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Odor perception between heterosexual partners: Its association with depression, anxiety, and genetic variation in odorant receptor OR7D4

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ABSTRACT

We performed a study on a sample of 856 individuals to answer whether the pleasantness/unpleasantness of the odor perception of their partners (rating of partner odor) is associated with depression and anxiety. To evaluate the influence of common genetic variation of the odorant receptor OR7D4 on the rating of partner odor, the variant rs8109935 was genotyped in the whole sample. The rating of partner odor was significantly associated with scores of anxiety and depression. Depression (OR: 0.75, 95%CI: 0.56–0.98, $p = 0.039$) and anxiety (Robust Coef ± SE: -13 ± 0.6, $p = 0.044$) were inversely associated with pleasantness rating of partner odor. Ordered probit regression analysis shows that the rating of partner odor was significantly associated with the rs8109935 genotypes (Coef ± robust SE: 0.19 ± 0.09, $p = 0.028$). These findings suggest that odor perception between heterosexual partners may have an impact on the development of depression and anxiety, and that it might be influenced by genetic variation in *OR7D4*.

1. Introduction

As odor perception is closely linked to the limbic system, it is directly involved in emotional perception and behavior. Odorants and pheromones have, for instance, an important role in mate selection. There is growing evidence to suggest the involvement of genes in the histocompatibility complex (MHC) of mate choice (Ziegler et al., 2005). In fact, the pleasantness of body odors from individuals correlates with a person's leukocyte antigens (HLA) phenotype (Santos et al., 2005). The physiological link between the MHC and body odors is known in other mammals as well (Fan et al., 1995; Potts et al., 1991; Ziegler et al., 2005). These observations are supported by the fact that MHC includes the most polymorphic loci in the genome, including a cluster of odorant receptor genes within the region of high linkage disequilibrium (LD) spanning more than 500 kb (Miretti et al., 2005). It, therefore, follows that the close linkage of the odorant receptor cluster with the HLA complex can account for human mate choices and odor preferences attributed to HLA (Wedekind et al., 1995; Ziegler et al., 2000).

Actually, both behavioral and molecular studies point to a potentially important role for odor receptor in thoughts and behavior (Axel, 2005; Buck, 2005). Interestingly, the functional role of odor perception is not restricted to behavioral preferences. On the contrary, human olfactory perception may play a role in human diseases (Martzke et al., 1997; Moberg et al., 1999). Prior research also demonstrates significant changes in olfactory performance of depressed patients (Amsterdam et al., 1987; Lombion-Pouthier et al., 2006). A recent report shows that odor identification ability is significantly reduced during the depressive state (Clepce et al., 2010).

Odorant receptors are seven-transmembrane domain G-protein-coupled receptors responsible for the detection of odorant molecules that are expressed on the cilia of olfactory sensory neurons in the nasal olfactory epithelium; they constitute the molecular basis for the sense of smell and to cope with the huge variety of odorants, mammals have developed nearly 1000 types of odorant receptors (Buck and Axel, 1991).

The odor perception has a strong inter-individual variability. Recent evidence demonstrates that genetic variations in ORs might explain why the same chemical smells pleasant to some, and unpleasant to others. The variations in olfactory receptor, family

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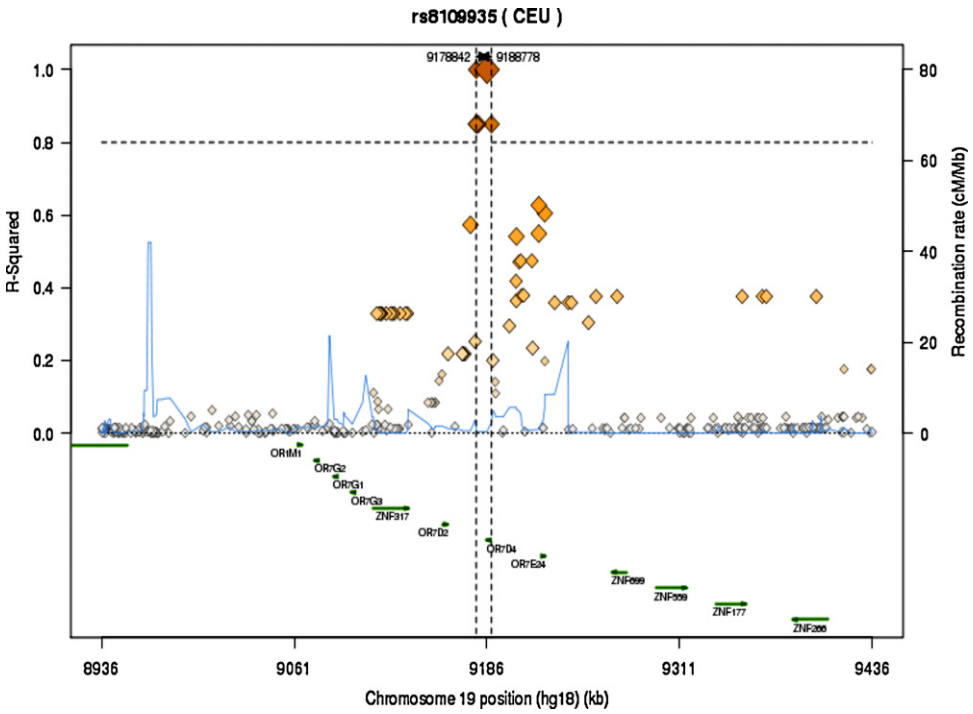

Fig. 1. Regional LD plot for SNP rs8109935 at chromosome 19. The SNP is plotted with their proxies (based on HapMap data for CEU) as a function of genomic location, annotated by the recombination rate across the locus (blue-line) and nearby genes (in green). The regional association plot of rs8109935 was performed by the SNAP server available at http://www.broad.mit.edu/mpg/snap/. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

7, subfamily D, member 4 (OR7D4), which selectively responds to two sex steroid-derived odors, androstenone and androstadienone, particularly account for a significant proportion of the variance in human odor perception (Keller et al., 2007). The variants responsible for the variance in the perception of these steroidal odors (two linked nonsynonymous SNPs, R88W and T133M) are, in addition, associated with a severe impairment of the in vitro function of the OR7D4 (Keller et al., 2007).

Genetic variants in OR7D4 receptor can modify the detection of the steroids androstenone and androstadienone, two pheromones that have the potential to evoke behavioral changes in many mammals, including humans, including mood changes (Kohl et al., 2001) and inter-sex communication (Kohl et al., 2001). We, therefore, hypothesized that OR7D4-related individual differences in odor perception of one's partner might exert a causal effect on depression and anxiety. To test this hypothesis, a population-based study was carried out on a sample of heterosexual individuals in which we measured the pleasantness/unpleasantness of the odor perception of their partners (rating of partner odor) and their levels of anxiety and depression. Also, it was explored if common genetic variations in *OR7D4* located in chr19 (p13.2) have any role in rating of partner odor.

2. Subjects and methods

2.1. Study design

Altogether, 856 participants (68.6% females) belonging to 630 European families (descent self-reported), mostly parent-offspring families (69.3%), aged 41.9 ± 0.7 (media ± robust SE) years, and who were willing to participate were included in this study. Recruitment of subjects was community-based and randomly selected by geographical location from a small rural community of the Province of Buenos Aires, Argentina. None of them was enrolled on the basis of phenotype. All participants went through a baseline evaluation of health status, medical history and physical examination and fasting blood samples for leukocytes DNA extraction and routine laboratory determinations.

All the investigations for this study were carried out in accordance with the guidelines of The Declaration of Helsinki. Written consents for the tests were obtained from all the subjects in accordance with the procedures approved by the Ethical Committee of the County of San Andres de Giles.

2.2. Measurement of depression and anxiety

The presence of depressive symptoms was evaluated by the Zung Self-Rating Depression Scale (ZSDS), a psychiatric instrument consisting of a self-administered, validated 20-item scale (Zung, 1965). The items on the scale include four common characteristics of depression: the pervasive affect, the physiological equivalents, other disturbances, and psychomotor activity. The ZSDS is a valid and sensitive measure of clinical severity of depression (Biggs et al., 1978). Summing of the individual item scores gives the total score, which ranges from 20 to 80. Score between 51 and 69 indicate depression and those between 70 and above severe depression.

The presence of anxiety was evaluated by the 14 items of the Hamilton Anxiety Scale (HAS) (Hamilton, 1959). The HAS covers the whole spectrum of the anxiety disorder: physical experience of anxiety, somatic and muscular symptoms, depressed mood, insomnia, and cognitive dysfunction. The level of anxiety is indicated by the HAS score thus: <18 mild; 18–24 mild to moderate; and 25–30 moderate to severe.

2.3. Evaluation of the rating of partner odor

All the subjects were asked to rate of pleasantness/unpleasantness of their partner odor (independent of particular situations as physical activity or other conditions) through self-assessment by answering the following question: "Rate the pleasantness of the odor of your partner (circle the corresponding number)" as 4 (very pleasant), 3 (pleasant), 2 (neutral), 1 (unpleasant), and 0 (very unpleasant). All the participants and technicians were unaware of the hypothesis being tested. The rating of partner odor was done as a general test, but excluding after exercising, or women experiencing menstrual cycle.

2.4. Genotype analysis

The genetic analyses were done on genomic DNA extracted from white blood cells by the standard method (Kawasaki, 1990).

Most of the common variants occur in all human populations; however, their frequencies differ among populations. HapMap provides information on the presence and relative allele frequency of many SNPs in the genome, and on the patterns of linkage disequilibrium among SNPs. The two previously mentioned non-synonymous SNPs, arginine-to-tryptophan R88W rs61729907 and threonine-to-methionine T133M rs5020278, described by Keller et al. (2007) have neither frequency data nor HapMap validation in any population (http://hapmap.ncbi.nlm.nih.gov/). Only one OR7D4 variant is present in HapMap, the rs8109935 G/A, a coding synonymous SNP located in codon 3, amino acid position 217 (chromosome 19, position 9185863, Fig. 1). This SNP was genotyped. This variant was predicted to show high risk of functional effect at the splicing regulation level (http://compbio.cs.queensu.ca/F-SNP/).

Genotyping was performed using a high-throughput genotyping method involving PCR amplification of genomic DNA with two-tailed allele-specific primers that introduce priming sites for universal energy-transfer-labeled primers, as previously described (Myakishev et al., 2001) (Prevention Genetics, Marshfield, WI, USA). To ensure genotyping quality, DNA samples were included as internal controls, hidden samples of known genotypes, and negative controls (water). No genotype with a signal below negative control was scored. The error analysis was performed by replicating a blinded sample (always belonging to the same individual) across the templates of the project six times.

The PLINK software (http://pngu.mgh.harvard.edu/purcell/plink/) was used in assessing the association of SNP with the affection status and related quantitative traits, and in testing for Hardy–Weinberg equilibrium (Purcell et al., 2007).

2.5. Statistical analysis

Quantitative data were expressed as mean ± robust SE to correct for relatedness (families as a cluster variable, which means that standard errors allow for intragroup correlation, relaxing the usual requirement that the observations must be independent). For univariate analysis, the differences between groups categorized by rating of partner odor scores were assessed by Kruskal–Wallis rank test and the correlations by Spearman's Rho correlation test, because most variables show no normal distribution.

For testing the association between the rating of partner odor as a categorical ordinal variable (scored from 0 to 4 from very unpleasant (0), unpleasant (1), neutral (2), pleasant (3) to very pleasant (4) and other variables), we used linear regression for an ordinal multinomial distribution (Probit Link function) for the rating of partner odor as the dependent (response) variable, with age, sex, ZSDS, and HAS scores as continuous or categorical predictor variables. In some analysis, rs8109935 genotype was added as a categorical factor for an additive model by coding the genotypes AA, AG, and GG as 0, 1, and 2 respectively. For analyzing the association of depression as a dichotomic variable (absence and presence of depression was rated by using ZSDS as 0 or 1, because only one individual reported severe depression and hence eliminated from the analysis) and anxiety as an ordinal categorical variable (anxiety was scored by using the HAS as absent 0, low 1, moderate 2, and severe 3 as explained earlier) with other variables including sex, age, and rating of partner odor, the logistic or ordered probit regression was used. In all these analyses, a correction for relatedness was included by using families as a cluster variable.

STATA program package 10.1 (StataCorp, College Station, TX, USA) was used to perform these analyses.

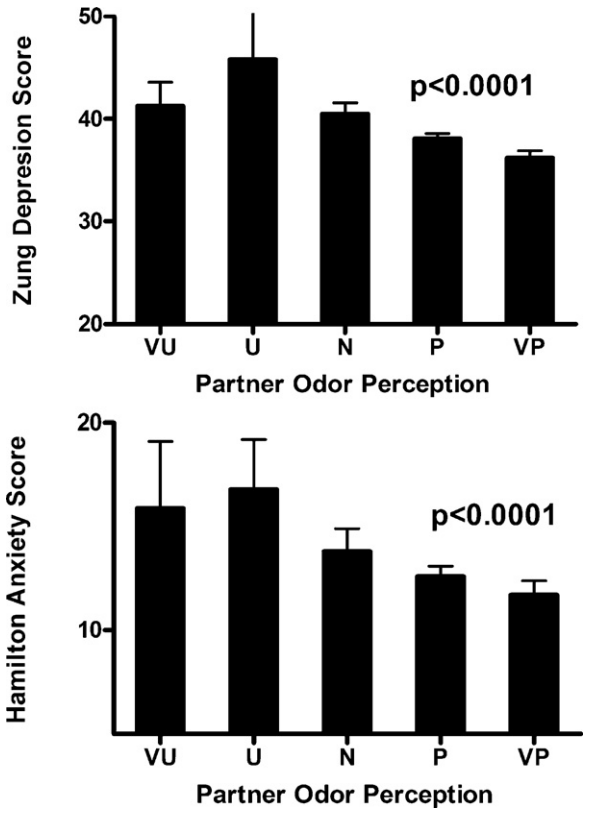

Fig. 2. ZSDS and HAS scores according to heterosexual partner odor perception. Subjects ($n = 629$) were asked to classify partner odor as very unpleasant (VU, $n = 15$), unpleasant (U, $n = 16$), neutral (N, $n = 95$), pleasant (P, $n = 299$), and very pleasant (VP, $n = 231$). p stands for statistical significance among groups using non-parametrical Kruskal–Wallis rank test.

3. Results

None of the subjects had neurological diseases, nor did any of them receive antidepressant medications during this study.

A total of 656 individuals (204 males and 452 females, age 41.9 ± 0.7) responded to the rating of partner odor questionnaire. Of the 856 participants enrolled in the study, 792 responded to the ZSDS and the HAS questionnaires; scores of ZSDS and HAS were 38.1 ± 0.3 and 12.6 ± 0.3, respectively.

The depressive symptoms were higher in women than in men. Among the 452 female participants, 11% suffered from depressive symptoms as indicated by the ZSDS score above 50; among the 204 male participants, 4.5% suffered from depressive symptoms. Likewise, anxiety symptoms were higher in women than in men. Fourteen percent of female participants and 8% of male participants suffered from anxiety symptoms. Evaluation of the relationship between the scores of depression and anxiety and the rating of partner odor shows that the rating of partner odor was significantly associated with anxiety and depression (Fig. 2, upper and lower panel). The association of the rating of partner odor with both depression and anxiety scores is not surprising, because both are highly correlated with each other (Spearman $R = 0.65$, $p = 0.00001$) in both the sexes (data not shown).

Using discriminant analysis with the rating of partner odor categories as the dependent variable in ordered probit regression corrected by families as a cluster variable, rating of partner odor from 0 to 4 was significantly and negatively correlated with being female, older, and scoring high in ZSDS independent of the score in the HAS (Table 1), the model explained a 3.4% of the total variance ($R^2 = 0.0335$). Accordingly, depression, as the dichotomic variable defined earlier, is inversely associated with pleasantness rating of partner odor (OR: 0.75, 95%CI: 0.56–0.98, $p = 0.039$) independent of sex (female vs. male: OR: 3.70, 95%CI: 1.60–8.54, $p = 0.002$) and age (OR: 0.98, 95%CI: 0.97–1.00, $p = 0.103$). Similarly, anxiety, as a categorical ordinal variable, is inversely associated with pleasantness rating of partner odor (Robust Coef ± SE: -13 ± 0.6, $p = 0.044$) independent of sex (0.38 ± 0.12, $p = 0.001$) and age (-0.002 ± 0.004, $p = 0.59$). Only depression remained, although marginally, associated with rating of partner odor after adjustment for sex, age, and the other psychological variable (OR: 0.73, 95%CI: 0.53–0.99, $p = 0.05$).

Finally, as rating of partner odor may be genetically determined, the hypothesis that this may be associated with genetic variability in the *OR7D4* was tested. We have genotyped rs8109935 A/G variant, which in our population showed allelic frequencies of 12% and 88% for the A and G alleles, respectively. Genotypes (AA: 2.1%, AG: 19.7% and GG: 78.2%) were in Hardy–Weinberg equilibrium ($p = 0.468$).

Table 1
Perception of heterosexual partner odor (rating of partner odor) score on a scale of 0–4 [very unpleasant (0), unpleasant (1), neutral (2), pleasant (3) to very pleasant (4)] was negatively and significantly correlated with being female, older, and scoring high in ZSDS, independent of the score in the HAS. Discriminant analysis was performed by an ordered probit regression test with rating of partner odor categories as the dependent variable and corrected by families as a cluster variable rendering a robust SE for the regression coefficient (Coef).

Variable	Coef.	Robust SE	p
Sex (female 2, male 1)	−0.3334787	0.0972457	0.001
Age	−0.0115856	0.0032261	0.0001
Zung depression score (ZSDS)	−0.0261402	0.0064569	0.0001
Hamilton anxiety score (HAS)	0.0063226	0.0066844	0.344

Ordered probit regression analysis corrected by relativeness (family cluster variable) showed that pleasantness rating of partner odor is significantly associated with the rs8109935 genotypes carrying the G allele (Coef ± robust SE: 0.19 ± 0.09, $p = 0.028$). In fact, the association (Coef ± robust SE: 0.20 ± 0.9, $p = 0.03$) remained even after adjusting for sex, age, ZSDS, and HAS scorings.

4. Discussion

There is plenty of evidence to show that odor perception of humans is distorted during illness, including psychiatric disorders. Conversely, the effect of a sustained unpleasant odor perception, such as the one in an interpersonal relationship, on mental health remains unknown. Hence, we explored the potential link between odor perception of heterosexual partners and symptoms of depression and anxiety.

This approach is justified by the fact that odor perception has an important role in conditioning social and reproductive behaviors. In addition, as odor perception can be affected by numerous factors, the possible role of genetic variation in the odor receptor gene was explored. The genetic analysis was focused on the common variation occurring in the *OR7D4* gene, as emerging evidence shows that it correlates with differences in the perception of androstenone and androstadienone, the two steroids believed to be human pheromones. To accomplish this, a population-based study was performed on a large, well-characterized sample of adult individuals and the following results were obtained. Measurement by two validated psychiatric instruments shows that the rating of partner odor was significantly and inversely associated with high scores of anxiety and depression. Depression – as a dichotomic variable – and anxiety – as a categorical ordinal variable – are inversely associated with positive rating of partner odor. Second, it was observed that the rating of partner odor was significantly associated with rs8109935 genotypes suggesting that genetic variability in the *OR7D4* might have a role in the odor perception between partners. In fact, the data of this study replicate, for the first time, the results of Keller et al. (2007) about the influence of genotype variations in *OR7D4* on odor perception.

Annotation of nearby SNPs in LD (proxies) with rs8109935, based on HapMap data, shows that the putatively associated variant is in fact in the *OR7D4* locus, and there is no other variant in LD in this gene (Fig. 1). Nevertheless, one cannot assume that the associated SNP is the causal variant as there are other SNPs in high LD in the intergenic region around rs8109935 (Fig. 1), despite the fact that this variant was predicted to show high risk of functional effect at the splicing regulation level.

The methodological strengths of this study are the large well-characterized population sample and the strategy adopted in selecting the participants. Although the findings are novel, they can be generalized only with some caveats. For example, a non-validated instrument was used to measure the rating of partner odor, because no standardized questionnaire was available. The instruments used so far are mostly suitable for measuring and evaluating the olfactory function or general olfactory impairment. Besides, it is worthwhile to evaluate the impact of short-term vs long-term rating of partner odor on depression and anxiety symptoms. A final caveat might be the cross sectional design of our study that does not allow to prove that the odor perception of one's partner have a causal effect on human disease. In fact, it seems at least equally plausible that emotional distress negatively affects odor perception or that a third factor (e.g. dissatisfaction in the relationship) affects both emotional wellbeing and odor perceptions. Hence, longitudinal studies may be very important to clarify this point.

A final comment regarding the importance of smell in interpersonal relationships is relevant here. For instance, previous studies show that women tend to smell out compatible HLA mates, detecting differences of one HLA allele among male odor donors with different MHC genotypes (Jacob et al., 2002). Further, women, and even some men, deliberately smell their partners' clothing when they are apart (McBurney et al., 2006). In conclusion, one may speculate that odor perception between heterosexual partners plays a role beyond that in sexual attraction and attachment. It can also potentially influence mood and the risk for psychiatric disorder.

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Conflict of interest statement

The authors have no conflict of interest to declare.

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