Summary

Olfactory receptors (ORs) belong to heptahelical G-protein coupled receptors as the largest group of membrane receptors in the human genome and they have evolved to detect a wide range of chemical structures. The first step in odour transduction in the olfactory system is mediated by olfactory receptors. Perception of odourants shows diversity within different human populations. It has been proposed that widespread phenotypic diversity in human olfaction is, partly, related to genetic variation in OR genes. In some examples it has been demonstrated that variation in OR genes by single nucleotide polymorphism (SNP) is at least partially responsible for this kind of difference. Segregating pseudogenes (SPGs) are known as genes that due to a disruptive SNP segregate in populations between intact genes and pseudogenes. This divider mutation can introduce a stop codon, or alter a highly conserved amino acid that is important for the proper functioning of the protein.

In this study, to gain more insight into the relation between olfactory receptors and chemical components, we carried out a large-scale investigation of ligand-receptor interactions for the human olfactory receptors as copy number variations (CNVs) and SPGs by a broad range of odourants related to anosmia with functional analysis and site-directed mutagenesis.

In this study OR1B1, OR2L8, OR4X2, OR8D2 and OR8B4 were deorphanized as SPGs with minor allel frequency (MAF of SNPs that convert active genes to pseudogene) of 33%, 22%, 16%, 50% and 26% respectively. Our data indicate that OR1B1 was activated significantly by 3-hydroxy-2-methyl-4pyran, Calone, androstenone and testosterone. OR2L8 had statistically significant responses to Yasamber and Timbrol and OR4X2 showed significant response to 2-aminoacetophenone. OR8D2 responded to anisic aldehyde and aldehyde C6 and OR8B4 to alcohol C6 and Muguet alcohol.
OR1B1 as SPGs display both functional and nonfunctional alleles in humans which make it a suitable candidate to explain variation of androstenone perception in human populations. Androstenone is a steroid and a metabolite of testosterone. Testosterone and androstenone have a similar general chemical structure with the same number of CAN. The relation between SNPs and odourant responses were studied by point mutations. OR1B1-574 as a mutation form of OR1B1 that converts the active gene of OR1B1 into a pseudogene, as was expected severely impairs OR1B1 function. OR10Q1 as one OR in the CNV group responds to ω-cyclopentadecalactone as an odourant in the musk group. The mutation form of OR10Q1 was produced according to unique single nucleotide variation with MAF> 10%. We investigated the ligand sensitivity of OR10Q1-540 (mutated variant) and OR10Q1 (wild type) receptor variants in vitro with Pentadecanolide as a suitable ligand for OR10Q1. The mutated variant did not show any different significant response in comparison with OR10Q1-WT.

We deorphanized 18 ORs including SPGs and CNVs and showed that there is no simple, direct relation between molecular features and the ability to respond to receptors. In our study, three groups were classified by OR activation: first, those ORs that only responded to one odourant; second, ORs that responded to more than one odourant according to special odourant descriptors; and third, ORs that responded to more than one odourant but did not follow any special rules.

It was hypothesised that similar functional residues between TM3 and TM6 may recognise the same or similar odourant ligands. So, we made multiple sequence alignments between all of the TM3-6 in deorphanized olfactory receptors. However, for some of the ligands, we found that the same odour activates closely related receptors from the same clade but for others, receptors from different clades are activated. It can be stated that by comparing the amino acid sequences of OR, no accurate prediction about OR response to odourants can be performed.
Although segregating pseudogenes seems to be a promising factor for genetic diversity in olfactory receptors, we could not show the role of individual SPGs in the variation of odourant perceptions, especially when similar odourants could activate more than one OR. The lack of one of them probably cannot interrupt the procedure of odourant perception. Also, according to our results, in addition to SPGs, CNVs responded to odourants related to specific anosmia. For instance, musk odourants only responded to ORs in the CNV group.