Minireview

The olfactory receptor family album

Chiquito Crasto, Michael S Singer and Gordon M Shepherd

Address: Department of Neurobiology, Yale University Medical School, 333 Cedar Street, New Haven, CT 06510, USA.

Correspondence: Gordon M Shepherd. E-mail: gordon.shepherd@yale.edu

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Abstract

Analysis of the human genome draft sequences has revealed a more complete portrait of the olfactory receptor gene repertoire in humans than was available previously. The new information provides a basis for deeper analysis of the functions of the receptors, and promises new insights into the evolutionary history of the family.

In the ten years since olfactory receptor genes were first identified [1], these genes and the receptors they encode have attracted growing interest. Olfactory or olfactory-like receptor genes are expressed in high numbers in the olfactory epithelium [1-3] but are also found in other tissues as far removed as the testes [4] and heart [5]. This is believed to reflect potentially varied functions for the proteins encoded by these genes.

The release of the first draft of the human genome, from the combined efforts of the public [6] and private [7] sectors, provides a new base for research on olfactory receptors and their genes. It is timely to synthesize the earlier work with this new information to provide new insights into the evolution of olfactory receptors and new directions for research on the molecular and neural basis of the sense of smell.

Olfactory receptors belong to the superfamily of G-protein-coupled receptors (GPCRs), which are characterized by seven transmembrane helical regions. For years it has been estimated that mammals have some 1,000 olfactory genes [8-10], making up the largest family in the genome. The family thus constitutes some 3% of the 31,000 genes now estimated to comprise the human genome. Before the complete draft genome sequence was available, Rouquier *et al.* [11] identified 72% of a sample of human olfactory receptor genes as pseudogenes (due to frame shifts and stop codons). A follow-up study [12] showed that loss of receptor function by the transformation of functional genes into pseudogenes is relatively common in human and prosimian primates, less common in lower primates, and is rarely found in mouse or zebrafish. The implication is that as species require less olfactory acuity, molecular disruptions accumulate and erode the functionality of olfactory receptor genes. The absence of functional olfactory receptors in the dolphin [13] and the deterioration of vision in moles [14] provide extreme examples of this mechanism, which is believed to account for the reduced olfactory acuity of humans compared to rodents and non-human primates.

Two recent publications [15,16] reporting data mining for olfactory receptors in the completed draft human genome have largely supported the earlier findings. Zozulya *et al.* [15] identified more than 347 full-length, functional olfactory receptor genes; Glusman *et al.* [16] reported 368 (see Figure 1). The number of pseudogenes identified in the two studies also compares well with the numbers of Rouquier *et al.* [11,12]. Glusman *et al.* [16] identified more than 900 olfactory receptor genes and pseudogenes, consistent with the earlier predictions [8-10]; of these, 681 are full-length, but only 322 are believed to be functional. They also detect some 70 full-length receptor genes that have yet to be assigned to any specific chromosome [16]. Detailed analysis of the human olfactory receptor family also enabled Glusman *et al.* [16] to infer diversification events in human evolution and a 'molecular clock' for the family, indicating the rate at which acquired mutations become fixed in the evolutionary lineage.

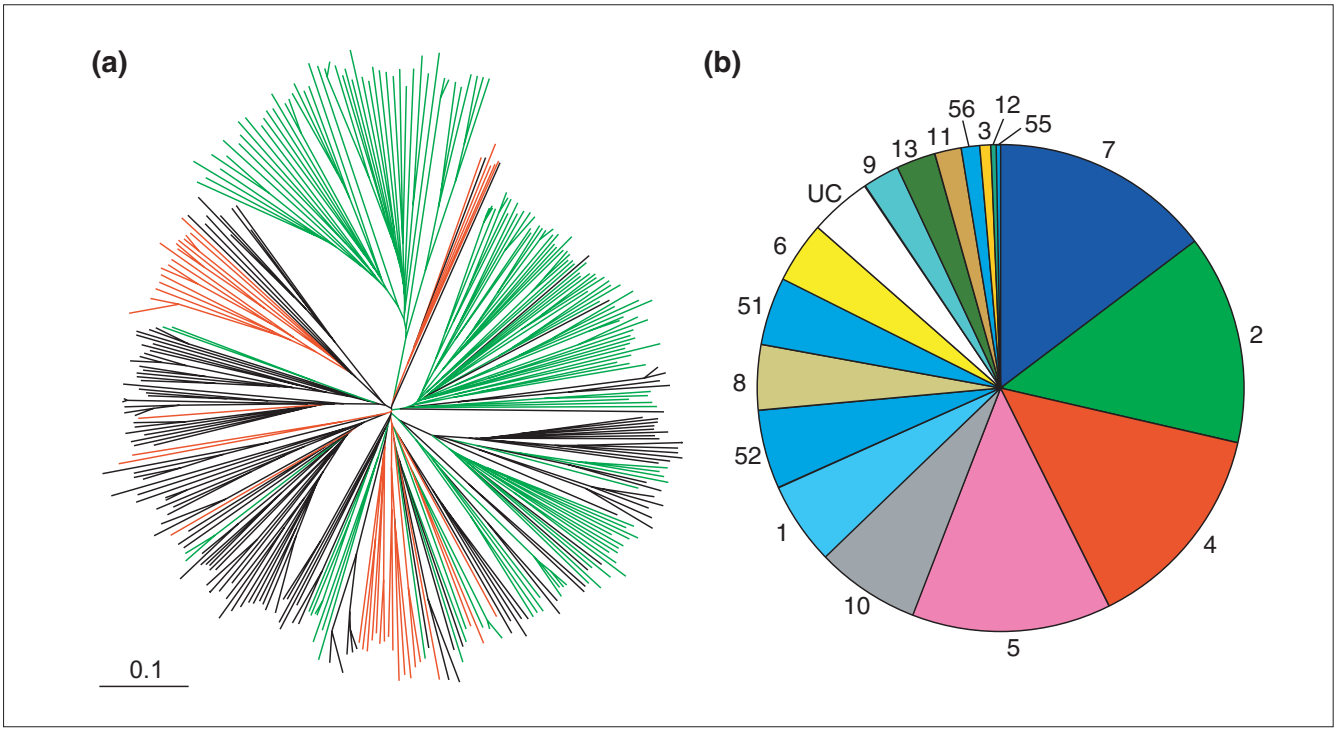

Figure 1
Classification of olfactory receptors. **(a)** An unrooted phylogenetic tree, reproduced with permission from [15]. Positions of olfactory receptor genes on chromosomes 11 (green) and 1 (red) are highlighted, and the scale bar corresponds to 10% sequence divergence. **(b)** The relative sizes of the various olfactory receptor gene families, reproduced with permission from [16]. Colors and numbers denote individual families, and those denoted UC are unclassified.

Using fluorescence *in situ* hybridization experiments, Rouquier *et al.* [12] determined that human olfactory receptor genes are distributed over all chromosomes except 20 and Y. Most of the receptor genes are present in two superclusters on chromosome 11, which is taken to reflect the evolutionary origin of this family as a repeated cluster on a single chromosome [15,16]. Next in order of frequency are receptors on chromosomes 1, 9 and 6 and 14; chromosomes 10, 22 and X each carry only one olfactory receptor gene. In addition, none of the 347 genes encoding functional receptors that were identified by Zozulya *et al.* [15] was found on chromosomes 2, 4, 18 or 21.

Developing a systematic classification and nomenclature for this enormous gene family is a daunting task: sequence identity has been a starting point. The sequence identity between any two randomly selected olfactory receptor genes is generally 40% or higher. The lowest reported percentage identity for a pair approaches 20% [15]. Glusman *et al.* [16], who reported average protein sequence identities among olfactory receptors in agreement with those reported by Zozulya *et al.* [15], also calculated sequence relationships with 55 non-olfactory GPCR sequences. The average percentage identity of 27% for olfactory: non-olfactory pairs was lower than that for pairs of olfactory receptors.

It was initially suggested that olfactory receptor sequences with a percentage sequence identity of 40% or greater constitute a family, and sequences with sequence identity of 60% or greater a subfamily [17,18]. This convention has been applied by Glusman *et al.* [16] to the entire olfactory subgenome (see the Human Olfactory Receptor Data Exploratorium (HORDE) website [19]); for instance: the name hOR11H11 means that this receptor belongs to family 11 and is the eleventh gene of subfamily H. The classification scheme of Zozulya *et al.* [15] at Senomyx, Inc. (La Jolla, USA) is based on the chromosome number, the family number and a unique member identifier; an example of an olfactory receptor name thus identified would be hOR14.03.03, where the gene is identified as localized on chromosome 14, and is the third member of family 3. In this scheme, the sequence identity required for an olfactory receptor to qualify as a member of a family was 43%.

The sequences of all the available olfactory genes and receptors, together with other chemosensory genes and receptors, are being made accessible on the web, at the Olfactory Receptor Database (ORDB) [20], a database sponsored by the Human Brain Project and the US National Institute on Deafness and Other Communication Disorders. The classification there is based simply on the chronological order in

which the sequences were published. The database also includes the terms from the other classification conventions so as to allow unambiguous identification, until a single system emerges by consensus within the field.

Earlier work identified olfactory receptor groupings characteristic of fish (Class 1) [21] and amphibians (Class 2) [22]. Glusman *et al.* [16] report that the genes for a large number of human olfactory receptors similar to Class I have fewer pseudogenes (52%) than those human olfactory receptors classified as similar to Class II (77%). On the basis of internal similarities between family members on different chromosomes, Glusman *et al.* [16] report evolutionary divergence and duplications depending on the subfamilies to which these receptor these genes belong.

Comparisons of the olfactory receptor gene sequences of humans and other species have been carried out to identify orthologs and map out the evolutionary patterns of the development of olfactory receptors. Lane *et al.* [23] identified seven functional orthologs between olfactory receptor genes of the mouse and human P2 clusters. Eighteen mouse and eight human olfactory receptors were identified, and the orthologs showed greater than 80% identity. The smaller size of the P2 gene cluster in humans supports the conclusions of Rouquier *et al.* [12] that the range and acuity of the human olfactory receptor array is diminished compared to rodents. Lane *et al.* [23] segregated these orthologs into four sections (based on their zonal location in the olfactory epithelium); the percentage identities of sequences within a section was over 80% and the inter-section identities were less than 60%. They also pointed out that receptors with similar sequences were more likely to be expressed in the same epithelial zone, compared to those with less similar sequences.

An important feature of olfactory receptor genes is that only one allele is expressed in any given olfactory receptor neuron [24]. The mechanisms underlying exclusion of the other allele and of other genes are not yet understood. A sequence search by Lane *et al.* [23] failed to reveal conserved motifs that might be related to transcription factor binding sites or promoter regions. This issue will undoubtedly attract increasing interest in the future.

Although a given cell expresses only one type of receptor, a receptor can interact, with varying affinities, with many different odor molecules [25]. Odor coding thus involves ensembles of olfactory receptor cells with varying degrees of activation in response to a given odor or odor mixture. The mechanisms of binding of odor molecules are being studied in expression systems, and are combined with molecular modeling of ligands [26] and receptors [27], which gives evidence of interactions between the odor molecules and a binding pocket in the receptor. The binding pocket resembles that in other GPCRs, although with specific residues that can interact more broadly with the odor ligands [27,28].

Mining of the human genome and of other genomes soon to be revealed will give further insights into the functional properties of the olfactory receptor sequences. It is remarkable that the diverse approaches summarized here have yielded largely convergent results. The next stage in studies of this fascinating family of molecules should produce critical evidence for the regulatory mechanisms involved in gene expression, and identification of residue motifs specific to the binding pockets of different receptors, providing the basis for a receptor classification on functional grounds. This information will need to be integrated with the results of a wealth of studies of odor maps and neuronal circuits in the olfactory pathway before an understanding of the brain mechanisms underlying smell perception can begin to emerge.

References

1. Buck L, Axel R: **A novel multigene family may encode odorant receptors: a molecular basis for odor recognition.** *Cell* 1991, **65:**175-181.
2. Parmentier M, Libert F, Schurmans S, Schiffmann S, Lefort A, Eggerickx D, Ledent C, Mollereau C, Gerard C, Perret J: **Expression of members of the putative olfactory receptor gene family in mammalian germ cells.** *Nature* 1992, **355:**453-455.
3. Weth F, Nadler W, Korsching S: **Nested expression domains for odorant receptors in zebrafish olfactory epithelium.** *Proc Natl Acad Sci USA* 1996, **93:**13321-13326.
4. Goto T, Salpekar A, Monk M: **Expression of a testis-specific member of the olfactory receptor gene family in human primordial germ cells.** *Mol Hum Reprod* 2001, **7:**553-558.
5. Hillier L, Lennon G, Becker M, Bonaldo MF, Chiapelli B, Chissoe S, Dietrich N, DuBuque T, Favello A, Gish W, *et al.*: **Generation and analysis of 280,000 human expressed sequence tags.** *Genome Res* 1996, **6:**807-828.
6. International Human Genome Sequencing Consortium: **Initial sequencing and analysis of the human genome.** *Nature* 2001, **409:**860-921.
7. Venter JC, Adams MD, Myers EW, Li PW, Mural RJ, Sutton GG, Smith HO, Yandell M, Evans CA, Holt RA *et al.*: **The sequence of the human genome.** *Science* 2001, **291:**1304-1351.
8. Mombaerts P: **Seven-transmembrane proteins as odorant and chemosensory receptors.** *Science* 1999, **286:**707-711.
9. Mombaerts P: **Molecular biology of odorant receptors in vertebrates.** *Annu Rev Neurosci* 1999, **9:**315-320.
10. Pilpel Y, Sosinsky A, Lancet D: **Molecular biology of olfactory receptors.** *Essays Biochem* 1998, **33:**93-104.
11. Rouquier S, Taviaux S, Trask B, Brand-Arpon V, van den Engh G, Demaille J, Giorgi D: **Distribution of olfactory receptor genes in the human genome.** *Nat Genet* 1998, **18:**243-250.
12. Rouquier S, Blancher A, Dominique G: **The olfactory receptor gene repertoire in primates and mouse: evidence for reduction of the functional fraction in primates.** *Proc Natl Acad Sci USA* 2000, **97:**2870-2874
13. Freitag J, Ludwig G, Andreini P, Roessler P, Breer H: **Olfactory receptors in aquatic and terrestrial vertebrates.** *J Comp Physiol* 1998, **183:**635-650.
14. Springer MS, Burk A, Kavanagh JR, Waddell VG, Stanhope MJ: **The interphotoreceptor retinoid binding protein gene in therian mammals: implications for higher level relationships and evidence for loss of function in the marsupial mole.** *Proc Natl Acad Sci USA* 1997, **94:**13754-13759.
15. Zozulya S, Echeverri F, Nguyen T: **The human olfactory receptor repertoire.** *Genome Biol* 2001, **2:**research0018.1-0018.12.
16. Glusman G, Yanai I, Rubin I, Lancet D: **The complete human olfactory subgenome.** *Genome Res* 2001, **11:**685-702.
17. Glusman G, Bahar A, Sharon D, Pilpel Y, White J, Lancet D: **The olfactory receptor gene superfamily: data mining, classification, and nomenclature.** *Mamm Genome* 2000, **11:**1016-1023.
18. Lancet D, Ben-Arie N: **Olfactory receptors.** *Curr Biol* 1993, **3:**668-674.

19. **Human Olfactory Receptor Data Exploratorium (HORDE)** [http://bioinformatics.weizmann.ac.il/HORDE]
20. **ORDB at Senselab** [http://senselab.med.yale.edu/ordb]
21. Ngai J, Dowling MM, Buck L, Axel R, Chess A: **The family of genes encoding odorant receptors in channel catfish.** *Cell* 1993, **72:**657-666.
22. Freitag J, Kreiger J, Strotmann J, Breer H: **Two classes of olfactory receptors in *Xenopus laevis*.** *Neuron* 1995, **15:**1383-1392.
23. Lane RP, Cutforth T, Young J, Athanasiou M, Friedman C, Rowen L, Evans G, Axel R, Hood L, Trask BJ: **Genomic analysis of orthologous mouse and human olfactory receptor loci.** *Proc Natl Acad Sci* 2001, **98:**7390-7395.
24. Chess A, Simon I, Cedar H, Axel R: **Allelic inactivation regulates olfactory receptor gene expression.** *Cell* 1994, **78:**823-834.
25. Malnic B, Hirono J, Sato T, Buck LB: **Combinatorial receptor codes for odors.** *Cell* 1999, **96:**713-723.
26. Araneda RC, Kini AD, Firestein S: **The molecular receptive range of an odorant receptor.** *Nat Neurosci* 2000, **3:**1248-1255.
27. Singer MS: **Analysis of the molecular basis for octanal interactions in the expressed rat I7 olfactory receptor.** *Chem Senses* 2000, **25:**155-164.
28. Floriano WB, Vaidehi N, Goddard WA 3rd, Singer MS, Shepherd GM: **Molecular mechanisms underlying differential odor responses of a mouse olfactory receptor.** *Proc Natl Acad Sci USA* 2000, **97:**10712-10716.