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Bitter Taste Genetics- the relationship to tasting, liking, consumption and health

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Abstract

Bitter is the most complex of human tastes, and is arguably the most important. Aversion to bitter taste is important for detecting toxic compounds in food; however, many beneficial nutrients also taste bitter and these may therefore also be avoided as a consequence of bitter taste. While many polymorphisms in TAS2R genes may result in phenotypic differences that influence the range and sensitivity of bitter compounds detected, the full extent to which individuals differ in their abilities to detect bitter compounds remains unknown. Simple logic suggests that taste phenotypes influence food preferences, intake and consequently health status. However, it is becoming clear that genetics only plays a partial role in predicting preference, intake and health outcomes, and the complex, pleiotropic relationships involved are yet to be fully elucidated.

Introduction

Taste is one of our most important senses. It allows the assessment of the nutritional value, safety and quality of our food. Consumption of foods is vital, but can also pose risks. Taste is vital for preventing intake of toxic substances and indigestible materials, ensuring adequate

intake of energy and providing enjoyment in food. However, there is significant variability in how effectively individuals can detect toxins, and modulate preferences for fruits and vegetables or high calorie foods¹⁻³.

Humans possess 5 basic tastes; salty, sour, sweet, umami and bitter. Sweet, umami and salt (low concentrations) are appetitive tastes, which are involved in the detection of the "desirable" components of food. Salt (high concentrations), sour and bitter are aversive tastes, and are important for defensive eating^{4, 5}. Humans detect "tastants" (chemicals stimulating the sense of taste) via stimulation of taste receptors on taste receptor cells, which cluster in taste buds. These cells are found in the soft palate, pharynx, larynx, epiglottis, and on the gustatory papillae on the tongue. Sour and salt receptors are channel type receptors⁶⁻¹⁰, while sweet, umami and bitter are all detected by G-protein coupled receptors (GPCRs)¹¹. Two classes of GPCRs have been identified in taste receptor cells, the TAS1R family of receptors detect sweet and umami¹² and TAS2Rs detect bitter compounds^{13, 14}.

Salt and sour tastes are triggered by elements or ions, and thus are referred to as mineral tastes¹⁵. Sour taste is responsible for the detection of unripe or spoiled foods and can be triggered by extracellular acidic pH and by organic acids that penetrate the cell membrane^{16, 17}. However, the precise contribution of each is not yet fully elucidated. Several cellular sour taste receptors have been proposed, including acid sensing ion channels^{18, 19}, HCNs²⁰, potassium channels^{21, 22}, transient receptor potential channels (PKD2L1 and PKD1L3)^{10, 23}. Salt receptors are important in the assessment of electrolyte and mineral content, and are dependent on concentration, and can be appetitive or aversive^{24, 25}. Appetitive salt receptors include the sodium specific amiloride sensitive epithelia channel (ENaC)²⁴ and non-specific amiloride-insensitive channels²⁶. Aversive salt taste is modulated by sour and bitter taste mechanisms²⁵.

The expression of 3 TAS1R genes combine to form heterodimers which detect sweet and umami ^{12, 21}. The TAS1R2/TAS1R3 heterodimer detects sweet, while the TAS1R1/TAS1R3 detects umami¹². The major role of both is to indicate the caloric content of food. Umami is responsible for savoury flavour in foods and is triggered by protein content, mainly L-amino acids^{12, 27}, while sweet receptors are triggered by sacchrides¹². However, calorie free artificial sweeteners and amino acids with little caloric content can also activate these receptors^{28, 29}.

Bitter taste, the focus of this review, is important in detection of potentially toxic compounds, bacterial metabolites and protein products of spoilage and/or food aging^{5, 30-32}. Bitter taste receptors are also activated by some artificial sweeteners, resulting in a bitter after-taste^{33, 34}, and contribute to the aversive response to high salt concentrations²⁵. Twenty five functional TAS2R genes have been identified in humans^{13, 35}, named numerically from TAS2R1 to TAS2R64, with gaps in the numbering due to non-existent genes, pseudogenes or proposed genes which are yet to be annotated. While all TAS2Rs are believed to detect bitter compounds, several receptors are yet to be deorphaned^{13, 14, 36} (Table 1).

The tasting experience is complex, interacting with olfaction, the detection of fats and oils, temperature, and the common chemical sense, which is responsible for irritation from chemicals substances (for example, capsaicin in chillies). Visual cues, touch, cultural and social norms, personality, and health attitudes can also impact upon food choices, in concert with the aforementioned 5 basic tastes^{1, 37}.

Bitter taste

Bitter taste is arguably the most important, and certainly the most complex and sensitive of all the taste types. There are more unique receptors dedicated to bitter than any other taste, and bitter molecules are able to be detected at much lower thresholds than others, up to 1000 times lower in some cases³¹. Bitter taste is generally thought to have evolved to protect humans from the consumption of toxic compounds³⁶.

Copious natural sources of bitter tasting compounds exist in nature. The substances are structurally and functionally diverse, and many are potentially toxic. Plants may produce bitter compounds as a defence mechanism against herbivores, and bitter taste receptors may have in turn evolved to protect herbivores against consumption of these toxins^{5, 11, 38}. Many bitter compounds are harmful and therefore need to be avoided. Common toxins in plant products include progoitrin (found in turnips), cyanide (found in cassava), saponin (found in soy beans). Interestingly, bitter substances such as denatonium benzoate are often added to consumer products to discourage accidental poisoning³⁹.

Whilst many bitter compounds are toxic if consumed in sufficient quantity, several also have beneficial nutritional or pharmaceutical properties⁴⁰⁻⁴³. Bitter tasting foods include highly

nutritious vegetables such as spinach, turnip, soy products and cruciferous vegetables (broccoli, bok choy, kale, cauliflower, rocket). Bitterness can be an acceptable or even desirable property in some foods, such as sharp cheeses, coffee, beer and tea⁴⁴⁻⁴⁶ and fermented food products⁴⁷. These foods contain a range of different bitter tasting chemicals such as isothiocyanates, polyophenols, methylxanthines, isoflavones, sulfamides, phenols, flavonoids, and catechins^{37, 38, 48, 49}. Bitter taste can also be imparted in food following heating and food degradation³¹. The bitter components of fermented foods come from protein breakdown, although bitterness is desirable in some contemporary foods, this may additionally act as an effective deterrent against the consumption of decayed food⁴⁷.

TAS2R receptors are low affinity compared to other families of GPCRs, with a functional detection range of high μ M to mM, however, this is similar to the concentration of most nutrients in food⁵⁰. All TAS2Rs are coupled to the same intracellular signalling molecules, with receptor activation leading to the activation of the heterotrimetric G protein, which consists of α , β and γ subunits^{51, 52}. The α -gustducin sub-unit activates a phosphodiesterase and is involved in the regulation of intracellular cAMP levels^{51, 52}. The β and γ subunits activate phospholipase CB₂ (PLC β_2), leading to the generation of inositol phosphate (IP₃), which mediates the release of intercellular calcium stores and the activation of the monovalent selective cation channel, TrpM5^{53, 54}. From the taste buds, three branches of three cranial nerves (CNVII, IX and X) then conduct these signals to the medulla⁵⁵. In addition some TAS2Rs require interaction with auxillary proteins for the receptor transport protein (RTP) and receptor expression enhancing protein (REEP) families⁵⁶.

TAS2R expression is not limited to the taste buds. TAS2R expression has been demonstrated in a range of cell types including, solitary chemosensory cells, endocrine cells, epithelial cells⁵⁷ and smooth muscle cells, in the airways⁵⁸, gastrointestinal tract⁵⁹, pancreas⁶⁰, reproductive organs⁶¹, and the brain⁶². The signalling pathways activated by TAS2Rs are conserved in different tissues. This implies that TAS2Rs not only respond to xenobiotics, but may also have physiological agonists⁶³.

Expression of TAS2Rs in non-gustatory tissues implies roles for these receptors in processes other than concious taste perception. Tastants may play a role in the regulation of digestion and metabolic processes following ingestion⁶⁴⁻⁶⁶. Functions may include modulation of cholesterol⁶⁵ and insulin levels⁶⁷ and regulation of gastric emptying⁶⁸⁻⁷². Interestingly,

intragastrically administered mixtures of bitter compounds has been shown to trigger some of the same centres in the brain stem as oral taste receptors⁶⁶.

In the airways TAS2Rs are thought to detect bitter metabolites produced by microorganisms and may act as a defence mechanism against the inhalation of irritants⁷³. TAS2R stimulation has been shown to have anti-inflammatory properties⁷⁴, and asthmatic children have been shown to exhibit increased TAS2R expression in peripheral blood cells⁷⁵. Paradoxically, activation of TAS2Rs has been shown to induce relaxation of smooth muscle cells, however, this was found to occur in a compound and species dependent manner^{58, 76}.

Multiple TAS2Rs are expressed on each taste receptor cell^{77, 78}. One TAS2R may be activated by several ligands, and the same ligand may activate multiple TAS2Rs^{36, 79}. Hundreds of molecules (for example; isothiocyanates, quinine, caffeine, strychnine, acesulfame K and erythromycin) have been described as TAS2R ligands (Table 1)^{36, 63, 80}. The tuning of TAS2Rs varies dramatically. Some are highly selective or narrowly tuned and only detect one or a limited number of bitter compounds. Others are broadly tuned and can detect a wide range of molecules (Table 1). TAS2R10, 14 and 46 are so broadly tuned, that combined, they have been suggested to detect half of the bitter compounds known³⁶. Sensitivity of bitter taste receptors also varies widely, with the EC50 values varying widely from the mid nM range to the low mM range⁸⁰.

Modulation of bitter taste by gene polymorphisms

A significant degree of variance exists in the TAS2R genes. Genetic polymorphisms have been identified and validated in nearly all of the intact and functional TAS2R genes (Table 2). The functional consequences of a majority of these polymorphisms are yet to be elucidated.

TAS2R38 variants and PTC/PROP tasting

The best studied of the TAS2R genes is *TAS2R38*. The TAS2R38 receptor detects compounds with thiocyanate moiety (N-C=S), including phenylthiocarbamide (PTC) and 6-n-propyl-2-thiouracil (PROP)⁸¹, the two most common compounds used in bitter taste research. Whilst neither of these compounds is known to occur naturally in foods, several related

compounds do occur naturally and activate the same receptor. This includes isothiocyanates, which are formed in cruciferous vegetables when glucosinolates are hydrolysed⁸².

Threshold tasting of PROP or PTC is often used as a surrogate marker for bitter taste in general. PROP/PTC tasting phenotypes are divided into non-tasters who are blind to the bitterness of PTC/PROP and tasters who find PTC/PROP bitter. Tasters can further be divided into medium tasters and supertasters, with supertasters finding PTC/PROP intensely bitter. PTC has been commonly used in laboratory studies, however, due to its slightly sulphurous odour⁸³ and reports of its toxicity⁸⁴, PROP has now become more common in laboratory studies. A very high degree of correlation has been demonstrated between PTC and PROP perception, although they do not completely match^{85, 86}.

13 non-synonymous single-nucleotide polymorphisms (SNPs) have been validated in the *TAS2R38* gene (http://www.ncbi.nlm.nih.gov/snp, 1000 genome project validated only, accessed May, 2014). Three of the SNPs (rs713598; rs1726866 and rs10246939; Table 3), resulting in three amino acid substitutions (A49P, A262V, V2961), have been identified as being primarily responsible for the variation in PTC/PROP tasting status. Despite the number of potential haplotypes, two common haplotypes Ala-Val-Ile (AVI) and Pro-Ala-Val (PAV)^{87, 88}, account for more than 90% of the Caucasian population ⁸⁷. The V262A and V291I polymorphisms are in perfect linkage disequilibrium, therefore some studies only assay the AV and PA sites. Other rare haplotypes (AAV, PVI and AAI) have been identified, but only occur in specific populations. The AAI haplotype is limited to sub-Saharan Africans⁸⁹.

There is a strong correlation between PAV/AVI status and sensitivity to PTC/PROP tasting⁹⁰. The PAV haplotype is associated with the PTC/PROP tasting phenotype and the AVI haplotype is associated with the non-tasting phenotype. The AAI haplotype has shown intermediate responses in *ex vivo* studies⁸⁸. AAV has been shown to have intermediate sensitivity *in vivo*⁸⁸. PAI responses are similar to PAV, and PAI may represent a rare secondary taster allele^{63, 88}. Percentages of PAV/PAV, PAV/AVI and AVI/AVI are similar to those of super tasters (30%), medium tasters (50%) and non-tasters (20%), respectively^{88, 91, 92}. However, proportions differ between populations around the world; in west Africa, only 3% of the population are non-tasters; in China the frequency of non-tasters ranges from 6-

23%; in India the incidence of non-tasters have been reported to be as high as $40\%^{93, 94}$; in Georgia, the frequency of non-tasters is more than $50\%^{95}$.

However, genotype and tasting phenotype do not overlap completely. The PAV and AVI diplotypes account for 45-80% of the variance of taste sensitivity to thiocyanate containing chemicals^{87, 96}. Additional variation may be explained by other SNPs in *TAS2R38*, SNPs in other TAS2R genes^{97, 98} or other modifying mechanisms, such as epigenetics and influences due to age⁹⁹, and sex⁹³.

TAS2R38 variants and broader bitter taste

Numerous psychophysiological studies have suggested that *TAS2R38* genotype and PTC/PROP tasting phenotype also correlate with sensitivity to a range of bitter compounds that are structurally unrelated to PTC or PROP. These include caffeine^{100, 101}, naringin^{100, 102}, isohumulones^{103, 104}, L-phenylalanine¹⁰⁰, epicatechins¹⁰⁰, salicin, methimazole⁶³, and, to a lesser extent, quinine^{100, 101, 105}. This is used to justify the use of PTC and PROP as markers of bitter taste in general. However, the strength of these relationships varies significantly between compounds and concentrations. Negative findings have also been reported for caffeine^{106, 107} and quinine¹⁰⁷⁻¹⁰⁹, and no clear pattern has been demonstrated between goitrin and singrin⁶³. The rare haplotypes (PVI, AAI, AAV) have displayed unexpectedly heterogeneous profiles in response to bitter stimuli, dependent on the stimulus used. The rare haplotypes match PAV in responses to methimazone, goitrin, singrin, and PTC, but not PROP⁶³. This suggests that minor differences in structure may translate into major differences in receptor affinity.

TAS2R38 variants and general taste acuity

Increased sensitivity to bitter tastants has been hypothesised to correlate with greater taste sensitivity to other tastants. PROP tasters have been shown to have heightened response to sweet¹¹⁰⁻¹¹⁴ and salty stimuli^{115, 116}. Oral irritation to capsaicin, ethanol and carbonation are also heightened amongst tasters^{111, 117}. Tasters also show increased responses to olfactory cues¹¹⁸ and to viscous substances such as fats and thickeners^{111, 119, 120}.

PROP tasting status and sensitivity to thermal taste have been proposed to serve as markers of enhanced global sensitivity^{121, 122}. This has been suggested to have an anatomical basis, as general taste acuity, PROP intensity and TAS2R38 genotype correlate with density of

fungiform papillae on the tongue^{105, 110, 119, 123, 124}. However, the relationship is complex and remains unclear. In TAS2R38 heterozygotes fungiform papillae density does not seem to predict PROP intensity⁸⁵. Others have found no correlation between PROP intensity, creaminess perception and fungiform papillae density¹²⁵. It has been suggested that differences in central nervous system processing are more important than fungiform papillae density¹²⁶. Additional studies are required to resolve the exact nature of the contribution of fungiform papillae density to bitter taste.

Other TAS2R variants and bitter taste

While TAS2R38 is the most studied of the bitter taste genes, others have been identified that contribute phenotypic variance in bitter taste. The TAS2R16 receptor is sensitive to Beta-glucopyranosides, a family of compounds that include salicin, a natural analgesic found in the bark of the willow⁸⁰. Polymorphisms in this gene are associated with subtle differences in bitter taste¹²⁷. The non-synonymous *TAS2R16* polymorphism, K172N (rs846664), confers significant phenotypic variance *in vitro*, with the N172 (common) allele conferring 2 fold greater sensitivity to salicin, arbutin (found in berries) and amygdalin (found in bitter almonds)¹²⁷.

Missense polymorphisms in the *TAS2R9* gene (rs3741845; V187A), and the *TAS31R31* gene (rs10772423; V240I) are significantly associated with the perceived bitterness of Acesulfame K, with the polymorphisms explaining 7% and 8.7% of the variance, respectively³³. *TAS2R31* polymorphisms are also associated with differential bitterness of aristolochic acid and saccharin³³. *In vitro* assays have confirmed the functional importance of four *TAS2R31* mutations, which had independent effects on receptor response¹²⁸.

Recently, it has been demonstrated that variants in the α -gustin gene further explain the variations in PROP tasting across individuals¹²⁹. Identification of additional variants in the intracellular signalling pathways of TAS2Rs may further explain the variance in bitterness perception, beyond the variation in the receptor alone.

Bitter compounds contained in foods

Influence of taster status on the detection of bitter compounds in food

Bitter compounds are not consumed in isolation, but in foods. In laboratory testing, bitter compounds are often dissolved in aqueous solutions or delivered on impregnated filter paper,

but consumption of bitter compounds in actual food may not always have the same response. Also, bitterness of foods can be altered dependent on season, cultivar, growth conditions, and food storage and preparation methods¹. These factors may confound results and complicate the analysis of associations.

Greater sensitivity to PROP has previously been associated with increased bitterness perception from glucosinolate and singrin containing vegetables , but not from non-glucosinolate producing vegetables¹³⁰. This demonstrates the detection of bitter compounds in whole food. However, the other components of the food may interact to mask or heighten tastes.

PROP tasters have also been shown to be more sensitive to the addition of the bitter compound caffeine to foods and beverages. PROP supertasters are better able to discriminate added caffeine in orange juice, and tasters were better able to detect caffeine in cream cheese than non-tasters¹³¹. While tasters were more likely to detect added caffeine in solution than not tasters, interestingly, addition of non-nutritive sweetener decreased bitterness intensity in both groups by similar degrees¹³².

PROP taster status has been shown to influence the bitterness perception of black coffee (containing caffeine)¹⁰⁰. Additionally, a haploblock across TAS2R3, TAS2R4, and TAS2R5 (rs765007, rs2234001, rs2234012, rs2227264) explains some variability in the bitterness of espresso coffee¹³³. PROP taster status also influences bitterness perception of dark chocolate (containing epicatechin)¹⁰⁰.

Conversely, others have reported no differences between PROP tasters and non-tasters in sensory responses to white, milk or dark chocolate¹³². Furthermore, no association was found between PROP taster status and bitter perception of white grapefruit (containing naringin)¹⁰⁰. However, a mutation in the TAS2R19 gene (rs10772420, resulting in the substitution of Arg for a Cys at amino acid position 299 appears to influence grapefruit juice bitterness¹³³ and accounting for this variation may account for the null result found when only considering PROP taster status.

Influence of TAS2R genotype and tasting status on "liking" of bitter foods

Taste sensitivity is logically hypothesised to play a role in food preferences^{38, 134}. Taste is arguably the most important determinant of food choices^{38, 135, 136} and genetics is powerfully positioned to influence intake¹³⁷. It has been reported that dislike of bitter taste is one of the primary reasons cited by consumers for rejecting certain foods, such as fruits, vegetables, whole grain products and soy¹³⁸. It is a logical hypothesis that those insensitive to bitter compounds would have less aversion to bitter foods, than those who find them more bitter. As many healthy vegetables have bitter components, this hypothesis has been extended to suggest that non-tasters would eat more healthy vegetables and therefore experience better health outcomes. Despite the neatness of this hypothesis, clear relationships between taste sensitivity and "liking" of bitter foods has not been consistently demonstrated^{111, 130, 139}, and has even been directly contradicted¹⁴⁰. Genetically mediated differences in bitter perception may not be sufficient alone to alter food acceptance, as bitterness perception is only one facet of the complex sensory profile of food. The extent to which genetic variance in taste receptors influences preference and intake is contentious. Genes that involve metabolism and satiety may also be involved¹⁴¹.

In children, taste perception strongly influences food preferences, and in this age group taste is one of the strongest mediators of fruit and vegetable consumption^{142, 143}. It has been demonstrated that non-taster children prefer sucrose less than those with the taster allele, however, the same association was not found to continue in adults¹¹². Similarly to children, adolescent PTC tasters had lower preference for raw cruciferous vegetables and other bitter tasting foods, but had a higher preference of sweet tasting foods¹⁴⁴.

In adults, it has been reported that PROP tasters have reported lower preference for a range of vegetables including brussel sprouts, cabbage, spinach²⁹, asparagus and kale¹³⁹. Conversely, two studies have shown no correlation between PROP status and the liking of broccoli^{44, 145}. In young women, a similar inverse correlation was demonstrated between PROP sensitivity and acceptance of tart citrus fruit, cruciferous vegetables, spinach, and coffee^{29, 146}. Female PROP tasters also show a decreased acceptance of sweet and fatty foods⁴⁶.

Interestingly, despite adults demonstrating no difference between tasters and non-tasters in the sensitivity of detection of bitterness in white grapefruit¹⁰⁰, PROP sensitivity correlated with a lower preference for grapefruit juice¹⁰² and other citrus fruits¹⁴⁶. Furthermore, taster

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children were observed to give a lower hedonic rating to a grapefruit/orange juice blend in a 3:1 ratio, compared to the same juices mixed in a 1:1 ratio¹⁴⁷. Similarly, supertasters gave a lower hedonic rating than non-tasters to naringin solutions sweetened with 4% sucrose¹⁰².

However, despite reported differences in sensitivity of taste perception to black coffee and dark chocolate, PROP taste status and TAS2R38 genotype were not shown to correlate with reported "liking" of these foods¹¹⁹. This may be because coffee and dark chocolate also include chemicals that enhance feelings of well-being¹⁴⁸. Studies have shown that pairing flavours with caffeine consumption can result in increased liking of those flavours. Liking of a tea spiked with caffeine was increased compared to the same tea when spiked with quinine, which affords similar bitterness, but lacks the stimulation of caffeine¹⁴⁹.

In the genetically and geographically isolated village of Carlantino in Italy, the community as a whole reports a high liking of vegetables in general, and degree of reported liking did not vary by PROP taster status. It may be that the high cultural acceptance of vegetables overrides that contribution of genetics or the cooking styles of this culture may reduce bitterness acceptability among all taster groups¹⁰⁰. In the same population, supertasters expressed a lower degree of liking of pungent and spicy foods and alcoholic beverages than non-tasters¹⁰⁰.

Different variants of the TAS2R43 gene have been associated with coffee liking, The TAS2R43 receptor is sensitive to caffeine. Possession of the wild-type allele, coding for the functional variant of the gene, was found to have a higher association with coffee liking, while the H212R polymorphism had an inverse association with coffee liking ¹⁵⁰. Interestingly, this SNP has been shown to reduce function of the expressed protein *in vitro* ¹¹⁴.

In fitting with increased general taste acuity; PROP tasting adults report a lower liking for salad dressing⁹⁴ sweetened milks⁸⁵, pungent foods¹⁵¹, alcoholic beverages¹⁵² and sweet taste¹¹³. However, in terms of sweet preference, children show the reverse response, with PROP tasting children preferring sweets^{112, 153}. This suggests that the psychophysiological response to tastants may evolve over time.

Influence of TAS2R genotype and tasting status on food intake patterns

Nutrients such as glucosinolates, isoflavones, phytonutrients are bitter tasting compounds found in health foods to which PROP tasters may be more sensitive. Therefore, PROP sensitive individuals may be more sensitive to the bitter tastes of "healthy" foods, which may influence health status^{29, 30, 138, 147}. However, although bitter taste sensitivity can affect the acceptance and liking in some foods, it appears to have a mixed influence on intake. Additional factors may alter how these factors relate to actual food intake. Influence may include cooking approaches and seasoning methods, health attitudes, personality traits, social and cultural influences, age and sex^{1, 95, 144, 154, 155}.

Several investigators have reported that adult PROP tasters and those with the PAV haplotype, consume fewer vegetables^{48, 139, 156-158} and fruit¹²⁴. Interestingly this has been shown not to be restricted to vegetables that are predominantly bitter¹⁵⁸. The AVI variation (non-taster) of the gene was associated with nutrient intake pattern indicative of healthy eating⁹⁶. PROP tasters have also been shown to eat fewer fats than non-tasters¹⁴⁷. However others have shown no relationship between vegetable intake and TAS2R38 genotype or taster status¹⁵⁹⁻¹⁶¹.

In a multifactorial study of vegetable intake, sensitivity to bitter and sweet tastes, and reported preferences, it was found these factors only partially predicted intake. A small, but direct, relationship with age was also found¹³⁹. A similar analysis found that PROP taster status, TAS2R38 genotype, sweetness perception, fungiform papillae density, age and sex all combine as a significant predictor of vegetable preference and explained 15.3% of the variation in males and 18.9% of the variation in females, with taster status and age being the most significant variables⁹⁶.

In a group of young women, perceived PROP bitterness was not associated with the frequency of consumption of 22 bitter foods¹⁶². Conversely, in a group of young males undergoing colonoscopy screening, those with the highest PROP sensitivity ate the least vegetables⁴⁸. A similar link between PROP sensitivity and cruciferous vegetable intake was found in an Italian population¹⁵⁶. However, in an Australian cohort it was found that *TAS2R38* diplotype did not predict intake of folic acid (methylfolic acid, pteroylmonoglutamic acid or total folic acid) or intake of vitamin C, which would be expected to be elevated with higher vegetable intake¹⁶⁰.

Taster children have been reported to eat less vegetables¹⁶³ and more sweets when offered a range of foods¹⁶⁴. When given the choice of snacks, taster children consumed approximately half as many vegetables as non-taster children. Furthermore, 32% of taster children consumed no fruit and vegetables at all during the experiment, compared to 8% of non-taster children¹⁶³. However, other studies have reported no association between PROP status and either liking or intake of vegetables in children^{49, 165}.

Vitamin B_6 , thiamine and folate intake were significantly higher and B12 intakes were significantly lower in women with the non-taster phenotype or AVI/AVI diplotype. This suggests that non-tasters consume more green leafy vegetables and fortified cereals (the main sources of dietary folate) and less animal products (the main source of B_{12})⁹⁶. Similar patterns of dietary intake between tasters and non-tasters have been described elsewhere¹⁶⁶. However, there were no significant differences in vitamin C, carotene or biotin⁹⁶ which would be expected if fruit and vegetable intakes differed considerably. A weak inverse correlation between TAS2R50 (rs1376251) C allele and the intake of dietary fibre and vegetables, has also been demonstrated¹⁵⁹. Super tasters have shown lower acceptance to whole grain breads¹⁶⁷.

Other genetic factors involved in these relationships may yet be defined. Modest linkage has been identified between TAS2R38 polymorphisms and other markers on chromosome $16p^{98}$. The peak association score being at the gene encoding G γ 13, one of the intracellular signalling molecules of TAS2Rs¹⁶⁸.

Alcohol consumption is complex and influenced by polymorphisms in a number of TAS2R genes. Taster phenotype and genotype are a significant predictor of alcohol consumption ^{111, 133}, Non-tasters consume alcoholic beverages more frequently^{111, 133, 152}. A polymorphism in the TAS2R16 gene (rs846672) significantly influenced consumption of alcohol beverages, with the minor allele homozygotes consuming twice as many alcoholic beverages¹³³. The same pattern was found for another polymorphism in the same gene (rs1308724). An additional polymorphism in the TAS2R13 gene (rs1015443 [C1040T, Ser259Asn]) showed a significant association with alcohol consumption, with those homozygous for the minor allele consuming the least alcohol¹⁶⁹.

Energy intake has been observed to be higher in those with the non-taster phenotype¹⁷⁰. In a study of young Japanese women, AI (non-tasters) had higher energy and carbohydrate intakes than the tasters. Neither vegetable nor dairy product intake was different between the homozygotes with AI haplotype and the carriers of PV haplotype¹⁶¹. In Amish women, those with the non-taster genotype displayed increased disinhibition of eating¹⁷¹

Individuals can modify the bitterness of foods with additives (e.g. adding milk and sugar to coffee) or cooking methods. There is strong motivation for food and pharmaceutical industries to control the bitterness of their products in order to increase acceptance¹⁷². This may be through a variety of mechanisms, including selective plant breeding, use of encapsulation, additional of salt, sweeteners and other flavours¹³⁸. Unfortunately, these methods may reduce the health value of some bitter foods. Addition of bitter taste receptor antagonists is a novel and innovative field of research, however the consequences of the mechanisms of action at play here are not yet clear^{100, 173}.

The links between bitter taste perception and health and disease

TAS2R38 mutations, and bitter taste responses have been linked to several adverse health events, such as alcoholism, smoking, and a range of chronic conditions including obesity, thyroid function and colon polyps^{38, 46, 111, 133, 137, 139, 174-176}. The simple logic is that increased sensitivity to bitterness, leads to an alteration in preferences and aversions, which in turn alters dietary choices and habits. Furthermore, it is thought that dietary habits subsequently influences nutritional status, predicting long term health and chronic disease risk. However, the interactions between taste and other dietary influences are complex and the complete nature of the interactions is yet to be fully elucidated.

It has often been hypothesised that individuals with increased bitter taste sensitivity might have lower health outcomes due to the avoidance of bitter tasting antioxidant and nutrient rich foods, resulting instead in increased consumption of sweet, fatty and nutrient poor foods, leading to a greater risk of cardiovascular disease³⁷. However, it appears that those with high sensitivity to bitter taste have heightened taste acuity in general, resulting in a parallel reduction in intake of sugars and fats^{86, 110, 111}. Non-tasters who are less sensitive to bitter vegetables, may eat more vegetables, but are also more likely to be "sweet likers"¹⁷⁷ and to

prefer high fat foods⁹⁴. This suggests that increased taste acuity may prevent overconsumption in all food groups. These relationships complicate the potential interactions between bitter taste and health.

Bitter taste and Body Mass

Several authors have suggested that bitter taste perception might alter body mass^{30, 153, 178, 179}. In the 1960's it was demonstrated that those with the lowest thresholds for PROP and quinine tasters were more likely to be Sheldonian ectomorphs (thinner), whilst those with the highest thresholds were more likely to be endomorphs (heavier)¹⁸⁰. This was replicated in a study of young Japanese females, where those with the taster haplotype were shorter and thinner than those with the non-taster haplotype¹⁶¹.

Several studies have observed an increased Body Mass Index (BMI) in female PROP nontasters^{30, 94, 96, 181, 182}. Similar patterns have been seen with overweight (but not obese) middle age and elderly subjects tasting PROP as less bitter than those of normal weight^{30, 96, 111, 152,} ¹⁸¹. However, this relationship was not maintained when genotype status replaced phenotype in the analysis^{30, 96}. Overall, no direct relationship was demonstrated between genotype, consumption and BMI in large epidemiological studies^{49, 165} or genome wide association studies^{141, 183}. This suggests phenotype is more influential than genotype in BMI, and it appears sex plays a role in the strength of this association.

In children, non-tasters tend to be heavier, but the effects are small¹⁵³. Others have shown an association only in girls, with non-tasters having higher BMIs and higher body fat¹⁸⁴. In a longitudinal study PTC taster girls had a higher bodyweight than non-tasters at age 11, but at 18 years this difference was lost¹⁴⁴. Additionally, non-taster children have been shown to be at higher risk of developing dental caries^{185, 186}. This may be due to increased preference for sweet foods, or due to decreased sensitivity to oral bacteria, resulting in a decreased drive for teeth brushing¹⁸⁶.

A history of middle ear infection, which has the capacity to cause damage to the chorda tympani nerve, and a reduction in taste acuity, is associated with obesity in adults¹⁸⁷ and children¹⁸⁸. Past damage leading to non-tasting could result in a greater preference for intake of sweets¹⁷⁷, fats^{46, 111, 119} and alcoholic beverages^{176, 189}.

Bitter taste and Metabolic Disorders

Metabolic disorders often have dietary links, and so logically the role of bitter taste genes has been considered. Functional variants of the TAS2R9 have been associated with metabolic disorders, the inactive variant of the gene has been associated with impaired glucose homeostasis ⁶⁴. The non-taster AVI/AVI genotype was associated with a slightly lower risk of diabetes, in a study of post-menopausal women. This suggests that these women may have consumed a more prudent diet over their lifetimes⁴⁹. However, no direct link between diet and TAS2R38 genotype were found. Importantly, this observation may be explained by changes in diet over time⁴⁹, which are not easily captured in cross-sectional studies, particularly as taste acuity has been shown to diminish with age^{190, 191}.

PTC non-tasters are at higher risk of thyroid disease than tasters³⁸. This may be due to increased intake of bitter thiocyanate compounds, which are dietary goitrogens, that can inhibit the amount of biologically available iodine, and can effect energy balance^{192, 193}.

Bitter taste and cardiovascular disease

A link between polymorphisms in the TAS2R50 gene and risk of myocardial infarction has been identified¹⁹⁴⁻¹⁹⁷, however no investigation of the link to dietary habits for this polymorphism have yet been undertaken. Conversely, others have found no link between *TAS2R38* genotypes and risk of cardiovascular disease⁴⁹.

Bitter taste and colon cancer

The findings into the relationship between colon polyps and PTC/PROP taster status have been mixed. Basson *et al.*,⁴⁸ found a weak but positive correlation between tasters and the number of colon polyps detected. Conversely, Carra *et al.*,¹⁹⁸ found that the AVI/AVI (non-taster) diplotype was associated with an increased risk of colon cancer. The authors hypothesised that rather than a dietary link existing, the AVI/AVI serves as a biomarker for impaired function of the gastrointestinal tract, resulting in slower elimination of toxins from the gut. However, Schembre *et al.*,¹⁵⁹ investigated colon cancer risk by polymorphisms in TAS2R38, TAS2R16 and TAS2R50 and found no significant relationships. Furthermore, Lucock *et al.*,¹⁶⁰ found that taste diplotype alone did not influence polyp risk, but red cell folate status did interact with bitter taste diplotype to predict polyp risk. The conflicting nature of these results means the relationship between bitter taste genotype, tasting phenotype and colon cancer are still unclear.

Bitter taste, alcoholism and smoking

PROP non-tasters also taste alcoholic beverages as less bitter, consume alcoholic beverages more frequently¹⁵², and have a higher risk of alcoholism. As such, bitter tasting may protect against some forms of alcoholism^{176, 199-201}. Polymorphisms in the TAS2R38 and TAS2R16 receptors are associated with alcohol dependence^{133, 199, 201}.

Smoking has long been associated with PROP/PTC tasting. Chronic smokers are more likely to be non-tasters^{202, 203}. In African American smokers, those with the non-taster haplotype smoked significantly more than those with the taster haplotype²⁰⁴. This may be because bitter chemicals are less offensive to non-tasters.

Bitter taste and affective disorders

Level of sensitivity to bitter tastants has been linked to levels of serotonin and noradrenaline. Enhancing noradrenaline levels significantly lower the tasting thresholds of quinine. In addition, anxiety level positively correlates with bitter taste thresholds. This suggests a level of plasticity to tasting parameters and may explain the relationship between affective disorders and diet²⁰⁵. Leptin has been shown to modulate sweet taste and alcohol intake, in mice^{206, 207}, but this has not been demonstrated for bitter taste directly.

The evolution of bitter taste

From an evolutionary point of view, it is interesting that there are considerably more receptors dedicated to detecting bitter than the other tastes. Just 3 genes have been identified in the TAS1R family, which are responsible for both sweet and umami taste, but for bitter taste humans have at least 25 intact TAS2R genes and several pseudogenes, making it the largest family of taste receptors. TAS2R genes are clustered together on chromosomes 5, 7 and 12^{13, 208, 209}. On average, species that eat plants (herbivores and omnivores) have more TAS2Rs than carnivores²¹⁰. Despite an increased number of receptors, behavioural studies have shown that plant eaters, are less sensitive to quinine hydrochloride (a natural bitter compound)²¹⁰. This suggests that although herbivores can detect a larger number of bitter to reject bitter foods²¹¹. Herbivores may also have developed additional mechanisms for detoxification, such as fermentation by microbes²¹².

The high sequence homology (30-70%) between TAS2R genes²¹³ suggests that the high number of TAS2R genes seems to have evolved from gene duplication events that expanded the range of bitter compounds to which humans are sensitive⁶⁹. A conspicuous feature of the TAS2R genes is an absence of introns^{13, 208, 209}.

Even subtle genetically determined differences can have several selective advantages that only become visible over evolutionary time spans¹²⁷. Taste is one of the primary means of determining the acceptability of foods, and might have been critical in the survival of early humans³⁰. Bitter taste is innate, and is present at birth in humans and other primates, and does not need to be learnt^{138, 211}. Great apes have a clearly identifiable TAS2R38 orthologue, although it contains several differences from the human gene. The few individual animals sequenced to date, are all homozygous for an allele containing a proline at position 42, an alanine at position 262 and a valine at position 296 (analogous to the major PAV taster allele in humans)^{87, 214}.

It may be logical to assume that more sensitive versions of the *TAS2R* genes would improve avoidance of toxins, and therefore would confer an evolutionary advantage. However, as PTC/PROP non-tasters are found in almost all human populations, this may be evidence that this polymorphism has been subject to "balancing selection" or selection for heterozygous individuals in the population^{89, 215}. Under balancing selection, both alleles are maintained in the population because they are both useful and heterozygosity may allow detection of a wider range of compounds⁸³. Non-tasting variants of TAS2R genes are more common in African populations in areas where Malaria is endemic. It has been suggested that lower sensitivity to bitter tastes allows increased consumption of bitter plant compounds that have antimalarial properties¹²⁷.

TAS2R genes show remarkable variation across species. For example, the human TAS2R38 gene shows only a 65% amino acid identity to the most closely related gene in the mouse²¹⁶. Given that the human taster and non-taster alleles of these genes are 99% identical at the amino acid level, it is possible that the most closely related gene in mice does not serve to detect PTC and its relatives at all. This may not be surprising, given the differences in the diet between the two species⁹³.

External influences on taste

The lack of concordance between the results of tasting studies suggests that the link between genetics, tasting, liking and consumption is complex, and not yet fully understood. Eating is a complex behaviour with multiple determinants, including age, sex, prior experiences, social and cultural norms, body, and health and weight attitudes. Few studies have factored all the these determinants into one study, but those that have done have generally found interesting associations^{85, 147, 151}.

Food Adventurousness

Only limited studies have investigated the role of personal characteristics in the relationships between taster status and food intake. "Food adventurousness" (the self-reported frequency of trying new foods) helped to clarify this relationship. PROP tasters who rated themselves as adventurous, liked more food per food group (fruits, vegetables, alcohol, non-fat condiments) than those who rated themselves as non-adventurous. However, in the non-taster group, adventurousness did not influence food liking¹⁵¹. In a study of Finnish twins those who rated themselves as adventurous expressed higher liking for sour and spicy foods, and fruit and vegetables. The adventurous group also had more tolerance for capsaicin burn in the sensory-hedonic test²¹⁷.

In the elderly, it is suggested that environmental factors are more important than genetic influences in food preferences. Instead, the elderly may be more inclined to try and accept novel foods because of diet-related attitudes and beliefs that are formulated throughout the years¹⁹⁰.

Age, sex and environmental influences

Age, sex, morphology and environment interact to produce major differences in bitter taste perception among individuals¹¹⁰. In fact, genetic predisposition may be outweighed by environmental influences. In one study taster status, age, and sex were found to be the most significant influences on food preferences, while genotype was less important⁹⁶. TAS2R38 status is not sufficient to explain PROP bitterness perception, particularly at suprathreshold levels, suggesting other environmental or genetic mechanisms may play a role^{85, 88}.

Taste changes across individual lifespans result from gene-environment interactions; genetic predispositions are modified with exposure to pathogens, medications or changes in hormonal

status. Bitterness and general taste acuity may show loss with aging. As previously discussed, tasting and liking of bitter substances appear to be more heavily influenced by genetics in children than in adults^{99, 112, 142, 143}. In the elderly, intensity of taste is reduced relative to young adults²¹⁸. This may be due to decreased olfactory senses influencing taste or reduction in fungiform papillae density in the elderly^{190, 219}.

Sex specific differences in influence of TAS2R38 genotype of bitter taste phenotype have been noted in both children¹⁷⁹ and adults^{46, 96}. Furthermore, the influence of gender on bitter taste perception may vary throughout the life cycle. Young women are more likely to be supertasters than young men¹¹⁰, and this may advantageously contribute to the avoidance of ingestion of toxins, tetratogens and abortifacients²²⁰. During pregnancy, bitter perception peaks during the first trimester and is lowest in the third trimester²²¹. Bitterness may decline in women following menopause and with aging in general – offering a potential advantage for the acceptance of bitter and nutrient rich food among the elderly¹³⁷.

Maternal diet may influence the tasting status of the fetus in later life. In rats, maternal consumption of ethanol led to increased acceptability of ethanol and quinine, but not sucrose in the offspring. This suggests that exposed offspring taste ethanol as less bitter²²². Another study showed that offspring of mothers who ate more fruit during lactation were more likely to accept peaches than babies fed formula. However, the mothers consumption of string beans did not have the same effect²²³. It has also been demonstrated that there is a sensitive period of exposure after birth in which exposure can alter perception. Infants fed bitter protein hydrolysates in their formula, led to a learned acceptance of the bitter flavour²²⁴.

Although it has not been directly demonstrated, it is likely that such early life programming occurs via epigenetic mechanisms. Significant variation in RNA expression levels of *TAS2R38* have been demonstrated to correlate with bitter taste detection and diet²²⁵. The mechanisms controlling this variation are yet to be identified.

Conclusion

Historically, the investigation of bitter taste genetics has focused on TAS2R38, the so called PTC gene. The improvement in genomic technologies in recent years has led to a dramatic increase in the number of TAS2R gene variants that have been identified and validated.

However, the functional significance of the majority of these polymorphisms are yet to be elucidated. Additionally, some TAS2R receptors remain as orphans, with no functional ligands identified.

It has long been hypothesised that TAS2R genotype and resulting taster phenotype would influence food preference, dietary intake and consequently health and disease. Despite the neatness of this logic, this relationship is yet to be completely understood. While it is clear that TAS2R genotype and taster phenotype contribute to food preference and choices of dietary intake, it is clear that other factors play a significant role. These additional factors include age, "food adventurousness", gender, fungiform papillae density and social and cultural influences. All these factors may also interact with genetic and epigenetics to alter outcomes.

Unravelling the interaction between these factors is complex, and at times data appear inconsistent. However, it is important to note that studies examining differences in perception and liking use a diverse array of methods to determine taster status, making it sometimes difficult to make comparisons across studies. Also, some studies rely on genotyping or PROP/PTC tasting alone, whilst others combine the two assessments to determine taster status. Another issue is that studies that examine differences in tasting, preference and intake often focus on specific population subsets, which though informative, do not reveal a true representation of the population as a whole.

Failure in earlier investigations to take into account the complexities of taste preference, intake and health outcomes may be limiting our understanding of the genetic contribution to these parameters, where pleiotropic effects are clearly present. Care should be taken to address this in future studies into bitter taste genetics.

	Breadth of	
Receptor	Tuning	Examples of Ligands
TAS2R1	Intermediate ²²⁶	Bitter Peptides ²²⁶ , Humulones, Picrotoxinin ³⁶
TAS2R3	Narrow ³⁶	Chloroquine ³⁶
TAS2R4	Intermediate ¹⁴	PROP, denatonium benzoate ¹⁴ , Epicatechin ²²⁷ , quinine ³⁶
TAS2R5	Narrow ³⁶	1,10-phenanthroline ³⁶ , Epicatechin ²²⁷
TAS2R7	Intermediate ²²⁸	Caffeine ⁶³ , Strychinine ⁸⁰ , cromolyn ³⁶ , malvidin-3- glucoside ²²⁷
TAS2R8	Narrow ¹⁴	chloramphenicol and denatonium benzoate ¹⁴
TAS2R9	Narrow-Intermediate ⁶⁴	Ofloxacin, procainamide and pirenzapine ⁶⁴
TAS2R10	Broad ²²⁹	Caffeine ⁶³ , Strychinine ⁸⁰ , Chloroquine, denatonium benzoate ²²⁹ , erythromycin, quinine ³⁶
TAS2R13	Narrow ³⁶	Denatonium Benzoate, Diphenidol ³⁶
TAS2R14	Broad ²²⁹	Caffeine ⁶³ , Alpha-thujone ²³⁰ , absinthin, quinine, aristolochic acid ³⁶
TAS2R16	Broad ⁸⁰	B-D-glucopyranoside group, eg salicin ⁸⁷ and amygdalin ³⁶
TAS2R19		Orphan
TAS2R20		Orphan
TAS2R30	Intermediate ²³¹	Denatonium benzoate, Picrotoxinin ⁶³
TAS2R31	Intermediate ³⁴	Saccharin ³⁴
TAS2R33		Orphan
TAS2R38	Broad ^{87, 88}	Isothiocyanates (including PROP and PTC) ^{87 88}
TAS2R39	Intermediate ³⁶	Epicatechin ²²⁷ , Colchicine, denatonium benzoate ³⁶
TAS2R40	Intermediate ²³²	Quinine, Chlorpheniramine ³⁶ , Humulone isomeres ^{36, 232}
TAS2R41	Narrow ²³³	Chloramphenicol ²³³
TAS2R42		Orphan
TAS2R43	Intermediate ^{34, 231}	Sulfonyl amide sweetners ³⁴ , Caffeine ⁶³ , Sacchrin (Pronin et al, 2004
TAS2R44	Intermediate ³⁶	Quinine, Acesulfame K, Saccharin ³⁶
TAS2R45		Orphan
TAS2R46	Broad ^{36, 229}	Qunine, Caffeine, Denatonium Benzoate, Hydrocortisone, Strychnine ^{36, 229}
TAS2R47	Broad ³⁶	Denatonium Benzoate, Diphenidol, Artemorin, Absinthin ³⁶
TAS2R48		Orphan
TAS2R49	Narrow ³⁶	Cromolyn ³⁶
TAS2R50	Narrow ²³⁴	Amarogentin, Andrographolide ³⁶
TAS2R60		Orphan

TABLE 1: Functional TAS2R receptors, their breadth of tuning, and some examples of ligands.

Gene	# Non synonymous variants
TAS2R1	15
TAS2R3	8
TAS2R4	14
TAS2R5	17
TAS2R7	20
TAS2R8	18
TAS2R9	18
TAS2R10	16
TAS2R13	11
TAS2R14	12
TAS2R16	7
TAS2R19	15
TAS2R20	16
TAS2R30	8
TAS2R31	20
TAS2R33	0
TAS2R38	13
TAS2R39	8
TAS2R40	8
TAS2R41	9
TAS2R42	0
TAS2R43	15
TAS2R44	20
TAS2R45	0
TAS2R46	7
TAS2R47	8
TAS2R48	15
TAS2R49	16
TAS2R50	14
TAS2R60	30

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<u>**TABLE 2:**</u> Number of non-synonymous nucleic acid variants in each intact and functional TAS2R gene. Only variants validated by the 1000 genomes project were included. Retrieved May 2014 from http://www.ncbi.nlm.nih.gov/snp

Reference SNP ID	Alleles	MAF	Variant Amino Acid Position	Alleles
rs713598	C/G	C=0.4692	49	Proline/Alanine
rs10246939	C/T	T=0.4509	262	Alanine/Valine
rs1726866	G/A	A=0.4082	296	Valine/Isoleucine

TABLE 3: Common and rare variants in the TAS2R38 gene (ref: <u>http://www.ncbi.nlm.nih.gov/snp</u>) *MAF; minor allele frequency*

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