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Better smelling through genetics: mammalian odor perception

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The increasing availability of genomic and genetic tools to study olfaction—the sense of smell—has brought important new insights into how this chemosensory modality functions in different species. Newly sequenced mammalian genomes—from platypus to dog—have made it possible to infer how smell has evolved to suit the needs of a given species and how variation within a species may affect individual olfactory perception. This review will focus on recent advances in the genetics and genomics of mammalian smell, with a primary focus on rodents and humans.

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Introduction

The ability to sense small molecules in the environment is an adaptation found in all living things from plants to humans. In animals, the chemical senses of smell and taste differ from the physical senses of vision, touch, and hearing in the diversity of possible stimuli that can be perceived to have a distinct taste or smell. Both small organic molecules and small proteins induce taste sensations. Volatile small molecule odorants as well as non-volatile proteins and non-volatile hydrocarbons all can induce olfactory sensations, depending on the species. Some animal species even perceive carbon dioxide [1].

Biomedical research in olfaction, which lagged behind investigation into vision in the past century, has experienced two recent phases of growth since the early 1990s. These growth phases correlate approximately with the discovery of the genes encoding the odorant receptors (ORs) by Buck and Axel in 1991 [2] and the subsequent completion of the sequencing of the human [3,4] and other mammalian genomes. The search for the receptors that mediate the detection of a large number of odorants was grounded in biochemical experiments in the mid-1980s that showed that odors stimulate the production of cyclic AMP (cAMP) via an olfactory-enriched adenylate cyclase [5,6]. Buck and Axel reasoned that odorants would be detected by a large family of G protein-coupled receptors (GPCRs), selectively expressed in the olfactory epithelium that would couple odor binding to the production of cAMP. These assumptions proved to be true and yielded not only a Nobel Prize to Buck and Axel for their discovery and subsequent characterization of the ORs but also a veritable boom of interest in tackling additional unsolved problems in the field of chemosensory perception. This review highlights recent discoveries concerning the olfactory organs, receptors, and ligands that mediate specific olfactory behaviors as well as detailing what comparative genomics has taught us about the sense of smell in diverse mammals.

Evolution of chemosensory receptor gene families in diverse mammalian species

The three most prominent genetic features of GPCRs that bind odors are the dramatic variation in the size of chemosensory receptor gene repertoires between species, the large number of pseudogenes in many species and especially humans, and the unparalleled genetic variability within species.

ORs were first identified in the rat [2], but subsequently found in a large number of mammalian species following genome sequencing and annotation. The size of the OR gene repertoire has been reported for the following mammals: opossum (1518) [7], platypus (~700) [8], mouse (~1500), human (~960), dog (~1100), and rat (~1500). The difference in the number of functional ORs between species is amplified by the differences in the ratio of pseudogenes, genes that have accumulated small deletions, point mutations, or frame shifts that prevent the expression of a functional OR protein. Among land mammals, humans have the highest pseudogene fraction with 51% (even excluding the large pseudogene family 7E), compared with 41% in chimpanzee, 30% in Old World monkeys, 15–20% in New World monkeys and lemurs, and around 20% in cattle, dog, rat, and mouse [9,10] (Table 1). The semi-aquatic platypus has a pseudogene fraction of 52% [8]. Marine mammals also have a high percentage of OR pseudogenes: Dall's porpoises (78%), dwarf sperm whales (77%), minke whales (58%), and Steller's sea lions (37%) [10]. The extremely high rate of OR pseudogenization in aquatic mammals may reflect the relative paucity of odor ligands encountered in such environments. The accumulation of OR pseudogenes in humans is presumed to be a consequence of relaxed

Table 1

Comparative genomics of mammalian chemosensory receptor genes.

Receptor type	Where expressed (in mice)	# Genes in humans	% Pseudogenes in humans	# Genes in mammals (range)	% Pseudogenes in mammals (range)	Ligands
OR	OE (most OSNs) SO (most OSNs) VNO (few OSNs)	960	51	700–1518	15–78	Diverse volatile odorants • General odorants • Pheromones
V1R	VNO (apical) OE (few OSNs in humans)	117	98	65–849	50–100	Diverse volatile odorants • General odorants • Pheromones
V2R	VNO (basal) GG (V2r83, most OSNs)	20	100	10–283	48–100	Diverse non-volatile pheromones • MHC peptides • MUPs • ESPs
TAAR	OE (few OSNs) GG (few OSNs)	6	0	3–22	Low	Volatile amines

purifying selection on many ORs. On the contrary it also has been shown that some ORs are adapting to human-specific odor perception requirements, as seen in signs of positive selection like human-specific subfamily extensions [11,12]. The ligands of the ORs that acquired a species-specific function in humans are not known but would be of interest to discover.

Recently, a second family of olfactory GPCRs known as trace amine-associate receptors (TAARs) were described and shown to respond to volatile amines present in urine [13••]. Humans have six TAAR genes [13••], but different tetrapod species have between three and 22 members of the TAAR gene family and some fish species have up to 109 TAAR genes [14]. The behavioral relevance of the TAARs as odorant receptors is not yet known, but based on the pharmacology of mouse TAARs they may be important for receiving social cues related to sexuality and fear.

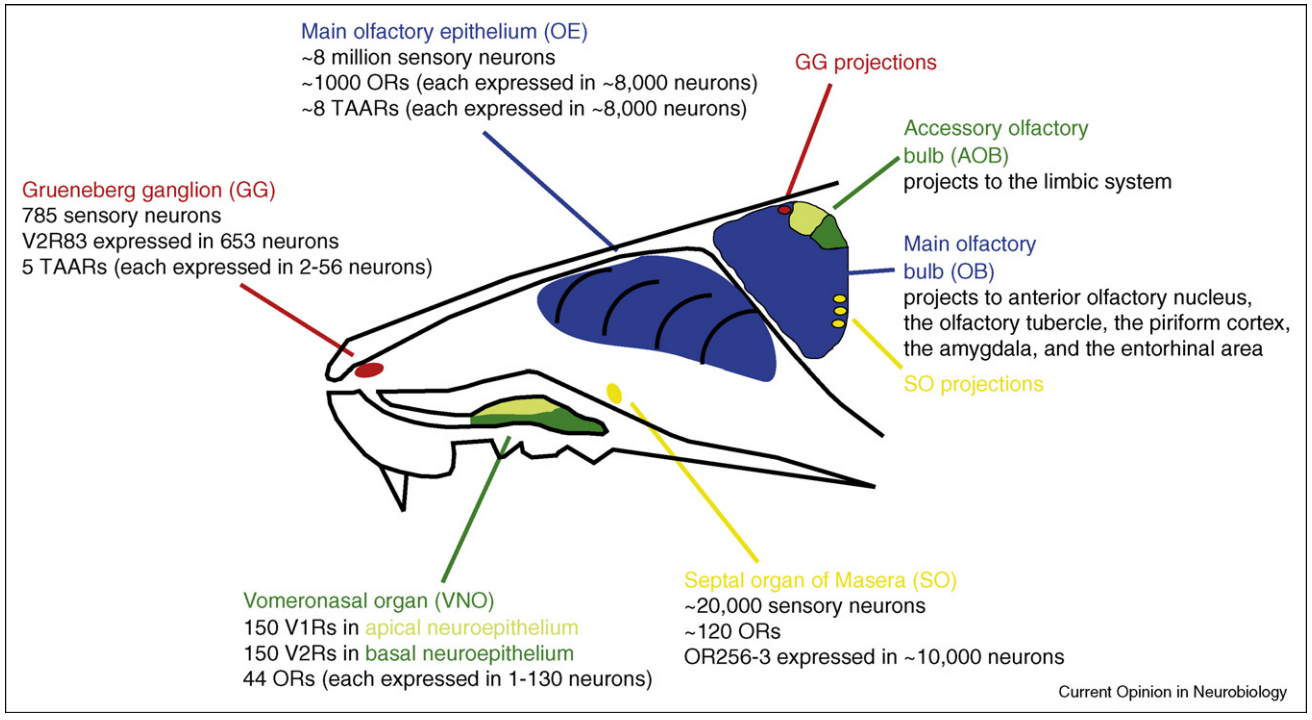
Two additional and distinct families of GPCRs, unrelated to ORs and TAARs, were found to be expressed in the vomeronasal organ (VNO) of some mammals and are called V1Rs and V2Rs, for class 1 and class 2 vomeronasal receptors. Both V1R and V2R gene families are exceptionally variable in gene number among mammals [15–17]. The V1R gene family has no functional member in the chimpanzee genome, whereas there are 270 in the platypus genome. Humans have two functional V1Rs but 115 pseudogenes. Dogs have only eight intact V1R genes and 54 pseudogenes, whereas the platypus has 579 V1R pseudogenes in addition to the 270 intact V1Rs [15,16]. The differences in size of the V1R repertoire are considered to be the largest variation in gene family size in all mammalian gene families [18]. V2R genes have completely degenerated in humans, chimpanzee, macaque, cow, and dog. In these species only V2R pseudogenes are found. By contrast, the opossum, the mouse, the rat, and the platypus have between 79 and 121 intact V2R genes and between 79 and 158 pseudogenes [16,17] (Table 1). The details of what ligands activate V1Rs and V2Rs have not yet been fully worked out, but the limited information available suggests that V1Rs detect volatile odorants and V2Rs are specialized for non-volatile protein ligands [19]. The underlying variability in VR number across species may reflect the relative importance of pheromone communication for a given mammal.

Genetic variability in humans and impact on the sense of smell

Beyond these differences in chemosensory GPCRs between species, large genetic differences between individuals of the same species have been found. Most work on intra-species variability has been performed in humans. However, it has been suggested that the considerable variability found for canine OR genes correlates with differences in olfactory abilities between individual dogs [20]. In mice, strain-specific sensitivity to isovaleric acid, the odor of sweaty socks, was mapped to a small genetic region [21], which was subsequently found to harbor a cluster of ORs [22]. It remains to be formally proven that some or all of these ORs are deleted or mutated in the isovaleric acid insensitive strains.

In humans, large genetic variability in OR genes has been shown on the sequence level, resulting in co-segregating pseudogenes [23], as well as on the structural level, resulting in copy-number variation that alters the number of copies of a given OR [24••,25••,26••]. These discoveries suggested that inter-individual differences in sensitivity to odors [27] are at least partially caused by genetic variability in odorant receptors. This hypothesis was recently confirmed for two odors, androstenone and isovaleric acid. Sensitivity to both odors was previously suggested to be genetically determined [28,29]. Genetic association studies showed that polymorphisms in

Figure 1



Schematic of the rodent olfactory system, indicating all four olfactory sensory organs, the classes of chemosensory receptor they express, and a summary of gene expression in each. Neurons in the VNO that express ORs project to the very rostral tip of the AOB (not indicated in the figure).

OR7D4 [30••] and OR11H7P [31••] influence the sensitivity to androstenone and isovaleric acid, respectively. In parallel with these targeted phenotype–genotype associations two groups have reported unbiased whole genome approaches that assigned genomic regions to specific aspects of odor perception [32,33••]. These screens turned up genome regions devoid of OR genes [33••] that presumably harbor genes affecting other aspects of peripheral olfactory function or more central aspects of odor perception.

Species-specific olfactory organs detecting different odorant stimuli

Mammals possess multiple organs for detecting odors: the olfactory epithelium (OE), the vomeronasal organ (VNO), the Grueneberg ganglion (GG), and the septal organ of Masera (SO) (reviewed in [34,35]). While rodents possess all four of these organs (Figure 1), some are missing in other mammals. There are increasingly strong data to support the idea that each organ has a specialized biological function, expresses specific chemosensory receptors and signal transduction components, and responds to chemically distinct stimuli (Figure 1; Table 1). The Grueneberg ganglion, which was only discovered in 1973, contains a small number of sensory neurons that were recently shown to mediate alarm pheromone detection in mice [36••]. The septal organ of Masera, first described in 1943, is a patch of olfactory epithelium at the ventral base of the nasal septum that is found in a variety of mammals [37]. Recent work suggests that the septal organ is both broadly tuned to various volatile odors and also shows a unique sensitivity to mechanical stimulation [38]. The olfactory epithelium is largely devoted to detecting general odors but also detects volatile pheromones, while the opposite is true for the VNO.

Just as there is some blurring of the chemical specificity of each of these organs, there is no strict segregation of a specific type of chemosensory receptor in each olfactory organ. The receptors that bind odors in most olfactory sensory neurons (OSNs) in the olfactory epithelium and in the septal organ are ORs. In the VNO, V1Rs and V2Rs are the principal receptors. The Grueneberg ganglion expresses V2R82 in the majority of sensory neurons, but TAARs are expressed in a subset of the neurons in the olfactory epithelium and the Grueneberg ganglion. Some neurons in the mouse VNO express ORs [39] and in humans, who lack a functional VNO, a V1R gene is expressed in the olfactory epithelium [40].

Not all sensory neurons in the olfactory epithelium appear to express olfactory GPCRs. Classic anatomical experiments indicated that the rodent olfactory epithelium has a specialized set of neurons with a characteristic projection pattern to olfactory bulb glomeruli known as the 'necklace glomeruli,' so named for their

appearance of linear beads on a string. Recent work from two groups suggests that these neurons express GC-D, a receptor guanylate cyclase [1,41], as well as carbonic anhydrase [1]. Behavior genetic and physiological experiments demonstrated that these GC-D neurons are extremely sensitive to carbon dioxide [1] but also required for responses to peptide hormones and urine components [41].

Genetic analysis of the mouse VNO confirms the behavioral importance of this organ in the social and reproductive biology of rodents. Targeted deletion of a large cluster of V1Rs produced mice with deficits in maternal and sexual behavior [42]. Mice lacking the TRPC2 ion channel, the transduction channel necessary for signaling of both V1Rs and V2Rs, show severe defects in male [43,44] and female sexual behavior [45]. By contrast, humans and other primates possess only a vestigial VNO by anatomical criteria [46]. The VNO is also vestigial by genetic criteria, as TRPC2 is non-functional in humans and other Old World primates [47]. Not only is the VNO vestigial in humans, but humans also lack other innovations in chemosensory signaling that mice retain. For instance, small secreted proteins in rodent urine called major urinary proteins (MUPs) and in tear secretions from the extraorbital lacrimal gland called exocrine gland-secreting peptides (ESPs) [48••] are likely to act as pheromone signals in mice [49••]. Humans entirely lack both the MUP and ESP gene families. Whether humans have largely lost the ability to communicate social cues with chemical signals typically received by the VNO or whether we use our olfactory epithelium for that purpose remains an open question.

Future prospects

The increasing scale and rapidly decreasing cost of genome sequencing opens up many new opportunities in olfaction. For instance, the advent of personal genome sequencing may make it feasible to correlate a given human's olfactory capacities with variation in her or his genome. Future sequenced genomes of mammals with interesting ecology and lifestyles—naked mole rats, bats, elephants, and others would be of interest—will provide further insights into selective pressures that shape the olfactory subgenome. As approaches to express odorant receptors in heterologous cells continue to match specific ORs with their ligands [50], it will become possible to do large-scale analysis relating the structure of an odorant receptor to its functional properties. Examining smell at angstrom resolution by solving crystal structures of ORs in various liganded states seems plausible now that several related non-olfactory GPCR structures have been solved [51]. Aside from the curiosities of this sensory modality, all of this ferment in the field of smell may ultimately provide translationally important advances in disease biomarkers, including early diagnosis of neurodegenerative and psychiatric diseases [52] and the detection of specific disease states by changes in body odor [53].

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