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Olfactory threshold for bourgeonal and sexual desire in young adult males

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ABSTRACT

Olfactory receptors were found to be expressed also in human sperm giving rise to the hypothesis that they might play a role in fertility and sexual behavior. For instance, bourgeonal was demonstrated to be an agonist of sperm cells olfactory receptor, OR1D2. OR1D2 has been found to be expressed in human olfactory epithelium and to play a critical role in human sperm chemotaxis. Recent preliminary evidence showed that olfaction sensitivity (determined by n-butanol olfactory threshold) and sexual desire were associated in young adult males. It is reasonable to hypothesize that bourgeonal olfactory threshold could be related with human sexual behavior and desire.

In 37 healthy young adult male volunteers (age range 20–36 years), the bourgeonal odor threshold and the intensity of sexual desire [the latter using the International Index of Erectile Function (IIEF) scale] were examined. In addition, samples of DNA were collected. Allele and genotype frequency of the OR1D2 single nucleotide polymorphisms (SNPs) were then evaluated in order to study the relationship between sexual desire and OR1D2 SNPs expression. The olfactory threshold was categorized as <10, 10 ⩽ threshold < 15, 15 ⩽ threshold < 20, ⩾20.

IIEF 1 and IIEF 2 scores were significantly associated. IIEF1 scores, but not IIEF2 scores were significantly associated with olfactory threshold. No statistically significant associations were found neither between genotypes frequency and sexual desire (IIEF1 and IIEF2), nor between genotypes frequency and olfactory threshold.

Hypothesizing for the first time the relationship between bourgeonal olfactory sensitivity and sexual desire in a group of young adult males, the present study found a significant association between lower olfactory threshold for bourgeonal and stronger sexual desire, in terms of IIEF1.

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Introduction

Animal noses can detect an enormous variety of odors at very low concentrations. This ability is mediated by olfactory receptors (OR) expressed in the olfactory epithelium [1]. OR expression is not restricted to sensory neurons in the nose as ectopic OR transcripts were found in different tissues such as myocardium, erythroid cells, ganglia of the autonomic nervous system, pyramidal neurons in the cerebral cortex, etc. [2–5]. Interestingly, Parmentier et al. [6] demonstrated the existence of some twenty human olfactory receptors (hORs) in sperm cells, and speculated that ORs might be implicated in chemotaxis of sperm cells during fertilization. Approximately 10 years later, Spehr et al. [7] identified and cloned the human olfactory receptor OR1D2 (also known as hOR17-4), which had a clear involvement in chemotaxis. In that study, Spehr at al. also showed that the OR1D2 receptor was activated by bourgeonal (a synthetic compound containing an aldehyde group connected to an aromatic ring via a carbon chain). Shortly thereafter, they found that OR1D2 was expressed in the olfactory mucosa [8]. It was hypothesized that olfaction might play an important role in fertility and human sexual behavior and desire.

It is thought that sexual behavior and sexual desire could be driven by pheromones which have been defined as chemical signals between organisms of the same species that communicate beneficial information from one individual to another [9,10]. However,

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pheromones and their role in humans are still a controversial issue and, so far, no pheromones have been conclusively identified [11,12]. Nevertheless, recent results have supported the hypothesis that the chemistry of axillary secretions (which can be considered a human pheromone source) and their effects on conspecifics in humans are analogous to other mammalian pheromone systems [13]. In particular, a compound in women's armpit extract, likely a human pheromone, apparently causes menstrual synchrony in females living in close quarters [12]. Thus, pheromones may have a direct impact on human mood by influencing hormone balance [14]. In fact, Wyart and co-workers [14] measured cortisol levels in so far as 4,16-androstadien-3-one (present in human male secretions such as sweat, saliva, and semen, and implicated as a putative human pheromone) influences arousal and mood, which are both linked to levels of this hormone.

The hypothesis

OR1D2 has been demonstrated to be expressed in human olfactory epithelium and to play a critical role in human sperm chemotaxis [7,8,15]. A recent preliminary study [16] disclosed that olfaction sensitivity (determined by n-butanol olfactory threshold) and sexual desire were significantly related in normosmic young adult males. It is reasonable to hypothesize that the olfactory threshold for bourgeonal (the most potent OR1D2 receptor agonist) could be related with human sexual behavior and desire.

In this prospective study, after determining the n-butanol olfactory threshold of the considered subjects, we investigated the association between olfactory sensitivity for bourgeonal, sexual desire and the frequency of 3 single nucleotide polymorphisms (SNPs) of OR1D2 gene in our cohort of volunteers. The study's primary aim was to test the hypothesis that there is an association between bourgeonal olfactory sensitivity and sexual desire intensity in young adult males. A secondary aim was to investigate the association between three SNPs [SNP reference ID number (rs) 769423, 769424 and 11078437], selected from 13 SNPs previously evaluated [17] as being the most promising in understanding unexplained male infertility, bourgeonal olfactory threshold, and sexual desire.

Evaluation of the hypothesis

The study was conducted in accordance with the guidelines of the Declaration of Helsinki and was approved by the University Hospital Ethics Committee (prot. n. 2244/2011). Written informed consent was obtained from all participants before undertaking any study-related procedure.

37 healthy, tobacco-free male volunteers ranging in age from 20 to 36 years (mean age 24.9 ± 3.6 years), recruited at the Department of Neurosciences, Padova University (Italy), were enrolled. All participants received a complete medical check in order to exclude ENT and urological disorders. They all exhibited normal nasal and paranasal sinus functions, having scored less than 1 on the Sinonasal Outcome Test (SNOT 22) [18], and did not suffer from asthma or allergies. None of them had a history of prior sinonasal surgery, head trauma, or used drugs that might have affected olfactory function. In addition, their genitourinary systems were normal. Finally, none of them was taking β-blockers or salicylic acid therapy (whose circulatory changes could cause erectile dysfunction affecting, in turn, sexual desire).

All participants underwent a quick olfactory screening with the Nez du Vin test, which involves identifying 6 aromas (lemon, mint, strawberry, pine, vanilla, smoke) by giving multiple-choice answers, according to McMahon and Scadding [19,20]. As all volunteers revealed a normal sense of smell (scores of 5 or 6 out of 6), they were then investigated to ascertain their odor threshold for n-butanol, using the Sniffin' Sticks® (Burghart Medical Technology, Wedel, Germany) test, as already described [20,21]. Odorants were presented in felt-tip pens. Instead of liquid dye, the pen's tampon is filled with 4 ml of liquid odorants or odorants dissolved in propylene glycol. For odor presentation the cap was removed for approximately 3 s and the pen's tip was placed approximately 2 cm in front of both nostrils. 16 dilutions were prepared in a geometric series starting from a 4% n-butanol solution (dilution ratio 1:2 in deionized aqua conservata as solvent). 3 pens were presented in a randomized order, with 2 containing the solvent and the 3rd the odorant: subjects had to identify the odor-containing pen. Threshold was defined as the mean of the last 4 of 7 staircase reversals. The subjects' scores ranged between 1 (the lowest olfactory performance) and 16 (the highest olfactory performance) [22]. Moreover, all subjects were tested to evaluate their odor threshold for bourgeonal [3-(4-tert-butylphenyl)-propanal; SantaCruzbiotechnology, Heidelberg, Germany]. This was achieved, similarly to the odor threshold for n-butanol, by presenting the odorant using sticks with a single-staircase, with a "three alternative forced choice" procedure. 29 dilutions were prepared in a geometric series starting from a 4% bourgeonal solution. The dilution ratio was 1:2 and 3 pens were presented in a randomized order. The bourgeonal (CAS# 18127-01-0) was of the highest available purity and was obtained from SIGMA Aldrich (Milan, Italy). For dilutions of bourgeonal, near odorless diethyl phthalate [SIGMA Aldrich (Milan, Italy)] was used as the solvent. The subjects' scores ranged between 1 (the highest olfactory threshold indicating the lowest olfactory performance) and 29 (the lowest olfactory threshold indicating the highest olfactory performance).

Participants' sexual desire was assessed using part of the International Index of Erectile Function (IIEF) scale, which is a brief, multidimensional, self-administered, validated method to measure several dimensions of male sexual functioning [23]. For the purposes of the present study, participants answered the following questions: "how often have you felt sexual desire?" (IIEF1) and "how would you rate your level of sexual desire?" (IIEF2). The questions were preceded by the phrase "over the past 4 weeks…". The volunteers were informed that the possible answers for IIEF1 were: 1 (almost never or never), 2 (occasionally), 3 (sometimes), 4 (often), 5 (almost always or always). For IIEF2 the answers were: 1 (very low), 2 (low), 3 (moderate), 4 (high), and 5 (very high).

Genomic DNA was obtained by brushing the oral mucosa. DNA samples were then quantified by measuring the adsorbance at 260 nm by means of nanodrop ND-1000 Spectrophotometer (Nanodrop Technologies, DE, USA).

Primers were designed using the Primer3 software (Whitehead Institute for Biomedical Research, Cambridge, UK) and specificity were tested using NCBI-Blastn and OligoAnalyzer 3.1 DT software. The resulting primers were suitable for amplifying and sequencing genomic DNA fragments of about 900 bp. The OR1D2 suitable primers of the 3 SNPs (rs769423, rs769424, rs11078437) were then used (Table 1). The Polymerase Chain Reaction (PCR) was performed following standard protocol: 40 cycles of 94 °C for 30 s, 59–61.3 °C for 30 s, and 72 °C for 30 s. The PCR products were analyzed on 1% agarose gels and, before sequencing performed in a core facility, they were purified using ExoSAP (GE Healthcare, Milan, Italy) to remove primers and PCR reagents.

Neither the olfactory detection threshold nor the two self-ratings of sexual desire were normally distributed. The olfactory detection threshold was categorized as <10, $10 \leqslant$ threshold < 15, $15 \leqslant$ threshold < 20, $\geqslant 20$ and all the analyses took into consideration the variable categorized. The association of the bourgeonal olfactory detection threshold with the two self-ratings of sexual desire and between the two self-ratings of sexual desire was analyzed with Fisher's exact test.

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