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Evolutionary Grass Roots for Odor Recognition

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Abstract

Considerable evidence supports the idea that odorant recognition depends on specific sequence variations in olfactory receptor (OR) proteins. Much of this emerges from in vitro screens in heterogenous expression systems. However, the ultimate proof should arise from measurements of odorant thresholds in human individuals harboring different OR genetic variants, a research vein that has so far been only scantly explored. The study of McRae et al., published in this issue of *Chemical Senses,* shows how the recognition of a grassy odorant depends on specific OR interindividual sequence changes. It provides a clear relevant example for the impact of genetics on olfaction and is an excellent portrayal of the power of human genomics to decipher olfactory perception.

Key Words: genotype, phenotype, haplotype, genetics, SNP, olfactory receptor

Commentary

Different human olfactory receptor (OR) paralogs (members of the receptor repertoire of our species) are widely believed to underlie the capacity to recognize a multitude of odorous chemicals. However, there is another dimension of OR variability that contributes importantly to our understanding of human chemosensory perception: interindividual differences. The pioneering work of John Amoore (1967) has clearly demonstrated that people's noses are widely different in threshold phenotypes for specific odorants and put forth the conjecture that this phenomenon is attributable to widely different personal arsenals of OR proteins. Further, a natural assumption, supported by evidence (Whissell-Buechy and Amoore 1973; Amoore 1974; Wysocki and Beauchamp 1984; Gross-Isseroff et al. 1992), was that olfactory threshold differences stem from genetics. Based on the huge body of biological data accumulated in the ensuing 60 years, this view is translated to the statement that different humans have different polymorphic versions of OR genes (Young and Trask 2002; Menashe et al. 2003; Hasin-Brumshtein et al. 2009).

In a species vista, each gene locus may harbor an appreciable number of genetic variants (alleles), coding for different protein sequences. An individual receives 2 of these: one from mother and the other from father. Thus, a person's OR repertoire is composed of pairs of alleles at each of the ~400 gene loci in the active olfactory subgenome. In theory, the allele count could reach ~800, that is, if every locus were heterozygously disposed. In fact, analyses of hundreds of human genomic sequences show that the total number of alleles per person ranges between 500 and 600 (Olender et al., unpublished data). Such a large repository of "personal receptors" is impressive and has profound implications to the variegation human olfaction. Importantly, because ORs are expressed in an allelically excluded mode (only one allele in every sensory cell; Chess et al. 1994), the brain receives a faithful sensory view of such personalized gamut of OR alleles.

An obvious premise is that different allelic protein variants would bind odorants with different affinity, but only one study had thus far provided direct evidence for this. Five years ago, Keller et al. (2007) have shown that the human perception of the steroid odorant androstenone is widely different for 2 allelic variants at the OR7D4 locus. Individuals homozygously carrying a variant with arginine at position 88 and threonine at position 113 (the RT allele) are adequately sensitive to androstenone, whereas individuals with both gene copies encoding tryptophan at position 88 and methionine at position 113 (the WM allele) are much less sensitive to the odorant and found it less unpleasant.

Androstenone is an unusual odorant as pertaining to the human odorant universe. It is a boar pheromone (Claus and Alsing 1976), with allusion of behavior-modifying effects in humans (Araneda and Firestein 2004). This may suggest a highly specialized mammalian receptor, potentially with atypical properties. The article of McRae et al. addresses an odorant much more frequently encountered in human general chemoreception. This is the compound *cis*-3-hexen-1-ol

(C3HEX), an unsaturated short-chain alcohol, described as "green grassy," found in a wide range of foods and beverages and constituting a key flavor of many fruits and vegetables (Jirovetz et al. 2002; Genovese et al. 2004). In an earlier study (Jaegera et al. 2010), a group of authors partly overlapping with the present one reported on a significant genetic association between the ability to detect the C3HEX and a genomic cluster of 25 OR gene on chromosome 6 (clusters 6@29; see Olender et al. (2004)). Such a "low-resolution" genetic signal is typically obtained in genotype–phenotype correlations as exemplified by some other association reports, for example, between isovaleric acid smelling and the receptor OR11H7P in cluster 14@20b on chromosome 14 (Menashe et al. 2007) and between the perception of methanethiol (excreted in human urine after eating asparagus) with a single nucleotide polymorphism near OR2M7 in cluster 1@248 on chromosome 1 (Eriksson et al. 2010).

In the current study, the authors aimed to go one step further and identify which of the OR genes in this cluster is the culprit. They sequenced the entire cluster's OR coding regions in 52 individuals with different C3HEX detection thresholds. They then asked which of the 147 OR-residing points of variation along the entire cluster showed the best (statistically significant) correlation to the observed phenotype. This entails identifying the point of variation for which the 3 possible genotypes (2 homozygous types and 1 heterozygous) show the strongest discrepancy in odorant threshold. Such procedure led to the identification of a previously documented but phenotypically anonymous missense variant in OR2J3 (threonine to alanine at position 113, T113A) as highly correlated with the phenotype divergence. An in vitro expressed OR with this variation on a background of otherwise reference genome sequence had a 20% reduction of the average C3HEX threshold. But because all assayed individuals with alanine at position 113 also had another variation, a substitution of arginine for glutamine at position 226 (R226Q), the authors went on to measuring in vitro the combined "haplotypic" sequence having both the T113A and R226Q substitution (the AQ haplotype). Rewardingly, they discovered that the AQ protein showed no odorant response. Indeed, individuals with such AQ sequence combination had a C3HEX threshold of 2.5 ppb, compared with 0.67 ppb for the carriers of the reference haplotype (X3.7 lower sensitivity), explaining the strong association signal seen in this cohort. That the individuals with nonresponsive haplotype could still smell C3HEX is explainable by the authors' finding that 2 other ORs in the same cluster, OR2J2 and OR2W1, also interact with the same odorant. This state of affairs, whereby odorants are typically recognized by more than a single OR (Malnic et al. 1999; Kajiya et al. 2001), also clarifies why the deleterious AQ haplotype reportedly explained only ~25% of the variation in C3HEX thresholds. Notably, the weakly reactive OR2J3 allele is much more prevalent in Africans, echoing other reported ethnogeographic differences in OR functionality (Menashe et al. 2003).

Integrating the take-home lessons of the 2 detailed molecular studies of OR genetics by Matsunami and colleagues, one observes that the discovered variations are not of a readily expected kind. The genetic variants found were not of the nonsense type, such as stop–gain and insertion–deletion (indels) (Menashe et al. 2003), or deletion copy number variations (Waszak et al. 2010). Instead, all were missense mutations (amino acid replacements), and except R88W of OR7D4 have had Polyphen (Adzhubei et al. 2010) prediction of "benign", that is, not highly deleterious. Such results suggest caution in passing a-priori judgment on the phenotypic outcome of OR genetic changes. Prudence along similar lines should be exerted when looking at the protein sequence position of observed OR sequence modifications. Among the 4 variations seen (Figure 1), 2 are in intracellular loops (positions 133 and 226), in the broadly defined region of interaction with the olfactory G-protein alpha subunit (Crasto 2009; Kato and Touhara 2009) but not in an obviously conserved or motif-containing region. The other 2 mutations also seem relatively unobstructive: R88W in OR7D4 is in the 1st extracellular loop, a region not hitherto implicated in receptor function, and T113A in OR2J3 is in the upper part of transmembrane helix 3 near to but not overlapping with a predicted odorant binding pocket (Man et al. 2004). Much more insight will become possible when more examples are reported and once a resolved 3-dimensional structure of an OR protein becomes available, as for other G protein–coupled receptors (Mustafi and Palczewski 2009).

The OR2J3 haplotypes reveal an intriguing evolutionary narrative (Table 1). The reference haplotype repetition time

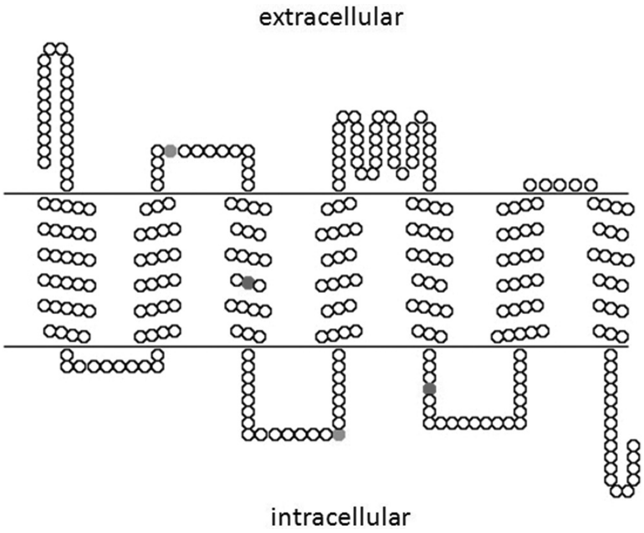

Figure 1 A schematic representation of the OR transmembrane topology, indicating the positions of the reported variants. The protein positions in OR2J3 associated with C3HEX threshold are highlighted in red, whereas those reported to underlie sensitivity to androstenone in OR7D4 are highlighted in orange (Keller et al. 2007). This figure appears in color in the online version of *Chemical Senses*.

Table 1 Evolutionary portrayal of OR positions 113 and 226 (p113 and p226), shown to affect C3HEX binding to OR2J3, as appearing in other OR homologs both in human and in other organisms documented in the HORDE database (http://genome.weizmann.ac.il/horde/)

OR gene	Species	lvt react	%id	p113	p226	Relation
OR2J3 HAP1*	human	278		T	R	var
OR2J3 HAP3	human	156	99.5	T	Q	var
OR2J3 HAP6	human	100	99.5	A	R	var
OR2J3 HAP2	human	0	99	A	Q	var
OR2J4P	human		89	T	Q	paralog
OR2J2	human		88	A	R	paralog
OR2J1P	human		89	A	R	paralog
C696.71-2984	chimp		98	T	R	ortholog
C696.65-251	chimp		95	T	R	ortholog
C696.63-9755	chimp		89	T	Q	homolog
C696.74-3848	chimp		90	A	R	homolog
NM_001000266	rat		83	T	W	ortholog
MOR256-18	mouse		82	T	W	ortholog
cOR2B2	dog		62	T	Q	homolog
Modo-OR2B8P	opossum		61	T	W	homolog
oa-OR2G22	platypus		60	T	R	homolog

%id is the percent amino acid sequence identity. The top 4 sequences represent the different human variants (var) whose analyses are reported by McRae et al., along with the nomenclature used by them for the haplotypes (HAPi) and their reported in vitro activity toward C3HEX (lvt react). The human reference genome variant is marked with "*". Sequences marked as "paralogs" are the 3 closest OR2J3 homologs in human. Sequences marked as "orthologs" are OR2J3 homologs in other species showing mutual best homology relationship. Sequences marked as "homologs" are the closest OR2J3 homologs in the other species, but without mutual best homology. For chimpanzee identifiers, "C" represents contig.

(TR) is also present in 2 very near chimpanzee orthologs, suggesting short-term conservation of the "smeller" version. However, in the presumed rodent orthologs, a completely different amino acid combination appears (TW), casting some doubt on the assumption that orthologous ORs have similar odorant binding underpinnings (Man et al. 2004). On the other hand, none of the species examined have the "null" haplotype AQ. Curiously, both human and chimp have other OR repertoire members (paralogs) with the intermediate haplotypic combinations TQ and AR, and the nearest OR2J3 dog sequence also has a TQ version. Finally, in the evolutionarily remote platypus, the most similar sequence carries the human reference combination TR. This type of evolutionary "lego" may further attest to the functional significance of these 2 sequence positions.

The human OR repertoire is currently defined mostly based on the reference human genome (Olender et al. 2004). The present study highlights the importance of establishing a complete compendium human OR alleles, expected soon in the HORDE database (Olender et al. 2004), together with extensive screening and psychophysics. This will likely generate a framework for additional genotype–phenotype association studies and prepare the arena for comprehensive future assessment of functional OR key residues both computationally (Crasto 2009) and experimentally. Such progress would hopefully lead to a "pharmacological" mapping of the entire OR repertoire, providing invaluable tools for the fragrance and flavor industries.

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