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Variation in the Human *TAS1R* Taste Receptor Genes

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Abstract

We have performed a comprehensive evaluation of single-nucleotide polymorphisms (SNPs) and haplotypes in the human *TAS1R* gene family, which encodes receptors for sweet and umami tastes. Complete DNA sequences of *TAS1R1*-, *TAS1R2*-, and *TAS1R3*-coding regions, obtained from 88 individuals of African, Asian, European, and Native American origin, revealed substantial coding and noncoding diversity: polymorphisms are common in these genes, and polymorphic sites and SNP frequencies vary widely in human populations. The genes *TAS1R1* and *TAS1R3*, which encode proteins that act as a dimer to form the umami (glutamate) taste receptor, showed less variation than the *TAS1R2* gene, which acts as a dimer with *TAS1R3* to form the sweet taste receptor. The *TAS1R3* gene, which encodes a subunit common to both the sweet and umami receptors, was the most conserved. Evolutionary genetic analysis indicates that these variants have come to their current frequencies under natural selection during population growth and support the view that the coding sequence variants affect receptor function. We propose that human populations likely vary little with respect to umami perception, which is controlled by one major form of the receptor that is optimized for detecting glutamate but may vary much more with respect to sweet perception.

Key words: evolution, human *TAS1R* genes, SNP, sweet taste, umami taste

Introduction

Molecular mechanisms of sweet perception have been elucidated by studies in rodents, which have identified taste receptors encoded by the *TAS1R* gene family (Bachmanov *et al.*, 2001; Kitagawa *et al.*, 2001; Max *et al.*, 2001; Montmayeur *et al.*, 2001; Nelson *et al.*, 2001; Sainz *et al.*, 2001). Humans carry three *TAS1R* (*T1R*) taste receptor genes in a single cluster on chromosome 1. The products of these genes are seven transmembrane domain G protein–coupled receptors that act as dimers, as *TAS1R2+3* to sense sugars, and as *TAS1R1+3* to perceive umami taste, a savory flavor exemplified by the taste of glutamate (Li *et al.*, 2002; Zhao *et al.*, 2003). The extent of variation in human *TAS1R* genes has not been well characterized, and potential functional consequences of such variation are unknown.

In contrast, substantial information has accumulated about variation in the *TAS2R* bitter receptor gene family. The *TAS2R* genes have been shown to be highly polymorphic (Wang *et al.*, 2004; Kim *et al.*, 2005), and both evolutionary genetic analyses (Wooding *et al.*, 2004) and biochemical activity assays (Bufe *et al.*, 2005; Soranzo *et al.*, 2005) have suggested that much of this DNA sequence variation results in altered receptor functions. In an effort to obtain a more complete understanding of genetic and functional variation in human taste perception, we have performed a survey of polymorphism in the three *TAS1R* genes. We have performed evaluations of the polymorphisms within these genes in worldwide populations and measured the frequency of the alleles and haplotypes of these genes in these populations. To address the question of the potential functional significance of these polymorphisms, we performed evolutionary genetic analyses to look for evidence of natural selection in the maintenance of this variation.

Materials and methods

Population samples

Human Genomic DNA was obtained from 88 unrelated individuals in eight different geographic populations, including 20 Cameroonians, 10 Northern Europeans, 10 Russians,

8 Pakistanis, 10 Hungarians, 10 Native Americans, 10 Chinese, and 10 Japanese (N = 176 chromosomes). All DNA samples except Cameroonian were purchased from Coriell Cell Repositories (http://locus.umdnj.edu/nigms/cells/humdiv.html).

Polymerase chain reaction and DNA sequencing

Single-nucleotide polymorphisms (SNPs) were discovered and assayed by sequencing genomic DNA, with sequence and genotypes assigned after sequencing both strands. Each of the six exons of all three TAS1R genes covering the coding region was amplified with the primers designed by software at the Primer3 Web site. Polymerase chain reaction (PCR) was performed in a total volume of 25 μl, containing 0.2 μM of each deoxynucleotide (Invitrogen), 15 pmol of each forward and reverse primers, 1.0–1.5 mM of $MgCl_2$, 10 mM Tris–HCl (pH 8.3), 50 mM KCl, 0.75 U of *Taq* DNA polymerase (PE Biosystems), and 100 ng of genomic DNA. PCR conditions (PE9700, PE Biosystems) were as follows: 35 cycles of denaturation at 94°C for 30 s; annealing at 55°C or 57°C, depending on the primers for 30 s; and extension at 72°C for 1 min. The first step of denaturation and the last step of extension were at 95°C for 2 min and 72°C for 10 min, respectively. Five microliters of the PCR products were separated and visualized in a 2% agarose gel. Fifteen microliters of this PCR product were then treated with 0.3 U of shrimp alkaline phosphatase (USB) and 3 U of exonuclease I (USB) at 37°C for 1 h, followed by incubation at 80°C for 15 min. This was diluted with an equal volume of dH_2O, and 6 μl was used for the final sequencing reaction. Sequencing reactions were performed in both directions on the PCR products in reactions containing 5 pmol of primer, 1 μl of Big Dye Terminator Ready Reaction Mix (PE Biosystems), and 1 μl of 5× dilution buffer (400 mM Tris–HCl, pH 9, and 10 mM $MgCl_2$). Cycling conditions were 95°C for 2 min and 35 cycles of 94°C for 20 s, 55°C for 20 s, and 60°C for 4 min. Sequencing reaction products were ethanol precipitated, and the pellets were resuspended in 10 μl of formamide loading dye. An ABI 3730 DNA sequencer was used to resolve the products, and data were analyzed by using ABI Sequencing Analysis (v. 5.0) and LASERGENE-SeqMan software.

Inference of haplotypes

Some haplotypes could be specified from genotypes of individuals, while other haplotypes were inferred. Haplotypes were inferred from unphased genotype data using the PHASE 2.0.2 computer program (Stephens *et al.*, 2001; Stephens and Donnelly, 2003).

Measures of genetic diversity

Levels and patterns of genetic diversity in each gene were measured using three statistics: π (the mean pairwise difference between sequences in a population sample), per nucleotide, S (the number of variable nucleotide positions in the sample), and F_{ST} (a measure of population differentiation). F_{ST} values were calculated using the method of Slatkin, treating the major continental regions as populations (Slatkin, 1991).

Because the *TAS1R* genes encode protein subunits that interact to form a functional receptor, we also estimated receptor diversity from a combinatorial standpoint, taking the different subunits into account. Here, diversity was measured as the expected total difference in amino acid sequence across both subunits, which is approximated estimated by the sum of the mean pairwise amino acid sequence differences within subunits.

Tests of evolutionary neutrality

The hypothesis of evolutionary neutrality was tested using Tajima's D statistic, which compares the mean number of nucleotide differences between sequences with the number of variable nucleotide positions in a population sample (Tajima, 1989). This test was originally designed for use in populations that have remained constant in size. However, several lines of evidence suggest that human population sizes have increased dramatically over the last 100,000 years. Such growth can have strong effects on tests of the D statistic.

Estimates of demographic parameters based on genetic data suggest that human populations have increased at least 100-fold over the last 75,000–100,000 years (Marth *et al.*, 1993; Rogers, 1995; Wall and Przeworski, 2000) The consensus of these studies is that ancient effective population sizes in humans were small—approximately 10,000. However, estimates of the time and magnitude of growth from that initial size vary substantially. For this reason, we used the DFSC program (Wooding *et al.*, 2004) to test the hypothesis of neutrality under a range of population histories. For each gene, Tajima's D test was performed under the assumption that the ancient effective population size in humans was 10,000, with the onset of population expansion ranging from 0 to 200,000 years before present and the magnitude of population expansion ranging from 0- to 500-fold. These were two-tailed tests, where P indicated the probability of observing a smaller value of D, given the population history parameters.

Results

DNA sequence variation

We identified variation in *TAS1R* genes by sequencing genomic DNA from different individuals. The polymorphisms observed in these genes are shown in Table 1.

Comparisons of aligned exonic sequences revealed 17 SNPs in *TAS1R1* (including 3 synonymous and 14 nonsynonymous variants), 18 SNPs in *TAS1R2* (8 synonymous and 10 nonsynonymous), and 12 SNPs in *TAS1R3* (6 nonsynonymous and 6 synonymous), for a total of 47 variant

Table 1 Details of SNPs within the human *TAS1R* genes

Gene	Chr	BAC clone	CDS	Size (bp)	Amino acid	dbSNP[a]	Exon	cSNP		Position of cSNP	Amino Acid encoded	Position in protein	Overall Allele frequency	Population-specific allele frequency							
														CAM	AME	NOR	JAP	RUS	HUN	CH	PAK
														n = 40	*n* = 20	*n* = 20	*n* = 20	*n* = 20	*n* = 20	*n* = 20	*n* = 16
TAS1R1	1	AL591866	BK000153	2526	841																
							2	c.C201 > T	C	201	Ser	67	Singleton	0.98	1	1	1	1	1	1	1
									T		Ser			0.02	0	0	0	0	0	0	0
							2	c.A284 > G	A	284	Asn	95	Singleton	1	1	1	1	1	1	1	0.94
									G		Ser			0	0	0	0	0	0	0	0.06
							2	c.C329 > T	C	329	Ala	110	Singleton	1	1	0.95	1	1	1	1	1
									T		Val			0	0	0.05	0	0	0	0	0
							2	c.C376 > A	C	376	His	126	0.99	0.95	1	1	1	1	1	1	1
									A		Asn		0.01	0.05	0	0	0	0	0	0	0
							2	c.T380 > C	T	380	Ile	127	0.98	1	1	1	1	1	1	0.85	1
									C		Thr		0.02	0	0	0	0	0	0	0.15	0
							3	c.T501 > C	T	501	Ile	167	0.98	1	1	1	1	1	1	0.85	1
									C		Ile		0.02	0	0	0	0	0	0	0.15	0
							3	c.C541 > G	C	541	Gln	181	0.99	0.95	1	1	1	1	1	1	1
									G		Glu		0.01	0.05	0	0	0	0	0	0	0
							3	c.A545 > G	A	545	Tyr	182	Singleton	1	1	1	1	1	1	1	0.94
									G		Cys			0	0	0	0	0	0	0	0.06
							3	c.A572 > G	A	572	Asn	191	0.98	1	1	1	1	1	1	0.8	1
									G		Ser		0.02	0	0	0	0	0	0	0.2	0
							3	c.A709 > C	A	709	Ile	237	0.98	1	1	1	1	1	1	0.85	1
									C		Leu		0.02	0	0	0	0	0	0	0.15	0
						rs10864628	3	c.G1039 > A	G	1039	Glu	347	0.95	0.78	1	1	1	1	1	1	1
									A		Lys		0.05	0.23	0	0	0	0	0	0	0
							3	c.G1114 > A	G	1114	Ala	372	0.83	0.9	1	0.75	0.65	0.95	0.65	0.6	0.94
									A		Thr		0.17	0.1	0	0.25	0.35	0.05	0.35	0.4	0.06
							3	c.C1117 > A	C	1117	His	373	Singleton	0.95	1	1	1	1	1	1	1
									A		Asn			0.05	0	0	0	0	0	0	0
							4	c.T1448 > C	T	1448	Ile	483	0.98	0.95	1	1	1	1	1	0.85	1
									C		Thr		0.02	0.05	0	0	0	0	0	0.15	0

Table 1 Continued

Gene	Chr	BAC clone	CDS	Size (bp)	Amino acid	dbSNP[a]	Exon	cSNP		Position of cSNP	Amino Acid encoded	Position in protein	Overall Allele frequency	Population-specific allele frequency							
														CAM	AME	NOR	JAP	RUS	HUN	CH	PAK
														n = 40	*n* = 20	*n* = 20	*n* = 20	*n* = 20	*n* = 20	*n* = 20	*n* = 16
							6	c.G1808 > A	G	1808	Arg	603	0.97	0.88	1	1	1	1	1	1	1
									A		His		0.03	0.12	0	0	0	0	0	0	0
							6	c.G2274 > A	G	2274	Glu	758	0.94	0.74	1	1	1	1	1	1	1
									A		Glu		0.06	0.26	0	0	0	0	0	0	0
							6	c.G2318 > A	G	2318	Trp	773	0.99	0.94	1	1	1	1	1	1	1
									A		Stop		0.01	0.06	0	0	0	0	0	0	0
TAS1R2	1	BX531760 (–)	BK000151	2520	839																
							1	c.C26 > G	C	26	Ser	9	0.25	0.25	0.3	0.4	0.4	0.35	0.4	0.35	0.14
									G		Cys		0.75	0.75	0.7	0.6	0.6	0.65	0.6	0.65	0.86
							1	c.C62 > T	C	62	Pro	21	Singleton	0.98	1	1	1	1	1	1	1
									T		Leu			0.02	0	0	0	0	0	0	0
		AL831755 (–)					3	c.A571 > G	A	571	Ile	191	0.75	0.71	0.7	0.78	0.85	0.56	0.75	1	0.69
									G		Val		0.25	0.29	0.3	0.22	0.15	0.44	0.25	0	0.31
							3	c.A634 > G	A	634	Ser	212	0.98	0.93	1	1	1	1	1	1	1
									G		Gly		0.02	0.07	0	0	0	0	0	0	0
							3	c.A700 > G	A	700	Ile	234	Singleton	0.93	1	1	1	1	1	1	1
									G		Val			0.07	0	0	0	0	0	0	0
						rs28470550	3	c.T882 > G	T	882	Thr	294	0.77	0.8	0.7	0.78	0.85	0.56	0.75	1	0.69
									G		Thr		0.23	0.2	0.3	0.22	0.15	0.44	0.25	0	0.31
							3	c.G949 > C	G	949	Gly	317	0.22	0.2	0.3	0.22	0.15	0.39	0.25	0	0.31
									C		Arg		0.78	0.8	0.7	0.78	0.85	0.61	0.75	1	0.69
							4	c.G1320 > A	G	1320	Pro	440	Singleton	1	1	1	1	1	0.95	1	1
									A		Pro			0	0	0	0	0	0.05	0	0
						rs9439751	4	c.G1344 > A	G	1344	Leu	448	Singleton	0.98	1	1	1	1	1	1	1
									A		Leu			0.02	0	0	0	0	0	0	0
						rs28374389	4	c.A1456 > G	A	1456	Ile	486	0.82	0.88	0.55	0.85	1	0.7	0.8	1	0.75
									G		Val		0.18	0.12	0.45	0.15	0	0.3	0.2	0	0.25

Table 1 Continued

Gene	Chr	BAC clone	CDS	Size (bp)	Amino acid	dbSNP[a]	Exon	cSNP		Position of cSNP	Amino Acid encoded	Position in protein	Overall Allele frequency	Population-specific allele frequency							
														CAM	AME	NOR	JAP	RUS	HUN	CH	PAK
														n = 40	*n* = 20	*n* = 20	*n* = 20	*n* = 20	*n* = 20	*n* = 20	*n* = 16
						rs11805253	6	c.C1719 > T	C	1719	Ala	573	0.95	0.85	1	1	1	1	0.89	1	1
									T		Ala		0.05	0.15	0	0	0	0	0.11	0	0
						rs6662276	6	c.G1720 > A	G	1720	Ala	574	0.93	1	1	0.94	0.75	1	0.89	0.94	0.88
									A		Thr		0.07	0	0	0.06	0.25	0	0.11	0.06	0.12
							6	c.A2065 > C	A	2065	Lys	689	Singleton	0.97	1	1	1	1	1	1	1
									C		Gln			0.03	0	0	0	0	0	0	0
							6	c.C2092 > T	C	2092	Leu	698	0.98	0.91	1	1	1	1	1	1	1
									T		Leu		0.02	0.09	0	0	0	0	0	0	0
							6	c.C2112 > T	C	2112	Pro	704	Singleton	1	1	0.94	1	1	1	1	1
									T		Pro			0	0	0.06	0	0	0	0	0
						rs12033832	6	c.C2319 > T	C	2319	Ser	773	0.66	0.68	0.75	0.55	0.65	0.88	0.65	0.4	0.75
									T		Ser		0.34	0.32	0.25	0.45	0.35	0.12	0.35	0.6	0.25
						rs12075191	6	c.C2370 > T	C	2370	Ile	790	0.93	1	1	0.9	0.75	1	0.9	0.95	0.88
									T		Ile		0.07	0	0	0.1	0.25	0	0.1	0.05	0.12
						rs9988418	6	c.G2513 > A	G	2513	Arg	838	0.96	0.87	1	1	1	1	0.9	1	1
									A		Lys		0.04	0.13	0	0	0	0	0.1	0	0
TAS1R3	1	AL139287 (+)	BK000152	2559	852																
							1	c.G13 > A	G	13	Ala	5	0.98	1	1	0.9	1	1	0.95	1	1
									A		Thr		0.02	0	0	0.1	0	0	0.05	0	0
							2	c.T284 > C	T	284	Leu	95	Singleton	0.98	1	1	1	1	1	1	1
									C		Pro			0.02	0	0	0	0	0	0	0
							3	c.G740 > A	G	740	Arg	247	0.94	0.75	1	1	1	1	1	1	1
									A		His		0.06	0.25	0	0	0	0	0	0	0
							3	c.G1099 > T	G	1099	Gly	367	0.99	0.95	1	1	1	1	1	1	1
									T		Cys		0.01	0.05	0	0	0	0	0	0	0
						rs3813210	3	c.C1248 > T	C	1248	Pro	416	0.88	0.58	1	1	0.85	1	1	0.95	1
									T		Pro		0.12	0.42	0	0	0.15	0	0	0.05	0

Table 1 Continued

Gene	Chr	BAC clone	CDS	Size (bp)	Amino acid	dbSNP[a]	Exon	cSNP		Position of cSNP	Amino Acid encoded	Position in protein	Overall Allele frequency	Population-specific allele frequency							
														CAM	AME	NOR	JAP	RUS	HUN	CH	PAK
														n = 40	*n* = 20	*n* = 20	*n* = 20	*n* = 20	*n* = 20	*n* = 20	*n* = 16
							4	c.G1323 > A	G	1323	Pro	441	0.77	0.88	1	1	1	0.95	1	1	1
									A		Pro		0.23	0.12	0	0	0	0.05	0	0	0
							4	c.C1362 > T	C	1362	Tyr	454	Singleton	0.97	1	1	1	1	1	1	1
									T		Tyr			0.03	0	0	0	0	0	0	0
							6	c.C1719 > T	C	1719	Leu	573	0.99	1	1	1	0.9	1	1	1	1
									T		Leu		0.01	0	0	0	0.1	0	0	0	0
							6	c.C1977 > T	C	1977	Phe	659	Singleton	1	1	1	0.95	1	1	1	1
									T		Phe			0	0	0	0.05	0	0	0	0
							6	c.G2203 > A	G	2203	Ala	735	0.94	0.75	1	1	1	1	1	1	1
									A		Thr		0.06	0.25	0	0	0	0	0	0	0
						rs307377	6	c.T2269 > C	T	2269	Cys	757	0.99	1	1	1	1	1	0.95	1	0.94
									C		Arg		0.01	0	0	0	0	0	0.05	0	0.06
							6	c.C2407 > T	C	2407	Leu	803	Singleton	1	1	1	1	0.94	1	1	1
									T		Leu			0	0	0	0	0.06	0	0	0

Chr, chromosome location; BAC clone, identifier of the bacterial artificial chromosome clone in the human genome assembly containing the *TAS1R* gene; CDS, cDNA sequence identifier; and singleton, variant allele was observed only once. Population designations: CAM, Cameroonian; AME, Amerindian; NOR, North American; JAP, Japanese; RUS, Russian; HUN, Hungarian; CH, Chinese; and PAK, Pakistani.

[a]SNP indentifier in the dbSNP public database (http://ncbi.nlm.nih.gov/SNP).

nucleotide and 30 variant amino acid sites. Thus, the majority (64%) of the changes are nonconservative and result in changes in the amino acids encoded at that position. Many of these sites are newly described (www.ncbi.nlm.gov/dbSNP). Examination of the distribution of polymorphisms across the different domains of the protein shows that most (77%) of the variant amino acid positions reside in the large predicted first extracellular domain of these three receptors. This domain is hypothesized to contain the ligand-binding site for carbohydrates and dipeptide sweeteners (Pin *et al.*, 2003; Xu *et al.*, 2004; Nie *et al.*, 2005). One SNP, which substitutes an A for the normal G at position 2318 in the *TAS1R1* cDNA sequence introduces a premature stop codon.

SNP population frequencies

The population frequencies of the *TAS1R* SNPs are summarized in Table 2.

The majority (68.%) of the SNPs in the *TAS1R* genes were observed in only one population, and all of the populations studied have at least one *TAS1R* SNP that was unique to that population. Only about half (47%) of the population-specific SNPs were uncommon within their population, with a minor allele frequency less than 10%. Only a few SNPs were widely distributed and observed in all populations, one in *TAS1R1* and two in *TAS1R2*.

TAS1R gene haplotypes

Haplotypes are the specific group of variant forms (alleles) present at polymorphic sites across a particular region of the genome. In humans, not all possible combinations of variant sites occur naturally. Instead, studies have shown that human genes typically exist in three to five major different haplotypes in most populations (The International HapMap Consortium, 2005). Haplotypes of the *TAS1R* genes are important because they determine the specific receptor proteins encoded by the different forms of these genes. We first enumerated haplotypes by evaluating individuals who were homozygous for all SNPs in each gene plus individuals who were heterozygous at one SNP, allowing explicit determination of two haplotypes. Across our entire sample, we explicitly observed a minimum of 11 haplotypes in *TAS1R1*, 17 haplotypes in *TAS1R2*, and 12 haplotypes in *TAS1R3*. As with the SNPs, African populations revealed the greatest haplotype diversity. Also as with the SNPs, some haplotypes were observed only within one population in our sample, although not all populations showed population-specific haplotypes.

Table 2 Population frequency of SNPs

Gene	Number of SNPs that were observed in only one population	Number of SNPs present in all populations surveyed
TAS1R1	16	1
TAS1R2	8	2
TAS1R3	8	0

Analysis of all genotypes using PHASE revealed 12 haplotypes in *TAS1R1*, 26 haplotypes in *TAS1R2*, and 13 haplotypes in *TAS1R3* (Tables 3, 4, and 5).

Minimum spanning trees were constructed to help visualize putative evolutionary relationships among haplotypes (Figure 1). Haplotype trees were similar for *TAS1R1* and *TAS1R3*, which were each characterized by a single common haplotype and several rare ones (Figure 1A,C). The minimum spanning tree relating *TAS1R2* haplotypes was different, characterized by a large number of rare haplotypes, with many found at similar frequencies (Figure 1B).

Measures of genetic diversity

We performed standard measures of genetic diversity to help determine how the variation we found in the *TAS1R* genes compares to that found in most human genes. The mean pairwise difference between sequences, per nucleotide (π) was highest in *TAS1R2* (2.76 nt/2520 nt = 0.110%), followed by *TAS1R1* (0.86 nt/2526 nt = 0.034%) and *TAS1R3* (0.62 nt/2559 nt = 0.024%). These values fall within the upper 95th and lower 5th percentiles of the distribution reported in a genome-wide analysis (Sachidanandam *et al.*, 2001), with the value for *TAS1R2* being much higher than average and the values for *TAS1R1* and *TAS1R3* lower than average. Another comparison with values for 3305 genes (Salisbury, 2003) revealed a similar pattern, with *TAS1R2* falling in the top 10th percentile and *TAS1R1* and *TAS1R3* falling in the lower 40th and 25th percentiles, respectively (Figure 2). Like π, S was highest in *TAS1R2* (n = 18), followed by *TAS1R1* (n = 17) and *TAS1R3* (n = 12).

F_{ST} values were highest in *TAS1R1* (F_{ST} = 0.160), followed by *TAS1R3* (F_{ST} = 0.068) and *TAS1R2* (F_{ST} = 0.019). Comparisons with the distributions reported for the 3305 genes by Salisbury (2003) along with values reported for ~25,000 SNPs by Akey *et al.* (2002) revealed that while F_{ST} in *TAS1R1* was slightly higher than the average across a large number of genes, F_{ST} was slightly lower than average in *TAS1R2* and *TAS1R3* which fell in the bottom 35th and 20th percentiles (Figure 2).

Comparisons across populations revealed that our continental samples differed substantially with respect to both nucleotide and amino acid diversity (Table 6).

In general, the patterns of diversity in the sample as a whole were reflected in the continental subsamples. For instance, *TAS1R2* was the most diverse gene both in the sample as a whole and in each subsample. Among the subsamples, Africa was the most diverse at all three loci. A preponderance of diversity in Africa is common and is generally attributed to a combination of the antiquity and substructuring of African populations (Tishkoff and Verrelli, 2003). Interestingly, while the Native American sample was the least

Table 3 Haplotypes of human *TAS1R01* gene

	Nucleotide position									
	1111122									
	22333555570114823									
	08278044703114071									
	14960115299478848									
	snnnnsnnnnnnnnsn	Population								
HT		Ca	Ch	Hu	Ja	NA	Eu	Pa	Ru	Total
1	CACCTTCAAAGGCTGGG	17	9	13	13	20	14	14	19	119
2	..............A.	0	0	0	0	0	1	0	0	1
3	..............AA.	0	0	0	0	0	0	1	0	1
4	............A....	1	0	0	0	0	0	0	0	1
5	...........A.....	3	0	0	0	0	0	0	0	3
6	........G..A.....	5	0	0	0	0	0	0	0	5
7	......G.........A	4	7	7	7	0	5	1	1	32
8	....CC..GC...C...	6	0	0	0	0	0	0	0	6
9	...A......A.....	0	1	0	0	0	0	0	0	1
10	..T..............	2	0	0	0	0	0	0	0	2
11	.G.....G.........	0	3	0	0	0	0	0	0	3
12	T.........A.A..A.	2	0	0	0	0	0	0	0	2
Total		40	20	20	20	20	20	16	20	176
Unique		6	2	0	0	0	1	1		10

HT, haplotype number; s, synonymous SNP; and n, nonsynonymous SNP. Nucleotide position of the SNP within the predicted coding sequence is indicated by number displayed vertically over each SNP position. Population designations: Ca, Cameroonian; Ch, Chinese; Hu, Hungarian; Ja, Japanese; NA, North American; Eu, European; Pa, Pakistani; and Ru, Russian.

Table 4 Haplotypes of human *TAS1R02* gene

	11111222222								
	5678933477001335								
	267308424512691171								
	621402905690522903								
	nnnnnsnssnsnnssssn	Population							
HT		Ca	Ch	Hu	Ja	NA	Eu	Pa	Ru	Total
1	GCGAAGGGGACGACCCCG	3	0	1	0	0	0	2	2	8
2	..............T..	3	0	2	0	7	1	2	3	18
3	...........A....T.	0	0	0	0	0	1	0	0	1
4	.....TC...T......A	1	0	0	0	0	0	0	0	1
5	..A..TC...........	1	0	0	0	0	0	0	0	1
6	..A..TC........T..	2	0	0	1	1	0	0	1	5
7	..A..TC......T.T..	1	0	0	1	3	2	0	0	7
8	..A..TC....A....T.	0	0	0	1	0	0	0	0	1
9	..A..TC...T......A	1	0	1	0	2	1	1	3	9
10	..A..TC..G........	0	0	1	0	0	0	0	0	1
11	..A..TC..G.....T..	0	0	0	0	0	0	0	1	1
12	..A.GTC......T.T..	0	0	2	0	0	1	1	1	5
13	.T...............	1	0	0	0	0	0	0	0	1
14	C................	2	3	3	1	0	4	0	2	15
15	C..............T..	0	0	1	0	0	0	0	0	1
16	C...........A....T.	0	0	0	0	0	1	0	0	1
17	C........G........	0	0	0	0	0	0	1	0	1
18	C.......A.G........	2	0	0	0	0	0	0	0	2
19	C.....C........T..	1	0	0	0	0	0	0	0	1
20	C....TC........T..	0	0	0	0	0	0	1	0	1
21	C.A..TC...........	2	0	0	0	0	0	0	0	2
22	C.A..TC....A....T.	6	4	1	6	5	3	4	5	34
23	C.A..TC....A..T.T.	8	12	5	6	2	5	3	2	43
24	C.A..TC..G........	2	0	0	0	0	0	0	0	2
25	C.AG.TC..G........	0	1	1	4	0	1	1	0	8
26	C.AG.TC.A...C.....	4	0	2	0	0	0	0	0	6
Total		40	20	20	20	20	20	16	20	176
Unique		7	0	2	1	0	2	2	1	15

HT, haplotype number; s, synonymous SNP; and n, nonsynonymous SNP. Nucleotide position of the SNP within the predicted coding sequence is indicated by number displayed vertically over each SNP position. Population designations: Ca, Cameroonian; Ch, Chinese; Hu, Hungarian; Ja, Japanese; NA, North American; Eu, European; Pa, Pakistani; and Ru, Russian.

diverse with respect to *TAS1R1* and *TAS1R3*, it was the second most diverse with respect to *TAS1R2*.

The combinatorial analysis of diversity confirmed that the majority of variation in the sweet and umami receptors is accounted for by *TAS1R1* and *TAS1R2*, as opposed to *TAS1R3*, which is more conserved. For example, although the Native American sample was the least diverse with respect to *TAS1R3*, it was the second most diverse with respect to *TAS1R2* and was thus the second most diverse with respect to overall variation in the sweet receptor (*TAS1R2* + *TAS1R3*).

Tests of evolutionary neutrality

Evolutionary genetic analysis can be used to distinguish genetic variation that has been influenced by natural selection (and thus is biologically functional) from other kinds of variations. Such analyses test for statistically significant deviation from evolutionarily neutral genetic drift. Tests of Tajima's *D* statistic were used to examine the hypothesis of evolutionary neutrality for the sequence variants observed in the *TAS1R* genes. We tested our hypotheses under several different models that account for different scenarios of population growth. The hypothesis of evolutionary

Table 5 Haplotypes of human *TAS1R03* gene

	Nucleotide position 111111222 27023379227 184942617060 340983297394 nnnnsssssnns	Population								
HT		Ca	Ch	Hu	Ja	NA	Eu	Pa	Ru	Total
1	GTGGCGCCCGCC	11	19	18	16	20	18	15	18	135
2	..........T	1	0	0	0	0	0	0	0	1
3	.........T.	10	0	0	0	0	0	0	0	10
4	........T...	1	0	0	0	0	0	0	0	1
5	......T.....	0	0	1	0	0	2	0	0	3
6	....T.....	0	0	0	0	0	0	0	1	1
7	....T..T....	0	0	1	0	0	0	1	0	2
8	....TA.....	0	0	0	1	0	0	0	0	1
9	...T.....	0	0	0	0	0	0	0	1	1
10	...T..T.....	13	1	0	1	0	0	0	0	15
11	..A.....A..	0	0	0	2	0	0	0	0	2
12	.C..TA.....	3	0	0	0	0	0	0	0	3
13	A.....	1	0	0	0	0	0	0	0	1
Total		40	20	20	20	20	20	16	20	176
Unique		5	0	0	2	0	0	0	1	8

HT, haplotype number; s, synonymous SNP; and n, nonsynonymous SNP. Nucleotide position of the SNP within the predicted coding sequence is indicated by number displayed vertically over each SNP position. Population designations: Ca, Cameroonian; Ch, Chinese; Hu, Hungarian; Ja, Japanese; NA, North American; Eu, European; Pa, Pakistani; and Ru, Russian.

neutrality of the variation in *TAS1R2* was rejected under realistic assumptions about population growth. For example, under the assumption that the human population size increased 100-fold, 100,000 years ago, the hypothesis of evolutionary neutrality was strongly rejected ($P > 0.99$). For *TAS1R1* and *TAS1R3*, the results were less striking but still similar. Under slightly larger magnitudes of growth (e.g., 200-fold, 100,000 years ago), the hypothesis of neutrality was rejected for *TAS1R1* and *TAS1R3* as well, and thus all three observed values of *D* were significantly greater than expected. We note, however, that the comparison of *D* values in these genes with values calculated for roughly 3300 genes reported by Salisbury *et al.* (2003) suggests that these values fall within the expected range, although the value for *TAS1R2* is somewhat higher than average. We hypothesize that such general conclusions regarding evolutionary neutrality using Tajima's *D* measures may reflect selective sweeps that have occurred commonly in human evolutionary history, and our Tajima's *D* measurements may indicate that such selective sweeps have occurred in the *TAS1R* genes.

Discussion

Our survey has revealed significant nucleotide and protein sequence diversity in the *TAS1R* taste receptor family. This information is important for understanding receptor function as different haplotypes encode significantly different proteins, and these protein subunits may, in turn, interact to further shape taste perception in human individuals. The different forms of *TAS1R2*, which encodes the sweet-specific component of the receptor, are unusually diverse compared to other human genes. These differences appear unlikely to be evolutionarily neutral, and we predict that they underlie interindividual variation in sensitivity to sweet compounds.

The site of amino acid variation in the *TAS1R* receptor gene family is quite dissimilar from that in the *TAS2R* bitter receptor gene family (Kim *et al.*, 2005). In the *TAS2R* family, much of the variation occurs in the transmembrane domains, and little exists in the first extracellular domain. Conversely, the *TAS1R*s carry the majority of amino acid sequence variation in their first extracellular domain. Because *TAS1R* receptors are thought to bind carbohydrate ligands in their large first extracellular domain while *TAS2R* receptors are thought to bind ligands in their transmembrane domains, we hypothesize that the amino acid sequence diversity in these receptors is related to their carbohydrate recognition and binding functions. Several structure–function studies and molecular modeling studies have suggested possible ligand-binding sites within *TAS1R* proteins (Nofre 2001; Jiang *et al.*, 2004, 2005; Morini *et al.*, 2005). For example, the binding of the artificial sweetener cyclamate to the *TAS1R3* subunit requires extracellular domain 3 and/or transmembrane domain 7 of this protein (Jiang *et al.*, 2005). Since none of the SNPs described here reside in these regions, we would predict that they would not result in differences in cyclamate perception in the population.

One important source of variation in protein structure, alternative splicing of the mRNA, has been reported for *TAS1R1* (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene&cmd=Retrieve&dopt=full_report&list_uids=80835). Neither did we observe any DNA sequence variants in this gene that might account for this alternative splicing nor did we observe any variants at or near splice sites in *TAS1R2* and *TAS1R3*.

It is not surprising that among the human *TAS1R* genes, *TAS1R3* reveals a relatively smaller degree of diversity. This gene encodes the subunit that is common to both sweet and umami tastes and thus is likely constrained by the requirement of maintaining functional interactions with both the *TAS1R1* and *TAS1R2* receptor proteins. *TAS1R1* is intermediate in the number of nonsynonymous SNPs and in the values of π and *S*. *TAS1R2* showed high levels of *S*, the pairwise differences between sequences, placing this level of variation in the top 5–10% of all human genes surveyed, depending on the study used for comparison.

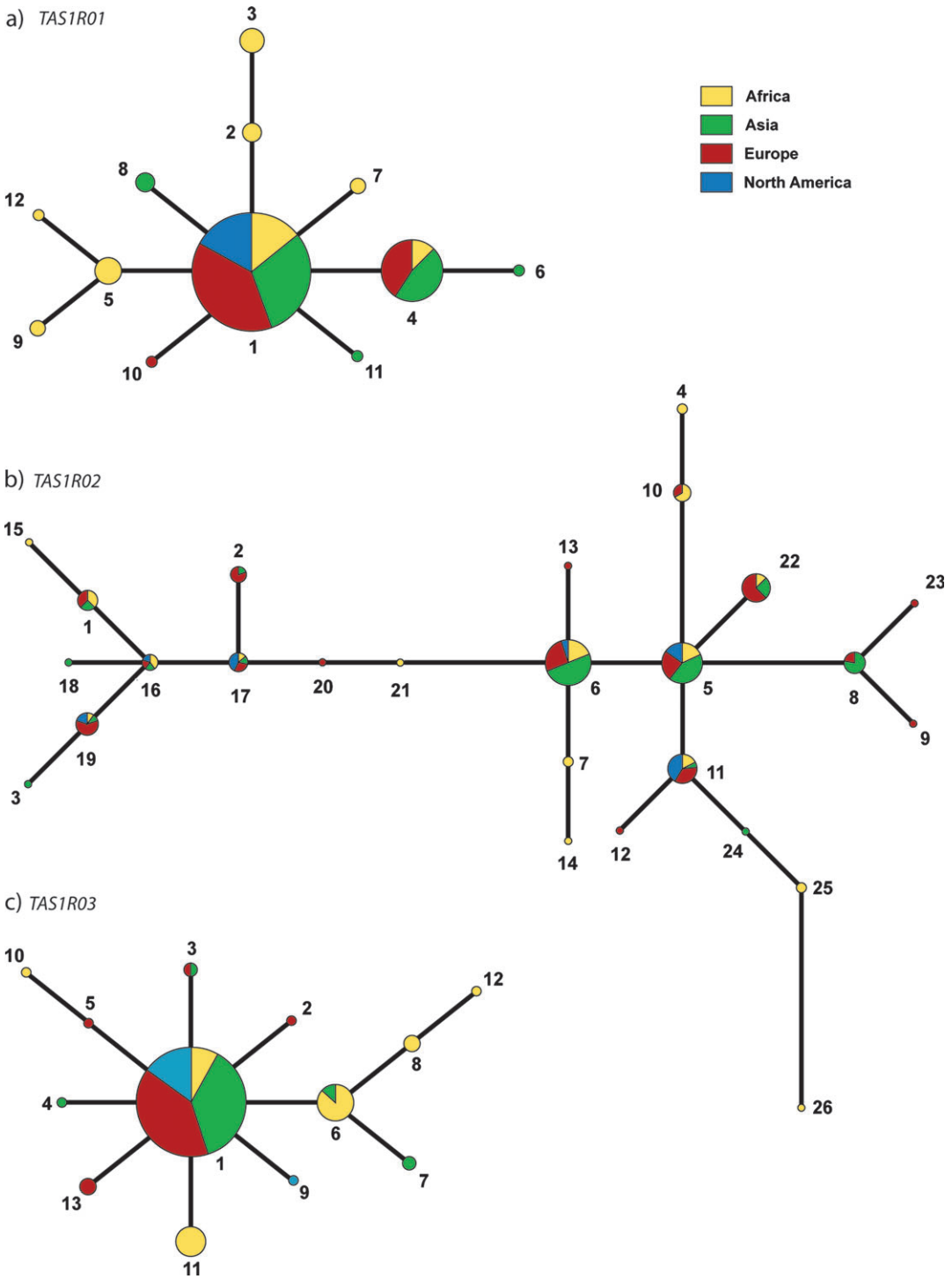

Figure 1 Minimum haplotype spanning trees for the *TAS1R* genes. Each circle represents a haplotype, and the size of each circle represents the haplotype's relative frequency. Haplotypes are numbered according to the numbers assigned to haplotypes in Tables 3, 4, and 5. Within a circle, shading indicates the fraction of observations in the populations indicated. Each connection between haplotypes corresponds to one nucleotide substitution.

The high levels of diversity observed in *TAS1R2*, including the presence of eight nonsynonymous nucleotide substitutions, are notable. The minimum spanning tree for this gene reveals many different haplotypes that are present at low to moderate frequency (Figure 1B). This pattern of variation, in conjunction with the rejection of evolutionary neutrality by Tajima's *D* test, suggests that human sweet taste perception mediated by this gene may have evolved to sense a wide variety of structurally different sweet substances.

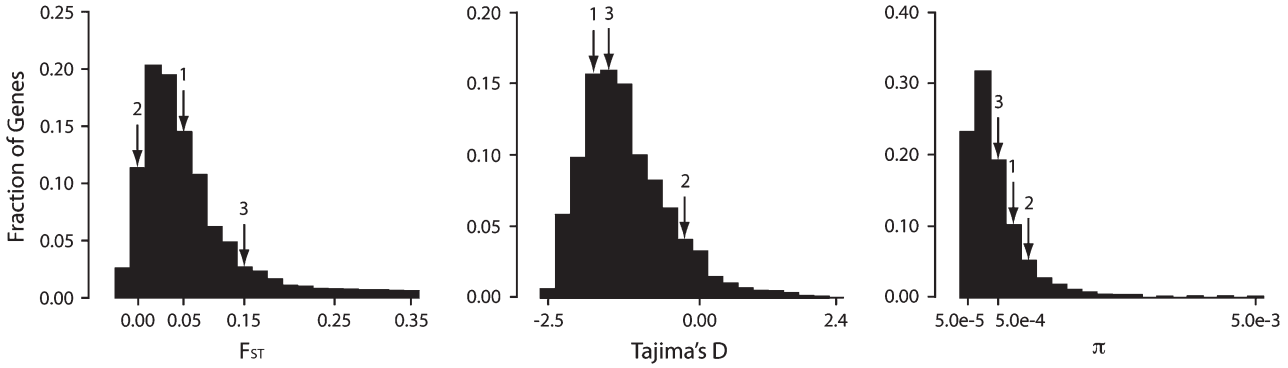

Figure 2 Observed F_{ST}, Tajima's *D*, and π values for human TAS1R genes. Numbered arrows indicate the respective values of each measure within distributions of each value reported by Akey *et al.* (2002). 1 = value for *TAS1R1*, 2 = value for *TAS1R2*, and 3 = value for *TAS1R3*.

Variation in *TAS1R* genes has recently been compared to variation in *TAS2R* genes across the phylogenetic spectrum (Shi and Zhang, 2006). This study examined a number of different evolutionary genetic parameters from those examined in our study and concluded that variation in the *TAS1R* gene family has been under positive natural selection. This conclusion is in agreement with our results and suggests that the variants we observe are functionally significant in taste perception.

Whatever the role of selection, the prevalence of amino acid variation in the *TAS1R* genes raises numerous questions about the patterns of phenotypic variance that might be associated with these variants. Although heritable interstrain differences in sweet taste sensitivity have been documented in mice (Reed *et al.*, 2004), little is known about heritable interindividual variation of sweet sensitivity in humans. The few surveys reported to date indicate that thresholds to sugars exhibit variability in the population (Blakeslee and Salmon, 1935; Pangborn, 1970, 1981), although no systematic surveys across different racial or ethnic groups have been reported.

Table 6 Nucleotide and amino acid diversity in continental populations

	Nucleotide	Per nucleotide	Amino acid	Per amino acid
TAS1R1				
Africa	1.51	-5.97×10^{-4}	1.11	-1.32×10^{-3}
Asia	1.04	-4.12×10^{-4}	0.93	-1.10×10^{-3}
Europe	0.38	-1.50×10^{-4}	0.38	-4.50×10^{-4}
North America	0	0	0	0
TAS1R2				
Africa	3.17	-1.30×10^{-5}	1.94	-2.31×10^{-3}
Asia	2.15	-8.53×10^{-4}	1.16	-1.38×10^{-3}
Europe	2.93	-1.20×10^{-5}	1.81	-2.15×10^{-3}
North America	2.68	-1.10×10^{-5}	1.85	-2.20×10^{-3}
TAS1R3				
Africa	1.65	-6.45×10^{-4}	0.92	-1.07×10^{-3}
Asia	0.27	-1.06×10^{-4}	0.04	-4.68×10^{-5}
Europe	0.19	-7.40×10^{-5}	0.13	-1.50×10^{-5}
North America	0	0	0	0

Columns indicate mean pairwise difference, within populations, with respect to nucleotide and amino acid sequences. Values in parentheses indicate values normalized for sequence length.

Our findings suggest that substantial amino acid variation is present in all three *TAS1R* genes. Variation in these genes is localized to domains hypothesized to be involved in ligand binding. Further, this variation is not distributed uniformly among human populations. Given our findings, we hypothesize that 1) human populations will harbor more heritable variation in sweet taste sensitivity than in umami taste sensitivity, 2) human populations will differ appreciably in heritable variation, with Africans being most diverse, and 3) *TAS1R3*, while less than diverse than other *TAS1R* genes, may be an important source of covariance in sweet and umami sensitivity by virtue of the fact that it harbors amino acid substitutions that could affect both phenotypes.

Recent studies in cats have shown that the Tas1R2 gene is a pseudogene (Li *et al.*, 2005), consistent with the observation that this species appears to be insensitive to sweet substances. While we did not observe any variants in human *TAS1R2* that resulted in stop codons, we did observe one such SNP in *TAS1R1*. This SNP, which converts a codon encoding tryptophan to a stop, was observed solely in African populations. The known variation in human umami taste sensitivity commonly occurs in non-African populations (Lugaz *et al.*, 2002). So, while the stop codon in *TAS1R1* is unlikely to be the cause of insensitivity to glutamate, other SNPs in this gene remain as candidates for the source of this trait in humans.

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