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Variations in the human olfactory receptor pathway

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Abstract. Of all five senses, olfaction is the most complex molecular mechanism, as it comprises hundreds of receptor proteins enabling it to detect and discriminate thousands of odorants. Until lately, the understanding of this highly sophisticated sensory neuronal pathway has been rather sketchy. The sequencing of the human genome and the consequent advent of new genomic tools have opened new opportunities to better understand this multifaceted biological system. Here, we present the relevant progresses made in the last decade and highlight the possible genetic mechanisms of human olfactory variability.

Keywords. Olfactory receptors, congenital general anosmia, specific anosmia, SNPs, pseudogenes, threshold sensitivity.

Introduction

Olfaction, the sense of smell, is one of the most curious systems of sensory information processing, where signals from multiple olfactory receptor (OR) types are converted into an accurate 'odor image'. Many organisms rely on olfactory cues for a wide range of activities such as food acquisition, reproduction, migration and predator alarming. For that, the olfactory system is characterized by a remarkable ability to detect and discriminate thousands of low molecular mass compounds (odorants). This sophisticated chemical detection apparatus, which has evolved over ~1 billion years, has long intrigued scientists attempting to understand its molecular facets.

Olfactory perception is a result of a cascade of biochemical and electrophysiological processes, in which the intrinsic information residing in the molecular structure of an odorant is converted into the perception of a characteristic odor quality and intensity. The human olfactory epithelium accommodates about 10 million olfactory sensory neurons (OSNs), each extending a single dendrite to the surface of the epithelium. The mucus-bathed dendritic ends bear specialized cilia, which enhance the receptive membrane surface. Odorants of various chemical configurations are inhaled and reach the olfactory epithelium, situated at the posterior region of the nasal cavity. There, they dissolve in mucus, and then interact with receptor proteins within the ciliary membrane of OSNs. This interaction, between the receptors and their ligands, is the first step in a signal transduction pathway which eventually produces an axonal electrical message that is transmitted to processing in the brain.

Olfactory signaling

Olfactory signaling begins with the recognition of an odorant by receptor proteins within the ciliary membrane of an OSN's dendrite. Following the binding of the odorant, the receptors undergo a conformational change, which initiates an intracellular cascade of signal transduction events, involving the G-protein-dependent elevation of cyclic AMP (cAMP), leading to opening of cation channels and membrane depolarization (Fig. 1). This process triggers action potential in the unmyelinated axons of the OSNs, leading to the olfactory bulb. There, the axons form synapses with apical dendrites of neurons of mitral cells within structures called glomeruli. Mitral axons leaving the olfactory bulb project widely to other brain structures, such as the olfactory cortex, where further information processing occurs.

It is generally accepted that the olfactory system employs a combinatorial strategy to discriminate among the millions of odorous compounds and their mixtures.

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Odor perception appears to be a multidimensional task, whereby every olfactory receptor (OR) binds numerous types of odorants with different affinities and vice versa [1–3]. Consequently, a unique combinatorial code is generated for every odor stimulus, suggesting that olfactory discrimination is a polygenic trait. However, based on the receptor affinity distribution (RAD) model [4], most of the odorant binding affinities of an OR are weak, and only a few have biological significance. Of these, the strongest affinity receptor determines the detection and recognition thresholds, which may therefore be treated as a single gene trait [2].

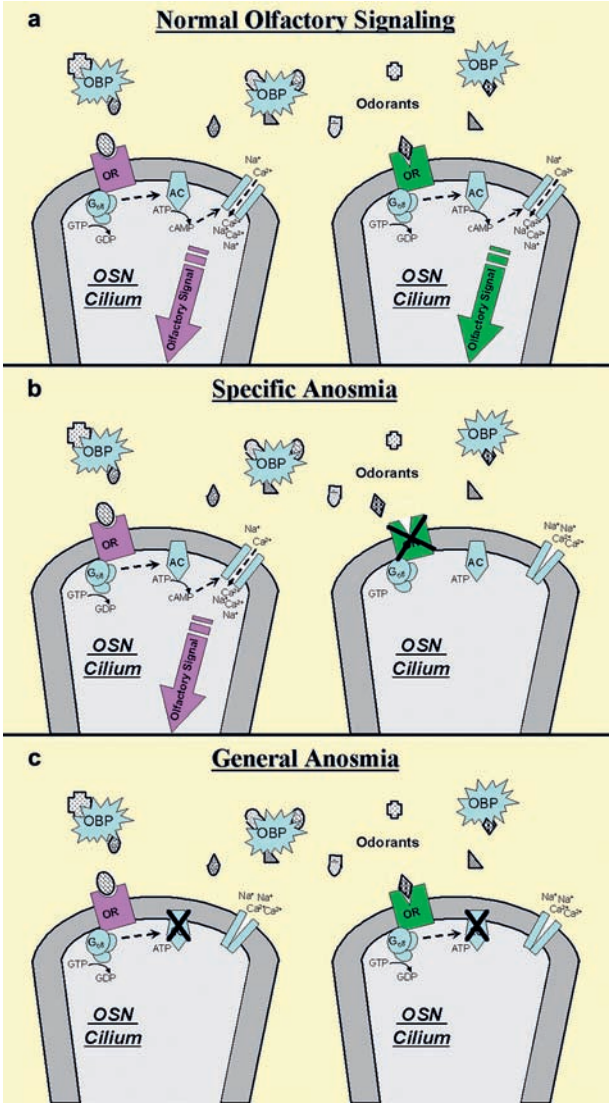

Figure 1. A schematic drawing of the olfactory signaling pathway and the putative underlying mechanisms of olfactory deficits. (*a*) In humans with a normal sense of smell, both the olfactory receptors (ORs) and the subsequent signaling cascade molecules are intact, allowing the perception of all available odorous volatile molecules (odorants). (*b*) Specific anosmia –damage to one OR would eliminate the response or significantly decrease the sensitivity towards one or a few odorants. Such specific OR inactivation would likely not affect perception of other odorants. (*c*) General anosmia – disruptive mutations in one or more of the olfactory signal transduction proteins would extinguish the olfactory signal stemming from all OR types.

The processing of the olfactory information is very precise and tightly regulated. Yet, much of the neuronal wiring of this complex system is still poorly understood. It is widely accepted that each OSN expresses only one type out of the hundreds possible OR genes, known as 'the one neuron-one receptor rule' [5, 6]. Also, every OSN selects only one allele for a given gene [7, 8]. For many years, OR gene expression regulation was a subject for many studies and scientific debates [9, 10]. Recently, it was demonstrated that the expressed OR protein itself elicits a feedback signal which prevents other OR alleles from being expressed in the same neuron [11, 12]. This monoallelic expression regulation is very important for the correct establishment of the olfactory signal. In this realm, it was suggested that the explicit OR protein of each OSN triggers its convergence to its particular glomeruli in the main olfactory bulb [13–16]. This means that each glomerulus is an amplified representation of the olfactory response of many OSNs that share the expression of the same OR gene and allele. The elucidation of these expression regulation mechanisms and their molecular consequences is only a small step in a long journey of understanding the basis of olfactory signal processing.

Olfactory receptors

Olfactory receptor proteins, which constitute the molecular basis of the sense of smell [17], belong to the G-protein-coupled receptor (GPCR) superfamily, transducers of a wide array of extracellular signaling molecules that constitute important targets in the pharmaceutical industry [18]. Members of this protein superfamily share features of sequence and structure, including seven hydrophobic transmembrane helices, as well as three intracellular and three extracellular loops that link these helices. ORs belong to GPCR family A, or rhodopsin-like GPCRs, which is the largest and most well studied. Although the overall sequence similarity between the various members of this family is very low, with mean amino acid pairwise identity of 17% [18], they share several highly conserved sequence motifs (Fig. 2), thought to play an essential role in either maintaining the structure and functional conformational transitions of these proteins, or in interacting with upstream and downstream partners [19]. In contrast, residues that are part of the hypothetical OR binding site display higher variability among OR genes [20, 21] to facilitate the recognition of a large and diverse repertoire of ligands (Fig. 2).

In most mammals, the olfactory repertoire has 1000–1400 OR genes, constituting the largest gene family within their genomes [22–24]. These are divide into two classes con-

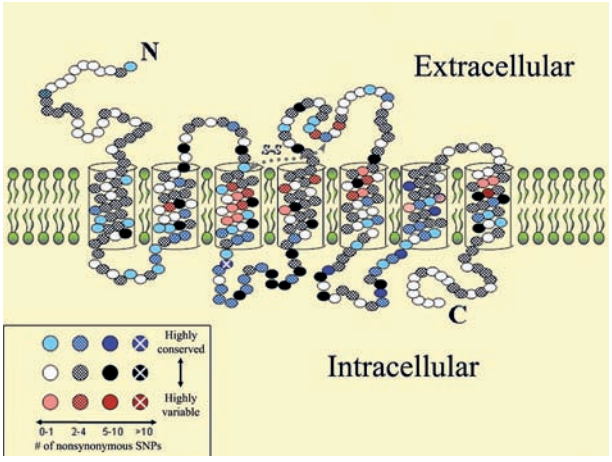

Figure 2. Genetic variability in olfactory receptors. Two levels of genetic variability are depicted along the OR protein consensus sequence diagram. Each residue (circle) positional conservation among human, mouse and dog ORs is indicated by a color (blue red and grey for highly conserved, highly variable and indifferent). In addition, the color intensity signifies the number of nonsynonymous SNPs that are found for each position in the HORDE database (http://bip.weizmann.ac.il/HORDE for details).

taining 17 families, which further divide into subfamilies, all based on sequence similarity scores. ORs with >40% protein sequence identity are considered within the same family and if they share >60% as belonging to the same subfamily [25]. It was suggested that ORs of the same subfamily might recognize molecules with similar chemical and/or physical characteristics [3, 20, 21]. However, more experimental evidence is needed to fully confirm this assumption [26, 27].

The OR coding region spans ~1 kb, almost always without introns, a property that facilitates their identification and cloning from genomic DNA. Vertebrate OR genes are organized in genomic clusters and are distributed on almost all chromosomes. As an example, in humans they are absent only on chromosomes 20 and Y. This wide genomic distribution is believed to have evolved from a single OR gene. This evolutionary process of OR gene migration was favored by strong selective pressure towards expanding and diversifying the OR gene repertoire, as an increased OR count likely enhances both sensitivity and selectivity [4]. Interestingly, this repertoire augmentation process appears to have been reversed in primates in the last 20 million years, probably because they became less dependent on olfactory cues [28–30]. This is manifested in the observation that in such species OR genes underwent a massive accumulation of pseudogenizing mutations, generating in-frame stop codons. This process of OR gene loss has remarkably accelerated in the human lineage, leaving less than half of the OR genes intact [22, 28, 29, 31, 32]. The high prevalence of defective human OR coding regions is a wide evolutionary deterioration whose phenotypic impact awaits elucidation. Each such pseudogene may be regarded as a natural knockout, potentially affecting the human ability to detect and discriminate odorants.

Human olfactory diversity

Unlike most mammals, humans do not depend on olfactory cues for survival and therefore are considered microsmatic organisms (organisms with a feeble sense of smell). Still, we use our nose to enjoy perfumes, food and beverage, avoiding poisons and stale food, as well as in subtle social interactions. The relatively minor importance of olfaction to human evolutionary fitness is reflected in a remarkable OR gene loss which has been thought to play a significant role in our somewhat inferior olfactory sensitivity [29, 31]. Nevertheless, a recent study demonstrated that in human and other primates, typical detection thresholds towards various odorants are comparable to those of rodents [33]. It appears that the considerable evolutionary change in human olfaction could underlie more subtle olfactory deficits.

It has indeed been known for decades that human beings are highly variable in their olfactory sensitivities. This interindividual variability is in the form of cases of significant threshold deficiencies towards particular odorants, termed specific anosmia or 'smell blindness' [1, 34–36]. Such human deficiencies have been studied for dozens of odorous chemicals [36]. An ambitious study in this field was carried out under the auspices of National Geographic magazine, examining six distinct odorants in nearly 1.5 million of its readers [37]. Pronounced variability was observed, with the most notable cases (~30% prevalence) of specific anosmia being towards androstenone and galaxolide (musk). This survey as well as other olfactory studies also demonstrated that olfactory sensitivity towards particular odorants may vary significantly according to age, gender, geographical location and various environmental factors, suggesting a complex trait [38–41].

Specific anosmia

Specific anosmia, the incapacity of an individual with otherwise normal smell sensitivity to detect particular odorants is seldom absolute. Most often a person has a 10–100 fold diminished sensitivity to a given odorant, hence the more exact term is specific hyposmia. In contrast, specific hyperosmia is described as enhanced sensitivity towards a specific odorant [36]. Both hyper- and hyposensitivity towards specific odorants are likely driven by the same molecular mechanism. Yet despite the wide variety of reports of specific anosmia, no comparable evidence has been reported for specific hyperosmia.

Considerable evidence indicates that specific anosmia is genetically determined. For example, various studies

showed that anosmia to androstenone (16-androsten-3-one), a steroid of gonadal origin that serves as a boar pheromone, is highly concordant in monozygotic twins [34, 42]. Whissell-Buechy et al. [43] demonstrated that anosmia to the odorant pentadecalactone behaves as a recessive trait in human beings. A similar Mendelian recessive inheritance was observed in mice with specific deficiency to detect isovaleric acid [44]. A subsequent linkage analysis study has associated this phenotype with two distinct genomic loci, on mouse chromosomes 4 and 6 [45]. Interestingly, a small fraction in the human population is also incapable of specifically detecting isovaleric acid [35]. A comparable linkage analysis in humans, potentially focusing on the human syntenic regions, would be advisable.

Although specific anosmia has a strong genetic element, other confounding factors contribute to the overall olfactory variability of human individuals. For example, olfactory faculties are age dependent, reaching a maximum in the late teenage years and then declining gradually [38]. This deterioration accelerates and becomes significant during the 6th decade. Similarly, olfactory sensitivity may be influenced by gender, whereby women have been reported to perform better than men in certain olfactory tests [38]. Other environmental and behavioral factors have also been suggested to affect olfactory performance, whereby subjects allegedly anosmic to androstenone became capable of smelling it following repeated exposure [46]. This phenomenon of 'olfactory plasticity' could explain part of the variability in androstenone perception, and in general, incomplete penetrance has to be taken into account for this odorant and its functional homologs. Overall, this evidence illustrates that human response to odorous molecules is a complex trait, regulated by genetic, developmental and environmental factors.

The molecular basis of 'odor blindness' (specific anosmia) is believed to be analogous to color blindness and specific taste deficits. Color vision is controlled by three genes encoding cone opsin receptors for long (red) and medium (green) wavelength (on chromosome X) and for short (blue) wavelengths (autosomal). Mutations that inactivate one of these genes significantly diminish our color discrimination ability and result in a different form of color blindness [47]. A similar phenomenon was recently observed in the more complex system of taste perception. A member of the taste receptors class II (*T2R38*) gene was associated with the gustatory capability to detect phenylthiocarbamide (PTC) [48]. Like many cases of specific anosmia, the ability to taste the bitter taste of PTC was subject to various psychophysical studies that indicated a Mendelian recessive trait [49] with possible genetic and environmental confounding factors [50]. Indeed, the two haplotypes of the *T2R38* gene that segregated significantly between tasters and non-tasters of PTC could not explain the entire diversity in the study. Thus, it was suggested that PTC detection is a complex trait with a major quantitative trait locus (QTL), with relatively high phenotypic effect. In this aspect, smell perception is more closely related to gustation than to color vision, since it also employs a group of chemoreceptors to distinguish between large varieties of chemicals. Still, as all three sensory pathways utilize the same operating principles based on GPCRs, it may be that their interindividual phenotypic variability is driven by broadly similar genetic mechanisms.

General anosmia

In addition to the human interindividual diversity in perceiving specific odorants, humans also vary in their general olfactory capabilities. This covers a wide range of phenomena, from general anosmia through hyposmia (diminished sensitivity to smell). At the other end of the scale is general hyperosmia (enhanced smell sensitivity) [51]. Moreover, during aging, a general olfactory loss occurs which is also a feature of several neurodegenerative diseases such as Alzheimer and Parkinson diseases [52].

It is estimated that about 1% of the Western world's population suffers from chemosensory disorders. Most of the people suffering from smell disorders have an acquired condition, which develops during life, due to allergy, viral upper respiratory tract infection, nasal sinus diseases, head trauma, inhalation of noxious chemicals or medicinal drug intake [53, 54]. A much smaller minority are born without a sense of smell, an affliction referred to here as congenital general anosmia (CGA). Two broad categories of CGA can be considered: CGA occurring with other anomalies (syndromic) and CGA seen as an isolated condition. Prevalence for isolated CGA is roughly estimated to be between 1:5000 and 1:20,000. Upon examination by biopsy of the olfactory region in several anosmic patients, their respiratory epithelium was found to be normal, but not their olfactory epithelium, some of them lacking it totally. In all cases, axonal abnormalities and the absence of mature olfactory sensory neurons were observed. It has been proposed that the olfactory epithelium may degenerate due to functional failure in olfaction [55, 56]. Genetic studies of CGA are very limited due to its low prevalence in the human population. And although no causative mutation was found for this phenotype, all familial cases described are consistent with an autosomal dominant mode of inheritance with partial penetrance [57–59].

The most well studied group of syndromic CGA is related to Kallmann syndrome. Such patients exhibit hypogonadotropic hypogonadism and anosmia, secondary to failure of gonadotropin-releasing hormone (GnRH)-producing neurons to migrate from the olfactory placode to the brain, and to agenesis of the olfactory bulbs. The prevalence of the disease has been estimated at one in

10,000 for males and five to seven times lower in females. Three different modes of inheritance have been reported in familial cases of Kallmann syndrome: X chromosome-linked, autosomal dominant and autosomal recessive [60]. The X-linked form of Kallmann syndrome has been well characterized, being caused by mutations in the gene *KAL1* (chromosome Xp22.3) [61–63]. The *KAL1* protein, anosmin-1, is a locally restricted component of basement membranes and/or extracellular matrices during the organogenesis period [60]. It has been found to enhance axonal branching from olfactory bulb output neurons [64] and to affect the migratory activity of gonadotropin releasing hormone (GnRH)-producing neurons [65].

To date, no causative genes have been described for isolated human CGA. However, in mouse, three transduction genes have revealed behavioral phenotypes consistent with general anosmia when they are inactivated. Knockout mice for the functional olfactory cyclic nucleotide-gated channel (Cnga2) [66], the stimulatory olfactory G-protein (Gnal) [67] or enzyme adenylyl cyclase III (Adcy3) [68] display profound reductions or even absence of physiological responses to odorants. Most of the homozygously deficient mice die within a few days after birth due to an apparent inability to locate their mother's nipple and suckle.

Congenital and progressive blindness have been studied much more extensively in human beings. Inherited forms of blindness can be caused by anomalies in the central nervous system [69] or in different parts of the eye: cornea, iris, lens, retina and the optic nerve. About 140 genomic loci have been associated with retinal dystrophies, and more than 60 genes have been identified. The partial similarity in the transduction processes between olfaction and vision may facilitate the future identification of some of the causative genes of CGA and its mode of genetic transmission.

The possible molecular basis of human olfactory variability

The molecular mechanisms of human olfactory variability may be deduced from the biochemical pathways of olfactory reception. The binding of an odorant to an olfactory receptor initiates a cascade of events leading to olfactory signaling (Fig. 1). Changing one or more components in this signal transduction pathway may lead to an abnormal olfactory phenotype. In this realm, the most promising candidate genes are the human orthologs of the three mouse knockout transduction genes described above. Similarly, mutations in other genes of olfactory signal transduction cascade may underlie changes in the membrane action potential and consequently in the olfactory neurological signal. For example, olfactory marker protein (OMP), a well-established marker of olfactory tissues [70], has been shown to modulate the kinetics of olfactory electrophysiological responses. In addition, there are two kinds of phosphodiesterases (PDEs): calmodulin-PDE (PDE1C) and cAMP-PDE (PDE4E), and various protein kinases [71] that were implicated in the termination of olfactory signal transduction. Other genes that may perturb initiation of olfactory signaling are those who facilitate the interaction between odorants and their receptors. Included in this group are biotransformation enzymes proposed to play a role in the post-signaling processing of the odorant molecules themselves, thus eliminating them from the vicinity of ORs. These include cytochrome P-450 (*CYP2G1*) and UDP glucoronosyl transferase (*UGT2A1*) [72–74]. In addition, there are odorant-binding proteins (*OBP2A*), lipocalins that may mediate the binding of odorants to olfactory receptors or prevent saturation of olfactory receptors by excessive odorant concentration [75]. It should be noted that some of these candidate genes are functionally expressed in other non-olfactory tissues, and mutations in these components may result in more compound disorders or syndromic anosmia. Finally, changes in proteins which participate in the transcription regulation of OR genes as well as other crucial genes in OSN may cause an inappropriate development and functionality of the olfactory system. Possible candidates of this kind are members of the Olf/Early B-cell factor and Nuclear Factor I transcription factor families, which are mainly expressed in post-mitotic olfactory neurons [76, 77] and control the expression of several genes (*ADCY3, CNGA2* and *OMP*).

In contrast to the diverse molecular mechanisms that could underlie general olfactory sensitivity, the main obvious candidate genetic determinants of olfactory perturbations towards specific odorants are OR genes. Moreover, these types of olfactory discrepancies are significantly more prevalent than general anosmia [36, 78] and hence are likely caused by more frequent genetic variations. Single-nucleotide polymorphism (SNP) sites at which two alternative bases occur at appreciable frequency are the most common genetic variation in the human genome. Consequently, they are believed to constitute the genetic component of most multifactorial human traits. In the case of specific anosmia, SNPs that modify a particular OR function could lead to significant threshold sensitivity differences towards particular odorants. This could be rationalized by the 'threshold hypothesis' [2]. According to this premise, the highest-affinity receptor towards a certain odorant is the one that determines the odorant's threshold sensitivity. If this OR is damaged, the threshold will be defined by the next highest affinity receptor. In the case of a large OR repertoire, as in rodents, a high level of functional redundancy is maintained, and the loss of the highest-affinity receptor will have a diminished probability of generating a recognizable olfactory deficit. In contrast, in humans, where the OR repertoire

has diminished significantly, affinity values would tend to be more widely spaced, and threshold variations could become more prevalent. In such cases, an inactivating polymorphism in an OR that encodes the best receptor for a certain odorant is expected to cause a significant threshold difference between individuals who carry the functional OR and those in whom it is deleted.

Various types of SNPs in OR genes might underlie odorant-specific olfactory deficits. The most obvious ones are the ~600 nonsynonymous SNPs (nsSNPs) that may change a residue crucial to protein function (Fig. 2) [79]. For example, changing the arginine (R) in the highly conserved MAYDRY motif that is believed to participate in the coupling of the G protein to the receptor was demonstrated to terminate the protein function [80]. Despite the relatively high conservation of this residue, it has been found to display polymorphisms in 15 different intact OR genes, which is significantly more than the average polymorphism count per residue along the protein sequence. Other types of candidate polymorphisms are those that change amino acids in the complementarity-determining region (CDR) for odorant recognition in a functional OR (Fig. 2) [20, 21]. These might not inactivate the receptor but rather change the affinity spectrum of its corresponding odorants. In addition, polymorphisms in the promoter or other regulatory regions of OR genes might underlie expression modifications which modify OR function [12]. The present partial knowledge about the genomic disposition and control mechanisms of ORs renders the detection of such important deleterious polymorphisms less straightforward.

The most promising candidates for underlying odorant-specific olfactory deficits are recently discovered SNPs of a highly unusual disposition. These generate a premature stop codon in the OR gene sequence and consequently segregate between an intact gene and an inactive one (pseudogene). Several dozens such Segregating PseudoGenes (SPGs) have been discovered in the human OR sub-genome [81]. These define a remarkable genetic variability whereby almost every human being possesses a unique assortment of intact and disrupted ORs. Furthermore, significant differences were observed among various ethnic groups in the degree to which certain polymorphic pseudogenes are conserved in their intact forms. While these ethnic differences in functional OR repertoires could be explained in terms of geographic isolation and bottleneck events, an alternative evolutionary mechanism is related to selection, suggesting that different intact ORs tend to be conserved more effectively depending on geography or lifestyle. These OR SPGs introduce a remarkable genetic diversity to the human genome, whose phenotypic correlate awaits elucidation.

Another type of potential causative genetic polymorphisms in human OR genes are the observed variations in copy number of several OR genes, mainly on chromosomal telomeric regions [82]. These polymorphisms are believed to have emerged via meiotic non-allelic homologous recombination (NAHR) events that are associated with genomic segmental duplication in the human genome. In rare meioses, these segmental duplications are mistaken for allelic sequences, with the result that chromosomes are incorrectly spliced. Consequently, NAHR could account for various types of genomic rearrangement in OR clusters, such as deletions, duplication and inversions. Similarly, it may account for gene conversion events that may underlie disruptive mutations by introducing non-functional sequences from a pseudogene into a functional gene [28, 83].

Finally, although ORs are the most straightforward candidates, the potential involvement of non-receptor genes in specific anosmia cannot be ruled out. A well-known example is the involvement of Acj6, a POU-domain transcription factor, in specific anosmia to a subset of odorants in the fruit fly *Drosophila melanogaster* [84].

Closing remarks

The significance of olfaction to the quality of human life is often underestimated. This sensory pathway plays a key role in food and beverage recognition and enjoyment, it enables people to avoid dangers such as smoke, spoiled food and poison, and it affects human social interactions. The realization of the underlying genetic mechanisms of human olfactory diversity would open new opportunities to both the food and fragrance industry. For example, human panels for the testing of new products could be selected such that their genetic profiles would fit those of the targeted consumers in an optimal way. Similarly, products could be directed to certain segments of the population according to their predictable olfactory capabilities. Alternatively, specific anosmia or hyperosmia could be artificially induced to eliminate or enhance specific odors. This could be done both at the genomic level, where specific OR genes would be silenced or overexpressed, and at the protein level, using agonists and antagonist. Thus, much of the future of olfactory research and development might depend on deciphering the genetic basis of specific human deficiencies.

Moreover, in the post-genome era, considerable efforts are being devoted to deciphering the genetic basis of human multi-factorial traits, attempting to correlate between phenotypes and genotypes. In this realm, genetic research on human olfactory diversity could be an excellent model system for understanding the genetic basis of human phenotypic variation. The current situation, where olfaction is no longer essential to human fitness and hence no selective constraints are acting on the related genes, allows the introduction of various genetic perturbations into the system. Consequently, it has become a substantial platform for genetic studies. As in olfaction, there are

many biological systems where gene families subserve a particular functional role in identification and processing various ligand libraries. We propose, therefore, that a better understanding, through genetic studies, of odorant-OR relations would significantly help to form a better picture of similar complex systems.

Summary

The sense of smell, a highly complex neurobiological system that facilitates the discrimination of millions of volatile compounds, is crucial for survival and well-being. Genetic polymorphisms in olfactory genes may result in two distinct, yet related phenotypic consequences. In mouse, mutations in the olfactory G protein transduction pathway result in complete olfactory deficiency. In humans, the counterpart phenotypes are relatively rare, and therefore their causative mutations have not yet been identified. It is likely that subtle variations in general olfactory performance are more common in the human population and hence constitute an auspicious field for future genetic studies. On the other hand, genetic polymorphisms in OR genes are prevalent and amply documented. Some of these genetic variants exchange between functional and non-functional forms of the coded OR protein (segregating pseudogenes), while others segregate between gene existence and absence. These genetic polymorphisms are promising candidates to underlie the abundant human olfactory threshold variations, namely the specific anosmia phenotypes. Future studies of human olfactory deficits would shed further light on the molecular mechanism of odor perception, as well as on the genetics of analogous biological systems.

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