Profiling of TAAR genes expression in human olfactory epithelium and functional characterization of the hTAAR1 and hTAAR5

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Abstract:

Profiling of trace amine-associated receptor (TAAR) genes expression was performed in 15 human olfactory epithelia from autopsy. After total RNA extraction, DNase treatment, RNA integrity evaluation and reverse transcription, the expression of the 6 targeted TAAR genes and reference genes for normalization was analyzed using a real-time reverse transcription PCR platform. Upon normalization and quantification, we observed that hTAAR5 was expressed at a relatively high level in most samples (14/15) whereas hTAAR1 was detected at a trace level in most samples (12/15). The expression of hTAAR2, hTAAR6, hTAAR8, and hTAAR9 -at a trace level- was noted in a limited number of samples.

An agonist screening of a 460 odorant compound library was performed on recombinant human TAARs expressed in HEK293T-hRTP1S/hRTP2 cells using a CRE-luciferase reporter assay. A series of new linear and cyclic alkylamines were identified as potent activators of TAAR1. Most of them are characterized by an unpleasant fishy or ammoniacal odor shared by many alkylamines. These findings suggest that hTAAR1, as well as hTAAR5, could work as chemosensory receptors in human olfaction.

Introduction:

TAARs belong to the family of G-protein coupled receptors (GPCR class I) whose first deorphanized member rat and human TAAR1 responds to trace amines [Borowsky et al, 2001 ; Bunzow et al, 2001]. These biogenic amines as phenylethylamine, tyramine, tryptamine and octopamine are present in mammalian nervous systems at very low concentrations [Zucchi et al, 2006]. TAARs are phylogenetically related to biogenic amine receptors. Human and murine TAAR1 are expressed in a variety of tissues such as brain, stomach, kidney, lung, and small intestine [Borowsky et al, 2001].

TAARs were suggested to be a second class of chemosensory receptors in the olfactory epithelium of vertebrates in addition to the large family of olfactory receptors [Fleischer et al, 2009]. Indeed, expression of all TAAR subtypes except TAAR1 has been reported in mouse olfactory epithelium [Liberles and Buck 2006]. More recently, Carnicelli et al. [2010] described the detection of all human TAARs (hTAAR5 mainly and hTAAR1 at trace level) in OMP-positive nasal biopsies. Several volatile amines were shown to interact with specific murine TAARs in heterologous cell models. Indeed, some mTAARs are activated by endogenous amines (derivatives of naturally occurring amino acids) that may play a role in behavioral response.

A recent publication reports the deorphanization of human TAAR5 specifically by trimethylamine [Wallrabenstein et al, 2013]. Trimethylamine is a bacterial metabolite found in some animal odors, and to humans it is a repulsive odor associated with bad breath and spoiled food [Mitchell and Smith, 2001]. Similar to other low-molecular-weight amines, phenylethylamine, a ligand of hTAAR1, has a fishy odor. From an industrial point of view, it is worth studying this family of receptors as potential human chemosensory receptors. No ligand for hTAAR2, hTAAR6, hTAAR8 and hTAAR9 has been identified so far. To clarify the functionality and their role in human olfaction, we performed a profiling of TAAR genes expression in 15 complete human olfactory epithelia. Additionally, an odorant molecules library has been screened on the 6 non-pseudogenic human TAARs.

Methods:

Profiling of TAAR genes expression: This project was approved by the ethics committee from Erasme Hospital. Olfactory epithelium obtained from 15 individuals of European origin were collected within ~24 hours after death. RNA was extracted, DNase treated, quantified and quality estimated. A customized TLDA microfluidic card was designed to investigate the hTAAR genes expression. The PCR instrument calculates a C_q value representing the PCR cycle at which the reaction reaches a fluorescent intensity above background.

Functional assays: Deorphanization screening was performed in HEK293T-hRTP1S/hRTP2 cells with CRE-luciferase reporter assay system [Zhuang and Matsunami 2008]. Briefly, cells plated one day before, were transfected with hTAAR and pGL4.29 plasmids using TransIT®-LT1 (Mirus) according to the manufacturer's protocol. Twenty hours after transfection and four hours after incubation with compounds, cells were lysed and processed for luminescence measurement using a Spectra Max M5 reader (Molecular Devices). Results were expressed as percentage of the response induced by 10 µM of Forskolin. Statistical analysis and curve fitting was done by the Hill equation using GraphPad Prism 4.

Results:

TAARs expression patterns in the human olfactory epithelium

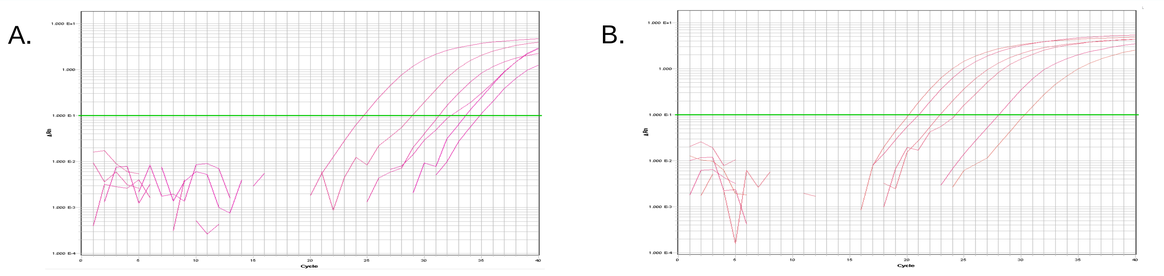

Figure 1. - TaqMan® Low Density Array (TLDA) amplification plots results for 6 hTAAR targets (A) and for 6 reference genes specific of OE (B) expressed in log ΔRn vs cycle number for one individual (female, 72 y-o). Threshold is set manually at ΔRn=0.1. The order of amplification plots for this sample is TAAR5 (Cq=25), TAAR8 (Cq=29), TAAR2 (Cq=31), TAAR9 (Cq=32), TAAR1 (Cq=34) and TAAR6 (Cq=35) for panel A, and ADCY3 (Cq=20), GNAL (Cq=21), CNGA2 (Cq=23), RTP1 (Cq=24), Ric8B (Cq=28), and OBP (Cq=30) for panel B with C_q from lower to higher i.e. in inverse proportion to the expression level.

A customized TLDA was designed to investigate 6 human TAAR genes expression in 15 human olfactory epithelia (OE) samples. Figure 1A shows amplification plots obtained for the 6 TAAR genes and figure 1B for the 6 reference genes specific of OE from one individual (female, 72 y-o). 3 stably expressed reference genes specific for olfactory epithelium (Ric8B, GNAL and ADCY3) were selected by the geNorm algorithm which allowed normalization with multiple reference genes for quantification studies. In average, for the 15 samples, C_q values of TAAR genes ranged between 31.0 ± 3.2 and 38.2 ± 2.3 (mean ± SD) (figure 2). These results reflect a low expression of the TAAR genes as compared to other genes involved in the olfactory cascade. Some targets gave an undetermined C_q value which is arbitrary assigned to 40 cycles to allow the calculation of an average C_q value. Average C_q value < 35 were observed only for TAAR1 (34.6 ± 2.9) and TAAR5 (31.0 ± 3.2). The 4 other TAAR genes giving average C_q value > 35 (indicating a too low expression for accurate detection) were considered as very low or non-expressed genes. After normalization and quantification, results showing TAAR5 expressed in 14/15 olfactory epithelia at a level ranging from 6 to 742 copies/20 ng RNA; and TAAR1 detected in 12/15 olfactory epithelia at trace level (1 to 11 copies/20 ng RNA). hTAAR2, 6, 8, and 9 were expressed at trace levels in 7, 4, 9 and 10 olfactory epithelia samples, respectively. No relation between the TAAR genes expressed and age or sex of individuals was observed.

Name \ Age	59	61	68	72	75	75	79	81	50	53	54	65	74	74	75	Mean	SD
TAAR1	33	31	36	34	40	33	32	34	34	33	33	33	40	34	40	34.6	2.9
TAAR2	35	40	35	31	35	40	40	35	35	35	40	35	40	40	40	37.1	3.0
TAAR5	30	31	33	25	40	32	30	30	29	29	32	31	31	29	33	31.0	3.2
TAAR6	34	37	35	35	35	40	40	40	40	40	37	40	40	40	40	38.2	2.3
TAAR8	35	40	40	29	40	33	34	32	34	36	34	34	40	34	34	35.3	3.3
TAAR9	34	33	33	32	31	33	40	34	40	34	40	40	35	40	32	35.4	3.4

Name \ Age	59	61	68	72	75	75	79	81	50	53	54	65	74	74	75	Mean	SD
TAAR1	2	11	1	1	0	5	7	1	3	2	5	5	0	3	0	3.2	3.2
TAAR2	0	0	2	8	1	0	0	1	2	1	0	1	0	0	0	1.0	1.9
TAAR5	15	18	6	742	0	11	28	21	99	56	9	12	15	58	7	73.2	186.8
TAAR6	1	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0.3	0.4
TAAR8	0	0	0	39	0	5	3	8	3	0	1	2	0	2	4	4.4	9.8
TAAR9	1	4	5	3	14	5	0	2	0	2	0	0	2	0	12	3.4	4.4

Figure 2. - Expression profile of human TAAR genes in OE. Age of the individuals are shown above the figure, females are colored in pink and males in blue. Upper panel: for each of the 6 TAARs (rows), the average C_q was calculated from the C_q values obtained for each of the 15 individuals (columns). Lower panel: normalization and quantification were calculated and indicated values correspond to the approximate number of copies of target for 20 ng RNA engaged in the RT-PCR reaction.

TAARs respond to volatiles amines

In an effort to deorphanize the hTAARs, an agonist screening of 460 odorant molecules encompassing 155 nitrogen-containing compounds was performed. Recombinant human 6 TAARs were expressed in HEK293T-hRTP1S/hRTP2 cells and tested using a cAMP dependent reporter gene assay (CRE-Luciferase).

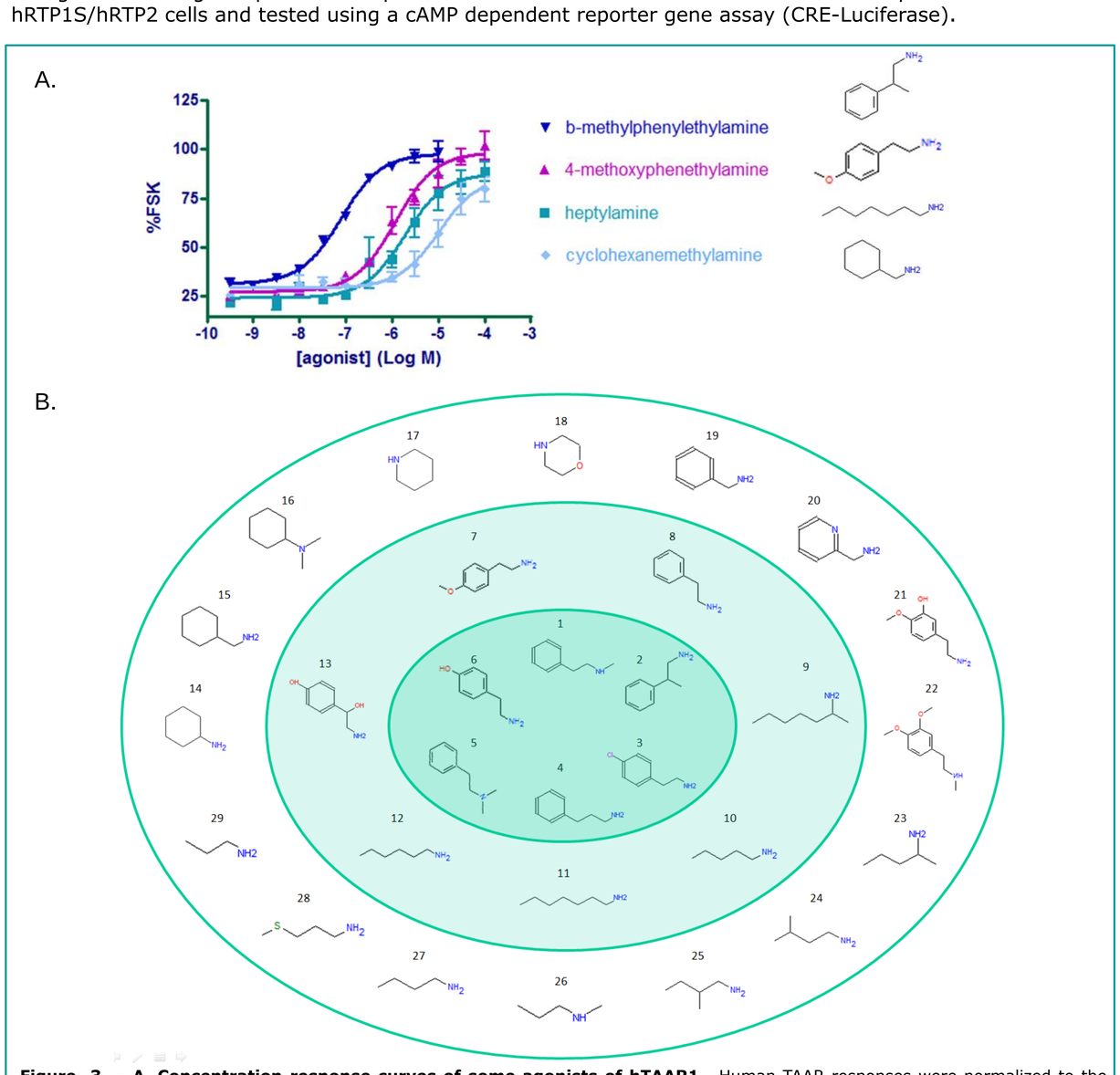

Figure 3. – A. Concentration-response curves of some agonists of hTAAR1. Human TAAR responses were normalized to the response of forskolin (10 µM; %FSK) in function of the agonist concentration in log M. Error bars are standard deviation over duplicates. Chemical structures of odorants are illustrated in the same order than in the legend of the graph.
B. Chemical structure of various hTAAR1 agonists. In the center of the ellipse are drawn the more potent agonists (EC_{50} < 0.5 µM). The intermediate portion contains potent agonists (0.5 µM <EC_{50} < 5 µM). The less potent agonists (5 µM <EC_{50} < 50 µM) are represented in the outer part.

The nitrogen-containing compounds library includes amine-, nitrile-, amine oxide-, pyrazine-, pyridine-compounds. The organoleptic properties are variable, comprising molecules with pleasant (fruity, floral, nutty, musky, green, herbal,…) and bad (animal, putrid, ammoniacal, fishy, musty,…) odors. In total, 42 agonists were identified for hTAAR1. Figure 3A illustrates concentration-response curves of some agonists of hTAAR1 and figure 3B shows chemical structures of the 29 most potent (EC_{50} < 50 µM). All the active molecules are alkylamines with a minimum of three carbons. The functional amine group can be a primary, secondary or tertiary amine. Some ligands are based on a benzene ring or a cyclohexane ring. Most of the agonists are characterized by an unpleasant fishy or ammoniac-like odor (figure 4). By contrast, hTAAR5 is activated by trimethylamine only, confirming recent results of Wallrabenstein et al, [2013]. hTAAR2, 6, 8 and 9 were not activated by any of the compounds tested.

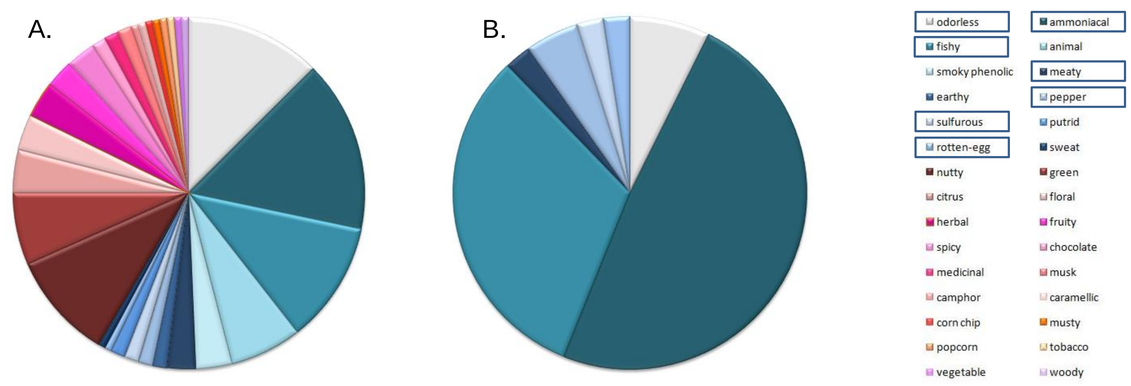

Figure 4. - Description of the organoleptic properties of 153 chemical structures containing a nitrogen function tested in functional assay (A) and of 42 compounds which are hTAAR1 agonists (B). The color code is white for odorless compounds; green-blue for malodors and red-pink-purple for pleasant odors. Legend shows the odor type classifications and the text boxes correspond to the odor type classifications of the hTAAR1 agonists.

Conclusions:

hTAAR5 and -at a trace level- hTAAR1, were expressed in the most studied olfactory epithelia. By screening a large odorant library, we discovered a wide range of chemical structures activating the receptor hTAAR1 in heterologous mammalian cells. All the active molecules are linear or cyclic alkylamines and mostly characterized by an unpleasant fishy and ammoniac-like odor. Previously published deorphanization of two human TAARs were confirmed: hTAAR5 can be activated with a high specificity by the tertiary amine trimethylamine [Wallrabenstein et al, 2013] and hTAAR1 responds to phenylethylamine, tyramine and 2-methylbutylamine [Liberles and Buck 2006]. The series of new odorant agonists identified shows that hTAAR1 is broadly tuned as compared to hTAAR5.

For the other family members, no responses were observed with hTAAR2, hTAAR6, hTAAR8 and hTAAR9. Although these genes are not considered as pseudogenes, we cannot exclude that missense mutated genes can be nonfunctional due to a substitution of key amino acids governing protein folding or interactions with signal transduction components as observed for olfactory receptors.

These study shows that the 2 human TAARs expressed in the olfactory epithelium recognize specifically odorant amines. These findings, suggest that the broadly activated hTAAR1, as well as the high narrowly activated hTAAR5, could work as chemosensory receptors in human olfaction.

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