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Olfactory-like receptor cDNAs are present in human lingual cDNA libraries ☆

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Abstract

Olfactory and pheromone receptors (ORs) constitute a large family of G-protein-coupled receptors involved in the detection and transduction of odorant signals. Using degenerated primers complementary to the highly conserved transmembrane domains II, III, VI, and VII within this protein family, Gaudin et al. have recently described the expression of several OR genes in foetal human tongue. Among the nine genes identified in human foetal tongue (*HTPCR06*, *HGMP07I*, *JCG6*, *TPCR85*, *JCGI1*, *JCG2*, *JCG3*, *JCG5*, and *JCG9*), only four (*HTPCR06*, *HGMP07I*, *JCG3*, and *JCG5*) were found to be expressed in adult tongue, suggesting that ORs might perform developmental functions in this organ. The objective of our work was to obtain additional information about the expression of olfactory-like genes in human tongue. In the present study, the synthesis and the screening of a cDNA library from epithelial cells of human adult tongue is reported. Two kinds of PCR analysis were performed. First, partial olfactory-like receptor cDNAs amplified with the degenerated primers used by Gaudin et al. were cloned and described. Second, a comparison of the expression profiles of the olfactory-like receptor genes previously identified before was carried out using specific primers. Among the genes studied we found that four genes (*HTPCR06*, *JCG3*, *JCG5*, and *JCG6*) are expressed in epithelial cells of the surface of the adult tongue. Additionally, we show that three olfactory-like receptor genes *OR7A5/HTPCR2*, *OR6Q1*, and *OR7C1/TPCR86* are also expressed in these cells.

Keywords: G-protein-coupled receptors; Epithelial cells; Taste tissues; Tongue

Olfactory receptors (ORs) were first identified in rats on the basis of sequence homology with other G-protein-coupled receptors (GPCRs), such as the adrenergic and dopaminergic receptors [1]. It is estimated that the murine genome contains almost 1400 OR genes, while the human genome carries only about 900 OR genes [2]. The repertoire of functional OR genes in humans is almost three times smaller than in mice. Indeed, ORs have intronless open reading frames and over 50% have derived (because of frameshifts, in-frame stop codons, or disrupting interspersed repeats) in pseudogenes in humans [3,4]. This extreme decrease in human functional olfactory repertoire can be qualified as a relatively recent genomic process, which is probably still continuing because of the disappearance of a selection pressure. A substantial fraction of the functional OR

☆ *Abbreviations:* bp, base pair; dNTP, desoxyribonucleotide triphosphate; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; GPCR, G-protein-coupled receptors; OLR, olfactory-like receptor; OR, olfactory receptor; ORF, open reading frame; PCR, polymerase chain reaction; RT, reverse transcription.

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genes encoded by human genome is organized in clusters distributed over the majority of human chromosomes except chromosomes 20 and Y [2]. Despite its relatively "small" size, the functional olfactory "subgenome" occupies nearly 1% of the human genome [5].

All members of the GPCR family, ORs are constituted of seven transmembrane α-helices separated by three extracellular and three intracellular loops [6]. It has been suggested that the region between second and sixth transmembrane domains of ORs forms a ligand-binding pocket [7,8]. Recently, a prediction of the odorant-binding site was made by comparing human and mouse ORs. Twenty-two amino acid positions were proposed to be essential for ligand-binding on the basis of their high conservation between orthologs and on the basis of their variability among paralogs. Nevertheless, up to date, no three-dimensional structure of any GPCR (except rhodopsin) has been established [9] and little is known about structural determinants of ligand recognition by the ORs. This is largely due to the problems with purification and crystallization of ORs and, to some extent, to the difficulty in functional expression of their genes in heterologous expression systems [6] where expression yields are low.

OR genes are expressed in olfactory neurons in a monoallelic fashion. This implies that only unique receptor gene, among the hundreds present in the genome, is expressed in a single neuron [10]. It is interesting to mention that the expression of OR-like mRNAs was also observed in spermatozoids [11,12], lingual epithelium [13,14], heart [15], and olfactory bulb [16] in different species. Several hypotheses were proposed concerning plausible functional roles of OR proteins produced in tissues different from olfactory epithelium. It has been proposed that they are involved: in neuronal guidance during embryogenesis [17], in chemotaxis in case of male germ cells [18] or in taste recognition [13,19]. Indeed, Abe et al. found a full-length cDNA (*GUST27*) encoding a protein structurally related to the olfactory receptors and that the GUST27 mRNA was expressed in taste buds and in the surrounding cellular tissues of the rat tongue papillae. For the time being, no trustworthy confirmation of this hypothesis was ever obtained.

The objective of our work was to obtain additional information about the expression of olfactory-like genes in human tongue by the characterization of additional partial OLR cDNAs in a cDNA library from adult human lingual epithelium and by the analysis of the expression profile in this library of the OLR cDNAs identified previously [14] in human tongue samples.

Materials and methods

Biological samples. Human lingual epithelial cells were brushed from the tongue surface of a Caucasian adolescent patient (regarded as an adult individual in the paper) submitted to papilloma tissue removal and stored in "RNAlater" buffer (Qiagen, Ambion, Austin, Texas, USA) at −20 °C for several days. After defrosting, the solution was centrifuged and mechanically homogenized with a Dounce homogenizer.

Total RNA extraction. Total RNA was extracted from homogenized human lingual epithelial cells from the patient using GenElute Direct mRNA kit (Sigma–Aldrich, 3050 Spruce St., St. Louis, USA) [20] and its integrity was checked by agarose gel electrophoresis. Forty-five microlitres of the sample was treated with DNase RNAse-free (Promega, USA) for 30 min at 37 °C. The RNA sample was submitted to acid phenol extraction and subsequently to ethanol precipitation to remove DNAse.

cDNA synthesis. cDNA synthesis was performed using Superscript Choice System for cDNA Synthesis (Invitrogen, Carlsbad, USA). The first and second strand synthesis of the cDNA was accomplished by Superscript II reverse transcriptase respecting the conditions defined by the supplier. An oligo d(T)*Not*I primer was used for reverse transcription (RT) (Table 1). The second strand was synthesized in the volume of 100 μl using the *Escherichia coli* DNA I polymerase in combination with *E. coli* DNA ligase and *E. coli* RNAseH.

The reaction was performed in the buffer supplied by the furnisher where (ss)cDNA, dNTPs, and DEPC water were added. The sample was incubated during 2 h at 16 °C, and then T4 DNA polymerase was added to ensure that the termini of the cDNA are blunt ended. The reaction was carried out for additional 5 min and finally stopped by EDTA (0.5 M). Double strand cDNA was precipitated with ethanol and ligated to an *Eco*RI adapter (Table 1). cDNAs were fractioned according to their size by column chromatography (Invitrogen). The neighbouring fractions (from 1 to 6, 7 to 12, and 13 to 20) were pooled and precipitated with ethanol.

cDNA library amplification. cDNAs were amplified using Expand Long Template PCR System (Roche, Basel, Switzerland) in the following conditions: 94 °C for 2 min (predenaturation); 10 cycles, 94 °C for 10 s, 46 °C for 30 s, and 68 °C for 5 min; 20 cycles, 94 °C for 10 s, 46 °C for 30 s, and 68 °C for 5 min (each elongation step longer by 20 s as the previous one); 68 °C for 7 min (final extension) [d(T)*Not*I and *Eco*RI primers were used]. The PCR was performed in the buffer supplied by the furnisher in the final volume of 50 μl.

PCR conditions were as follows: 95 °C for 3 min (predenaturation); (30 cycles), 95 °C for 1 min, 55 °C for 1 min, and 72 °C for 1 min; 72 °C for 7 min (final extension).

Detection of OLR cDNAs. In order to clone the partial OLR cDNA contained in our library, the former was screened by PCR using degenerated primers (RS1, RS2, RAS3, and RAS4) complementary to the conserved transmembrane domains (II, III, VI, and VII) of ORs. These primers were designed on the basis of olfactory receptor gene homology [14]. *RS1* and *RS2* were used as 5′ primers, and *RAS3* and *RAS4* as 3′ primers (Table 1).

All PCR were performed in the total volume of either 10 or 50 μl using l0× PCR buffer, Finzyme polymerase 0.4 U/10 μl or HotStartTaq polymerase 0.5 U/10 μl (Qiagen SA), 200 μM of each dNTP; 1.5 mM $MgCl_2$ in case of HotStart polymerase buffer, 1 μM degenerated primers or 0.3 μM specific primers.

PCR for OLR genes [14] was performed as follows: predenaturation—15 min at 95 °C; 30 cycles, denaturation 30 s at 94 °C; annealing 1 min 30 s at 55 °C, and elongation 2 min at 72 °C; final extension 7 min at 72 °C.

Amplification of full-length ORFs of OLRs *JCG5*/*OR10A4*, *OR7A5*, and *OR6Q1*, chosen on the basis of the results obtained with degenerated primers, was performed as follows:

JCG5/*OR10A4:* predenaturation—5 min at 95 °C; 30 cycles, denaturation—1 min at 95 °C, annealing for 30 s at 48 °C, and elongation for 1 min 15 s at 72 °C; final elongation for 10 min at 72 °C.

OR7A5: predenaturation—5 min at 95 °C; 30 cycles, denaturation—l min at 95 °C, annealing—30 s at 46 °C, and elongation—1 min l5 s at 72 °C; final elongation—10 min at 72 °C.

Table 1
List of the primers used

cDNA synthesis (5′ → 3′)	
oligo(dT) *Not*I	5′-TTT-TGC-GGC-CGC-$(TTT)_6$-3′
*Eco*RI adapter	5′-CGA-C AG-C AA-CGG-3′
(double strand)	3′-GCT-GTC-GTT-GCC-TTAA-5′

Degenerated primers
RS1: 5′-CA(AGCT)AC(AGCT)CC(AGCT)ATGTA(CT)(ACT)T(AGCT)TT(CT)(CT)-3′
RS2: 5′-ATG GC (AGCT)TA(CT)GA(CT)(AC)G(AGCT)TA(CT)GT(AGCT)GC-3′
RAS3: 5′-(GC)(CT)RCA(AGCT)GT(AGCT)(GC)(AT)RAA(AGCT)GC(CT)TT-3′
RAS4: 5′-TA(AGT)AT(AGCT)AR(AGCT)GGRTT(AGCT)ARCAT(AGCT)GG-3′

Gene name	Full-length coding sequence amplification (5′ → 3′) (Gaudin et al.)
HGMP07I/OR1E1	5′-ATG ATG GGA CAA AATCAA AC-3′ 5′-TCAGAG AGA GAA GAA AGT T-3′
JCG1/OR5P3	5′-ATG GGG ACT GGA AAT GA-3′ 5′-TCA AGA AAA TAT TTT TTA TTC TAA G-3′
JCG2/OR8D2	5′-ATG GCT ACT TCA AAC CAT TCT TC-3′ 5′-TCA GGA TGA CTG CCT TCC C-3′
JCG3/OR5P2	5′-ATG AAT TCC CTG AAG GAC G-3′ 5′-CTA TGT AAT ATC ATT ATT TGA AGT TC-3′
JCG5/OR10A4	5′-ATG ATG TGG GAA AAC TGG-3′ 5′-TCA TAG TTT CTG AGA GCC-3′
JCG6/OR10A5	5′-ATG GCT ATA GGA AAC TGG-3′ 5′-CTA TGG GAT ACA GTT TCT G-3′
JCG9/OR8D1	5′-ATG ACC ATG GAA AAT TAT TCT A-3′ 5′-TCA TTT TCC TAC TAA GAC CT-3′
TPCR85/OR8B8	5′-ATG GCT GCT GAG AAT TC-3′ 5′-TCA GGA GAA TGC ATT TTT G-3′
HTPCR06/OR2K2	5′-ATG CAA GGA GAA AAC TTC AC-3′ 5′-TCA GAG ATG TTC GTG TGT TT-3′
GAPDH	5′-GAA ATC CCA TCA CCA TCT-3′ 5′-TCC ACA GTC TTC TGG GTG -3′
	Full-length coding sequence amplification (5′ → 3′) (this work)
JCG5/OR10A4	5′-CTC GAG ATG ATG TGG GAA AAC TGG ACA-3′ 5′-AAG CTT TCA TAG TTT CTG AGA GCC CAG-3′
HTPCR2/OR7A5	5′-CTC GAG ATG GAA CCA GGA AAT GAT ACA-3′ 5′-AAG CTT TGG GCA CTT CTT GAA AAA TTG-3′
OR6Q1	5′-CTC GAG ATG GGC TTT GCT GGC ATC CAT-3′ 5′-AAG CTT TCA CTG TCC CTT CCA AAAGCT-3′
TPCR86/OR7C1	5′-CTC GAG ATG GAA ACA GGA AAT CAA ACA-3′ 5′-AAG CTT TGA AAG TCC TGC AGT GAT GTC-3′

OR6Q1: predenaturation—5 min at 95 °C; 30 cycles, denaturation—1 min at 95 °C, annealing—30 s at 65 °C, and elongation—1 min at 72 °C; final elongation—10 min at 72 °C.

Amplification of *OR7C1* cDNA has been unsuccessful although many PCR conditions were tested. Primer sequences were synthesized for positional cloning so they include additional *Xho*I and *Hin*dIII restriction sites.

Cloning and sequencing of PCR products. PCR fragments were separated by 1.2% agarose gel electrophoresis, purified from agarose gel using the Qiaex II Gel Extraction Kit (Qiagen), and cloned into the pGEM-Teasy vector in the conditions suggested by the supplier (Promega). Fifty nanograms of vector and ~100 ng of insert DNA were used for each reaction. The final volume of ligation was either 10 or 20 μl depending on the DNA concentration in the samples. Ligation reaction was carried out at room temperature for 1 h or overnight at 4 °C.

Sequencing of recombinant vectors was performed using T7 and SP6 primers by the sequencing facilities of the Institute of Biophysics and Biochemistry in Warsaw.

Bioinformatics. Human OLR partial sequences were analysed in HORDE ("The Human Olfactory Receptor Data Exploratorium"), a database dedicated to OLRs (Weizmann Institute of Science, Rehovot, Israel) (http://bioinformatics.weizmann.ac.il/HORDE), and in GenBank from National Library of Medicine, Bethesda, MD, USA (http://www.ncbi.nlm.nih.gov/Genbank). Similarity-based searches were performed using GenBank Blast 2.1 software (NCBI, http://www.ncbi.nlm.nih.gov/BLAST).

Translation in six reading frames of obtained human OLR partial sequences was done using the software from INFOBIOGEN (France) (http://www.infobiogen.fr/services/analyseq/). Measurement of the distance between protein sequences was done using the Jotun Hein method (MegAlign) and phylogenic tree was built using the Clustal method within the DNASTAR software.

Results

cDNA quality

The lack of genomic DNA in the sample containing cDNAs synthesized with Superscript system was

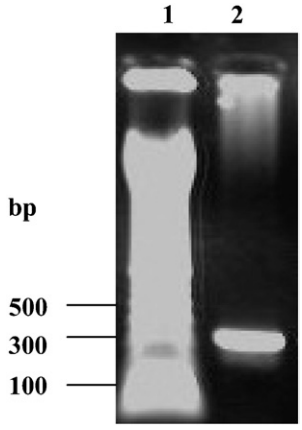

Fig. 1. Confirmation of cDNA quality. The amplification of GAPDH cDNA by PCR with specific primers (Table 1) was performed to prove that cDNA from epithelial cells was not contaminated by genomic DNA (the presence of band of 355 bp means that the amplified fragment does not contain intron—lane 2). Otherwise it would be 855 bp long. The molecular weight marker (lane 1) was 100 Base-Pair Ladder from Amersham Biosciences (Amersham Biosciences UK Limited, Amersham Place, Little Chalfont, Buckinghamshire, HP7, 9NA, UK). The PCR products were loaded on 1% agarose gel and submitted to electrophoresis.

confirmed by PCR using primers for the amplification of *GAPDH* (Table 1). They flank a region of *GAPDH* with an intron. Thus, the expected size of the cDNA amplicon is shorter by 500 bp compared to the size of the PCR product obtained from genomic DNA. As one may see in Fig. 1, cDNAs were devoid of any contamination by genomic DNA.

Cloning of partial olfactory-like receptor cDNAs from human lingual epithelial cells

The cDNA synthesized with the SuperScript system was amplified by PCR using degenerated primers RS1, or RS2 and RAS3 or RAS4. As it was observed before [14] we observed that only PCRs realized with RS2 and RAS4 primers were specific enough to be used for further analysis (data not shown). Results obtained with this primer set are presented in Fig. 2. A major PCR product of about 520 bp was obtained. Additional slightly visible bands (~300 and 900 bp) were not taken into consideration because it was reported earlier by subsequent cloning that only amplicons of ~520 bp are worth further analysis [14], and that ~300 and 900 bp bands come from artefactual amplifications. The PCR products were ligated into the pGEM-Teasy vector. After transformation, all the clones obtained were screened by PCR (using M13/pUC primers) (results not shown) and all PCR products corresponding to about 700 bp were analysed by *Alu*I restriction digestion in order to eliminate identical clones. *Alu*I digestion (numerous recognition sites in human genome) was a rough method used to differentiate the amplified OLR

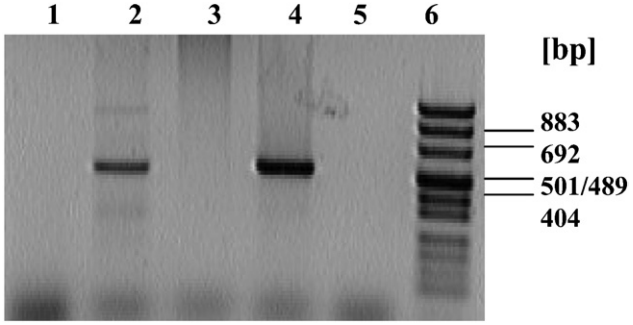

Fig. 2. PCR on cDNA libraries from adult tongue epithelial cells. PCR was performed on different cDNA chromatographic fractions with degenerated primers. PCR products were separated by 1% agarose gel electrophoresis: lane 1, cDNAs from fractions 1–6; lane 2, cDNAs from fractions 7–12; lane 3, cDNAs from fractions 13–20. A positive control was made by the amplification of *JCG2* cDNA from a plasmid containing the ORF (lane 4) and a negative control was made using water instead of cDNA (lane 5). In lane 6 was loaded a molecular weight marker (pUC Mix Marker 8, Fermentas, Lithuania,UAB V.Graiciuno 8,Vilnius 2028).

partial fragments one from another. Table 2 summarizes the results obtained from sequence alignments.

Among the clones obtained five of them contained cDNA of genes and seven contained cDNAs of pseudogenes. For instance, clones ROLF 2.1, ROLF 2.2, ROLF 3.3, ROLF 7.3 (containing *OR5G3P* cDNA), ROLF 16.2 (containing *OR7E24P* cDNA), and ROLF 18.1, ROLF 31.1 (containing *OR7A3P* cDNA) contained pseudogene cDNAs.

The clones ROLF 5.2 and ROLF 8.1 contained *JCG5* cDNA (also found as *OR10A4* in the HORDE database) previously described [14]. As compared to *JCG5 cDNA*, the cDNAs contained in ROLF 8.1 have one nucleotide change causing amino acid substitution Arg262 → Gln. ROLF 5.2 cDNA carries three nucleotide changes causing amino acid substitutions Argl82 → Ser, Vall97 → Glu, and Arg262 → Gln. This sequence is strictly identical to the *OR10A4* sequence.

The clone 12.2 contained the *OR6Q1* cDNA. By comparison to the sequence found in the HORDE database, two nucleotide changes were detected causing amino acid substitutions Vall02 → Phe and Tyrl03 → Cys.

The clone 19.2 contained the *OR7C1* cDNA initially described as *TPCR86* by Vanderhaeghen et al. [18]. It carries one silent mutation (C328 → T) as compared to *OR7C1*.

The clone 20.3 contained the *OR7A5* cDNA described as *HTPCR2* by Vanderhaeghen et al. [22]. It carries a single mutation, resulting in amino acid change Tyr123 → Phe.

The PCR fragments contained in two clones ROLF 2.1 and 18.1 (they differ from each other only by four nucleotides) localized in the same chromosomal clone RP11-430C17 correspond to highly homologous GPCR sequences but do not seem to be related to any OR sequences (96% identity at the nucleotide level with the

Table 2
Results of alignment of partial OLR clones with sequences available in databases

Clone name	Results of Blastn search	Differences with published sequences	Total number of mutations	Gene or pseudogene localization
ROLF 2.1	*Genomic clone RP11-430C17*	—	*19*	*pseudogene (11q)*
ROLF 2.2	*Genomic clone RP11-560B16*	—	*92*	*pseudogene (11)*
ROLF 3.3	*Genomic clone RP11-782L18*	—	*12*	*pseudogene (11)*
ROLF 5.2	**JCG5/OR10A4**	**Arg262 → Gln**	**1**	**gene (11pl5.3)**
ROLF 7.3	*OR5G3P*	—	*7*	*pseudogene (11q12.1)*
ROLF 8.1	**JCG5/OR10A4**	**Arg182 → Ser Val197 → Glu Arg262 → Gln**	**3**	**gene (11p15.3)**
ROLF 12.2	**OR6Q1**	**Val102 → Phe Tyrl03 → Cys**	**2**	**gene (11q12.3)**
ROLF 16.2	*OR7E24P*	—	*5*	*pseudogene (13p13.13)*
ROLF 18.1	*Genomic clone RP11-430C17*	—	*19*	*pseudogene (11)*
ROLF 19.2	**TPCR86/OR7C1**	—	**1**	**gene (19p13.1)**
ROLF 20.3	**HTPCR2/OR7A5**	**Tyr123 → Phe**	**1**	**gene (19p13.1)**
ROLF 31.1	*OR7A3P*	—	*1*	*pseudogene (19q13.1)*

Pseudogenes are presented in italic, genes in bold letters. The chromosomal localization is indicated in brackets. About the genes, the differences observed with the protein sequences found in the databases are indicated. The "total number of mutations" refers to the comparison of a given ROLF sequence to the "best match" from databases.

human seven transmembrane helix receptor). Two clones, ROLF 2.2 and ROLF 3.3, contained cDNAs very homologous to OR cDNAs (to *JCG5* with 81% identity and to *OR6Q1* with 97% identity, respectively) but correspond to the partial sequences of pseudogenes as they bear numerous mutations and stop codons. Other sequenced clones ROLF 31.1, ROLF 16.2, and ROLF 7.3 contained cDNAs of pseudogenes *OR7A3P*, *OR7E24P*, and *OR5G3P*, respectively. They all bear from one (in the case of ROLF 31.1) to several mutations (five and seven mutations for ROLF 16.2 and ROLF 7.3, respectively) compared to the sequences in HORDE.

Three genes *JCG5*/*OR10A4*, *HTPCR2*/*OR7A5*, and *OR6Q*1 first identified on the basis of their partial sequences were amplified from the same cDNA template with specific primers for the full-length ORF (Fig. 3). The full-length ORF cDNA of *TPCR86*/*OR7C1* could not be amplified although many PCR thermal profiles and reaction conditions were tested. *JCG5*/*OR10A4* and *HTPCR2*/*OR7A5* cDNAs were cloned into pGEM-Teasy vector and sequenced. The presence of point mutations described in Table 2 and mentioned above was confirmed. This result indicated that these mutations were not due to PCR mistakes but correspond to the genetic variants of the OR genes, confirming the relatively high degree of polymorphism previously observed for this family of genes [14,21].

Amino acid sequences deduced from new olfactory receptor genes (*HTPCR2*/*OR7A5*, *OR6Q1*, and *TPCR86*/*OR7C1*) cloned from human tongue are presented in Fig. 4

Expression profile of full-length ORFs of OLR genes from lingual cDNAs from different origins

Among the nine OR genes found to be expressed in the foetal tongue by Gaudin et al. (*HTPCR06*/*OR2K2*, *HGMP07I*/*OR1E1*, *JCG6*/*OR10A5*, *TPCR85*/*OR8B8*, *JCG1*/*OR5P3*, *JCG2*/*OR8D2*, *JCG3*/*OR5P2*, *JCG5*/*OR10A4*, and *JCG9*/*OR8D1*), four of them were also found to be expressed in the adult tongue: *HTPCR06*/*OR2K2*, *HGMP07I*/*0R1E1*, *JCG3*/*OR5P2*, and *JCG5*/*OR10A4*. Using PCR on a cDNA library obtained from adult human tongue epithelial cells we found that *HTPCR06*/*OR2K2*, *JCG3*/*OR5P2*, *JCG5*/*OR10A4*, and *JCG6*/*OR10A5* are expressed in the epithelial cells of the tongue whereas *HGMP07I*/*OR1E1* was not found to be expressed (Fig. 5). This expression pattern was compared with the data described by Gaudin et al. [14] (Table 3).

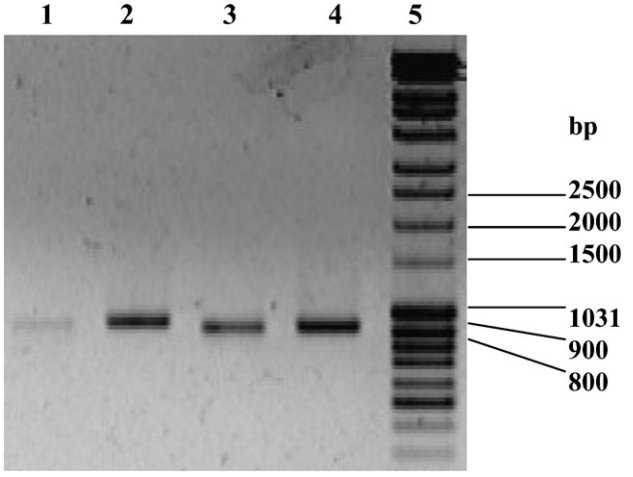

Fig. 3. Detection of *OR10A4*/*JCG5*, *OR7A5*/*hTPCR*, and *OR6Q1* full-length ORFs in a library from adult tongue epithelial cells. PCR was carried out on a cDNA library from epithelial cells of an adult tongue using specific primers for full-length ORF of *OR10A4*/*JCG5* (lane 1), *OR7A5*/*hTPCR2* (lane 2), and *OR6Q1* (lane 3), and *JCG2* used as a positive control (lane 4). The molecular weight marker (lane 5) was the Mass Ruler TM DNA Ladder Mix (Fermentas, Vilnius, Lithuania). The PCR products were loaded on 1% agarose gel and submitted to electrophoresis.

HTPCR2/OR7A5

MEPGNDTQISEFLLLGFSQEPGLQPFLFGLFLSMYLVTVLGNLLIILATISDSHLHTPMYFFLSNLSFADICVTSTTIPKMLMNIQTQNKVITYIACL
MQMYFFILFAGFENFLLSVMAYDRFVAICHPLH**F**MVIMNPHLCGLLVLASWTMSALYSLLQILMVVRLSFCTALEIPHFFCELNQVIQLACSDSFL
NHMVIYFTVALLGGGPLTGILYSYSKIISSIHAISSAQGKYKAFSTCASHLSVVSLFYGAILGVYLSSAATRNSHSSATASVMYTVVTPMLNPFIYS
LRNKDIKRALGIHLLWGTMKGQFFKKCP

OR6Q1

MQPYTKNWTQVTEFVMMGFAGIHEAHLLFFILFLTMYLFTLVENLAIILVVGLDHRLRRPMYFFLTHLSCLEIWYTSVTVPKMLAGFIGVDGGK
NISYADCLSQL**FC**FTFLGATECFLLAAMAYDRYVAICMPLHYGAFVSWGTCIRLAAACWLVGFLTPILPIYLLSQLTFYGPNVIDHFSCDASPLLA
LSCSDVTWKETVDFLVSLAVLLASSMVIAVSYGNIVWTLLHIRSAAERWKAFSTCAAHLTVVSLFYGTLFFMYVQTKVTSSINFNKVVSVFYSV
VTPMLNPLIYSLRNKEVKGALGRVFSLNFWKGQ

TPCR86/OR7C1

METGNQTHAQEFLLLGFSATSEIQFILFGLFLSMYLVTFTGNLLIILAICSDSHLHTPMYFFLSNLSFADLCFTSTTVPKMLLNILTQNKFITYAGCLS
QIFFFTSFGCLDNLLLTVMAYDRFVAVCHPLHYTVIMNPQ*LCGLLVLGSWCISVMGSLLETLTVLRLSFCTEMEIPHFFCDLLEVLKLACSDTFINNVVI*
*YFATGVLGVISFTGIFFSYYKIVFSILRISSAGRKHKAFSTCGSHLSVVTLFYGTGFGVYLSSAATPSSRTSLVASVMYTMVTPMLNPFI*YSLRNTDMKRALG
RLLSRATFFNGDITAGLS

Fig. 4. Protein sequences deduced from new olfactory receptor genes cloned from adult human tongue (established using www.infobiogen.fr.services/analyseq/). Amino acid substitutions (Table 2) are marked in bold letters. The entire amino acid sequence of *TPCR86/OR7C1* was established on the basis of translation of its partial cDNA sequence (italic letters) with help of HORDE database—full-length of *TPCR86/OR7C1* cDNA was never amplified with specific oligonucleotides.

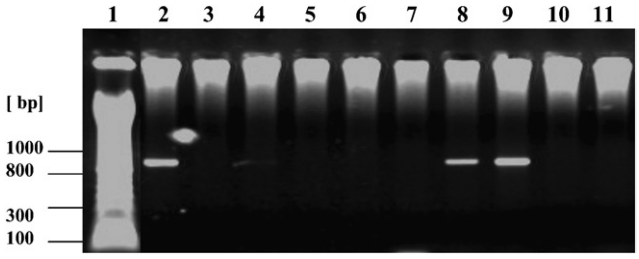

Fig. 5. Detection of ORLs transcripts in epithelial cells of adult tongue. PCR was carried out on a cDNA library from epithelial cells of an adult tongue using specific primers for full-length ORF of *HTPCR06/OR2K2* (lane 2), *HGMPO7I/OR1E1* (lane 3), *JCG6/OR10A5* (lane 4), *TPCR85/OR8B8* (lane 5), *JCG1/OR5P3* (lane 6), *JCG2/OR8D2* (lane 7), *JCG3/OR5P2* (lane 8), *JCG5/OR10A4* (lane 9), and *JCG9/OR8D* (lane 10). The molecular weight marker (lane 1) was 100 Base-Pair Ladder from Amersham Biosciences (Amersham Biosciences, Little Chalfont, UK). A negative control in which the cDNA template was omitted was added (lane 11). The PCR products were loaded on 1% agarose gel and submitted to electrophoresis.

Sequence similarities of all studied putative olfactory receptors expressed in human tongue are presented in Table 4 and a dendrogram reflecting protein sequence distances is presented in Fig. 6.

Discussion and conclusions

In the present study, we have cloned cDNAs of four OR genes (*JCG5/OR10A4*, *OR7A5/HTPCR2*, *OR7C1/TPCR86*), and *OR6Q1* and seven pseudogenes expressed epithelial cells of the tongue. The partial cDNA of *JCG5/OR10A4* is contained in two different clones: ROLF 8.1 and ROLF 5.2. As compared to the sequence of *OR10A4* found in the HORDE database, the first one carried three mutations leading to amino acid replacement Arg182 → Ser, Val197 → Glu, and Arg262 → Gln whereas the second one carried only one mutation leading to amino acid replacement Arg262 → Gln. Single point mutations in *JCG5* cDNA (a silent mutation at position 880 and a mutation at position 617 resulting in Leu206 → Pro replacement) were also observed before. It means that several alleles of OR10A4 are expressed in human population. Our results suggest that this gene may be highly polymorphic in human population. Three nucleotide changes in *OR7C1* cDNA contained in the clone ROLF 19.2 are silent mutations since they have no incidence on amino acid sequence of the translated protein. Thus, this represents a so-called silent polymorphism. In the case of *OR7A5* (also reported as *HTPCR2* by Parmentier et al. [11] and Vanderhaeghen et al. [22]), only one mutation was detected. In the case of *OR6Q1* cDNA, two mutations were found (Table 2).

Single nucleotide polymorphism (SNP) is the most common source of variation in the human genome and a particular attention is drawn to the identification of SNPs within coding regions (cSNPs). Sharon et al. [21] estimated the mean nucleotide diversity of OR coding regions at the level of 0.078% (ranging from 0% to 0.16%), which is twice higher than in the case of other GPCRs, and similar to the nucleotide diversity levels of noncoding regions along the human genome. This might be the source of interindividual differences in odour perception. Such a high incidence of cSNPs in olfactory subgenome is explained by a weak positive selection pressure acting on OR genes [21].

The percentage of pseudogene cDNAs present in the clones analysed in our laboratory reached 58%. It is consistent with our previous observations [14]. Surprisingly, the proportion of expressed pseudogenes is almost identical to the proportion of 60% of pseudogenes [23] in

Table 3
Comparison of the expression profiles of OLR cDNAs

Gene name	*HTPCR06/OR2K2*	*HGMP071/OR1E1*	*TPCR85/OR8B8*	*JCG1/OR5P3*	*JCG2/OR8D2*	*JCG3/OR5P2*	*JCG5/OR10A4*	*JCG6/OR10A5*	*JCG9/OR8D1*	*HTPCR2/OR7A5*	*OR6Q1*	*TPCR86/OR7C1*
ORF size (bp)	951	944	936	936	936	969	948	954	927	957	954	960
Foetal cDNA (Gaudin et al. [14])	+	+	+	+	+	+	+	+	+	No data	No data	No data
Adult cDNA (Gaudin et al. [14])	+	+	–	–	–	+	+	–	–	No data	No data	No data
Adult cDNA (the present work)	+	–	–	–	–	+	+	+	–	+	+	+

"+" stands for the presence of a transcript and "–" for its absence.

human olfactory subgenome. This proportion is estimated to be the highest among primates and other animal species [24].

In this study, the expression of full-length ORFs of chosen OLR genes was analysed in epithelial cells of the adult tongue and compared to the results of previous expression analyses [14]. As one may see in Table 3, some differences can be spotted. When comparing two different adult cDNA expression profiles of OLR genes (Table 3), it was observed that two genes (*HGMP07I/OR1E1* and *JCG6/OR10A5*) are not expressed equally. It should be mentioned that in previous work [14] the tissue samples contained epithelial cell on the surface of the tongue and deeper tissue layers while, in this work, only upper epithelial cells were used. Therefore, it is possible that the differences observed are due to the cell type content of the samples. In this case, different OLRs would be expressed in different cell types, most of them being expressed in epithelial cells of the surface of the tongue. The differential expression profiles can also result from spatial differences in OLR expression. However, the finding that most of the OLRs expressed in adult human tongue are expressed in the epithelial cell of the surface of the tongue indicates that the expression of OLR in tongue is probably not related to taste perception because taste buds are not included in this cell population.

Reassuming, these data indicate that the compared human cDNA tongue libraries are slightly different. In this study, the screening of a cDNA library from tongue cells with degenerated primers leads to the cloning of four OLR genes (*JCG5/OR10A4*, *OR7A5/HTPCR2*, *OR7C1/TPCR86*, and *OR6Q1*). *JCG5/OR10A4* which has been cloned from a tongue cDNA library and three other genes (*OR7A5/HTPCR2*, *OR7C1/TPCR86*, and *OR6Q1*) were identified as being expressed in human lingual epithelium for the first time. They belong, however, to different olfactory gene subfamilies and are localized in different chromosomes (*OR7A5/HTPCR2*, *OR7C1/TPCR86* are localized in the cluster 19@14.843 and *OR6Q1* in the cluster in 11@57.73). No other genes from these two clusters were described to be expressed in the lingual epithelium.

Although no quantitative analysis was performed in this study, according to PCR results (strong bands and thin bands observed), it can be suspected that some OLR genes are expressed at higher levels than others. For instance, *JCG6/OR10A5* is very poorly expressed as compared to *HTPCR06/OR2K2*, *JCG3/OR5P2*, and *JCG5/OR10A4*. These differences in the expression levels of OLR in tongue were also observed previously. Young et al. [25] have also shown that the expression of certain OR genes is stronger (even up to 300 times) as compared to other OR genes in olfactory epithelium.

In conclusion, it appears that most of the OLRs expressed in human adult tongue are expressed in the

Table 4
Distances of protein sequences by the Jotun Hein Method (DNASTAR software, MegAlign)

Identity (%)	Divergence (%)											
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
1		43.3	53.5	43.9	42.0	46.0	47.6	45.6	45.0	47.4	56.2	45.8
2	99.9		45.3	39.9	40.5	46.8	46.8	41.9	42.3	41.8	42.3	41.2
3	70.9	93.2		38.6	40.5	44.9	46.2	43.0	41.7	43.1	67.4	40.8
4	97.8	100	100		48.2	42.3	43.3	70.2	39.7	58.3	39.1	48.1
5	100	100	100	84.8		42.6	42.3	50.3	44.6	48.5	43.0	65.2
6	91.2	88.8	94.7	100	100		73.4	42.4	46.6	42.3	44.1	43.9
7	86.5	88.8	90.7	99.9	100	32.8		44.0	46.6	42.3	45.4	45.5
8	92.3	100	100	37.9	79.0	100	97.4		43.7	61.2	43.7	50.8
9	94.1	100	100	100	95.6	89.3	89.3	98.5		42.0	44.4	47.4
10	87.0	100	100	60.0	83.9	100	100	54.2	100		43.3	49.4
11	64.6	100	42.7	100	100	97.2	93.1	98.5	96.2	99.9		43.9
12	91.7	100	100	85.1	46.5	97.8	92.7	77.7	87.0	81.6	77.8	

Percent divergence is calculated by comparing sequence pairs in relation to the phylogeny reconstructed by MegAlign. Percent Similarity compares sequences directly, without accounting for phylogenetic relationships.
Bold numbers stand for the names of proteins. Protein IDs are given in brackets. **1**, HGMP07I/OR1E1 (CAA 46127.1); **2**, HTPCR06/OR2K2 (NP 995581.1); **3**, HTPCR2/OR7A5 (NP 059976.1); **4**, JCG2/OR8D2 (NP 001002918.1); **5**, JCG3/OR5P2 (NP 703145.1); **6**, JCG5/OR10A4 (NP 997069.1); **7** JCG6/OR10A5 (NP 835462.1); **8**, JCG9/OR8D1 (NP 001002917.1); **9** OR6Q1 (NP 001005186.1); **10**, TPCR85/OR8B8 (NP 036510.1); **11**, TPCR86/OR7C1 (NP 945182.1); and **12**, JCG1/OR5P3 (NP 703146.1).

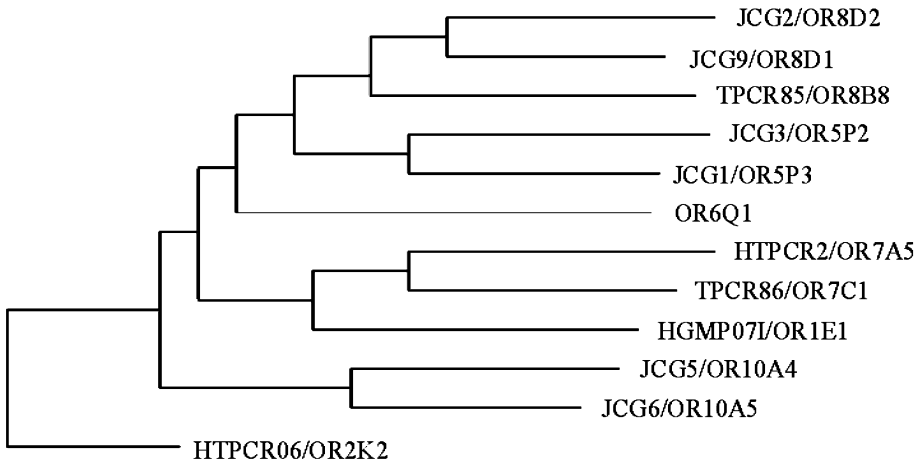

Fig. 6. Dendrogram representing the sequence similiarities between all putative olfactory receptors presented in this article—established using Clustal Method from DNASTAR (MegAlign) software.

epithelial cells of the surface of the tongue and that their presence there is probably independent of taste perception. On the other hand, these OLRs may be involved in putative perception of still unknown taste ligands or odorants. It was also proposed [17] that they might be responsible for guidance of cells assuring the specificity of cell migration and tissue assembly that occurs throughout embryogenesis. This hypothesis proposes that cells assemble organisms, including their brains and nervous systems, with the aid of a "molecular-addressing code" in which OLRs would play a crucial role. If this were true, they could play a role during organization of the embryonic taste cells of part or whole lingual epithelium.

Finally, since lower vertebrates (Reptilians) perceive odours with their tongues, it cannot be excluded that the expression of OLRs in lingual epithelium is only an evolutionary atavism and they do not play any special role in mammalian epithelial cells.

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