A polymorphism in the human gene encoding *OBPIIa* affects the perceived intensity of smelled odors

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Abstract

Among the factors that contribute to the physiological variability of the olfactory function of individuals, an important role seems to be played by the OBPs present in the mucus that bathes the ciliated terminals of the olfactory sensory neurons, facilitating the access of odorants to the olfactory receptors. It was recently highlighted that the *rs2590498* polymorphism in the odor binding-protein (OBPIIa) gene it is associated with the olfactory threshold in healthy individuals. Aim of this study was to evaluate: 1) the presence of a relationship between the threshold olfactory performance of healthy subjects and the intensity with which they perceive the smelled odorants, and 2) the effect of the *rs2590498* polymorphism of the *OBPIIa* gene on perceived intensity. We found a positive correlation between threshold olfactory and perceived intensity, and that AA homozygous subjects reported a perceived intensity higher than heterozygous and GG homozygous subjects. By showing a positive effect of the *rs2590498* polymorphism of the *hOBPIIa* gene on the intensity perceived, these results suggest that it allows a larger number of molecules in an odorous mixture to reach the olfactory receptors.

Keywords: physiological variations of perceived intensity, OBPIIa odorant binding proteins, olfactory threshold, olfactory function.

The ability to perceive odors allows individuals to locate and choose foods, identify and avoid potential sources of danger and select a partner for mating, thus playing an important role in social life and for the survival of individuals [1]. Individuals can be classified as normosmic, hyposmic or anosmic depending on whether they show a normal, reduced or absent ability to detect odors [2]. The reasons of this individual variability are multiple and can be traced back to personal experience, environmental factors and genetic ones [3-6].

Olfactory perception is a process that begins with the bond between odorants and olfactory receptors (ORs), located on the cilium membrane of olfactory sensory neurons (OSNs), immersed in a thin layer of mucus rich in water and glycoproteins [7, 8]. In this region, known as the "perireceptor space", "perireceptor events" are observed which consist of variation in the composition and thickness of the mucus, presence of enzymes that modify the concentration and/or structure of the odorant and presence of "Odorant Binding Proteins" (OBPs) which facilitate the accessibility of OSNs to odorants [9-12]. Since most odorants are hydrophobic, some authors suggested that OBPs have the task of capturing odors, transporting them through the mucus layer to the ORs, thus initiating the transduction of the olfactory signal [13-16].

The *rs2590498* (*A/G*) polymorphism of the gene encoding human *OBPIIa*, the only OBP found in the olfactory mucosa of humans [8, 17], has been associated with individual variations in orthonasal perception of both simple and complex odors: subjects who were homozygous for the major allele A showed a significantly higher olfactory performance than subjects heterozygous or homozygous for the minor allele G [18-20]. In particular, healthy individuals with the AA genotype achieved higher threshold olfactory scores and perceived a significantly higher number of single molecules than heterozygous or GG homozygous ones, while patients affected by inflammatory bowel disease (IBD) and Parkinson's disease (PD) with genotype AA were protected from olfactory dysfunction even when confronted with higher skills such as odor discrimination and odor identification [18, 19, 21, 22]. In addition, perceived intensity for an odor mixture is known to be related to the number of mixture constituents smelled, which in turn is correlated with the olfactory threshold [23].

With the aim of understanding whether a greater expression of OBP protein may have an increasing effect on the number of the mixture molecules that reach the receptor sites, as well as on the minimum perceived concentration, the main goal of this study was to evaluate the effect of the *rs250498* polymorphism of the gene coding for human *OBPIIa* on the perceived intensity for each of the odors smelled during the Identification test (I-test). However, since this hypothesis arises from the known relationship between *OBPIIa* genotype and threshold olfactory score, we first assessed whether the subjects of our panel showed a correlation between olfactory threshold and perceived intensity.

As shown in **Figure 1**, Pearson's correlation results indicate that a positive correlation exists between the T olfactory score obtained by each individual and his/her intensity perceived for each of 16 odors smelled during the I-test ($r > 0.287$, $p \leq 0.017$), thus highlighting that the intensity reported by individuals, when perceiving odors, is closely connected with their olfactory threshold: lower olfactory threshold means higher perceived intensity and the other way around.

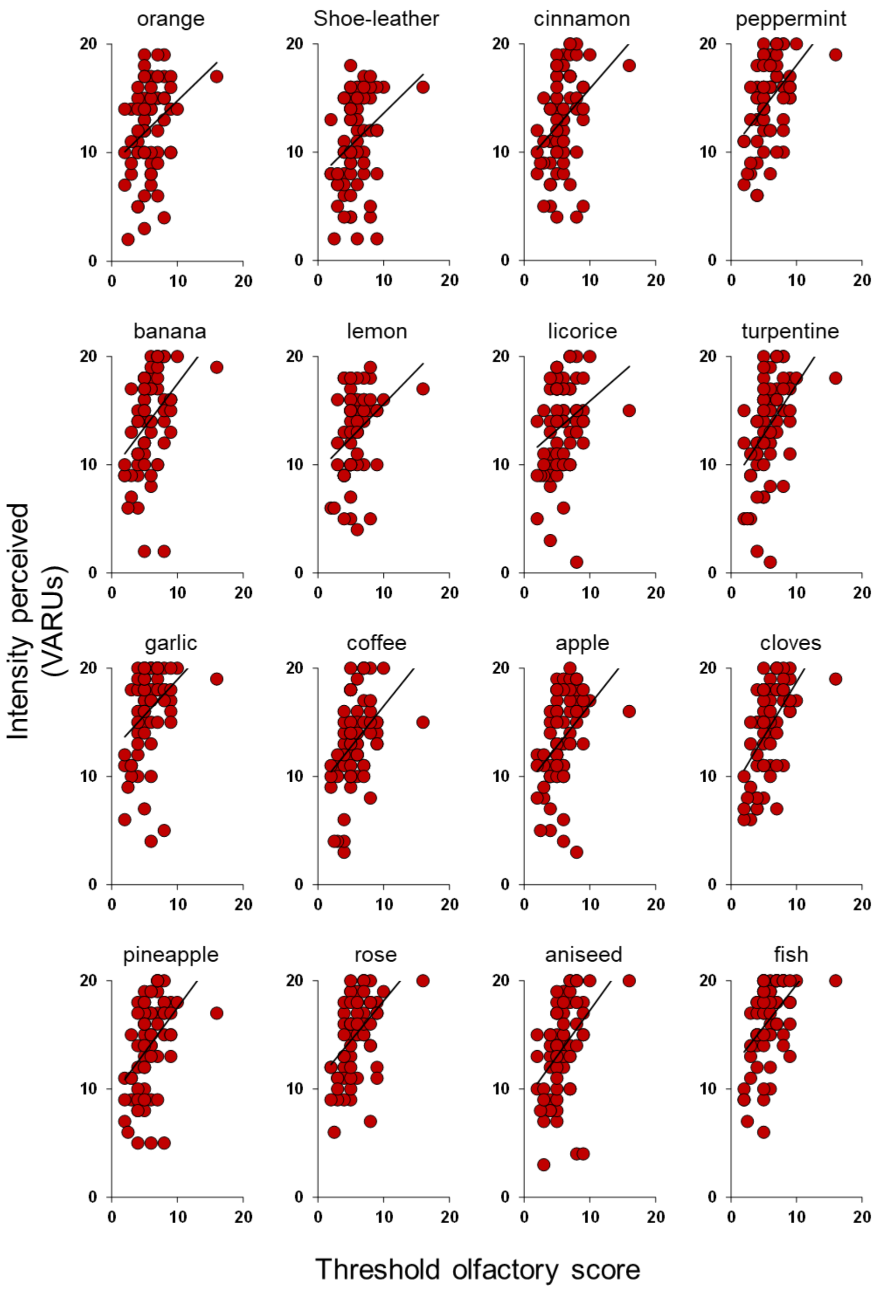

Fig. 1. Pearson's correlation analysis between threshold olfactory score and odor intensity perceived by subjects. Orange: $r = 0.324$, $p = 0.007$; shoe-leather: $r = 0.309$, $p = 0.009$; cinnamon: $r = 0.367$, $p = 0.002$; peppermint: $r = 0.472$, $p < 0.0001$; banana: $r = 0.413$, $p < 0.001$; lemon: $r = 0.335$, $p = 0.005$; licorice: $r = 0.287$, $p = 0.017$; turpentine: $r = 0.4699$, $p < 0.0001$; garlic: $r = 0.368$, $p = 0.002$; coffee: $r = 0.422$, $p < 0.001$; apple: $r = 0.4259$, $p < 0.001$; cloves: $r = 0.552$, $p < 0.0001$; pineapple: $r = 0.440$, $p < 0.001$; rose: $r = 0.464$, $p < 0.0001$; aniseed: $r = 0.442$, $p < 0.001$; fish: $r = 0.473$, $p < 0.0001$.

Most odors are more or less complex molecule mixtures and recently it has been shown that the intensity of an odor mixture perceived by individuals is related to the number of mixture constituents smelled by each of them, which in turn is correlated with their olfactory threshold [23]. By considering that the olfactory threshold is affected by the *OBPIIa* genotype, our main goal was to verify the effect of the rs250498 polymorphism on the perceived intensity of the I-test odors. One-way ANOVA showed a significantly effect of the *OBPIIa* genotype on the perceived intensity for all odors ($F_{2.66} > 3.332$, $p < 0.05$) except for the smell of fish ($F_{2,66} = 1.929$, $p = 0.15$). Post-hoc analysis revealed that: a) individuals with AA genotype perceive a significantly higher intensity than GG individuals for all odors except for fish ($p < 0.05$); b) individuals who are homozygous for the allele A perceive a significantly higher intensity than those who are heterozygous for all odors except cinnamon, peppermint, banana, garlic, apple, cloves, rose, aniseed and fish ($p < 0.05$); c) between individuals who are heterozygous or GG homozygous, the only significant difference found is for the odor of garlic ($p = 0.003$) (Fig. 2).

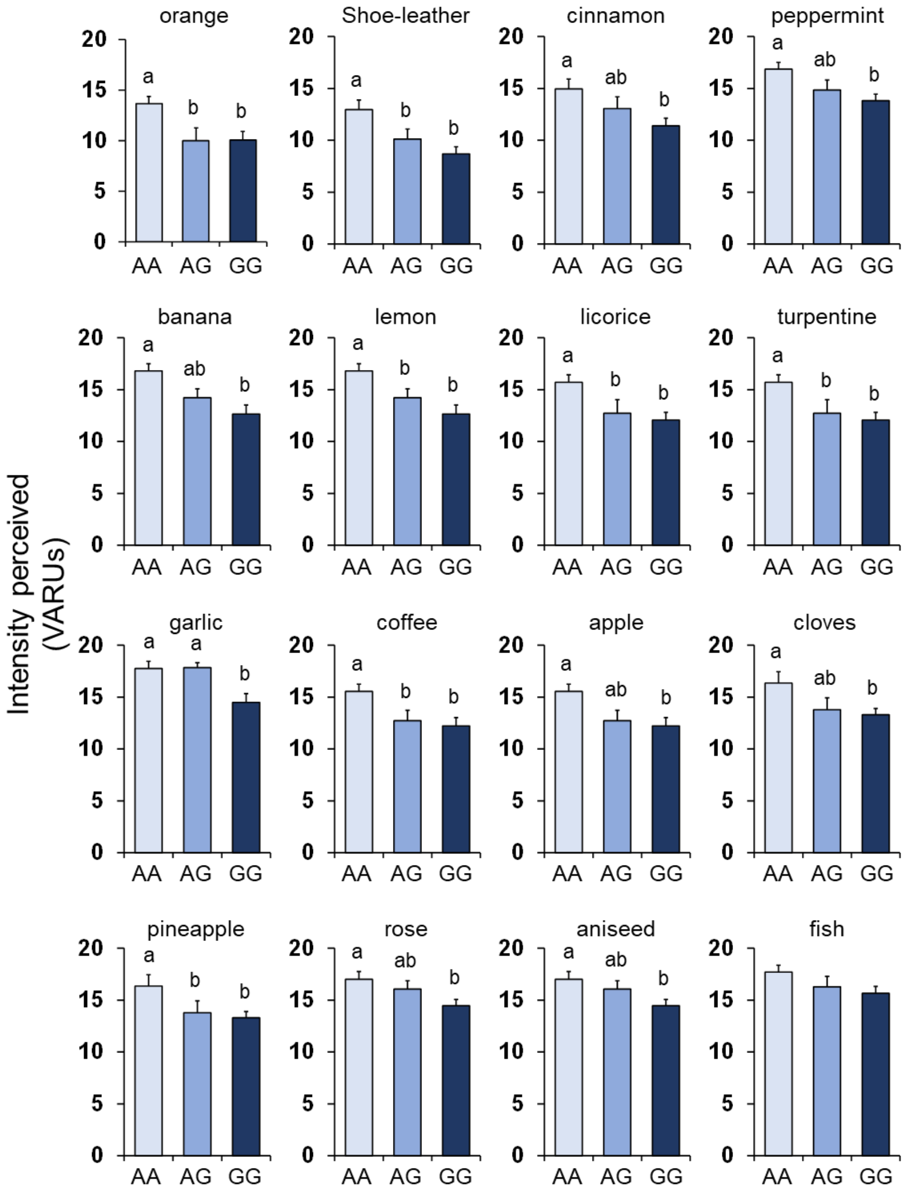

Fig. 2. Mean values ± s.e.m. of odor intensity perceived by subjects according to genotype of the *OBPIIa* locus. N = 69 (AA = 20; AG = 18; GG = 31). Different letters indicate significant differences ($p < 0.05$; Fisher's LSD test subsequent to one-way ANOVA).

The results of this study confirm that individuals carrying both major A alleles are generally more sensitive than heterozygotes and homozygotes ones for the minor G allele. These findings suggest that the allele A of OBPIIa gene, by having an effect on the intensity as well as on the olfactory threshold, may allow a larger number of molecules in an odorous mixture to reach the receptor sites. In agreement, recent bioinformatics data suggest that the mutation in the OBPs gene decreases the expression of OBPs in the olfactory epithelium [21], by reducing the ability to transport odorants through the mucus layer and, consequently, making the odorant/OR binding more difficult. In fact, the number of action potentials reaching the mitral cells in the olfactory bulb and hence the higher centers of olfactory perception, depends on the amplitude of the receptor potential generated in the OSNs, which in turn depends on the number of ORs activated. As a consequence, individuals with the AA genotype may perceive odors with a higher intensity because of the larger number of OBPs expressed in the mucus. In these individuals, the process of transduction and coding of the olfactory information takes place at higher intensity than that characterizing AG and GG individuals, who possess a smaller number of OBPs in the mucus layer.

Materials and methods

Subjects

Sixty-nine healthy, non-smoking Caucasian volunteers (53 females and 16 males), aged 19 to 55 (31.7 ± 1.29 years), recruited in Cagliari (Sardinia, Italy), took part in this study. All subjects were in good health and none of them reported head trauma, sinusitis or nasal septal disorders; the exclusion criteria were diabetes, pregnancy or breastfeeding and the use of drugs that interfere with olfactory perception. The volunteers were chosen on the basis of information collected through questionnaires or interviews to make sure that no one had evident olfactory dysfunctions, did not suffer from colds or any ailment that could interfere with perceptual skills. All procedures were approved by the Local Ethical Committee and each subject gave an informed written consent before participation.

Olfactory function screening and intensity of perceived odors

The olfactory threshold for each subject was assessed by means of the test for the evaluation of the olfactory threshold (Threshold test, T-test) of the "Sniffin 'Sticks Extended Test" (SSET); Burghart Instruments, Wedel, Germany) [24]. Briefly, the experimenter has 16 triplets of pens available (with 16 increasing concentrations), each of which consists of two pens containing a solvent and a third (target pen) filled with the test odor (n-butanol). The participant's task is to identify the pen containing n-butanol, with a forced choice mechanism. It starts from a very low concentration and goes up until the participant identifies the target pen twice in a row. This is the starting point and represents the first reversal. Then you step down by a triplet at a time until the participant makes a mistake, at which point you step up (second reversal) and so on for seven reversals: the olfactory threshold is defined as the average of the dilution steps of the last 4 reversals. For each of the odors presented during the identification test of the same kit as the Sniffin 'Sticks, the subjects also had to give a personal assessment of the perceived intensity, putting a mark on the "Visual Analogue Rating Units" (VARUs) scale for values from 0 to 20 VARUs [25].

Genetic analysis

Subjects were genotyped for the *rs2590498* (*A/G*) polymorphism of the *OBPIIa* gene using a conventional TaqMan® SNP Genotyping Assay (Applied Biosystems by Life-Technologies Italia, Europe BV) according to the data reported in the literature [19, 20] (Sollai et al. 2019; Tomassini Barbarossa et al. 2017). The following two primers were used: Forward PCR GCCAGGCAGGGACAGA and Reverse PCR CTACACCTGAGACCCCACAAG. Two TaqMan probes were designed according to the *OBPIIa* gene (bold and underlined), probe/reporter 1: VIC-TCGGTGAC**A**TGAACC and probe/reporter 2: FAM-TCGGTGAC**G**TGAACC. After the PCR runs, the samples were read by a gene sequence reading system at 60 °C for 1 min and the allelic discrimination results were analyzed by means of a software associated with the gene sequence reading system (Applied Biosystems). In order to verify the success of the allelic discrimination, all samples were analyzed in duplicate and compared with positive controls (whose polymorphism was already known) and negative (without DNA) controls. Molecular analyses revealed that 20 subjects were homozygous AA, 18 were heterozygous AG and 31 were homozygous GG.

Statistical analysis

Pearson's correlation test was used to assess for a relationship between the subjects' perceived intensity of odors presented during the I-test and their threshold olfactory score. Statistical analyses were conducted using GraphPad Prism 6 software (GraphPad Software, San Diego, CA, USA). Values of $p < 0.05$ are considered significant.

One-way ANOVA was used to analyze the effect of the *OBPIIa* genotype on the intensity perceived by the subjects for each of the odors smelled during the identification test. Post-hoc comparison was conducted with Fisher's LSD test; p values < 0.05 were considered significant. The statistical analysis was carried out using STATISTICA for WINDOWS (version 7.0; StatSoft Inc, Tulsa, OK, USA).

Acknowledgements

The Authors thank the volunteers, without whose contribution this study would not have been possible.

Conflict of interest

There are no financial and personal relationships with other people or organizations that may lead to a conflict of interest.

Funding: This work was supported by a grant from the University of Cagliari (Fondo Integrativo per la Ricerca, FIR 2019-2020).

Author contributions:

Giorgia Sollai: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Resources, Software, Validation, Roles/Writing - original draft, Writing - review & editing. **Melania Melis**: Data curation, Methodology, Software. **Iole Tomassini Barbarossa:** Funding acquisition, Resources, Validation, Writing - review & editing. **Roberto Crnjar:** Funding acquisition, Resources, Validation, Writing - review & editing.

[1] R.J. Stevenson, An initial evaluation of the functions of human olfaction, Chem Senses 35(1) (2010) 3-20.
[2] I. Croy, S. Nordin, T. Hummel, Olfactory Disorders and Quality of Life—An Updated Review, Chemical Senses 39(3) (2014) 185-194.
[3] Y. Hasin-Brumshtein, D. Lancet, T. Olender, Human olfaction: from genomic variation to phenotypic diversity, Trends in genetics : TIG 25(4) (2009) 178-84.
[4] A. Sorokowska, P. Sorokowski, T. Hummel, Cross-Cultural Administration of an Odor Discrimination Test, Chemosens Percept 7(2) (2014) 85-90.
[5] W.S. Cain, J.F. Gent, Olfactory sensitivity: reliability, generality, and association with aging, Journal of experimental psychology. Human perception and performance 17(2) (1991) 382-91.
[6] G. Sollai, R. Crnjar, Age-Related Olfactory Decline Is Associated With Levels of Exercise and Non-exercise Physical Activities, Frontiers in aging neuroscience 13 (2021) 695115.
[7] H. Breer, Odor recognition and second messenger signaling in olfactory receptor neurons, Seminars in cell biology 5(1) (1994) 25-32.
[8] C.S. Silva Teixeira, N.M. Cerqueira, A.C. Silva Ferreira, Unravelling the Olfactory Sense: From the Gene to Odor Perception, Chem Senses 41(2) (2016) 105-21.
[9] T.V. Getchell, F.L. Margolis, M.L. Getchell, Perireceptor and receptor events in vertebrate olfaction, Progress in neurobiology 23(4) (1984) 317-45.
[10] A. Nagashima, K. Touhara, Enzymatic conversion of odorants in nasal mucus affects olfactory glomerular activation patterns and odor perception, The Journal of neuroscience : the official journal of the Society for Neuroscience 30(48) (2010) 16391-8.
[11] P. Pelosi, Perireceptor events in olfaction, Journal of neurobiology 30(1) (1996) 3-19.
[12] J. Pevsner, P.M. Hwang, P.B. Sklar, J.C. Venable, S.H. Snyder, Odorant-binding protein and its mRNA are localized to lateral nasal gland implying a carrier function, Proceedings of the National Academy of Sciences of the United States of America 85(7) (1988) 2383-7.
[13] G. Archunan, Odorant Binding Proteins: a key player in the sense of smell, Bioinformation 14(1) (2018) 36-37.
[14] H. Biessmann, E. Andronopoulou, M.R. Biessmann, V. Douris, S.D. Dimitratos, E. Eliopoulos, P.M. Guerin, K. Iatrou, R.W. Justice, T. Kröber, O. Marinotti, P. Tsitoura, D.F. Woods, M.F. Walter, The Anopheles gambiae odorant binding protein 1 (AgamOBP1) mediates indole recognition in the antennae of female mosquitoes, PloS one 5(3) (2010) e9471.
[15] J.D. Laughlin, T.S. Ha, D.N.M. Jones, D.P. Smith, Activation of pheromone-sensitive neurons is mediated by conformational activation of pheromone-binding protein, Cell 133(7) (2008) 1255-1265.
[16] P. Pelosi, Odorant-binding proteins, Critical reviews in biochemistry and molecular biology 29(3) (1994) 199-228.
[17] L. Briand, C. Eloit, C. Nespoulous, V. Bézirard, J.C. Huet, C. Henry, F. Blon, D. Trotier, J.C. Pernollet, Evidence of an odorant-binding protein in the human olfactory mucus: location, structural characterization, and odorant-binding properties, Biochemistry 41(23) (2002) 7241-52.
[18] M. Melis, I. Tomassini Barbarossa, T. Hummel, R. Crnjar, G. Sollai, Effect of the rs2890498 polymorphism of the OBPIIa gene on the human ability to smell single molecules, Behavioural brain research 402 (2021) 113127.

[19] G. Sollai, M. Melis, S. Magri, P. Usai, T. Hummel, I. Tomassini Barbarossa, R. Crnjar, Association between the rs2590498 polymorphism of Odorant Binding Protein (OBPIIa) gene and olfactory performance in healthy subjects, Behavioural brain research 372 (2019) 112030.
[20] I. Tomassini Barbarossa, M.H. Ozdener, Melania, L. Love-Gregory, M. Mitreva, N.A. Abumrad, M.Y. Pepino, Variant in a common odorant-binding protein gene is associated with bitter sensitivity in people, Behavioural brain research 329 (2017) 200-204.
[21] M. Melis, G. Sollai, C. Masala, C. Pisanu, G. Cossu, M. Melis, M. Sarchioto, V. Oppo, M. Morelli, R. Crnjar, T. Hummel, I. Tomassini Barbarossa, Odor Identification Performance in Idiopathic Parkinson's Disease Is Associated With Gender and the Genetic Variability of the Olfactory Binding Protein, Chem Senses 44(5) (2019) 311-318.
[22] G. Sollai, M. Melis, M. Mastinu, D. Paduano, F. Chicco, S. Magri, P. Usai, T. Hummel, I.T. Barbarossa, R. Crnjar, Olfactory Function in Patients with Inflammatory Bowel Disease (IBD) Is Associated with Their Body Mass Index and Polymorphism in the Odor Binding-Protein (OBPIIa) Gene, Nutrients 13(2) (2021).
[23] G. Sollai, I. Tomassini Barbarossa, P. Usai, T. Hummel, R. Crnjar, Association between human olfactory performance and ability to detect single compounds in complex chemical mixtures, Physiology & behavior 217 (2020) 112820.
[24] T. Hummel, B. Sekinger, S.R. Wolf, E. Pauli, G. Kobal, 'Sniffin' sticks': olfactory performance assessed by the combined testing of odor identification, odor discrimination and olfactory threshold, Chem Senses 22(1) (1997) 39-52.
[25] M. Fischer, Y. Zopf, C. Elm, G. Pechmann, E.G. Hahn, D. Schwab, J. Kornhuber, N.J. Thuerauf, Subjective and objective olfactory abnormalities in Crohn's disease, Chem Senses 39(6) (2014) 529-38.