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Genetic Variation in the Odorant Receptor *OR2J3* Is Associated with the Ability to Detect the ''Grassy'' Smelling Odor, *cis*-3-hexen-1-ol

Jeremy F. McRae[1,*], Joel D. Mainland[2,5,*], Sara R. Jaeger[1], Kaylin A. Adipietro[2], Hiroaki Matsunami[2] and Richard D. Newcomb[1,3,4]

[1]Food Innovation, The New Zealand Institute for Plant & Food Research Limited, Private Bag 92169, Auckland 1142, New Zealand, [2]Department of Molecular Genetics and Microbiology, Duke University Medical Center, 261 CARL Building, Box 3509 DUMC, Durham, NC 27710, USA, [3]School of Biological Sciences, University of Auckland, Private Bag 92019, Auckland 1142, New Zealand and [4]Allan Wilson Centre for Molecular Ecology and Evolution, Centre of Research Excellence, New Zealand
[5]Present address: Monell Chemical Senses Center, 3500 Market Street, Philadelphia, PA 19104, USA

Correspondence to be sent to: Richard D. Newcomb, The New Zealand Institute for Plant & Food Research Limited, Private Bag 92169, Auckland 1142, New Zealand. e-mail: Richard.Newcomb@plantandfood.co.nz

*These authors contributed equally to the work.

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Abstract

The ability to detect many odors varies among individuals; however, the contribution of genotype to this variation has been assessed for relatively few compounds. We have identified a genetic basis for the ability to detect the flavor compound *cis*-3-hexen-1-ol. This compound is typically described as "green grassy" or the smell of "cut grass," with variation in the ability to detect it linked to single nucleotide polymorphisms (SNPs) in a region on human chromosome 6 containing 25 odorant receptor genes. We have sequenced the coding regions of all 25 receptors across an ethnically mixed population of 52 individuals and identified 147 sequence variants. We tested these for association with *cis*-3-hexen-1-ol detection thresholds and found 3 strongly associated SNPs, including one found in a functional odorant receptor (rs28757581 in *OR2J3*). In vitro assays of 13 odorant receptors from the region identified 3 receptors that could respond to *cis*-3-hexen-1-ol, including *OR2J3*. This gene contained 5 predicted haplotypes across the 52 individuals. We tested all 5 haplotypes in vitro and several amino acid substitutions on their own, such as rs28757581 (T113A). Two amino acid substitutions, T113A and R226Q, impaired the ability of OR2J3 to respond to *cis*-3-hexen-1-ol, and together these two substitutions effectively abolished the response to the compound. The haplotype of *OR2J3* containing both T113A and R226Q explains 26.4% of the variation in *cis*-3-hexen-1-ol detection in our study cohort. Further research is required to examine whether *OR2J3* haplotypes explain variation in perceived flavor experience and the consumption of foods containing *cis*-3-hexen-1-ol.

Key words: *cis*-3-hexen-1-ol, genetic association, odor, odorant receptor, threshold of detection

Introduction

Aroma compounds make up a significant component of overall flavor of food and beverages. However, for some aromas, there is substantial variation among individuals in their ability to detect them (Brown et al. 1968; Stevens et al. 1988), with some individuals unable to detect the compound at all. These cases are known as smell blindness or specific anosmias (Amoore 1977; Lison et al. 1980; Wysocki and Beauchamp 1984). Considering the range of variation in the ability to detect some aroma compounds, there are still very few cases where genetic variation has been linked to variation in olfactory detection (Keller et al. 2007; Menashe et al. 2007; Eriksson et al. 2010). These instances include the boar taint, androstenone, the sweaty-smelling compound isovaleric acid, and the metabolites eliminated in the urine after consumption of asparagus.

Detection thresholds for the steroidal derivative androstenone have been linked to 2 substitutions within the odorant

receptor OR7D4, R88W, and T113M, which both impair the ability to detect the compound (Keller et al. 2007). Individuals that were heterozygous at both these variants were less likely to find androstenone ''sickening'' and more likely to attribute the compound as smelling like ''vanilla'' than individuals that were homozygous for the more sensitive haplotype. In the case of isovaleric acid, detection thresholds for the compound were linked to a segregating pseudogene, OR11H7P (Menashe et al. 2007). However, the same study was unable to find significant associations between detection thresholds for isoamyl acetate, L-carvone and cineole and variation within odorant receptor genes. Finally, the ability to detect metabolites of asparagus has been linked to variation within a large family of odorant receptors on chromosome 1 using a genome-wide association approach and a questionnaire-based phenotyping method (Eriksson et al. 2010). Pelchat et al. (2011) successfully replicated this association using a more rigorous two-alternative forced choice test. The causal variant and exact OR likely to be responsible are yet to be identified however.

This small number of cases is somewhat surprising given the large size of the olfactory receptor repertoire in humans, comprising approximately 400 intact genes (Olender et al. 2008) and the amounts of observed variation within these genes, particularly associated with segregating pseudogenes (Gilad et al. 2000). Furthermore, there is little work reporting links between genetic variation and flavor perception, and it remains to be shown whether genetic variation that underpins sensory acuity for a flavor compound is also associated with acceptability and liking for foods and beverages where the compound is a key flavor note.

Here, we investigate the genetic basis of the ability to detect the ''green grassy'' smelling compound, *cis*-3-hexen-1-ol (C3HEX). Variation in the ability to detect C3HEX is normally distributed, spanning 2 orders of magnitude (Jaeger et al. 2010), and as such, is not considered a specific anosmia. C3HEX is found in a wide range of foods and beverages and is a key flavor in many fresh fruits (Genovese et al. 2004) and vegetables (Jirovetz et al. 2002), such as raspberries (Klesk et al. 2004), broccoli (Forney and Jordan 1998), and beverages, including white wines (Reynolds et al. 1994). Furthermore, C3HEX is widely used as an added flavor in processed food to provide a fresh grassy note. In plants, C3HEX and related acetate esters are produced from linolenic acid, often in response to wounding; the classic example being the smell generated when grass is cut. Many insects can detect C3HEX and are attracted by the compound (James 2005). Finally, more recent evidence, mainly from rodent models, suggests that C3HEX, sometimes together with the green odor *trans*-2-hexen-1-al, may reduce stress and anxiety through modulating levels of biogenic amines in the brain (Tokumo et al. 2006).

Previously, we reported that detection threshold concentrations for C3HEX varied across almost 2 orders of magnitude (0.3–19.2 ppm) in a cohort of 48 participants of mixed sex and ethnicity (Jaeger et al. 2010). Using a genome-wide association (GWA) approach, we identified a region on chromosome 6 that was associated with detection thresholds for the compound and overlapped with a cluster of 25 odorant receptor genes (Jaeger et al. 2010). Here, we report the investigation of all sequence variants within the coding regions of all 25 odorant receptor genes within the cluster across all the participants of the original study. We go on to identify the receptors within the clusters that are able to detect C3HEX and then the causal variants within the odorant receptor, OR2J3, that affect sensitivity to C3HEX.

Materials and methods

Participants and detection threshold testing

Fifty-two voluntary participants took part in the research, which included 48 from the original Jaeger et al. (2010) study. All were employees at the Mt Albert campus of Plant & Food Research (then HortResearch) in Auckland, New Zealand. The sample of 52 was of mixed gender (38% male), age (22–53 years old: mean = 35.4 years old, standard deviation [SD] = 8.1 years old), and ethnicity. The majority of the participants were Caucasian (73.6%), with Indian (13.2%), Asian (11.3%), and Maori (1.9%) also present. None of the participants were related at the first or second degree.

Potential participants with general anosmia and existing medical conditions were screened out of the study. Immediately prior to threshold testing participants suffering from upper respiratory tract infections were asked to return when the infection had cleared. Total time commitment was 2–3 h, with movie tickets, wine, and chocolate used as incentives. The study was approved by the New Zealand Northern Y Regional Ethics Committee (Ref: NYT/07/08/092).

Individual orthonasal detection thresholds for C3HEX in water were estimated using the R-index method as described in Jaeger et al. (2010).

Sequencing ORs

The coding sequence of genes encoding odorant receptors and ~50 to 100 bp of flanking sequence were amplified by polymerase chain reaction (PCR) from genomic DNA extracted from blood samples provided by the 52 participants, including the 48 participants reported previously (Jaeger et al. 2010) plus 4 additional participants for which genotype data were not available at the time of the previous study. PCRs contained 1× Platinum *Taq* polymerase buffer (Invitrogen), 2.5 mM $MgCl_2$, 200 nM dNTPs, 500 nM forward and reverse primers, 0.05 U Platinum *Taq* DNA polymerase (Invitrogen), and 80 ng genomic DNA. Primer sequences used for amplifying and sequencing the odorant receptor genes are provided in Supplementary Table 1. The amplicons were sequenced bidirectionally by Sanger sequencing.

Sequence chromatograms were imported into Geneious (Drummond et al. 2011) and built into contigs for each sample. The consensus sequences from the contigs were aligned

using Muscle (Edgar 2004). The population alignments were exported as FASTA files and phased using Phase in DnaSP Version 5.10.00 (Librado and Rozas 2009). The phased alignments were exported as NEXUS formatted files. Variants identified in the population alignments were mapped to rsIDs (dbSNP build 132). Genotypes were exported from alignments and converted to pedigree format.

Genetic analysis

Summary statistics and dN/dS rates were determined with DnaSP Version 5.10.00 (Librado and Rozas 2009). Association testing was carried out with PLINK (Purcell et al. 2007). The genotypes obtained by sequencing were merged with the Affymetrix SNP6 genotypes derived previously (Jaeger et al. 2010). All variants were tested for association with the $\log_{10}$ transformed C3HEX detection thresholds using the Wald test assuming an additive quantitative trait. No corrections were made of gender, ethnicity, or any other form of population stratification. Reported r^2 values are the adjusted coefficients of determination. Linkage disequilibrium was calculated using HaploView 4.2 (Barrett et al. 2005). Box plots of threshold concentration for C3HEX by *OR2J3* genotype were constructed in R (R Development Core Team 2011). The allele and haplotype frequencies of HapMap populations were obtained from the 1000 Genomes phase 1 June 2011 genotype data release (The 1000 Genomes Project Consortium 2011).

Cell assays

Odorant receptors were subcloned into pCI expression vectors (Promega) with the first 20 amino acids of human rhodopsin as an N-terminal fusion. The sequences of receptors were verified by Sanger sequencing (3100 Genetic Analyzer; ABI Biosystems). The Dual-Glo Luciferase Assay System (Promega) was used for the luciferase assays (Zhuang and Matsunami 2008). CRE-luciferase (Stratagene) was used to assess receptor activation, and Renilla luciferase driven by an SV40 promoter was used as an internal control. Hana3A cells were plated on poly-D-lysine–coated 96-well plates (Nunc). Receptors were transfected into Hana3A cells along with 5 ng/well of RTP1S, 10 ng/well of CRE-luciferase, 5 ng/well of pRL-SV40, 2.5 ng/well of M3 muscarinic receptor (Li and Matsunami 2011), and 5 ng/well of the odorant receptor using Lipofectamine2000 (Invitrogen). Approximately 24 h post-transfection, the medium was replaced with 25 μL of odorant solution diluted in CD293 and incubated for 4 h at 37 °C and 5% CO_2. We followed the manufacturer's protocols for measuring luciferase and Renilla activities. Luminescence was measured using a Polarstar Optima plate reader (BMG). We tested the concentration response by applying 7 concentrations of C3HEX in triplicate, ranging from 10 nM to 10 mM. We fitted the resulting data with a three-parameter logistic model. We counted an odorant as activating a receptor if the 95% confidence intervals of the top and bottom parameter did not overlap, the SD of the fitted log EC_{50} was less than 1 log unit, and the extra sums of squares test confirmed that the odorant activated the receptor significantly more than the vector-only transfected control. Data were analyzed with Microsoft Excel and GraphPad Prism 4.

Results

Association studies

We hypothesized that variation in the coding region(s) of odorant receptor(s) activated by C3HEX causes functional and perceptual differences in the compound's detection. We first sought to identify all variants in the coding regions of all 25 odorant receptor genes that make up the cluster of odorant receptors around rs9295791 because previous polymorphisms that have been found to impact olfactory detection thresholds have been identified as nonsense or missense mutations within the coding region of olfactory receptor genes (Keller et al. 2007; Menashe et al. 2007). Across the cluster, 10 of the 25 genes have been classified as pseudogenes and an additional 4 as segregating pseudogenes (both the functional and pseudogenized alleles are present in the population) (Safran et al. 2003). Across the sequences of the 25 genes over the approximately 104 chromosomes from our participants, we identified 147 sequence variants, of which 144 were single nucleotide polymorphisms (SNPs) and the remaining 3 variants were insertion/deletion polymorphisms (indels). We detected both the intact and pseudogenized allele for 3 of the 4 segregating pseudogenes in our population (2 SNPs, rs2394517 and rs2073153, and 1 indel, rs66589491, but not the low-frequency SNP, rs17184009). Approximately, two-thirds of the SNPs were nonsynonymous (Table 1).

We then tested for association between the 144 SNPs within the coding regions, combined with 136 SNPs from the previous study across the odorant receptor cluster, and the threshold concentrations for the ability to detect C3HEX (Figure 1). Three SNP variants within odorant receptor genes in the cluster, rs72863513, rs7766902, and rs28757581, were strongly associated with C3HEX detection threshold concentrations (Supplementary Table 1), all of which are in high linkage disequilibrium with each other (r^2 = 0.759–0.918). Of these, rs28757581, which encodes a T113A substitution in OR2J3 (NM_001005216.2), is the only strongly associated variant that lies within a functional odorant receptor. The other 2 associated variants fall in the coding regions of the pseudogenes OR2P1P and OR2U2P but are not predicted to restore function. OR2U2P is missing 3 of the 7 transmembrane domains due to a nonsense mutation, and OR2P1P, which has homology with the TM7 domain of odorant receptors, has several stop codons earlier in same reading frame. The variants found in these genes do not return either gene to an intact protein in any of the tested subjects. Most of the odorant receptor genes in the cluster did not contain strongly associated variants, suggesting that

Table 1 Summary statistics for odorant receptor sequences within the chromosome six cluster

Gene	*n*	Sites	*S*	Eta	Hap	Hd	Pi	d*S*	d*N*	d*N*/d*S*
OR10C1	104	928	17	18	12	0.705	0.001507	5	12	2.4
OR11A1	104	947	6	6	6	0.214	0.000307	0	6	—
OR12D1P	104	945	10	10	5	0.393	0.000686	2	8	4
OR12D2	104	921	9	9	6	0.591	0.003476	1	8	8
OR12D3	104	951	5	5	6	0.727	0.001121	3	2	—
OR2AD1P	102	952	8	8	9	0.671	0.000989	4	4	1
OR2B3	104	939	4	4	5	0.112	0.000122	1	3	3
OR2B4P	104	954	1	1	2	0.486	0.000510	1	0	0
OR2G1P	104	991	2	2	3	0.111	0.000130	0	2	—
OR2H1	104	951	4	4	5	0.214	0.000233	2	2	1
OR2H2	104	939	4	4	5	0.734	0.001324	1	3	3
OR2H4P	102	921	1	1	2	0.502	0.000545	0	1	—
OR2H5P	104	937	6	7	8	0.368	0.000503	3	4	1.3
OR2I1P	104	972	9	9	8	0.572	0.001090	7	2	0.3
OR2J1P	102	933	9	9	8	0.678	0.002400	3	6	2
OR2J2	102	939	8	8	8	0.712	0.002574	2	6	3
OR2J3	104	936	5	5	6	0.695	0.001431	1	4	4
OR2J4P	104	933	6	6	6	0.709	0.002542	1	5	5
OR2N1P	104	934	4	4	5	0.305	0.000345	3	1	0.3
OR2P1P	102	735	6	6	7	0.753	0.002096	1	5	5
OR2U1P	104	1400	7	7	7	0.747	0.001218	1	6	6
OR2U2P	104	945	5	6	7	0.712	0.001044	n/a	n/a	—
OR2W1	104	960	4	4	6	0.431	0.000690	1	3	3
OR5U1	104	963	3	3	4	0.532	0.000624	1	2	2
OR5V1	102	963	4	4	4	0.475	0.000568	2	2	1
Total			144	147				46	97	

n, number of chromosomes; sites, number of nucleotides; *S*, number of polymorphic sites; Eta, number of mutations; Hap, number of haplotypes; Hd, haplotype diversity; Pi, nucleotide diversity; d*S*, number of synonymous sites; d*N*, number of nonsynonymous sites;d*N*/d*S*, the ratio of synonymous over nonsynonymous sites; and n/a, not applicable.

these genes either do not contain variation that affects the ability to detect C3HEX or that these odorant receptor genes do not play a major role in detecting C3HEX.

In vitro studies

In a complementary approach, we examined the 13 intact receptors in the cluster surrounding rs9295791 for their ability to respond to C3HEX in vitro. We tested 29 clones that represent 70% of the unique protein variants seen in the 1000 Genomes data (The 1000 Genomes Project Consortium 2011) for the 13 receptors. Each clone was tested for the ability to respond to C3HEX. Three odorant receptors responded to the compound in a concentration-dependent manner, OR2W1, OR2J2, and OR2J3 (Figure 2A); however, we cannot rule out the possibility that the other receptors are not functional in our assay. None of the variants within *OR2W1* or *OR2J2* was significantly associated with the ability to detect C3HEX in vivo. The 5 haplotypes of *OR2J3* found in the subject population showed varying responses to C3HEX. Two variants, rs28757581 (T113A) and rs3749977 (R226Q), had a reduced response to C3HEX. When both variants were present in the same haplotype, the response to C3HEX was abolished (Table 2; Figure 2B). The 2 amino acid substitutions in the nonresponsive haplotype, T113A and R226Q, are predicted to be located at different regions within OR2J3 (Figure 3). T113A lies within the predicted third transmembrane helix, whereas R226Q lies within the predicted third intracellular loop region. In live-cell staining, all haplotypes except for one (Hap3), which contains the R226Q variant, showed low levels of cell-surface expression (Supplementary Figure 1). These data suggest that only small amounts of receptor are required at the cell surface to produce responses by C3HEX in this luciferase assay and that in this case, the observed variation in C3HEX-induced receptor activation in the different haplotypes is not related to levels of cell-surface expression.

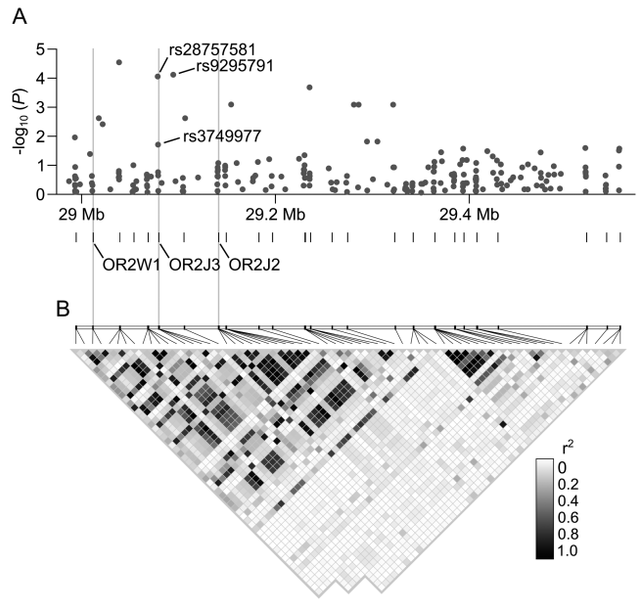

Figure 1 Plot of associations for selected SNP along the cluster of 25 odorant receptors at position 28.9–29.4 Mb along chromosome 6 (**A**). The locations of the odorant receptor genes are shown below the association plot. A full list of association statistics for all SNPs in the region are provided in Supplementary Table 2. Plot of linkage disequilibrium scores among variants with minor allele frequencies greater than 0.05 within the coding regions of the 25 odorant receptors (**B**). The linkage disequilibrium between pairs of variants is shown as squares shaded by r^2, with r^2 values of zero shaded white and higher r^2 values shaded as gray through to black for full linkage disequilibrium.

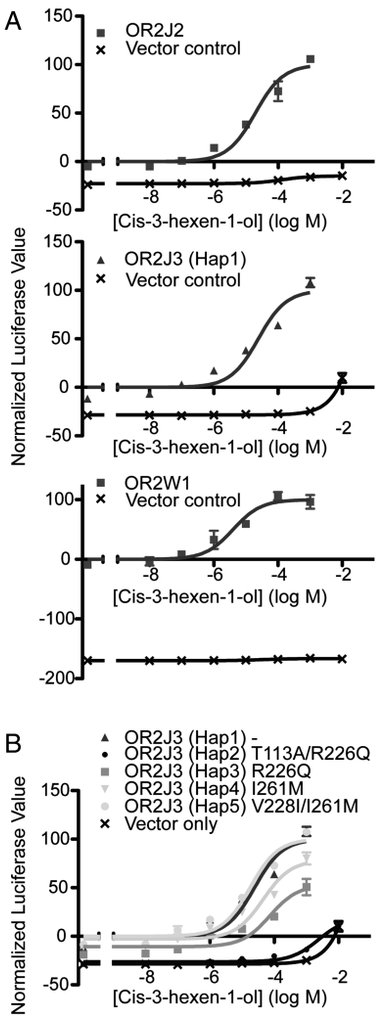

Figure 2 Concentration response curves of OR2J2, OR2J3, and OR2W1 for *cis*-3-hexen-1-ol (**A**). Concentration response curves of haplotypes of OR2J3 (**B**). Error bars are standard errors of 3 replicates. *y* axis values are normalized to the baseline response of the receptor (A) or Hap 1 (B). This figure appears in color in the online version of *Chemical Senses*.

The responses of the different haplotypes of *OR2J3* to C3HEX are consistent with the detection thresholds of participants in our subject cohort for this compound. Participants with the less sensitive haplotype, containing both T113A and R226Q, had significantly higher threshold concentrations than participants carrying more sensitive haplotypes ($T = 4.234$; $P = 9.83 \times 10^{-5}$) (Figure 4). The less sensitive haplotype explains 26.4% of the variance in C3HEX threshold concentrations in the subject cohort. A block of high linkage disequilibrium extends throughout most of the odorant receptor cluster (Figure 1B). *OR2J3* lies within this block, and rs28757581 and rs3749977 are in weak linkage disequilibrium with each other ($r^2 = 0.305$).

The HapMap populations genotyped during the 1000 Genomes Project have varying allele and haplotype frequencies (Figure 5), with African populations (ASW, LWK, and YRI) having higher proportions (>0.4) of the nonresponsive haplotype than other populations (<0.17) ($\chi^2 = 219.8$, degrees of freedom = 3, $P = 2.19 \times 10^{-47}$).

Discussion

Previously, we have linked the ability to detect the green grassy smelling compound *cis*-3-hexen-1-ol (C3HEX) to a cluster of odorant receptor genes on chromosome 6 (Jaeger et al. 2010). We hypothesized that one or more of these odorant receptors may respond to C3HEX and that genetic variation within odorant receptor gene/s at this locus may underlie variation in the ability to detect C3HEX.

We have examined sequence variation within the coding regions of all 25 odorant receptors across the study cohort. Association tests revealed 3 SNPs located within odorant receptor coding regions that are strongly associated with threshold concentrations for C3HEX. One of these was the nonsynonymous T113A substitution in the odorant receptor OR2J3, whereas the other 2 were variants found in predicted pseudogenes. Furthermore, when we tested the functional odorant receptors in the cluster for their ability to detect C3HEX in vitro, OR2J3 was one of only 3 receptors that responded to C3HEX. The T113A substitution resulted in a lowered sensitivity to the compound. This variant was in high linkage disequilibrium with another nonsynonymous variant in OR2J3, R226Q, which also compromises the ability to detect C3HEX, and together, they abolish the ability of OR2J3 to respond to C3HEX in cell assays.

Based on the predicted positions in the receptor, the T113A and R226Q are likely impacting different aspects of OR2J3 function. The T113A substitution lies in middle of the third transmembrane helix and is therefore more likely to affect binding of odors. In comparison, the R226Q lies within the third intracellular loop and is more likely to impact on general signal transduction as this loop is involved in the dynamics of G protein binding. Certainly, in vitro, the R226Q substitution reduces the response to not just C3HEX but all odors tested (Mainland JD, Li YR, Zhou T, Liu W-LL, Adipietro KA, Zhuang H, Zhan S, Lee SS, Matsunami H, submitted). The 2 substitutions are additive and producing the observed phenotype of reduced C3HEX sensitivity. We note that in our cohort and, indeed, in all 1000 Genomes HapMap individuals, the T113A substitution is only ever found on a R226Q background, allowing the detection of the variant by SNP-based genome-wide association. If T113A was not also linked to R226Q, we would have been less likely to detect an association with C3HEX detection and either of the substitutions. A similar situation is present for the androstenone anosmia where 2 substitutions in OR7D4 are additive in their negative impact on the receptor's response to the steroid derivative. The issue of multiple substitutions of small effect impacting on the power of whole-genome association has been widely

Table 2 Concentration/response analysis for different variants of *OR2J3* against *cis*-3-hexen-1-ol

Haplotype of *OR2J3*	Hap number	Amino acid position						Haplotype frequency	EC_{50} ($\times 10^{-5}$ M)	Max activation
		66	113	139	226	228	261			
—	Hap1	L	T	M	R	V	I	0.471	2.43	278
T113A/R226Q	Hap2	L	A	M	Q	V	I	0.125	NR[a]	—
R226Q	Hap3	L	T	M	Q	V	I	0.192	7.37	156
I261M	Hap4	L	T	M	R	V	M	0.010	4.50	222
V228I/I261M	Hap5	L	T	M	R	I	M	0.202	1.78	279
T113A		L	A	M	R	V	I	—	1.93	100
L66P		P	T	M	R	V	I	—	NR[a]	—
T113A/I261M		L	A	M	R	V	M	—	0.82	174
T113A/M139I/R226Q		L	A	I	Q	V	I	—	NR[a]	—

[a]NR = no response to *cis*-3-hexen-1-ol.

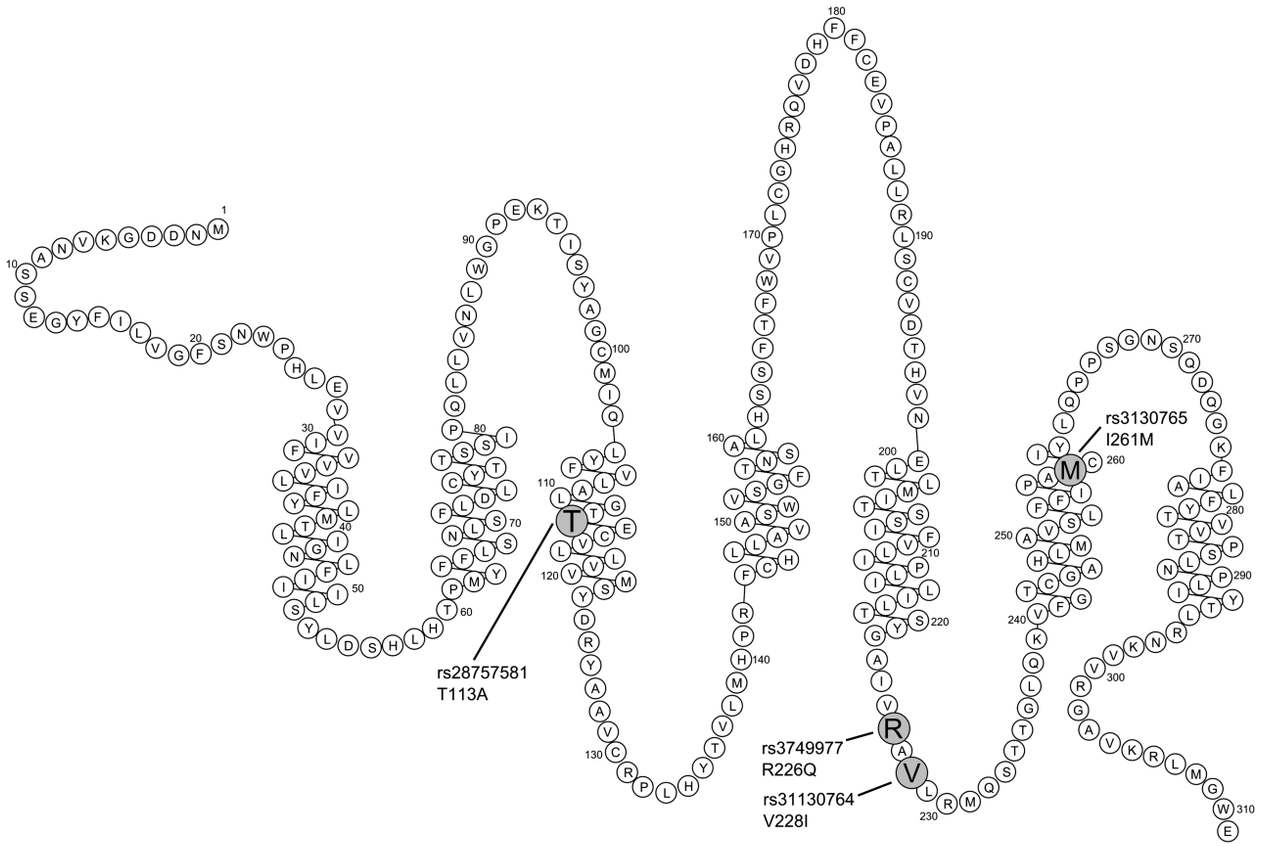

Figure 3 Snakeplot diagram of OR2J3 generated using RbDe (http://icb.med.cornell.edu/crt/RbDe/index.xml). The positions of the 4 nonsynonymous substitutions are indicated.

discussed (Yang et al. 2010), but whether this issue is a common feature of the molecular basis of olfactory differences awaits further investigation.

The T113A/R226Q haplotype, which does not respond to C3HEX in vitro, explained 26.4% of the variance in detection thresholds to the compound in vivo. This amount of variance that is explained by genetic variation is quite high, in comparison with other examples where estimates of the genetic contribution to odor detection and perception have been assessed. In a twin study, for example, genetic inheritance

explained 16% and 18% of the intensity ratings for the flavors cinnamon and isovaleric acid, respectively (Knaapila et al. 2008). In the cases, where the genetic variants are known, variation in OR11H7P explained approximately 8% of the variation in detection thresholds to isovaleric acid (Menashe et al. 2007), and OR7D4 explained approximately 19% and 39% of the valence and intensity ratings for androstenone, respectively (Keller et al. 2007). Furthermore, the contribution of the T113A/R226Q haplotype to genotypes of individuals within the study cohort correlates well with the in vitro results. Our cohort contained a single individual who was homozygous for the less sensitive haplotype. This individual had the highest C3HEX threshold concentration within the subject cohort. The remaining individuals with the nonresponsive haplotype were heterozygotes, and these individuals could still detect C3HEX, although at significantly higher threshold concentrations. Two other receptors within the cluster of 25 odorant receptors also respond to C3HEX. These receptors may play a role within individuals containing the less sensitive haplotype of OR2J3 but not enough to completely compensate for the loss of sensitivity at OR2J3 in our cohort. Although it seems very likely that T113A and R226Q in OR2J3 contribute to the changes in sensitivity to C3HEX in vivo, we cannot rule out the possibility that linked changes in noncoding regions around these genes cause the less sensitive phenotype due to, for example, reduced expression of OR2J3 or even OR2W1 or OR2J2. This is because we did not sequence the noncoding regions surrounding these genes or assess levels of expression of these genes within the olfactory epithelium in humans carrying the different haplotypes.

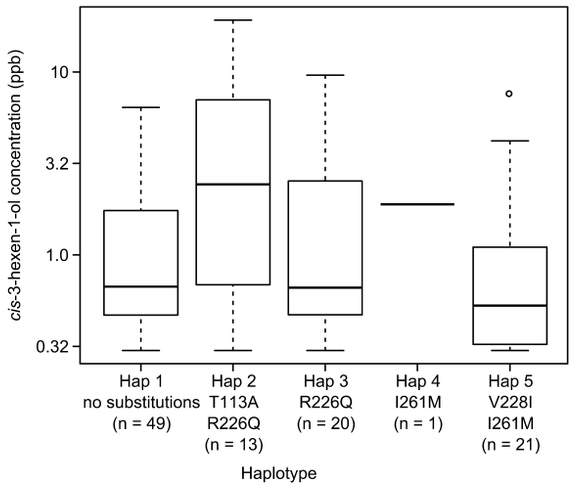

Figure 4 Median detection threshold concentrations in humans for *cis*-3-hexen-1-ol by haplotype (Hap) of *OR2J3*. Each subject is represented twice—once for the maternal and once for the paternal allele. The NCBI refseq ID for OR2J3 is NM_001005216.2, which in our haplotype designation is Hap 5. Boxes represent the upper and lower quartiles around the median value which is depicted as a bold line. The outermost lines connected via dashes represent the extremes of the range, while the single circle in Hap 5 is an outlier. Note that this outlier carries the Hap 2 and Hap 5 allele.

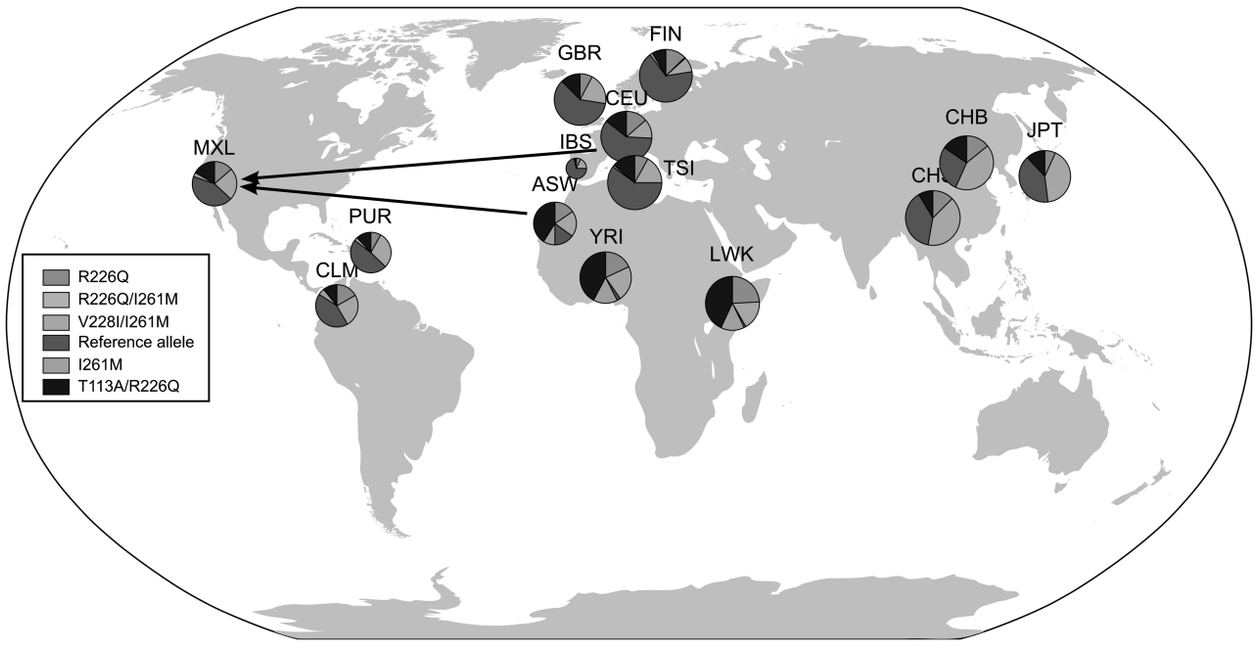

Figure 5 Worldwide distributions of *OR2J3* haplotypes. The pie charts show the frequency of the haplotypes in each population. Ethnic groups are displayed at the place of origin; arrows point to the location of sample collection. ASW, African ancestry in Southwest USA; CEU, Utah residents with Northern and Western European ancestry from the CEPH collection; CHB, Han Chinese in Beijing; CHS, Han Chinese South; CLM, Colombian in Medellin, Colombia; FIN, Finnish; GBR, British individuals from England and Scotland; IBS, Iberian populations in Spain; JPT, Japanese in Tokyo; LWK, Luhya in Webuya, Kenya; MXL, Mexican ancestry in Los Angeles, California; PUR, Puerto Rican; TSI, Tuscanians in Italy; and YRI, Yoruba in Ibadan, Nigeria. This figure appears in color in the online version of *Chemical Senses*.

Polymorphisms affecting sensory acuity may be more likely to impact receptors that are narrowly tuned, able to detect just a few compounds, as other receptors would not provide a redundant representation. Consistent with this expectation, OR7D4 is narrowly tuned to androstenone and androstadienone from a panel of 66 odorants (Keller et al. 2007). On the other hand, the C3HEX receptor, OR2J3, is more broadly tuned, responding to 10 of 55 compounds tested (Mainland JD, Li YR, Zhou T, Liu W-LL, Adipietro KA, Zhuang H, Zhan S, Lee SS, Matsunami H, submitted). Furthermore, C3HEX is not the most sensitively activating compound of OR2J3, and other receptors also respond to C3HEX. Despite this, polymorphisms in the receptor significantly affect the ability to detect C3HEX. This suggests that even though an odor can activate multiple ORs, the combinatorial code for detection thresholds may not be as redundant as previously thought. Perhaps, relatively few receptors are able to detect a given compound at relatively low concentrations; otherwise, odorant receptors containing variants responsible for differences in odor acuity would not be able to be identified using a GWA approach.

A broad survey of genetic variation in human populations (The 1000 Genomes Project Consortium 2011) found that haplotypes and allele frequencies of variants within *OR2J3* differ among populations around the world. African populations have a relatively high proportion (>40%) of the T113A/R226Q nonresponsive haplotype. This high frequency may translate into African populations, including African-American populations, being less able to detect C3HEX. Alternatively, in African populations, other polymorphisms in other receptors may compensate for the loss in C3HEX sensory acuity caused by T113A/R226Q at *OR2J3*.

C3HEX represents an important odor in food because it is found in many fruits, vegetables, and beverages and is added to processed foods and beverages to give them a fresh green quality. Other food compounds have been identified that have genetic associations with the ability to detect them. However, these examples are for tastants and not flavors. For example, individuals are more sensitive to the bitter compound PROP because of the dosage of the PAV haplotype within a bitter taste receptor, *TAS2R38* (Bufe et al. 2005). More sensitive individuals who have 2 copies of the PAV haplotype consume less of bitter-tasting foods, including many brassicas (Bell and Tepper 2006; Duffy et al. 2010). We might expect to find that variation in odor detection also plays a role in determining food preferences. In this case, individuals with the nonresponsive haplotype of *OR2J3* may have different food preferences for foods containing C3HEX from those of individuals without this haplotype. Future research to test these hypotheses will ultimately address whether genetic variation in sensory acuity for aromas contributes to food preference and consumption patterns.

Supplementary material

Supplementary material can be found at http://www.chemse.oxfordjournals.org/

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