Current Biology 17, 1403–1408, August 21, 2007 ©2007 Elsevier Ltd All rights reserved DOI 10.1016/j.cub.2007.07.046

Report

Specific Alleles of Bitter Receptor Genes Influence Human Sensitivity to the Bitterness of Aloin and Saccharin

Alexey N. Pronin,[1,*] Hong Xu,[1] Huixian Tang,[1] Lan Zhang,[1] Qing Li,[1] and Xiaodong Li[1]
[1] Senomyx
4767 Nexus Centre Drive
San Diego, California 92121

Summary

Variation in human taste is a well-known phenomenon [1]. However, little is known about the molecular basis for it. Bitter taste in humans is believed to be mediated by a family of 25 G protein-coupled receptors (hT2Rs, or TAS2Rs) [2–7]. Despite recent progress in the functional expression of hT2Rs in vitro, up until now, *hT2R38*, a receptor for phenylthiocarbamide (PTC), was the only gene directly linked to variations in human bitter taste [8]. Here we report that polymorphism in two *hT2R* genes results in different receptor activities and different taste sensitivities to three bitter molecules. The *hT2R43* gene allele, which encodes a protein with tryptophan in position 35, makes people very sensitive to the bitterness of the natural plant compounds aloin and aristolochic acid. People who do not possess this allele do not taste these compounds at low concentrations. The same *hT2R43* gene allele makes people more sensitive to the bitterness of an artificial sweetener, saccharin. In addition, a closely related gene's (*hT2R44*'s) allele also makes people more sensitive to the bitterness of saccharin. We also demonstrated that some people do not possess certain *hT2R* genes, contributing to taste variation between individuals. Our findings thus reveal new examples of variations in human taste and provide a molecular basis for them.

Results and Discussion

Bitter taste is one of the five basic taste qualities. A classic discovery in 1931 revealed a dramatic difference in individual sensitivity to the bitterness of phenylthiocarbamide (PTC)—some people taste its bitterness at low micromolar concentrations, whereas others do not taste it even at millimolar concentrations [9]. The inability to taste PTC appeared to be a simple Mendelian trait [10]. This discovery was the first demonstration that differences in human taste could be genetically determined. However, the nature of that gene remained unknown until very recently. In 2000, a novel family of G protein-coupled receptors, termed T2Rs, was identified and proposed to mediate bitter taste in humans and rodents [3, 11, 12]. One of the mouse T2Rs, *mT2R5*, proved to be a strong candidate for *Cyx*, a genetic locus influencing mouse sensitivity to cycloheximide [3, 11]. It was soon demonstrated that variation in one of the human *T2R* genes, *hT2R38*, is mainly responsible for the differences in individual sensitivity to the bitterness of PTC [8]. Until now, mT2R5 and hT2R38 remained the only two bitter taste receptors with a clearly proven function in vivo. Although several other human T2Rs have been reported to recognize bitter molecules in heterologous expression systems [4, 7, 13–16], their role in human taste has not yet been established.

Human *T2R* genes display a high degree of polymorphism: Many genes have multiple different haplotypes found in the human population [17, 18]. However, with the exception of *hT2R38*, it is not known whether variation in *hT2R* genes has any functional consequences. Recent attempts that used traditional human genetic analysis failed to establish new links between variation in bitter taste and human genes [19]. We took a different approach by using an in vitro functional assay as a guide to identify differences in human bitter taste.

The hT2R43-W35 Allele Is the Most Sensitive to Aloin In Vitro and In Vivo

By using an HEK293 cell-based assay, we identified aloin as a novel ligand for hT2R43 (also called hT2R61 in our previous publication [7]; GeneID: 259289) (Figure 1A). Aloin is a compound found in aloe plants. hT2R43 responded robustly to aloin, with the half-maximal response (EC_{50}) at ~1.2 μM. No other hT2R showed any significant response to aloin in our assay. Even the most closely related hT2R, hT2R44 (hT2R64 in our previous publication [7]; GeneID: 259290), demonstrated virtually no response to aloin (up to 128 μM, Figure 1A).

Based on published [17, 18] and our own data, there are two major variants for hT2R43 (Figure 1B; Table S1 in the Supplemental Data available online). The hT2R43-SR variant has serine at position 35 and arginine at position 212. The hT2R43-WH variant has tryptophan and histidine at the respective positions. hT2R43-WH was used in our earlier studies ([7], Figure 1A). Among the 55 subjects, we also identified two rare alleles—hT2R43-SH and hT2R43-Δ6 (Figure 1B; Table S1). Interestingly, genomic DNA from ten subjects yielded no polymerase chain reaction (PCR) product for the *hT2R43* gene (Figure 1C). This suggests that a significant minority (~18% in our subject pool) of people does not have the *hT2R43* gene or is missing a large part of it (homozygous for the *null* allele). Our observation is supported by one of the two chromosome 12 assemblies (NW_925328.1) that lacks the *hT2R43* gene. We determined that one other *hT2R* gene could be missing in some people. Genomic PCR demonstrated that one of the subjects was missing the *hT2R45* gene (Figure 1C). Thus, individuals can differ from each other not only because they express different hT2R allele variants, but also because some people do not possess certain bitter receptors at all.

To determine whether different hT2R43 variants respond differently to ligands, we tested all hT2R43 variants by using aloin. Among the four variants,

*Correspondence: alexey.pronin@senomyx.com

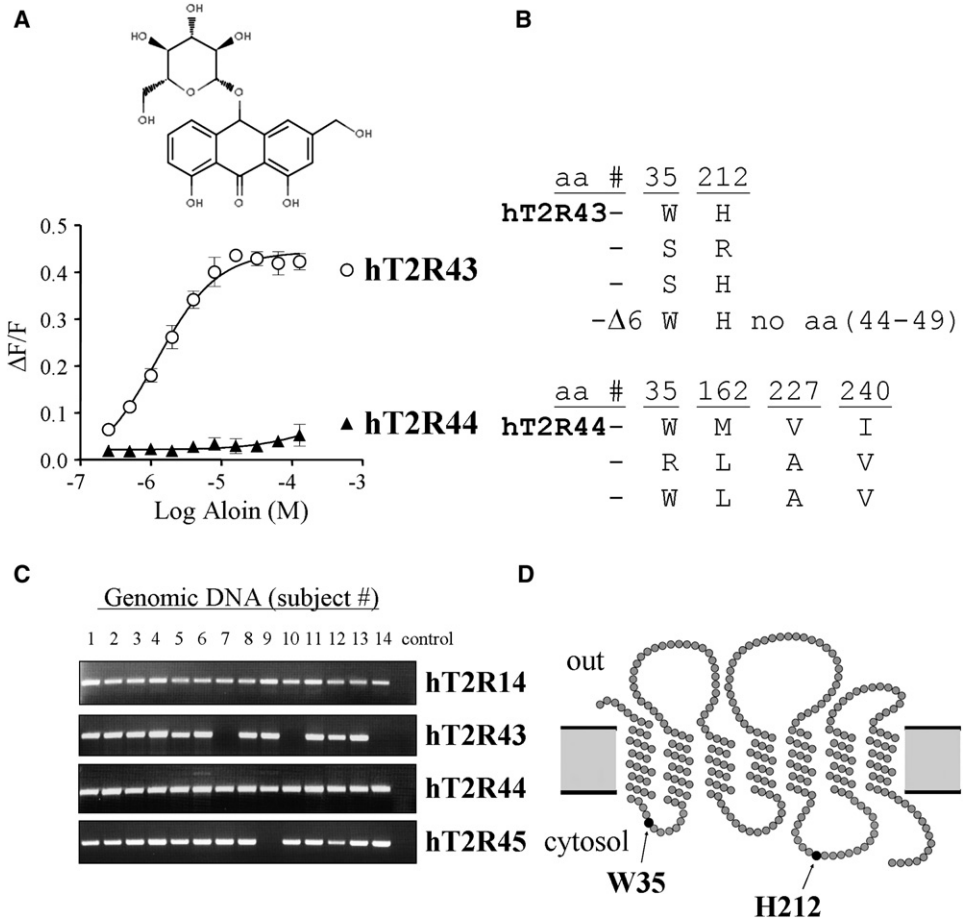

Figure 1. Polymorphism in the Gene for the Receptor Activated by Aloin (hT2R43)

(A) Dose-response relationships of the effects of aloin on calcium concentration in transfected HEK293 cells expressing hT2R43 and hT2R44. Calcium traces were recorded in the FLIPR and analyzed as described in the Supplemental Data. No hT2R other than hT2R43 and hT2R44 was seen activated at concentrations up to 60 μM. Each point represents the mean ± the standard deviation of the mean of at least three independent experiments carried out in quadruplicate.

(B) hT2R43 and hT2R44 allele variants used in this study. The specific amino acid residue at the indicated position ("aa #") is shown for each receptor variant. hT2R43-Δ6 has a deletion of six amino acid residues (#44–49) and five additional differences from hT2R43-WH.

(C) Some people do not have the *hT2R43* or *hT2R45* gene. Genomic DNA from the indicated subjects was analyzed for the presence of *hT2R* genes by the amplification of the coding region with primers specific to either *hT2R14*, *hT2R43*, *hT2R44*, or *hT2R45*. "Control" indicates samples with no template. Note that the *hT2R14* and *hT2R44* genes can be amplified from all subjects, suggesting that genomic DNA was present in all samples. However, the *hT2R43* gene cannot be amplified from subjects 7, 10 and 14, whereas the *hT2R45* gene cannot be amplified from subject 9. Gene amplification from all subjects was performed with three (*hT2R43*) or two (*hT2R45*) primer sets with results identical to those shown here. The photographs are representative of at least two independent experiments. The conclusion that some people lack all or a big part of the *hT2R43* gene is supported by the fact that, whereas *hT2R43* gene sequence can be found in the chromosome 12 assembly NT_009714.16 (Human Genome Sequencing Consortium), it is missing in one of the two chromosome 12 assemblies based on Celera Genomycs sequencing (NW_925328.1). Conversely, the *hT2R45* gene sequence can be found in the chromosome 12 assembly based on Celera Genomycs sequencing (NW_925328.1) but not in the chromosome 12 assembly NT_009714.16 (Human Genome Sequencing Consortium).

(D) Schematic representation of a predicted hT2R topology. Positions of amino acid residues that are variable in hT2R43 are indicated with arrows.

hT2R43-WH stood out in both the EC_{50} and efficacy (Figure 2A). Both hT2R43-SH and hT2R43-SR variants displayed low activity, indicating that W35 is the critical residue for receptor function. W35 is located within a predicted intracellular loop 1 of the receptor (Figure 1D) and is conserved among hT2Rs (21 out of 25 hT2Rs). Replacement of this tryptophan with either serine or arginine in hT2R16 results in dramatic reduction of the receptor activity (data not shown), suggesting that it plays an equally important role in other bitter receptors. Interestingly, this residue is located only five positions upstream of proline 49 in hT2R38. Replacement of this proline with alanine results in a PTC-nontaster phenotype [8, 15]. This suggests that these residues might play a similar role in a receptor function. Based on the predicted location, this residue is likely to be involved in coupling to a G protein. It could also be involved in receptor trafficking. In either case, amino acid replacement that negatively affects this function should cause a reduction in the maximum activity of the receptor—the result we observed in our assay (Figure 2A, Figure S2). In addition, an approximately 10-fold difference in the EC_{50} between hT2R43-WH and hT2R43-SR suggests that the replacement of this tryptophan might alter protein folding and indirectly affect ligand binding.

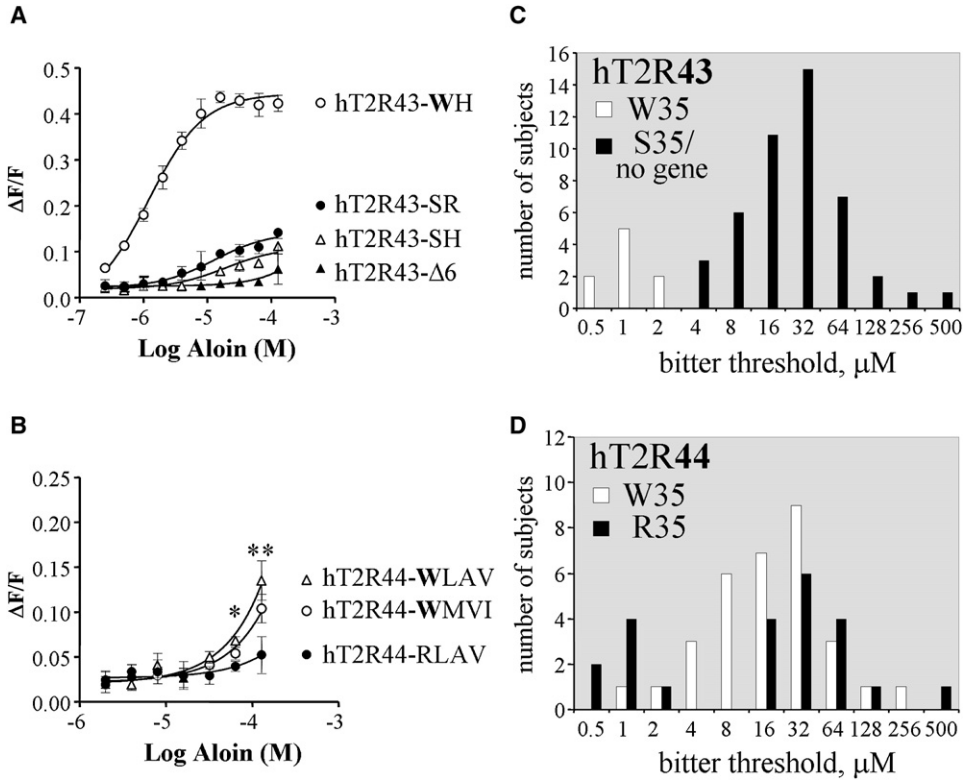

Figure 2. The hT2R43 Variant with W35 Is the Most Sensitive to Aloin both In Vitro and In Vivo

(A and B) Dose-response relationships of the effects of aloin on calcium concentration in transfected cells expressing indicated variants of hT2R43 (A) and hT2R44 (B). EC_{50} values for activation by aloin were 1.2 μM for hT2R43-WH, 13 μM for hT2R43-SR, 23 μM for hT2R43-SH and >128 μM for hT2R43Δ6 and hT2R44 variants. Asterisks indicate a statistically significant difference from the hT2R44-RLAV response ("*" indicates p = 0.05; "**" indicates p = 0.01). Each point represents the mean ± the standard deviation of the mean of at least three independent experiments carried out in quadruplicate.

(C) Distribution of bitter taste thresholds for aloin for groups defined by *hT2R43* genotypes. Groups with individuals that have at least one sensitive (W35) allele (homo- or heterozygous) are shown as white bars. The mean bitter threshold for aloin for the *hT2R43-W35* group was significantly lower than that for the group with individuals homozygous for the *hT2R43-S35* allele or lacking the *hT2R43* gene (1 μM versus 26 μM, respectively; $\alpha = 0.01$, $p < 0.00006$).

(D) Distribution of bitter taste thresholds for aloin for the same individuals but defined by *hT2R44* genotypes. Groups with individuals that have at least one sensitive (W35) allele (homo- or heterozygous) are shown as white bars. There is no significant difference in mean sensitivity to aloin between *hT2R44-W35* and *hT2R44-R35* groups (17 and 14 μM, respectively).

The *hT2R43* gene is highly homologous to the *hT2R44* gene (89% identity at the amino acid level), and the two receptors share some ligands in the in vitro assays [7, 14]. We observed a high degree of polymorphism in the *hT2R44* gene, resulting in variation at 11 different amino acid positions. We identified at least 15 different haplotypes (Table S2), four of which were previously described [18]. Interestingly, variations were observed at amino acid 35 (W or R) (Figure 1B), the residue crucial for activity in hT2R43. However, the two polymorphisms were probably the result of independent mutations because nucleotide change in the *hT2R44* gene occurs at nucleotide 103 instead of 104 in *hT2R43*.

We tested two of the more common variants of hT2R44 with aloin. Overall, hT2R44 was significantly less responsive to aloin than was hT2R43. However, the hT2R44-WMVI variant displayed higher activity than did hT2R44-RLAV (Figure 2B). The difference is likely due to W35 because the single mutation R35W brought the hT2R44-RLAV activity up to the same level as hT2R44-WMVI.

If hT2R43 is the main bitter taste receptor for aloin, then one would expect significant variation in individuals' sensitivity to aloin and that people with the hT2R43-W35 variants would be more sensitive than are others. To test this hypothesis, we conducted taste tests to determine the bitter recognition thresholds of individuals for aloin. Indeed, the taste tests revealed great differences in human sensitivity to aloin (Figure 2C). Furthermore, the taste sensitivity correlated with the *hT2R43* genotype. The most sensitive individuals had at least one *hT2R43-W35* allele (homo- or heterozygous; mean bitter taste threshold 1.0 μM), whereas individuals homozygous for either the *hT2R43-S35* allele or the *null* allele formed a significantly less-sensitive group (mean bitter taste threshold 26 μM; $\alpha = 0.01$, $p < 0.00003$). This observation correlates well with our in vitro assay data and strongly argues that hT2R43 is the main bitter taste receptor for aloin. The *hT2R43* haplotype effect explains ~62% of the total variance in sensitivity to the bitterness of aloin ($F = 28.0$, $p < 0.001$).

In contrast, at first glance, *hT2R44* genotypes did not correlate with the taste-test data (Figure 2D). There is no significant difference in the mean sensitivity to aloin between *hT2R44-W35* and *hT2R44-R35* groups (p = 0.99). However, when individuals with the *hTAS2R43-W35*

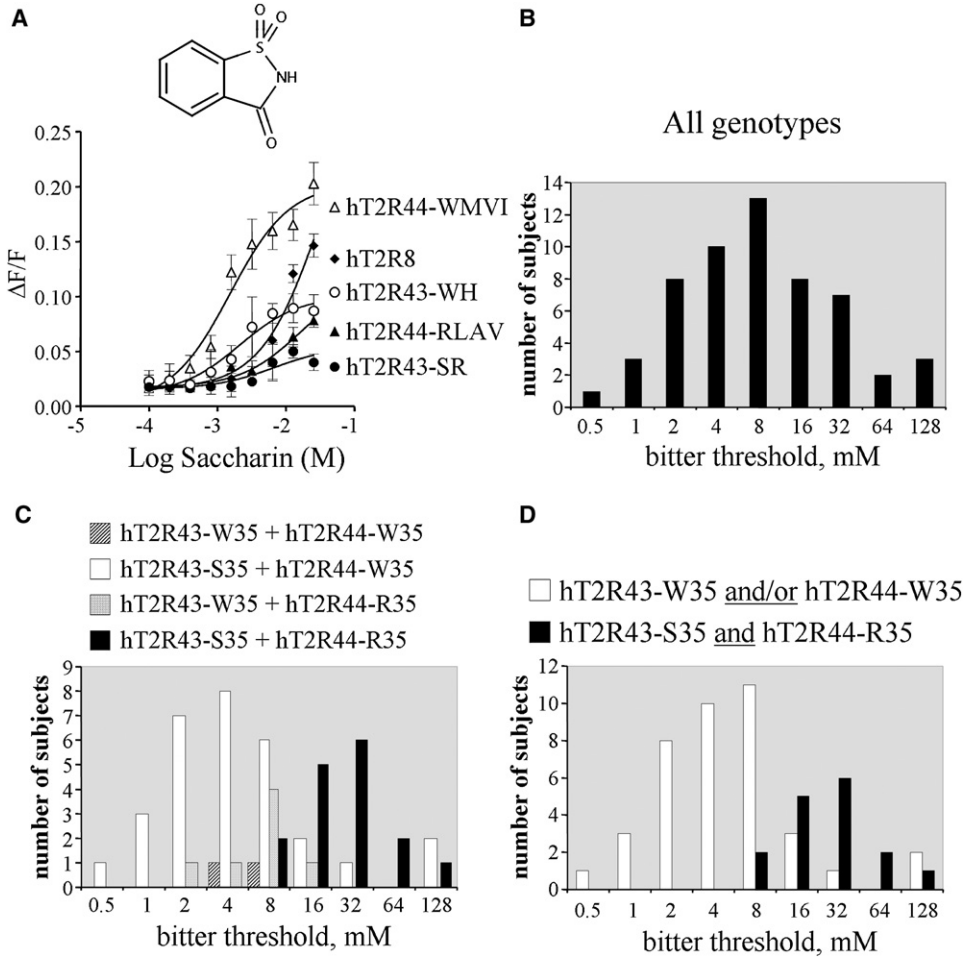

Figure 3. hT2R43 and hT2R44 Alleles with W35 Are Responsible for High Sensitivity to Saccharin

(A) Dose-response relationships of the effects of saccharin on calcium concentration in transfected cells expressing indicated variants of hT2R43, hT2R44 and hT2R8. EC_{50} values for activation by saccharin were 2.1 mM for hT2R43-WH, >20 mM for hT2R43-SR, 1.7 mM for hT2R44-WMVI, 16 mM for hT2R44-RLAV, and 32 mM for hT2R8. No other hT2R was activated by saccharin at concentrations up to 50 mM. Each point represents the mean ± the standard deviation of the mean of at least three independent experiments carried out in quadruplicate.

(B) Distribution of individual bitter taste thresholds for saccharin (all genotypes).

(C) Distribution of bitter taste thresholds for saccharin for groups defined by *hT2R43* and *hT2R44* genotypes. Individuals with at least one sensitive (W35) allele (homo- or heterozygous) were grouped together. Individuals lacking the *hT2R43* gene were grouped together with individuals homozygous for the *hT2R43-S35* allele.

(D) Distribution of bitter taste thresholds for saccharin for groups defined by *hT2R43* and *hT2R44* genotypes. Individuals with at least one sensitive (W35) allele (homo- or heterozygous) of either *hT2R43* or *hT2R44* gene were grouped together. Individuals homozygous for the insensitive alleles of both genes were grouped together. Individuals lacking the *hT2R43* gene were grouped together with individuals homozygous for the *hT2R43-S35* allele.

allele were excluded from the analysis, the effect of *hTAS2R44* genotype could be observed: Individuals with at least one *hTAS2R44-W35* allele were noticeably more sensitive compared to the group homozygous for the *hTAS2R44-R35* allele (mean bitter thresholds 20 μM and 42 μM, respectively, p = 0.025) (Figure S3B). Still, individuals with the *hT2R44-W35* allele were much less sensitive than were individuals with the *hTAS2R43-W35* allele. Thus, differences in the *hTAS2R44* gene contribute to variation in taste sensitivity among less-sensitive people, whereas the *hTAS2R43-W35* allele makes people supersensitive to aloin. This observation correlates well with our in vitro data showing that hT2R43 is much more sensitive to aloin than is hT2R44.

To confirm our findings, we used aristolochic acid, another ligand that activates hT2R43 in vitro [14]. By using aristolochic acid, we got results nearly identical with the results obtained with aloin (Supplemental Results; Figure S4). The individual bitter thresholds for aristolochic acid and aloin correlated very well (r = 0.85, p < 0.01), consistent with the idea of a common receptor for these compounds (hT2R43).

Both *hT2R43* and *hT2R44* Are Responsible for Increased Sensitivity to the Bitterness of Saccharin

According to in vitro studies [14], another common ligand shared by hT2R43 and hT2R44 is saccharin, a synthetic sweetener with a considerable bitter side taste to some people. We confirmed these results. In addition, we found that saccharin activated hT2R8 (Figure 3A), but at higher concentrations. Unlike aloin and aristolochic acid, saccharin activated the hT2R43

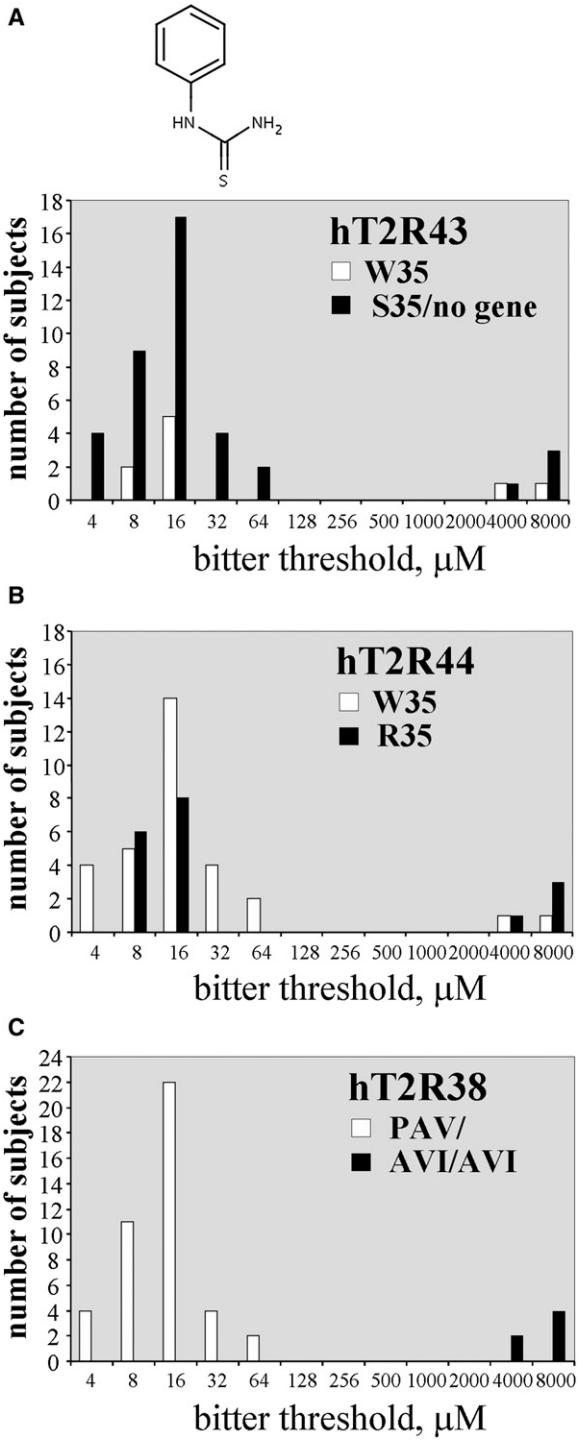

Figure 4. Sensitivity to the Bitterness of PTC Is Not Affected by *hT2R43* or *hT2R44* Genotypes

Distribution of bitter taste thresholds for PTC for groups defined by *hT2R43* (A), *hT2R44* (B), and hT2R38 (C) genotypes. Individuals that have at least one W35 allele (homo- or heterozygous) of the *hT2R43* or *hT2R44* genes were grouped together. Individuals with at least one "taster" *hT2R38-PAV* allele (P49-A262-V296; homozygous or heterozygous) were grouped together.

and 44 variants with W35 similarly to, but still significantly better than, the other alleles (Figure 3A). Consistent with the in vitro observation, taste tests showed that bitter taste thresholds for saccharin varied significantly (Figure 3B). Analysis of the *hT2R43* and *hT2R44* genes revealed that individuals that have at least one sensitive allele of either of these genes form a group that is considerably more sensitive to the bitterness of saccharin than are people carrying insensitive alleles of both genes (mean threshold 4.2 mM versus 25.7 mM respectively; $\alpha = 0.01$, $p < 0.0003$) (Figures 3C and 3D). Therefore, sensitivity to the bitter taste of saccharin appears to be controlled by both *hT2R43* and *hT2R44*. The combined *hT2R43* and *hT2R44* haplotype effect explains ~34% of the total variance in sensitivity to the bitterness of saccharin ($F = 6.36$, $p < 0.001$). It was proposed that the bitter taste of saccharin could be mediated by some intracellular proteins [20, 21]. Our findings argue that taste receptors play an important role in sensing saccharin bitterness. Saccharin and a chemically related sweetener, acesulfame K, are used in certain diet beverages. Like saccharin, acesulfame K tastes bitter to some people, and individual sensitivities to the bitterness of these sweeteners correlate very well [22]. Similar to saccharin, acesulfame K activates mainly hT2R44 in vitro [14]. Based on our results, we can speculate that individuals with the *hTAS2R44-W35* allele should be more sensitive to the bitterness of acesulfame K than are individuals with the *hTAS2R44-R35* allele, providing a possible explanation as to why some people find the taste of certain diet beverages unpleasant whereas others enjoy them.

Our data strongly indicate that *hT2R43* controls human taste sensitivity to aloin and aristolochic acid, whereas both *hT2R43* and *hT2R44* are involved in mediating bitter taste sensitivity to saccharin. However, there was a remote possibility that individuals with the *hT2R43-W35* variant were generally more sensitive to any bitter molecules. We tested this possibility with PTC, which does not activate either hT2R43 or hT2R44 in heterologous assay systems. Our taste tests revealed that individuals with different *hT2R43* or *hT2R44* alleles showed the same distribution in sensitivity (no significant difference; p value 0.54 and 0.9, respectively)—people with both sensitive and insensitive alleles can be found among PTC tasters and nontasters (Figure 4). This indicates that the *hT2R43-W35* and *hT2R44-W35* alleles make people more sensitive to their specific ligands only. In contrast, consistent with earlier reports, individuals with the taster allele of the *hT2R38* gene (PAV homo- or heterozygous) were clearly separated from individuals homozygous for the nontaster allele (AVI/AVI) (Figure 4C).

The saccharin sensitive genes, *hT2R43* and *hT2R44*, are the most closely related genes in the *hT2R* family (89% identity at the protein level). It is likely they are products of gene duplication. These receptors share some ligands, although they respond differently to them. Interestingly, both genes have alleles with the substitution of the same amino acid—W35. However, these variants appear to arise independently in these genes. This amino acid change is caused by a different nucleotide polymorphism—position 104 in *hT2R43* and position 103 in *hT2R44*. This independent rise of insensitive T2R alleles is similar to a recently reported case with the *hT2R38* gene. Similar to humans, chimpanzees vary in their sensitivity to the bitterness of PTC, and such

variation is caused by a polymorphism in their T2R38 gene [23]. However, the mutation causing lower sensitivity in chimpanzees is different from that in humans. We analyzed the available information about *T2R* gene sequences from other species. Most primate gene orthologs of *hT2R43* and *hT2R44* (from chimpanzees, orangutans, agile gibbons, and rhesus monkeys) encode proteins with W35, suggesting that this sensitive version of the proteins is the earlier one in evolution. However, there are sequences from gorillas and rhesus monkeys with polymorphism at amino acid 35, like humans. Therefore, the loss of the sensitive alleles for these two genes has roots in prehuman evolution. Our results show that only a small minority (~16%) of people posses the sensitive allele of the *hT2R43* gene, and a significant number (~18%) of people do not have the functional gene at all. Thus, as earlier research suggested [17, 24], it appears that the evolutional pressure to retain sensitive *T2R* genes was relaxed in humans. Interestingly, though, all individuals in our pool that lack the *hT2R43* gene are homozygous for the sensitive *hT2R44* allele (W35) (data not shown). We can thus speculate that it was important for survival of early humans to ensure that individuals retained at least some sensitivity to potentially toxic compounds recognized by the hT2R43 and hT2R44 receptors (such as the plant toxins aloin and aristolochic acid).

In summary, we used in vitro studies as guidance and discovered drastic variation in human taste sensitivities to three bitter molecules. We demonstrated that the taste variations can be traced to polymorphisms in two human *T2R* genes, providing only the second example of direct correlation between human bitter taste and specific genes. Studies of human taste have an inherent difficulty because of the limited scope of possible experiments. Our approach of combining in vitro and in vivo studies to exploit human T2R polymorphisms could prove useful in revealing more of the molecular mechanisms of human bitter taste.

Supplemental Data

Additional Results and Discussion, Experimental Procedures, seven figures, and twelve tables are available at http://www.current-biology.com/cgi/content/full/17/16/1403/DC1/.

Acknowledgments

Authors would like to thank Drs. Tanya Ditschun and Nicholas Schork for help with the statistical analysis of data. We are also thankful to Drs. Lubert Stryer, Mark Zoller, Bryan Moyer, Guy Servant, Vladlen Slepak, and Albert Zlotnik for valuable discussions and advice.

Received: March 15, 2007
Revised: July 1, 2007
Accepted: July 13, 2007
Published online: August 16, 2007

References

1. Snyder, D., Prescott, J., and Bartoshuk, L. (2006). Modern psychophysics and the assessment of human oral sensation. In Taste and Smell: An Update. Adv. Otorhinolaryngol, *volume 63*, T. Hummel and A. Welge-Lüssen, eds. (Basel, Switzerland: Karger), pp. 221–241.
2. Chandrashekar, J., Hoon, M.A., Ryba, N.J.P., and Zuker, C.S. (2006). The receptors and cells for mammalian taste. Nature *444*, 288–294.
3. Adler, E., Hoon, M.A., Mueller, K.L., Chandrashekar, J., Ryba, N.J., and Zuker, C.S. (2000). A novel family of mammalian taste receptors. Cell *100*, 693–702.
4. Bufe, B., Hofmann, T., Krautwurst, D., Raguse, J.D., and Meyerhof, W. (2002). The human TAS2R16 receptor mediates bitter taste in response to β-glucopyranosides. Nat. Genet. *32*, 397–401.
5. Conte, C., Ebeling, M., Marcuz, A., Nef, P., and Andres-Barquin, P.J. (2002). Identification and characterization of human taste receptor genes belonging to the TAS2R family. Cytogenet. Genome Res. *98*, 45–53.
6. Shi, P., Zhang, J., Yang, H., and Zhang, Y.P. (2003). Adaptive diversification of bitter taste receptor genes in mammalian evolution. Mol. Biol. Evol. *20*, 805–814.
7. Pronin, A.N., Tang, H., Connor, J., and Keung, W. (2004). Identification of ligands for two human bitter T2R receptors. Chem. Senses *29*, 583–593.
8. Kim, U.K., Jorgenson, E., Coon, H., Leppert, M., Risch, N., and Drayna, D. (2003). Positional cloning of the human quantitative trait locus underlying taste sensitivity to phenylthiocarbamide. Science *299*, 1221–1225.
9. Fox, A.L. (1931). Six in ten 'tasteblind' to bitter chemical. Sci. News. Lett. *9*, 249.
10. Blakeslee, A.F. (1932). Genetics of sensory thresholds: Taste for phenyl thio carbamide. Proc. Natl. Acad. Sci. USA *18*, 120–130.
11. Chandrashekar, J., Mueller, K.L., Hoon, M.A., Adler, E., Feng, L., Guo, W., Zuker, C.S., and Ryba, N.J. (2000). T2Rs function as bitter taste receptors. Cell *100*, 703–711.
12. Matsunami, H., Montmayeur, J.P., and Buck, L.B. (2000). A family of candidate taste receptors in human and mouse. Nature *404*, 601–604.
13. Behrens, M., Brockhoff, A., Kuhn, C., Bufe, B., Winning, M., and Meyerhof, W. (2004). The human taste receptor hTAS2R14 responds to a variety of different bitter compounds. Biochem. Biophys. Res. Commun. *319*, 479–485.
14. Kuhn, C., Bufe, B., Winnig, M., Hofmann, T., Frank, O., Behrens, M., Lewtschenko, T., Slack, J.P., Ward, C.D., and Meyerhof, W. (2004). Bitter taste receptors for saccharin and acesulfame K. J. Neurosci. *24*, 10260–10265.
15. Bufe, B., Breslin, P.A., Kuhn, C., Reed, D.R., Tharp, C.D., Slack, J.P., Kim, U.-K., Drayna, D., and Meyerhof, W. (2005). The molecular basis of individual differences in phenylthiocarbamide and propylthiouracil bitterness perception. Curr. Biol. *15*, 322–327.
16. Sainz, E., Cavenagh, M.M., Gutierrez, J., Battley, J.F., Northup, J.K., and Sullivan, S.L. (2007). Functional characterization of human bitter taste receptors. Biochem. J. *403*, 537–543.
17. Wang, X., Thomas, S.D., and Zhang, J. (2004). Relaxation of selective constraint and loss of function in the evolution of human bitter taste receptor genes. Hum. Mol. Genet. *13*, 2671–2678.
18. Kim, U., Wooding, S., Ricci, D., Jorde, L.B., and Drayna, D. (2005). Worldwide haplotype diversity and coding sequence variation at human bitter receptor loci. Hum. Mutat. *26*, 199–204.
19. Hansen, J.L., Danielle, R., Reed, D.R., Wright, M.J., Martin, N.G., and Breslin, P. (2006). Heritability and genetic covariation of sensitivity to PROP, SOA, quinine HCl, and caffeine. Chem. Senses *31*, 403–413.
20. Peri, I., Mamrud-Brains, H., Rodin, S., Krizhanovsky, V., Shai, Y., Nir, S., and Naim, M. (2000). Rapid entry of bitter and sweet tastants into liposomes and taste cells: Implications for signal transduction. Am. J. Physiol. Cell Physiol. *278*, C17–C25.
21. Reed, D.R., Tanaka, T., and McDaniel, A.H. (2006). Diverse tastes: Genetics of sweet and bitter perception. Physiol. Behav. *88*, 215–226.
22. Horne, J., Lawless, H.T., Speirs, W., and Sposato, D. (2002). Bitter taste of saccharin and acesulfame-K. Chem. Senses *27*, 31–38.
23. Wooding, S., Bufe, B., Grassi, C., Howard, M.T., Stone, A.C., Vazquez, M., Dunn, D.M., Meyerhof, W., Weiss, R.B., and Bamshad, M.J. (2006). Independent evolution of bitter-taste sensitivity in humans and chimpanzees. Nature *440*, 930–934.
24. Go, Y. (2006). Lineage-specific expansions and contractions of the bitter taste receptor gene repertoire in vertebrates. Mol. Biol. Evol. *23*, 964–972.