.82	2.00e-2	0.01	1.06	9.89e-1	0.00
Accumbens	L	554	-7.13	3.28	3.04e-2	0.20	0.20	3.11e-1	0.04
Accumbens	R	554	-2.77	2.73	3.10e-1	0.18	0.16	2.54e-1	0.05
Amygdala	L	556	0.61	5.27	9.08e-1	0.37	0.32	2.52e-1	0.05
Amygdala	R	553	-9.28	5.67	1.03e-1	0.23	0.34	5.06e-1	0.03
Caudate	**L**	**552**	**15.25**	**12.00**	**2.07e-1**	**-1.44**	**0.57**	**1.23e-2**	**-0.09**
Caudate	**R**	**547**	**4.23**	**12.71**	**7.40e-1**	**-1.37**	**0.63**	**3.33e-2**	**-0.08**
Hippocampus	L	554	19.36	10.25	6.04e-2	1.09	0.57	5.72e-2	0.08
Hippocampus	**R**	**554**	**10.11**	**10.05**	**3.16e-1**	**1.52**	**0.54**	**5.19e-3**	**0.11**
Pallidum	L	554	9.15	5.50	9.71e-2	0.28	0.32	3.84e-1	0.04
Pallidum	R	552	-1.05	3.80	7.82e-1	-0.08	0.22	7.12e-1	-0.01
Putamen	L	555	4.32	16.87	7.98e-1	1.26	0.88	1.56e-1	0.05
Putamen	R	552	-1.83	14.94	9.02e-1	-0.21	0.80	7.95e-1	-0.01
Thalamus	L	550	-2.29	14.68	8.76e-1	0.62	0.87	4.78e-1	0.03
Thalamus	R	556	17.47	13.23	1.88e-1	1.04	0.72	1.53e-1	0.06

Supplementary Table 6-5. Association between intensity ratings of PROP and brain volumes. Significant associations are bold. Interval is the time interval between taste test and brain scan. Taste is the perceived intensity ratings of PROP solutions. r is the correlation coefficient between taste and brain phenotypes. L and R indicate the volume is extracted for left and right hemisphere respectively.

Region of interest		n	Beta Interval	SD Interval	P Interval	Beta Taste	SD Taste	P Taste	r Taste
Superiorfrontal	L	553	-52.31	53.95	3.33e-1	3.40	2.54	1.82e-1	0.06
Superiorfrontal	R	553	-65.63	51.41	2.03e-1	1.27	2.44	6.04e-1	0.02
Rostralmiddlefrontal	L	552	-115.19	47.10	1.49e-2	-0.88	2.28	6.99e-1	-0.02
Rostralmiddlefrontal	R	550	-74.13	47.29	1.18e-1	-2.64	2.25	2.41e-1	-0.05
Caudalmiddlefrontal	L	553	-11.93	30.68	6.98e-1	-0.75	1.48	6.12e-1	-0.02
Caudalmiddlefrontal	R	553	-3.48	30.62	9.10e-1	0.19	1.52	9.01e-1	0.01
Parsopercularis	L	555	-33.89	22.97	1.41e-1	0.06	1.11	9.60e-1	0.00
Parsopercularis	R	552	-7.48	16.71	6.55e-1	0.32	0.83	7.04e-1	0.02
Parstriangularis	L	553	-4.79	16.55	7.72e-1	0.22	0.81	7.88e-1	0.01
Parstriangularis	R	554	6.05	20.44	7.67e-1	0.27	1.02	7.93e-1	0.01
Parsorbitalis	L	554	5.73	8.97	5.23e-1	-0.46	0.44	2.98e-1	-0.04
Parsorbitalis	R	555	-2.18	11.09	8.44e-1	0.89	0.54	1.02e-1	0.07
Lateralorbitofrontal	L	553	17.79	21.61	4.11e-1	-1.20	1.04	2.47e-1	-0.05
Lateralorbitofrontal	R	554	-13.80	19.94	4.89e-1	-0.65	0.98	5.08e-1	-0.03
Medialorbitofrontal	L	555	-32.89	18.40	7.46e-2	0.10	0.89	9.09e-1	0.01
Medialorbitofrontal	R	554	18.62	16.71	2.66e-1	0.07	0.83	9.37e-1	0.00
Precentral	L	550	-31.46	36.44	3.89e-1	-2.32	1.72	1.77e-1	-0.06
Precentral	R	548	-49.39	36.35	1.75e-1	-2.54	1.77	1.52e-1	-0.06
Paracentral	L	551	-21.97	14.71	1.36e-1	-0.52	0.73	4.73e-1	-0.03
Paracentral	R	550	-37.83	17.85	3.48e-2	-0.06	0.87	9.49e-1	0.00
Frontalpole	L	554	-0.31	5.09	9.51e-1	-0.23	0.25	3.63e-1	-0.04
Frontalpole	R	554	3.03	6.01	6.14e-1	-0.14	0.30	6.43e-1	-0.02
Superiorparietal	L	552	-11.60	37.92	7.60e-1	2.88	1.79	1.08e-1	0.07
Superiorparietal	R	551	0.41	39.24	9.92e-1	2.60	1.89	1.69e-1	0.06
Inferiorparietal	L	552	-44.41	42.25	2.94e-1	-1.17	2.01	5.62e-1	-0.02
Inferiorparietal	R	555	-55.58	48.31	2.51e-1	-0.42	2.34	8.59e-1	-0.01
Supramarginal	L	546	10.71	37.83	7.77e-1	1.86	1.80	3.01e-1	0.04
Supramarginal	R	545	-2.46	37.74	9.48e-1	-1.34	1.81	4.58e-1	-0.03
Postcentral	L	550	-29.01	30.09	3.36e-1	-1.55	1.44	2.82e-1	-0.05
Postcentral	R	548	19.28	31.88	5.46e-1	-1.33	1.55	3.89e-1	-0.03
Precuneus	L	551	12.00	25.99	6.45e-1	0.69	1.24	5.80e-1	0.02
Precuneus	R	552	-5.32	27.78	8.48e-1	1.14	1.32	3.89e-1	0.04
Lateraloccipital	L	553	5.00	34.74	8.86e-1	0.57	1.66	7.34e-1	0.01
Lateraloccipital	R	554	27.50	38.50	4.76e-1	1.23	1.85	5.06e-1	0.03
Lingual	L	552	-29.53	25.46	2.47e-1	0.50	1.21	6.81e-1	0.02
Lingual	R	553	-14.25	23.81	5.50e-1	-1.47	1.13	1.92e-1	-0.06
Cuneus	L	553	7.28	12.95	5.75e-1	0.23	0.61	7.07e-1	0.02
Cuneus	R	552	3.57	13.25	7.88e-1	0.40	0.64	5.33e-1	0.03
Pericalcarine	L	553	2.43	11.08	8.26e-1	0.12	0.51	8.15e-1	0.01
Pericalcarine	R	556	14.55	11.81	2.20e-1	0.17	0.54	7.50e-1	0.01
Superiortemporal	L	555	88.33	37.53	1.94e-2	1.11	1.76	5.28e-1	0.03
Superiortemporal	R	552	18.32	33.90	5.89e-1	-2.11	1.63	1.95e-1	-0.06
Middletemporal	L	555	76.68	43.03	7.61e-2	-0.44	2.00	8.26e-1	-0.01
Middletemporal	R	554	41.54	40.78	3.09e-1	-0.53	1.95	7.87e-1	-0.01
Inferiortemporal	L	554	-0.46	37.40	9.90e-1	1.26	1.79	4.82e-1	0.03
Inferiortemporal	R	554	112.69	38.37	3.57e-3	1.18	1.84	5.23e-1	0.03
Bankssts	L	554	-9.71	11.55	4.01e-1	-0.04	0.57	9.39e-1	0.00
Bankssts	R	553	-13.92	10.69	1.93e-1	-0.17	0.53	7.45e-1	-0.01
Fusiform	L	548	-9.71	26.67	7.16e-1	1.35	1.33	3.08e-1	0.04
Fusiform	R	553	5.79	27.69	8.35e-1	0.37	1.35	7.84e-1	0.01
Transversetemporal	L	553	7.53	6.06	2.15e-1	0.52	0.30	8.60e-2	0.07
Transversetemporal	R	553	4.30	4.71	3.62e-1	0.34	0.23	1.48e-1	0.06
Entorhinal	L	552	7.93	8.75	3.66e-1	-0.50	0.43	2.47e-1	-0.05
Entorhinal	R	549	28.28	10.54	7.65e-3	-0.76	0.51	1.33e-1	-0.06
Temporalpole	L	555	60.35	13.56	1.16e-5	-0.56	0.66	3.98e-1	-0.04
Temporalpole	R	552	34.27	13.50	1.15e-2	-0.41	0.66	5.36e-1	-0.03
Parahippocampal	L	550	-12.59	8.82	1.55e-1	-0.12	0.43	7.76e-1	-0.01
Parahippocampal	R	552	8.70	7.83	2.67e-1	0.15	0.37	6.98e-1	0.02
Rostralanteriorcingulate	L	554	-17.53	13.35	1.90e-1	0.30	0.65	6.45e-1	0.02
Rostralanteriorcingulate	R	553	-12.12	13.08	3.55e-1	-0.08	0.65	9.03e-1	-0.01
Caudalanteriorcingulate	L	551	-0.51	12.92	9.68e-1	0.24	0.64	7.07e-1	0.02
Caudalanteriorcingulate	R	554	-3.35	15.15	8.25e-1	0.12	0.74	8.70e-1	0.01
Posteriorcingulate	L	553	-0.68	13.77	9.61e-1	-0.51	0.67	4.46e-1	-0.03

Posteriorcingulate	R	552	-10.08	13.29	4.49e-1	-0.63	0.65	3.37e-1	-0.04
Isthmuscingulate	L	552	-4.96	11.12	6.56e-1	0.01	0.54	9.79e-1	0.00
Isthmuscingulate	**R**	**552**	**-7.79**	**11.49**	**4.98e-1**	**-1.09**	**0.55**	**4.94e-2**	**-0.08**
Insula	L	552	34.15	17.82	5.63e-2	-1.11	0.85	1.92e-1	-0.06
Insula	R	550	41.63	17.85	2.04e-2	-0.34	0.85	6.89e-1	-0.02
Accumbens	L	552	-7.45	3.31	2.50e-2	-0.02	0.16	9.17e-1	0.00
Accumbens	R	552	-3.08	2.75	2.64e-1	-0.02	0.13	8.65e-1	-0.01
Amygdala	L	554	0.48	5.31	9.28e-1	0.11	0.26	6.72e-1	0.02
Amygdala	R	551	-9.14	5.68	1.09e-1	0.32	0.27	2.41e-1	0.05
Caudate	L	550	16.36	12.14	1.81e-1	-0.16	0.50	7.43e-1	-0.01
Caudate	R	545	5.17	12.84	6.88e-1	-0.12	0.53	8.19e-1	-0.01
Hippocampus	L	552	20.10	10.35	5.37e-2	0.09	0.47	8.53e-1	0.01
Hippocampus	R	552	10.20	10.12	3.15e-1	0.18	0.45	6.93e-1	0.02
Pallidum	L	552	8.87	5.54	1.11e-1	-0.21	0.26	4.23e-1	-0.03
Pallidum	R	550	-0.47	3.82	9.02e-1	0.10	0.18	5.65e-1	0.02
Putamen	L	553	4.94	16.99	7.72e-1	0.29	0.74	7.02e-1	0.02
Putamen	R	550	-0.29	15.05	9.85e-1	0.01	0.67	9.86e-1	0.00
Thalamus	L	548	0.50	14.71	9.73e-1	0.71	0.70	3.12e-1	0.04
Thalamus	**R**	**554**	**20.37**	**13.24**	**1.26e-1**	**1.47**	**0.60**	**1.42e-2**	**0.10**

Supplementary Table 6-6. Association between intensity ratings of PROP and brain volumes adjusted for TAS2R38 genotype. Significant associations are bold. Interval is the time interval between taste test and brain scan. Taste is the perceived intensity ratings of PROP solutions. r is the correlation coefficient between taste and brain phenotypes. L and R indicate the volume is extracted for left and right hemisphere respectively.

Region of interest		n	Beta Interval	SD Interval	P Interval	Beta Taste	SD Taste	P Taste	r Taste
Superiorfrontal	L	527	-44.48	54.30	4.14e-1	5.11	3.45	1.39e-1	0.06
Superiorfrontal	R	527	-63.82	51.60	2.17e-1	3.87	3.36	2.50e-1	0.05
Rostralmiddlefrontal	L	526	-112.89	47.67	1.84e-2	0.23	3.13	9.42e-1	0.00
Rostralmiddlefrontal	R	524	-72.23	47.84	1.32e-1	-2.93	3.11	3.46e-1	-0.04
Caudalmiddlefrontal	L	527	-8.47	30.90	7.84e-1	-1.52	2.02	4.53e-1	-0.03
Caudalmiddlefrontal	R	527	-1.75	31.12	9.55e-1	0.28	2.11	8.94e-1	0.01
Parsopercularis	L	529	-35.27	22.84	1.24e-1	1.10	1.51	4.66e-1	0.03
Parsopercularis	R	526	-3.71	16.66	8.24e-1	0.89	1.13	4.30e-1	0.03
Parstriangularis	L	527	-6.47	16.57	6.96e-1	1.03	1.11	3.55e-1	0.04
Parstriangularis	R	528	4.54	20.46	8.24e-1	0.99	1.40	4.77e-1	0.03
Parsorbitalis	L	528	5.80	9.10	5.25e-1	-0.79	0.61	2.01e-1	-0.06
Parsorbitalis	**R**	**529**	**-0.26**	**11.13**	**9.81e-1**	**1.59**	**0.74**	**3.24e-2**	**0.09**
Lateralorbitofrontal	L	527	18.90	21.76	3.86e-1	-0.45	1.42	7.53e-1	-0.01
Lateralorbitofrontal	R	528	-10.89	20.19	5.90e-1	0.50	1.34	7.09e-1	0.02
Medialorbitofrontal	L	529	-30.94	18.49	9.52e-2	0.67	1.23	5.84e-1	0.02
Medialorbitofrontal	R	528	20.72	16.90	2.21e-1	1.40	1.14	2.20e-1	0.05
Precentral	L	524	-25.64	36.84	4.87e-1	0.82	2.34	7.28e-1	0.01
Precentral	R	522	-42.07	36.59	2.51e-1	-0.04	2.43	9.87e-1	0.00
Paracentral	L	525	-19.99	14.80	1.78e-1	-0.17	0.99	8.60e-1	-0.01
Paracentral	R	524	-38.26	18.17	3.60e-2	-0.18	1.18	8.80e-1	-0.01
Frontalpole	L	528	-0.72	5.18	8.90e-1	-0.03	0.35	9.33e-1	0.00
Frontalpole	R	528	3.35	6.07	5.81e-1	0.20	0.41	6.18e-1	0.02
Superiorparietal	L	526	-17.21	38.01	6.51e-1	3.02	2.45	2.18e-1	0.05
Superiorparietal	R	525	-0.57	39.64	9.89e-1	3.76	2.58	1.47e-1	0.06
Inferiorparietal	L	526	-49.18	42.37	2.47e-1	-2.07	2.75	4.52e-1	-0.03
Inferiorparietal	R	529	-52.02	49.27	2.92e-1	1.00	3.23	7.57e-1	0.01
Supramarginal	L	520	13.60	38.42	7.24e-1	2.63	2.47	2.87e-1	0.04
Supramarginal	R	519	-6.83	38.06	8.58e-1	-1.72	2.48	4.89e-1	-0.03
Postcentral	L	525	-26.18	30.44	3.90e-1	0.06	1.98	9.75e-1	0.00
Postcentral	R	523	22.55	32.42	4.87e-1	-0.73	2.13	7.32e-1	-0.01
Precuneus	L	525	7.22	26.10	7.82e-1	0.46	1.69	7.86e-1	0.01
Precuneus	R	525	-7.74	27.16	7.76e-1	1.27	1.75	4.69e-1	0.03
Lateraloccipital	L	527	15.07	35.07	6.68e-1	-0.72	2.25	7.49e-1	-0.01
Lateraloccipital	R	528	32.17	38.89	4.09e-1	1.02	2.53	6.88e-1	0.02
Lingual	L	526	-28.09	25.77	2.77e-1	-1.05	1.66	5.25e-1	-0.03
Lingual	R	527	-12.43	23.59	5.99e-1	-2.82	1.51	6.34e-2	-0.08
Cuneus	L	527	9.52	13.05	4.67e-1	0.49	0.83	5.53e-1	0.03
Cuneus	R	526	4.50	13.37	7.37e-1	1.35	0.87	1.22e-1	0.07
Pericalcarine	L	527	2.59	11.19	8.18e-1	0.16	0.69	8.18e-1	0.01
Pericalcarine	R	530	14.96	12.03	2.15e-1	0.86	0.72	2.33e-1	0.05
Superiortemporal	L	529	89.45	37.78	1.87e-2	2.96	2.39	2.17e-1	0.05
Superiortemporal	R	526	21.70	34.12	5.25e-1	-1.89	2.20	3.91e-1	-0.04
Middletemporal	L	529	72.63	43.34	9.51e-2	-2.61	2.71	3.37e-1	-0.04
Middletemporal	R	529	32.00	41.59	4.42e-1	0.36	2.68	8.93e-1	0.01
Inferiortemporal	L	528	6.49	37.75	8.64e-1	0.98	2.45	6.90e-1	0.02
Inferiortemporal	R	528	113.34	39.21	4.20e-3	1.93	2.47	4.36e-1	0.03
Bankssts	L	528	-10.03	11.68	3.91e-1	-0.30	0.80	7.02e-1	-0.02
Bankssts	R	527	-11.61	10.63	2.76e-1	-0.80	0.72	2.72e-1	-0.05
Fusiform	L	523	-10.36	26.62	6.97e-1	2.64	1.81	1.46e-1	0.06
Fusiform	R	527	5.60	27.93	8.41e-1	-0.61	1.87	7.43e-1	-0.01
Transversetemporal	**L**	**527**	**7.54**	**6.08**	**2.16e-1**	**0.82**	**0.41**	**4.70e-2**	**0.08**
Transversetemporal	R	527	4.29	4.75	3.67e-1	0.59	0.32	6.61e-2	0.08
Entorhinal	L	526	8.78	8.69	3.13e-1	-1.03	0.59	8.27e-2	-0.07
Entorhinal	R	524	30.04	10.72	5.41e-3	-1.09	0.70	1.19e-1	-0.06
Temporalpole	L	529	60.33	13.48	1.04e-5	-0.39	0.89	6.62e-1	-0.02
Temporalpole	R	526	36.82	13.62	7.22e-3	0.10	0.90	9.13e-1	0.00
Parahippocampal	L	525	-11.44	8.80	1.94e-1	-0.52	0.58	3.75e-1	-0.04
Parahippocampal	R	526	10.00	7.89	2.06e-1	0.16	0.51	7.49e-1	0.01
Rostralanteriorcingulate	L	528	-16.11	13.49	2.33e-1	0.09	0.90	9.25e-1	0.00
Rostralanteriorcingulate	R	527	-8.78	13.24	5.08e-1	0.01	0.89	9.95e-1	0.00
Caudalanteriorcingulate	L	526	-3.26	13.19	8.05e-1	-0.54	0.89	5.48e-1	-0.03
Caudalanteriorcingulate	R	528	0.21	15.37	9.89e-1	0.34	1.03	7.40e-1	0.01
Posteriorcingulate	L	528	-1.14	14.01	9.35e-1	-0.42	0.92	6.47e-1	-0.02

Posteriorcingulate	R	526	-7.12	13.68	6.03e-1	-0.56	0.89	5.32e-1	-0.03
Isthmuscingulate	L	527	-3.29	11.29	7.71e-1	-0.22	0.74	7.65e-1	-0.01
Isthmuscingulate	R	526	-6.21	11.60	5.93e-1	-1.04	0.75	1.66e-1	-0.06
Insula	L	526	35.67	18.02	4.88e-2	-0.59	1.15	6.08e-1	-0.02
Insula	R	524	42.01	18.04	2.06e-2	-0.41	1.16	7.24e-1	-0.02
Accumbens	L	526	-7.63	3.33	2.25e-2	-0.20	0.22	3.63e-1	-0.04
Accumbens	R	526	-2.68	2.79	3.37e-1	-0.05	0.18	7.67e-1	-0.01
Amygdala	L	528	-0.17	5.38	9.74e-1	0.00	0.35	9.95e-1	0.00
Amygdala	R	525	-9.27	5.73	1.07e-1	0.03	0.37	9.42e-1	0.00
Caudate	L	524	15.86	12.32	2.01e-1	0.06	0.65	9.24e-1	0.00
Caudate	R	521	4.83	13.14	7.14e-1	-0.96	0.72	1.87e-1	-0.05
Hippocampus	L	527	20.98	10.51	4.74e-2	-0.16	0.64	7.98e-1	-0.01
Hippocampus	R	526	10.37	10.21	3.11e-1	0.14	0.61	8.20e-1	0.01
Pallidum	L	526	9.17	5.54	9.92e-2	-0.47	0.35	1.81e-1	-0.06
Pallidum	R	524	-0.51	3.83	8.93e-1	-0.02	0.24	9.36e-1	0.00
Putamen	L	528	4.16	17.23	8.10e-1	-0.10	0.99	9.22e-1	0.00
Putamen	R	526	5.09	15.28	7.40e-1	-0.15	0.90	8.66e-1	-0.01
Thalamus	L	523	0.77	14.52	9.58e-1	0.96	0.94	3.09e-1	0.05
Thalamus	**R**	**528**	**18.86**	**13.21**	**1.55e-1**	**1.89**	**0.79**	**1.86e-2**	**0.10**

Supplementary Table 6-7. Association between intensity ratings of quinine and brain volumes in Human Connectome Project (HCP). Significant associations are bold. Interval is the time interval between taste test and brain scan. Taste is the perceived intensity ratings of quinine solutions ('Taste_Unadj' in HCP). r is the correlation coefficient between taste and brain phenotypes. L and R indicate the volume is extracted for left and right hemisphere respectively.

Region of interest		n	Beta Sex	SD Sex	P Sex	Beta Age	SD Age	P Age	Beta BrainSeg	SD BrainSeg	P BrainSeg	Beta Taste	SD Taste	P Taste	r Taste
Superiorfrontal	L	1097	-358.89	140.71	1.10e-2	-113.00	14.61	4.35e-14	1.96e-2	5.81e-4	2.52e-142	0.58	3.38	8.65e-1	0.00
Superiorfrontal	R	1095	-188.85	139.30	1.76e-1	-103.49	14.42	1.78e-12	1.91e-2	5.78e-4	8.12e-146	-2.09	3.42	5.41e-1	-0.01
Rostralmiddlefrontal	L	1096	48.53	125.20	6.98e-1	-80.80	12.98	8.43e-10	1.38e-2	5.17e-4	1.13e-107	2.03	3.05	5.05e-1	0.01
Rostralmiddlefrontal	R	1096	11.92	133.05	9.29e-1	-83.25	13.82	2.84e-9	1.48e-2	5.49e-4	1.17e-108	2.59	3.24	4.25e-1	0.01
Caudalmiddlefrontal	L	1096	-168.47	86.12	5.08e-2	-28.00	8.88	1.68e-3	6.53e-3	3.55e-4	2.74e-62	1.88	2.12	3.74e-1	0.02
Caudalmiddlefrontal	R	1097	-246.29	83.50	3.29e-3	-33.36	8.67	1.30e-4	6.41e-3	3.42e-4	3.10e-64	1.56	2.06	4.49e-1	0.02
Parsopercularis	L	1095	-161.24	63.39	1.12e-2	-23.59	6.57	3.56e-4	4.36e-3	2.59e-4	4.87e-54	0.01	1.57	9.96e-1	0.00
Parsopercularis	R	1092	-115.65	52.85	2.89e-2	-12.90	5.48	1.87e-2	3.68e-3	2.15e-4	2.53e-56	-0.15	1.32	9.10e-1	0.00
Parstriangularis	L	1094	63.21	46.12	1.71e-1	-17.37	4.77	2.92e-4	2.33e-3	1.89e-4	1.42e-31	-0.59	1.13	6.01e-1	-0.01
Parstriangularis	R	1096	35.81	58.76	5.45e-1	-20.02	6.10	1.07e-3	3.10e-3	2.39e-4	5.30e-35	-0.37	1.46	8.01e-1	-0.01
Parsorbitalis	L	1096	-9.52	21.65	6.63e-1	-8.67	2.27	1.43e-4	1.40e-3	8.93e-5	4.73e-48	-0.67	0.54	2.15e-1	-0.03
Parsorbitalis	R	1096	1.53	26.97	9.55e-1	-11.49	2.80	4.52e-5	1.72e-3	1.10e-4	1.92e-47	-0.23	0.67	7.28e-1	-0.01
Lateralorbitofrontal	**L**	**1100**	**-65.50**	**49.41**	**1.86e-1**	**-32.16**	**5.13**	**7.41e-10**	**6.35e-3**	**2.04e-4**	**1.80e-122**	**-2.94**	**1.16**	**1.16e-2**	**-0.04**
Lateralorbitofrontal	**R**	**1096**	**-62.97**	**49.68**	**2.05e-1**	**-34.25**	**5.15**	**6.05e-11**	**6.15e-3**	**2.04e-4**	**5.14e-127**	**-3.25**	**1.21**	**7.67e-3**	**-0.05**
Medialorbitofrontal	L	1096	129.13	47.13	6.29e-3	-6.02	4.88	2.18e-1	4.07e-3	1.93e-4	1.11e-77	-0.29	1.17	8.02e-1	-0.01
Medialorbitofrontal	R	1096	-33.36	43.23	4.40e-1	-9.86	4.48	2.81e-2	4.25e-3	1.78e-4	2.07e-93	-0.74	1.07	4.87e-1	-0.01
Precentral	L	1095	-91.04	88.65	3.05e-1	-31.13	9.20	7.57e-4	1.04e-2	3.63e-4	9.08e-119	-3.35	2.16	1.22e-1	-0.03
Precentral	R	1096	-14.62	90.29	8.71e-1	-30.63	9.32	1.07e-3	1.10e-2	3.72e-4	8.42e-126	1.15	2.21	6.01e-1	0.01
Paracentral	L	1092	-125.87	39.35	1.44e-3	0.16	4.07	9.68e-1	2.75e-3	1.62e-4	2.16e-54	0.91	0.97	3.49e-1	0.02
Paracentral	R	1096	-108.78	47.10	2.12e-2	-3.26	4.89	5.06e-1	3.38e-3	1.93e-4	2.91e-57	1.01	1.16	3.83e-1	0.02
Frontalpole	L	1093	-19.57	10.22	5.58e-2	-5.34	1.06	6.08e-7	5.37e-4	4.13e-5	1.57e-35	0.14	0.26	5.93e-1	0.01
Frontalpole	R	1097	-15.50	13.51	2.52e-1	-8.80	1.40	5.26e-10	6.91e-4	5.48e-5	1.45e-33	-0.18	0.34	5.99e-1	-0.01
Superiorparietal	L	1098	-556.12	106.98	2.73e-7	-30.93	11.09	5.47e-3	1.13e-2	4.42e-4	8.56e-100	2.66	2.58	3.02e-1	0.02
Superiorparietal	R	1098	-333.53	105.16	1.59e-3	-49.12	10.89	7.74e-6	1.10e-2	4.32e-4	4.63e-100	3.63	2.55	1.55e-1	0.03
Inferiorparietal	L	1096	-42.31	118.54	7.21e-1	-37.02	12.30	2.72e-3	1.18e-2	4.89e-4	2.62e-93	5.19	2.88	7.23e-2	0.04
Inferiorparietal	R	1098	243.17	129.57	6.10e-2	-74.35	13.44	4.40e-8	1.31e-2	5.31e-4	1.27e-98	3.26	3.20	3.08e-1	0.02
Supramarginal	L	1097	25.20	101.68	8.04e-1	-28.41	10.53	7.17e-3	1.02e-2	4.18e-4	6.79e-96	0.91	2.51	7.17e-1	0.01
Supramarginal	R	1096	-113.46	104.28	2.77e-1	-11.69	10.82	2.80e-1	9.34e-3	4.26e-4	3.29e-82	1.41	2.57	5.83e-1	0.01
Postcentral	L	1095	-220.31	77.20	4.44e-3	-25.76	8.02	1.37e-3	8.66e-3	3.17e-4	1.46e-113	0.25	1.91	8.96e-1	0.00
Postcentral	R	1095	-143.36	82.07	8.11e-2	-19.90	8.50	1.95e-2	7.89e-3	3.38e-4	1.32e-90	3.78	2.03	6.25e-2	0.04
Precuneus	L	1098	-154.32	71.43	3.11e-2	-39.70	7.39	1.11e-7	9.08e-3	2.94e-4	5.89e-129	-0.36	1.73	8.33e-1	0.00
Precuneus	R	1098	-215.21	76.42	5.00e-3	-30.34	7.91	1.36e-4	1.01e-2	3.14e-4	6.67e-141	-3.53	1.87	5.97e-2	-0.03
Lateraloccipital	L	1097	162.17	104.54	1.21e-1	4.05	10.83	7.09e-1	9.27e-3	4.33e-4	1.30e-75	-4.17	2.49	9.48e-2	-0.03
Lateraloccipital	**R**	**1100**	**95.93**	**109.66**	**3.82e-1**	**1.47**	**11.37**	**8.97e-1**	**1.00e-2**	**4.49e-4**	**1.26e-82**	**-6.71**	**2.66**	**1.18e-2**	**-0.05**
Lingual	L	1098	-34.74	79.73	6.63e-1	7.38	8.28	3.73e-1	5.48e-3	3.28e-4	1.53e-51	0.62	1.91	7.44e-1	0.01
Lingual	R	1098	-2.10	76.46	9.78e-1	-8.94	7.92	2.60e-1	5.25e-3	3.18e-4	3.03e-49	-0.77	1.77	6.64e-1	-0.01
Cuneus	L	1098	47.01	35.31	1.84e-1	-4.47	3.66	2.23e-1	2.34e-3	1.45e-4	1.86e-48	-0.57	0.84	5.00e-1	-0.02
Cuneus	R	1090	6.57	38.82	8.66e-1	-1.47	4.04	7.16e-1	2.76e-3	1.59e-4	2.41e-55	1.22	0.94	1.97e-1	0.03
Pericalcarine	L	1098	-45.31	36.22	2.12e-1	1.32	3.77	7.27e-1	2.04e-3	1.50e-4	8.11e-35	-1.41	0.81	8.33e-2	-0.04
Pericalcarine	R	1099	32.88	39.46	4.05e-1	0.03	4.10	9.95e-1	2.18e-3	1.63e-4	2.69e-34	-0.30	0.89	7.40e-1	-0.01
Superiortemporal	L	1099	43.17	89.94	6.31e-1	-16.07	9.33	8.54e-2	9.99e-3	3.68e-4	1.19e-110	-0.17	2.20	9.38e-1	0.00
Superiortemporal	R	1096	-87.53	85.21	3.05e-1	-26.13	8.84	3.24e-3	1.00e-2	3.52e-4	1.08e-113	1.34	2.04	5.12e-1	0.01
Middletemporal	L	1098	-10.05	89.81	9.11e-1	-50.49	9.32	8.22e-8	9.75e-3	3.69e-4	1.13e-106	4.27	2.20	5.26e-2	0.04
Middletemporal	R	1099	-20.18	98.13	8.37e-1	-53.33	10.19	2.25e-7	1.12e-2	4.04e-4	2.60e-110	1.27	2.37	5.92e-1	0.01
Inferiortemporal	L	1097	-116.22	109.50	2.89e-1	-36.76	11.34	1.25e-3	1.08e-2	4.50e-4	4.74e-94	0.08	2.70	9.77e-1	0.00
Inferiortemporal	R	1098	-109.34	109.72	3.19e-1	-29.67	11.39	9.36e-3	1.09e-2	4.50e-4	2.74e-95	-3.46	2.71	2.02e-1	-0.03
Bankssts	L	1090	12.64	34.03	7.10e-1	-3.47	3.53	3.26e-1	2.25e-3	1.39e-4	1.63e-51	1.16	0.85	1.76e-1	0.03
Bankssts	R	1095	-33.49	30.79	2.77e-1	-6.94	3.19	2.98e-2	2.07e-3	1.25e-4	1.90e-52	0.16	0.77	8.35e-1	0.01
Fusiform	L	1097	130.87	98.88	1.86e-1	-20.75	10.24	4.32e-2	8.54e-3	4.08e-4	1.22e-76	-3.05	2.44	2.12e-1	-0.03
Fusiform	R	1095	187.07	92.17	4.28e-2	-12.54	9.55	1.90e-1	8.75e-3	3.82e-4	1.53e-87	-1.98	2.26	3.83e-1	-0.02
Transversetemporal	**L**	**1100**	**-27.05**	**18.44**	**1.43e-1**	**3.42**	**1.91**	**7.41e-2**	**1.18e-3**	**7.53e-5**	**1.42e-47**	**-1.32**	**0.45**	**3.82e-3**	**-0.07**
Transversetemporal	R	1095	-29.55	14.39	4.03e-2	1.95	1.50	1.92e-1	9.65e-4	5.88e-5	1.19e-51	-0.23	0.35	5.16e-1	-0.02
Entorhinal	**L**	**1087**	**24.59**	**24.29**	**3.12e-1**	**0.91**	**2.53**	**7.20e-1**	**1.27e-3**	**1.00e-4**	**2.32e-33**	**-1.40**	**0.60**	**2.00e-2**	**-0.06**
Entorhinal	R	1089	51.37	25.64	4.55e-2	2.49	2.66	3.49e-1	1.12e-3	1.05e-4	1.32e-24	-0.77	0.63	2.23e-1	-0.03
Temporalpole	L	1098	-45.60	26.94	9.09e-2	0.71	2.79	7.98e-1	1.01e-3	1.09e-4	8.89e-20	-0.52	0.68	4.47e-1	-0.02
Temporalpole	R	1095	-57.36	28.22	4.25e-2	-0.27	2.93	9.28e-1	8.16e-4	1.15e-4	2.56e-12	0.14	0.70	8.39e-1	0.01
Parahippocampal	L	1091	-29.19	24.47	2.33e-1	2.70	2.53	2.87e-1	1.44e-3	1.01e-4	3.32e-40	0.15	0.59	7.97e-1	0.01
Parahippocampal	R	1096	-48.10	20.81	2.11e-2	4.23	2.16	4.99e-2	1.59e-3	8.55e-5	5.46e-63	-0.34	0.51	5.10e-1	-0.02
Rostralanteriorcingulate	L	1098	-62.90	38.13	9.94e-2	-8.76	3.95	2.70e-2	3.53e-3	1.56e-4	2.90e-86	-0.47	0.94	6.20e-1	-0.01
Rostralanteriorcingulate	R	1095	92.28	37.38	1.38e-2	-2.67	3.88	4.91e-1	1.99e-3	1.52e-4	1.20e-35	0.11	0.94	9.06e-1	0.00
Caudalanteriorcingulate	L	1095	-53.83	38.35	1.61e-1	-4.71	3.98	2.37e-1	1.83e-3	1.56e-4	2.43e-29	0.12	0.96	8.99e-1	0.00
Caudalanteriorcingulate	R	1094	-128.60	41.60	2.05e-3	-8.76	4.33	4.32e-2	2.17e-3	1.68e-4	6.66e-35	0.75	1.06	4.79e-1	0.02
Posteriorcingulate	L	1094	10.47	35.96	7.71e-1	-6.77	3.73	7.02e-2	2.54e-3	1.47e-4	4.06e-56	-0.52	0.89	5.58e-1	-0.01
Posteriorcingulate	R	1098	-25.83	39.85	5.17e-1	-13.06	4.13	1.63e-3	2.93e-3	1.64e-4	1.72e-59	0.96	0.98	3.26e-1	0.02
Isthmuscingulate	L	1096	9.12	32.80	7.81e-1	-17.01	3.40	7.26e-7	2.56e-3	1.34e-4	1.83e-66	-0.60	0.81	4.55e-1	-0.02
Isthmuscingulate	R	1089	-24.38	29.46	4.09e-1	-10.40	3.06	7.07e-4	2.04e-3	1.21e-4	4.63e-54	0.09	0.73	9.06e-1	0.00
Insula	L	1097	171.38	45.11	1.58e-4	-4.77	4.70	3.10e-1	5.12e-3	1.85e-4	2.15e-113	-1.37	1.10	2.14e-1	-0.02
Insula	R	1097	300.76	49.42	2.00e-9	10.05	5.14	5.07e-2	5.25e-3	2.04e-4	7.75e-101	-0.04	1.20	9.70e-1	0.00
Accumbens	L	1094	6.62	6.07	2.76e-1	-1.82	0.63	4.09e-3	4.11e-4	2.50e-5	5.55e-51	0.09	0.15	5.28e-1	0.01
Accumbens	R	1099	4.84	6.63	4.66e-1	-2.94	0.69	2.11e-5	4.58e-4	2.72e-5	5.41e-53	0.10	0.16	5.35e-1	0.01
Amygdala	L	1096	74.78	11.27	7.10e-11	-1.18	1.17	3.14e-1	9.74e-4	4.68e-5	4.35e-73	-0.17	0.27	5.36e-1	-0.01
Amygdala	R	1095	50.21	12.13	4.02e-5	0.02	1.26	9.87e-1	1.10e-3	5.05e-5	1.12e-77	0.12	0.29	6.89e-1	0.01
Caudate	L	1098	-96.41	30.30	1.60e-3	-11.88	3.17	2.10e-4	2.55e-3	1.26e-4	9.07e-60	0.10	0.63	8.70e-1	0.00
Caudate	R	1097	-77.24	30.49	1.18e-2	-12.21	3.19	1.53e-4	2.62e-3	1.26e-4	1.59e-61	-0.20	0.64	7.48e-1	-0.01
Hippocampus	L	1091	73.00	27.69	8.63e-3	3.80	2.88	1.88e-1	2.32e-3	1.15e-4	3.53e-67	-0.92	0.65	1.59e-1	-0.03
Hippocampus	R	1096	72.88	26.60	6.42e-3	4.61	2.77	9.73e-2	2.34e-3	1.10e-4	3.47e-67	-1.08	0.59	6.60e-2	-0.03
Pallidum	L	1096	47.02	17.45	7.24e-3	-9.07	1.81	6.98e-7	5.76e-4	7.14e-5	3.57e-15	0.22	0.42	6.07e-1	0.01
Pallidum	R	1091	2.42	12.98	8.52e-1	-6.79	1.35	7.15e-7	8.72e-4	5.34e-5	3.53e-49	0.43	0.31	1.65e-1	0.03
Putamen	L	1098	101.13	50.12	4.41e-2	-22.44	5.20	1.94e-5	2.78e-3	2.07e-4	2.24e-35	0.60	1.16	6.04e-1	0.01
Putamen	R	1098	129.64	37.99	7.27e-4	-24.95	3.97	1.10e-9	2.81e-3	1.58e-4	5.80e-50	0.34	0.79	6.72e-1	0.01
Thalamus	L	1099	-95.84	48.18	4.71e-2	3.55	4.99	4.77e-1	6.02e-3	1.97e-4	1.21e-130	0.78	1.18	5.07e-1	0.01
Thalamus	R	1097	-36.09	41.97	3.90e-1	-4.76	4.35	2.75e-1	4.78e-3	1.75e-4	9.21e-105	-0.83	0.99	4.02e-1	-0.02

Supplementary Table 6-8. Association between intensity ratings of sucrose octaacetate and brain volumes. Significant associations are bold. Interval is the time interval between taste test and brain scan. Taste is the perceived intensity ratings of sucrose octaacetate solutions. r is the correlation coefficient between taste and brain phenotypes. L and R indicate the volume is extracted for left and right hemisphere respectively.

Region of interest		n	Beta Interval	SD Interval	P Interval	Beta Taste	SD Taste	P Taste	r Taste
Superiorfrontal	L	555	-65.07	53.76	2.27e-1	-4.37	3.27	1.83e-1	-0.06
Superiorfrontal	R	555	-76.85	51.20	1.34e-1	-4.12	3.16	1.93e-1	-0.05
Rostralmiddlefrontal	L	554	-113.89	47.01	1.59e-2	-1.00	2.96	7.35e-1	-0.01
Rostralmiddlefrontal	R	552	-74.85	47.17	1.14e-1	-2.10	2.90	4.70e-1	-0.03
Caudalmiddlefrontal	L	555	-7.54	30.89	8.07e-1	-0.05	1.92	9.81e-1	0.00
Caudalmiddlefrontal	R	555	-4.51	30.69	8.83e-1	-0.96	2.01	6.34e-1	-0.02
Parsopercularis	L	557	-33.18	22.92	1.49e-1	-0.76	1.45	6.02e-1	-0.02
Parsopercularis	R	554	-4.99	16.74	7.66e-1	0.49	1.09	6.53e-1	0.02
Parstriangularis	L	555	-6.77	16.58	6.83e-1	-0.87	1.07	4.17e-1	-0.03
Parstriangularis	R	556	7.62	20.42	7.09e-1	1.14	1.33	3.92e-1	0.04
Parsorbitalis	L	556	5.61	8.98	5.32e-1	-0.35	0.58	5.45e-1	-0.03
Parsorbitalis	R	557	-2.98	11.11	7.88e-1	0.32	0.71	6.48e-1	0.02
Lateralorbitofrontal	L	555	18.13	21.64	4.03e-1	-1.87	1.35	1.67e-1	-0.06
Lateralorbitofrontal	R	556	-13.11	19.95	5.12e-1	-0.79	1.28	5.35e-1	-0.03
Medialorbitofrontal	L	557	-31.28	18.30	8.82e-2	1.02	1.16	3.81e-1	0.04
Medialorbitofrontal	R	556	18.75	16.71	2.62e-1	0.00	1.09	9.96e-1	0.00
Precentral	L	552	-34.23	36.51	3.49e-1	-2.93	2.22	1.88e-1	-0.05
Precentral	R	550	-51.34	36.32	1.58e-1	-4.45	2.30	5.41e-2	-0.08
Paracentral	L	553	-24.05	14.65	1.01e-1	-1.61	0.94	8.84e-2	-0.07
Paracentral	R	552	-39.77	17.77	2.58e-2	-1.45	1.13	2.00e-1	-0.05
Frontalpole	L	556	0.14	5.08	9.77e-1	-0.12	0.33	7.16e-1	-0.02
Frontalpole	R	556	3.29	5.99	5.83e-1	-0.04	0.39	9.26e-1	0.00
Superiorparietal	L	554	-20.57	37.84	5.87e-1	-1.61	2.31	4.88e-1	-0.03
Superiorparietal	R	553	-9.25	39.18	8.14e-1	-1.89	2.45	4.41e-1	-0.03
Inferiorparietal	L	554	-38.52	42.19	3.62e-1	1.26	2.61	6.30e-1	0.02
Inferiorparietal	R	557	-59.27	48.53	2.23e-1	-1.63	3.04	5.92e-1	-0.02
Supramarginal	L	548	6.49	37.78	8.64e-1	0.31	2.33	8.94e-1	0.01
Supramarginal	R	546	0.82	37.66	9.83e-1	0.82	2.34	7.27e-1	0.01
Postcentral	**L**	**552**	**-32.13**	**29.98**	**2.85e-1**	**-4.04**	**1.87**	**3.13e-2**	**-0.09**
Postcentral	R	550	24.20	31.79	4.47e-1	1.14	2.02	5.72e-1	0.02
Precuneus	L	553	9.06	25.96	7.27e-1	-0.53	1.61	7.40e-1	-0.01
Precuneus	R	554	-5.53	27.75	8.42e-1	1.03	1.69	5.42e-1	0.03
Lateraloccipital	L	555	-2.57	34.60	9.41e-1	-1.95	2.14	3.63e-1	-0.04
Lateraloccipital	R	556	17.99	38.44	6.40e-1	-0.95	2.38	6.89e-1	-0.02
Lingual	L	554	-30.49	25.50	2.33e-1	-0.69	1.57	6.63e-1	-0.02
Lingual	R	555	-12.26	23.84	6.07e-1	-0.26	1.46	8.58e-1	-0.01
Cuneus	L	555	7.23	12.89	5.75e-1	0.88	0.78	2.59e-1	0.05
Cuneus	R	554	2.61	13.23	8.44e-1	0.51	0.82	5.33e-1	0.03
Pericalcarine	L	555	3.07	11.05	7.81e-1	0.85	0.64	1.84e-1	0.05
Pericalcarine	R	558	13.11	11.80	2.68e-1	-0.23	0.67	7.33e-1	-0.01
Superiortemporal	L	557	84.26	37.44	2.53e-2	0.01	2.26	9.96e-1	0.00
Superiortemporal	R	554	17.83	34.10	6.01e-1	-0.94	2.09	6.54e-1	-0.02
Middletemporal	L	557	81.75	43.00	5.85e-2	1.15	2.56	6.54e-1	0.02
Middletemporal	R	556	48.18	40.79	2.39e-1	2.05	2.51	4.15e-1	0.03
Inferiortemporal	L	556	0.89	37.35	9.81e-1	2.41	2.31	2.98e-1	0.04
Inferiortemporal	**R**	**556**	**119.07**	**38.45**	**2.14e-3**	**4.86**	**2.37**	**4.14e-2**	**0.08**
Bankssts	L	556	-10.27	11.53	3.74e-1	-0.06	0.75	9.37e-1	0.00
Bankssts	**R**	**555**	**-9.57**	**10.47**	**3.61e-1**	**1.85**	**0.69**	**7.75e-3**	**0.11**
Fusiform	L	550	-8.45	26.56	7.50e-1	2.96	1.74	8.99e-2	0.07
Fusiform	R	555	5.89	27.65	8.31e-1	1.85	1.77	2.95e-1	0.04
Transversetemporal	**L**	**555**	**8.35**	**5.97**	**1.63e-1**	**1.38**	**0.39**	**4.71e-4**	**0.14**
Transversetemporal	R	555	3.64	4.70	4.40e-1	0.11	0.30	7.17e-1	0.02
Entorhinal	L	554	7.68	8.73	3.79e-1	-0.47	0.57	4.09e-1	-0.03
Entorhinal	R	551	28.26	10.51	7.53e-3	-0.48	0.66	4.67e-1	-0.03
Temporalpole	L	557	60.30	13.54	1.15e-5	-0.39	0.86	6.46e-1	-0.02
Temporalpole	R	554	35.36	13.55	9.44e-3	0.83	0.86	3.35e-1	0.04
Parahippocampal	L	552	-13.73	8.77	1.18e-1	-1.01	0.55	6.75e-2	-0.08
Parahippocampal	R	554	9.80	7.87	2.14e-1	0.11	0.49	8.25e-1	0.01
Rostralanteriorcingulate	L	556	-17.92	13.27	1.78e-1	0.47	0.85	5.82e-1	0.02
Rostralanteriorcingulate	R	555	-13.60	13.05	2.98e-1	-0.53	0.85	5.34e-1	-0.03
Caudalanteriorcingulate	L	553	-0.62	12.93	9.61e-1	0.22	0.84	7.91e-1	0.01
Caudalanteriorcingulate	R	556	-3.01	15.11	8.42e-1	0.21	0.98	8.32e-1	0.01
Posteriorcingulate	L	555	1.76	13.74	8.98e-1	0.75	0.88	3.90e-1	0.04

Posteriorcingulate	R	554	-9.30	13.26	4.83e-1	-0.45	0.85	5.95e-1	-0.02
Isthmuscingulate	L	554	-5.48	11.07	6.21e-1	-0.71	0.70	3.10e-1	-0.04
Isthmuscingulate	R	554	-4.81	11.56	6.78e-1	-0.19	0.71	7.89e-1	-0.01
Insula	L	554	33.01	17.91	6.63e-2	-0.48	1.10	6.60e-1	-0.02
Insula	R	552	42.24	17.88	1.88e-2	0.30	1.10	7.85e-1	0.01
Accumbens	L	550	-2.66	14.72	8.57e-1	-0.01	0.91	9.88e-1	0.00
Accumbens	**R**	**552**	**-1.94**	**3.81**	**6.11e-1**	**-0.52**	**0.23**	**2.66e-2**	**-0.09**
Amygdala	L	556	18.47	13.28	1.66e-1	1.34	0.75	7.60e-2	0.07
Amygdala	**R**	**554**	**20.89**	**10.32**	**4.44e-2**	**1.18**	**0.59**	**4.77e-2**	**0.08**
Caudate	L	552	14.02	12.12	2.50e-1	0.10	0.58	8.60e-1	0.01
Caudate	R	554	10.66	10.12	2.94e-1	0.86	0.56	1.29e-1	0.06
Hippocampus	L	547	3.71	12.82	7.73e-1	-0.27	0.64	6.77e-1	-0.01
Hippocampus	R	556	1.17	5.30	8.25e-1	0.42	0.33	2.05e-1	0.05
Pallidum	**L**	**555**	**6.15**	**16.93**	**7.17e-1**	**1.92**	**0.91**	**3.65e-2**	**0.08**
Pallidum	R	553	-8.50	5.69	1.36e-1	0.55	0.35	1.20e-1	0.06
Putamen	L	552	-1.60	15.05	9.15e-1	0.48	0.83	5.65e-1	0.02
Putamen	R	554	-6.73	3.27	4.04e-2	0.34	0.21	9.67e-2	0.07
Thalamus	L	554	9.89	5.53	7.49e-2	0.52	0.33	1.19e-1	0.06
Thalamus	R	554	-2.48	2.74	3.66e-1	0.22	0.17	1.82e-1	0.05

Supplementary Table 6-9. Association between intensity ratings of denatonium benzoate and brain volumes. Significant associations are bold. Interval is the time interval between taste test and brain scan. Taste is the perceived intensity ratings of denatonium benzoate solutions. r is the correlation coefficient between taste and brain phenotypes. L and R indicate the volume is extracted for left and right hemisphere respectively.

Region of interest		n	Beta Interval	SD Interval	P Interval	Beta Taste	SD Taste	P Taste	r Taste
Superiorfrontal	L	552	-58.49	53.73	2.77e-1	-2.17	3.04	4.76e-1	-0.03
Superiorfrontal	R	552	-71.87	51.41	1.63e-1	-4.33	2.95	1.44e-1	-0.06
Rostralmiddlefrontal	L	551	-113.42	47.01	1.63e-2	1.32	2.77	6.33e-1	0.02
Rostralmiddlefrontal	R	549	-71.75	47.32	1.30e-1	-1.86	2.72	4.95e-1	-0.03
Caudalmiddlefrontal	L	552	-12.22	30.64	6.90e-1	-1.95	1.78	2.74e-1	-0.05
Caudalmiddlefrontal	R	552	-6.05	30.52	8.43e-1	-3.01	1.87	1.08e-1	-0.07
Parsopercularis	L	554	-34.72	22.97	1.32e-1	-0.66	1.35	6.27e-1	-0.02
Parsopercularis	R	551	-7.82	16.70	6.40e-1	0.23	1.02	8.17e-1	0.01
Parstriangularis	L	552	-4.96	16.53	7.64e-1	0.03	1.00	9.79e-1	0.00
Parstriangularis	R	553	6.58	20.39	7.47e-1	1.03	1.24	4.10e-1	0.04
Parsorbitalis	L	553	6.34	8.96	4.79e-1	-0.08	0.54	8.78e-1	-0.01
Parsorbitalis	R	554	-1.51	11.04	8.91e-1	1.26	0.65	5.45e-2	0.08
Lateralorbitofrontal	L	552	16.98	21.52	4.31e-1	-2.10	1.25	9.39e-2	-0.07
Lateralorbitofrontal	R	553	-14.37	19.90	4.71e-1	-1.70	1.19	1.54e-1	-0.06
Medialorbitofrontal	L	554	-32.66	18.40	7.68e-2	0.38	1.09	7.28e-1	0.01
Medialorbitofrontal	R	553	17.78	16.69	2.87e-1	-0.52	1.02	6.08e-1	-0.02
Precentral	L	549	-30.98	36.17	3.92e-1	-1.52	2.06	4.63e-1	-0.03
Precentral	R	548	-47.24	36.30	1.94e-1	-1.94	2.15	3.69e-1	-0.04
Paracentral	L	550	-22.10	14.69	1.33e-1	-0.94	0.89	2.90e-1	-0.04
Paracentral	R	549	-39.19	17.82	2.85e-2	-0.63	1.05	5.50e-1	-0.02
Frontalpole	L	553	0.07	5.09	9.89e-1	0.09	0.31	7.65e-1	0.01
Frontalpole	R	553	3.45	6.01	5.66e-1	-0.03	0.36	9.35e-1	0.00
Superiorparietal	L	551	-17.71	37.87	6.40e-1	-2.16	2.16	3.18e-1	-0.04
Superiorparietal	R	550	-2.45	39.26	9.50e-1	0.73	2.30	7.52e-1	0.01
Inferiorparietal	L	551	-40.64	42.23	3.37e-1	3.29	2.42	1.74e-1	0.06
Inferiorparietal	R	554	-55.95	48.22	2.47e-1	-0.55	2.85	8.48e-1	-0.01
Supramarginal	L	545	3.69	37.80	9.22e-1	-2.15	2.17	3.21e-1	-0.04
Supramarginal	R	544	0.49	37.68	9.90e-1	1.07	2.20	6.28e-1	0.02
Postcentral	L	549	-28.41	29.87	3.42e-1	-0.47	1.75	7.90e-1	-0.01
Postcentral	R	547	17.95	31.66	5.71e-1	-1.37	1.88	4.68e-1	-0.03
Precuneus	L	550	11.42	25.88	6.59e-1	1.63	1.50	2.76e-1	0.05
Precuneus	R	551	-4.62	27.66	8.68e-1	-0.09	1.57	9.54e-1	0.00
Lateraloccipital	L	552	1.81	34.63	9.58e-1	-1.48	2.00	4.62e-1	-0.03
Lateraloccipital	R	553	27.09	38.52	4.83e-1	1.09	2.22	6.22e-1	0.02
Lingual	L	551	-31.02	25.44	2.24e-1	-0.72	1.46	6.24e-1	-0.02
Lingual	R	552	-13.36	23.76	5.74e-1	-1.01	1.35	4.56e-1	-0.03
Cuneus	L	552	7.84	12.96	5.46e-1	0.83	0.73	2.54e-1	0.05
Cuneus	**R**	**551**	**4.46**	**13.19**	**7.35e-1**	**1.69**	**0.76**	**2.77e-2**	**0.09**
Pericalcarine	L	552	2.31	11.08	8.35e-1	0.08	0.59	8.88e-1	0.01
Pericalcarine	R	555	13.70	11.81	2.47e-1	-0.55	0.62	3.77e-1	-0.04
Superiortemporal	L	554	87.05	37.51	2.11e-2	0.79	2.10	7.05e-1	0.02
Superiortemporal	R	551	20.09	33.96	5.55e-1	-0.41	1.94	8.32e-1	-0.01
Middletemporal	L	554	77.43	43.03	7.32e-2	-0.34	2.37	8.87e-1	-0.01
Middletemporal	R	553	42.73	40.66	2.94e-1	1.32	2.33	5.72e-1	0.02
Inferiortemporal	L	553	-3.08	37.37	9.34e-1	-0.63	2.15	7.70e-1	-0.01
Inferiortemporal	R	553	112.52	38.26	3.52e-3	1.89	2.22	3.95e-1	0.04
Bankssts	L	553	-10.88	11.55	3.47e-1	-0.99	0.70	1.60e-1	-0.06
Bankssts	R	552	-13.39	10.63	2.08e-1	0.56	0.65	3.90e-1	0.04
Fusiform	L	547	-12.46	26.68	6.41e-1	0.07	1.63	9.66e-1	0.00
Fusiform	R	552	3.13	27.61	9.10e-1	-0.65	1.64	6.94e-1	-0.02
Transversetemporal	**L**	**552**	**7.86**	**6.01**	**1.92e-1**	**1.17**	**0.37**	**1.55e-3**	**0.13**
Transversetemporal	R	552	3.70	4.71	4.33e-1	-0.03	0.28	9.10e-1	0.00
Entorhinal	**L**	**551**	**7.21**	**8.66**	**4.05e-1**	**-1.57**	**0.53**	**2.99e-3**	**-0.12**
Entorhinal	R	548	28.89	10.53	6.41e-3	-0.43	0.61	4.81e-1	-0.03
Temporalpole	L	554	60.01	13.53	1.24e-5	-0.89	0.79	2.61e-1	-0.05
Temporalpole	R	551	34.61	13.49	1.07e-2	-0.38	0.80	6.38e-1	-0.02
Parahippocampal	**L**	**549**	**-13.51**	**8.78**	**1.25e-1**	**-1.38**	**0.51**	**7.43e-3**	**-0.11**
Parahippocampal	R	551	7.94	7.82	3.11e-1	-0.43	0.45	3.45e-1	-0.04
Rostralanteriorcingulate	L	553	-18.50	13.31	1.65e-1	-0.01	0.79	9.87e-1	0.00
Rostralanteriorcingulate	R	552	-13.04	13.07	3.19e-1	-0.90	0.79	2.57e-1	-0.05
Caudalanteriorcingulate	L	550	-1.29	12.82	9.20e-1	0.55	0.78	4.80e-1	0.03
Caudalanteriorcingulate	R	553	-3.83	15.08	8.00e-1	-0.86	0.91	3.43e-1	-0.04
Posteriorcingulate	L	552	-0.14	13.70	9.92e-1	0.11	0.82	8.91e-1	0.01

Posteriorcingulate	R	551	-10.23	13.28	4.42e-1	-0.60	0.79	4.48e-1	-0.03
Isthmuscingulate	L	551	-5.21	11.08	6.38e-1	-0.52	0.65	4.22e-1	-0.03
Isthmuscingulate	R	551	-5.71	11.53	6.21e-1	0.06	0.66	9.32e-1	0.00
Insula	L	551	34.48	17.81	5.39e-2	-1.30	1.02	2.04e-1	-0.05
Insula	R	549	42.04	17.86	1.93e-2	0.04	1.02	9.70e-1	0.00
Accumbens	L	547	-0.21	14.72	9.89e-1	0.14	0.84	8.72e-1	0.01
Accumbens	R	549	-1.03	3.81	7.86e-1	-0.35	0.21	1.02e-1	-0.07
Amygdala	L	553	18.65	13.28	1.62e-1	0.96	0.69	1.66e-1	0.05
Amygdala	R	551	20.59	10.34	4.79e-2	0.57	0.55	2.99e-1	0.04
Caudate	**L**	**549**	**15.71**	**12.10**	**1.97e-1**	**-1.15**	**0.54**	**3.53e-2**	**-0.07**
Caudate	R	551	10.41	10.11	3.05e-1	0.51	0.52	3.25e-1	0.04
Hippocampus	**L**	**544**	**4.12**	**12.79**	**7.48e-1**	**-1.52**	**0.59**	**1.09e-2**	**-0.09**
Hippocampus	R	553	0.86	5.31	8.71e-1	0.35	0.31	2.53e-1	0.05
Pallidum	L	552	5.56	16.96	7.43e-1	1.29	0.85	1.28e-1	0.06
Pallidum	R	550	-9.67	5.68	8.97e-2	0.07	0.33	8.30e-1	0.01
Putamen	L	549	-0.26	15.05	9.86e-1	0.04	0.77	9.59e-1	0.00
Putamen	R	551	-7.37	3.31	2.67e-2	0.08	0.19	6.65e-1	0.02
Thalamus	L	551	9.11	5.54	1.01e-1	0.04	0.31	8.97e-1	0.01
Thalamus	R	551	-3.06	2.75	2.67e-1	-0.01	0.16	9.29e-1	0.00