LETTERS

Genetic variation in a human odorant receptor alters odour perception

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Human olfactory perception differs enormously between individuals, with large reported perceptual variations in the intensity and pleasantness of a given odour. For instance, androstenone (5α-androst-16-en-3-one), an odorous steroid derived from testosterone, is variously perceived by different individuals as offensive ("sweaty, urinous"), pleasant ("sweet, floral") or odourless[1–3]. Similar variation in odour perception has been observed for several other odours[4–6]. The mechanistic basis of variation in odour perception between individuals is unknown. We investigated whether genetic variation in human odorant receptor genes accounts in part for variation in odour perception between individuals[7,8]. Here we show that a human odorant receptor, OR7D4, is selectively activated *in vitro* by androstenone and the related odorous steroid androstadienone (androsta-4,16-dien-3-one) and does not respond to a panel of 64 other odours and two solvents. A common variant of this receptor (OR7D4 WM) contains two non-synonymous single nucleotide polymorphisms (SNPs), resulting in two amino acid substitutions (R88W, T133M; hence 'RT') that severely impair function *in vitro*. Human subjects with *RT/WM* or *WM/WM* genotypes as a group were less sensitive to androstenone and androstadienone and found both odours less unpleasant than the *RT/RT* group. Genotypic variation in OR7D4 accounts for a significant proportion of the valence (pleasantness or unpleasantness) and intensity variance in perception of these steroidal odours. Our results demonstrate the first link between the function of a human odorant receptor *in vitro* and odour perception.

We investigated the hypothesis that polymorphisms in odorant receptors contribute to variability in human odour perception by combining a cell-based assay technique to identify active ligands for odorant receptors[9] with an olfactory psychophysical study of a diverse population of human subjects[10]. A total of 66 odorants were used to measure both odorant receptor responses *in vitro* and psychophysical responses in human subjects, with a focus on odorous steroids because the perception of these odours is exceptionally variable[3,11]. We cloned a panel of 335 putative human odorant receptors, representing more than 85% of the odorant receptors with full open reading frames, and expressed them in Hana3A cells, an HEK293T-derived cell line stably expressing accessory factors for odorant receptor expression[9,12]. We screened for androstenone-mediated stimulation with a luciferase reporter[9]. Among the receptors tested, that encoded by *OR7D4* showed the strongest responses to androstenone (Fig. 1a). Recent expression analysis shows that *OR7D4* is selectively expressed in human nasal epithelium[13]. Several other receptors showed smaller responses to androstenone that may be relevant *in vivo* (Supplementary Table 1), but these were not investigated further. The failure of a specific odorant receptor to respond in this assay must be interpreted with caution because it may reflect a failure of the odorant receptor to be functional in the assay rather than a lack of sensitivity to the tested odour.

A search for polymorphisms in *OR7D4* in SNP databases and sequencing the coding region of *OR7D4* in 391 subjects identified 13 non-synonymous SNPs in this receptor, with four occurring at

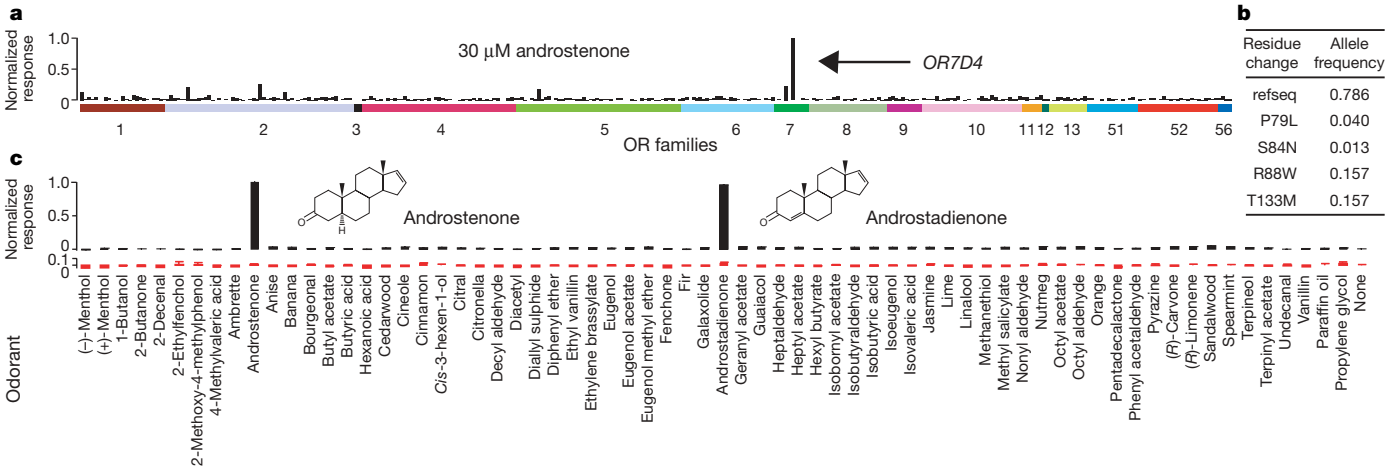

Figure 1 | *OR7D4* is selectively activated by androstenone and androstadienone. **a**, Hana3A cell luciferase assays of 335 unique human odorant receptors; the concentration of androstenone used was 30 μM. Numbers and coloured bars indicate different OR families. **b**, Allele frequencies of common variants. refseq, reference sequence. **c**, *OR7D4 RT* (black columns) and *WM* (red columns) tested against 66 odours and 2 solvents (30 μM, or 1/30,000). Normalized responses are shown as means and s.e.m. ($n = 4$).

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frequencies greater than 1% (Supplementary Table 2). Two non-synonymous polymorphisms in complete linkage disequilibrium in this population occurred at the highest frequency and led to two amino-acid changes (R88W and T133M; Fig. 1b). We refer to the most common allele of this receptor, or the reference sequence, as *RT*, and to the other as *WM*.

We investigated the ligand specificity of RT and WM receptor variants *in vitro* with a panel of 66 odours and 2 solvents. OR7D4 RT responds selectively to androstenone and the closely related odorous steroid androstadienone but shows no response to any other stimuli tested (Fig. 1c, top). OR7D4 WM shows no response to any compound at the concentrations tested here (Fig. 1c, bottom). Dose–response curves with RT and WM show that the paired SNPs in the WM variant, which affect amino acids in extracellular loop 2 and intracellular loop 2 (Fig. 2a), severely impair function (Fig. 2b). We generated odorant receptors with each of the SNPs and found that OR7D4 R88W and OR7D4 T133M retained an intermediate level of function, suggesting that both residues are important for OR7D4 function (Fig. 2b).

We examined two other SNP variants found at frequencies greater than 1%, which led to amino acid changes P79L and S84N, respectively (Fig. 1b and 2a). P79L and S84N possess residues R88 and T133 and are referred to by the single variant residue. Analysis of P79L function *in vitro* showed severely impaired function at all concentrations of either steroidal odour tested (Fig. 2c). In contrast, OR7D4 S84N showed remarkable sensitivity to both odours *in vitro*, exceeding the activity of the common RT variant at every concentration tested, with a concentration giving half-maximal response (EC_{50}) to androstadienone less than one-fifth of that of the RT variant (Fig. 2c).The functional differences between these two variants are not due to the amino-terminal epitope tag (Supplementary Fig. 1). The other non-synonymous substitutions in *OR7D4* result in varying receptor functions (Fig. 2d).

OR7D4 is situated in a cluster of seven intact odorant receptor genes, but we found that none of the polymorphisms of the six intact odorant receptors in the *OR7D4* gene cluster showed significant linkage with the *OR7D4* SNPs (Supplementary Fig. 2a and Supplementary Table 3). We tested the responses of all the major variants of odorant receptors in the *OR7D4* cluster and found that none showed responses to androstenone and androstadienone exceeding that of the impaired WM variant (Supplementary Fig. 2b, c).

The chimpanzee *OR7D4* orthologue differs from the human RT reference sequence at five amino acid residues: at the S84N substitution also found in humans and at four additional non-synonymous substitutions not found in humans (V26I, G171V, G227R and K232E). A dose–response analysis of the chimpanzee OR7D4 orthologue *in vitro* showed robust responses to both steroidal odours, exceeding the activity of the human S84N variant (Fig. 2c; compare purple and green curves).

What accounts for the functional differences between OR7D4 variants? We found no obvious difference in subcellular distribution or expression level in permeabilized Hana3A cells expressing RT, WM, P79L or S84N (Supplementary Fig. 3). Western blot analysis confirms that all are expressed at comparable levels (Fig. 2e), and RT, WM and P79L have similar low levels of surface staining as measured by flow cytometry of live cells stained to reveal the N-terminal epitope (Fig. 2f). S84N showed considerably more surface expression (Fig. 2f), suggesting that the increased function of this variant may stem from enhanced stability at the cell surface or from enhanced cell-surface trafficking.

We next asked whether variation in *OR7D4* is correlated with variation in the perception of androstenone and androstadienone measured in human subjects. Psychophysical data on 391 subjects performing three different tasks were collected: subjects rated the perceived intensity and valence of 66 different odours at two concentrations (Supplementary Fig. 4); detection thresholds were measured to androstenone and androstadienone in a subset of subjects, and to three control odours in all subjects[14,15] (Supplementary Fig. 5); subjects profiled four odours with 146 semantic labels[10,16] (see Supplementary Methods).

Psychophysical data on the subjects were subsequently divided according to genotype and assessed for the influence of *OR7D4* genotype on perceptual phenotype (Supplementary Table 2). Of the 66 odours and two solvents rated by *RT*/*RT* and *RT*/*WM* subjects, only androstenone and androstadienone showed a significant effect of genotype (Fig. 3a and Supplementary Fig. 6). The steroids were rated as less intense by the *RT*/*WM* group (Fig. 3a); the proportion of *RT*/*WM* subjects rating the high concentration of androstenone as "extremely weak" was fourfold that of *RT*/*RT* subjects (Supplementary Fig. 6). This phenotype was specific for these two compounds, as the perception of all the other odours was not affected by *OR7D4* genotype (Fig. 3a, b). The significant effect of *OR7D4* genotype on steroidal odour intensity perception was replicated in both males and females (Supplementary Fig. 7) and in the largest racial category, Caucasians (Supplementary Fig. 8). Although the *WM* allele strongly affected androstenone intensity perception even in heterozygous subjects, the group of *WM*/*WM* subjects showed an even stronger effect on intensity ratings, rating both steroidal odours as less intense

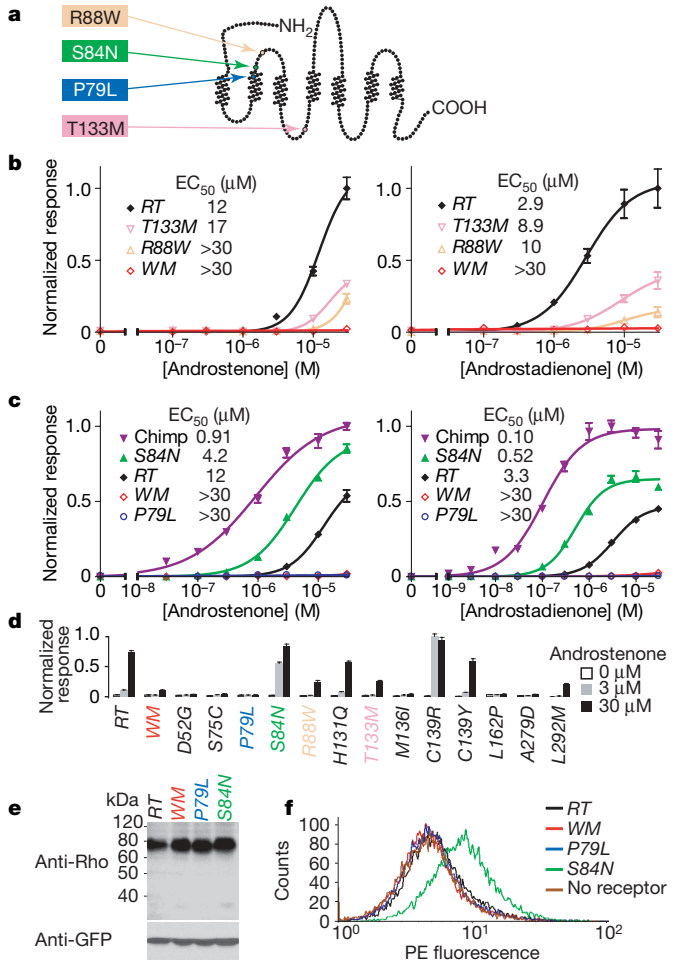

Figure 2 | Functional characterization of *OR7D4* polymorphisms. a, OR7D4 snake plot with amino acid changes indicated. **b**, **c**, Dose–response curves and EC_{50} values of OR7D4 RT, WM, R88W and T133M (**b**) and of OR7D4 RT, WM, P79L and S84N and chimpanzee OR7D4 (**c**) to androstenone (left) and androstadienone (right). **d**, Activity of 13 SNP variants compared with that of RT and WM variants. In **b**–**d**, normalized responses are shown as means and s.e.m. ($n = 4$–6). **e**, Western blot analysis of whole-cell lysates from HEK293T cells transfected with *OR7D4 RT*, *WM*, *P79L* or *S84N* and co-transfected with green fluorescent protein (*GFP*). **f**, Flow cytometry analysis of cell-surface OR7D4 RT, WM, P79L and S84N expression as measured by intensity of phycoerythrin (PE) signal among GFP-positive cells.

than the group of *RT/RT* subjects (Fig. 3b). The few subjects among our population carrying the less frequent *P79L* and *S84N* variants were examined, and *RT/P79L* subjects as a group showed a trend to perceive both androstenone and androstadienone as less intense than the group of *RT/RT* subjects (Fig. 3c). Conversely, *RT/S84N* subjects as a group showed a trend to rate both odours as more intense (Fig. 3c). However, these differences measured in subjects with the rare alleles were not statistically significant.

Detection thresholds of 121 subjects were determined for both steroidal odours (Fig. 3d, e). *RT/WM* subjects as a group had a higher detection threshold—and were therefore less sensitive—to both compounds than the group of *RT/RT* subjects (Fig. 3d) but had normal thresholds to three control odours (data not shown). The threshold for androstenone in the *RT/WM* group was 11-fold, and that for androstadienone 16-fold, of that in the *RT/RT* group. In addition, 46% of *RT/WM* subjects but only 28% of *RT/RT* subjects could not detect the highest concentration of androstenone we provided ($P < 0.05$; χ^2 test). Detection thresholds were also obtained from the few *RT/P79L*, *WM/WM*, *WM/P79L* and *RT/S84N* subjects (Fig. 3e and Supplementary Fig. 9). The proportion of *RT/P79L* subjects unable to detect the highest concentration of androstenone and androstadienone provided was more than double that of *RT/RT* subjects ($P < 0.05$; χ^2 test) (Fig. 3e). It is unclear why the loss of one functional allele has such a profound effect on the median detection threshold. The same trend was found with the *WM/WM* and *WM/P79L* groups (Supplementary Fig. 9), whereas the *RT/S84N* group was more sensitive to both steroids, with lower detection thresholds than the group of *RT/RT* controls. However, these differences were not statistically significant (Fig. 3e).

We next examined whether variation in *OR7D4* affects the perception of androstenone and androstadienone odour quality. The *RT/WM* group rated both steroidal odours as less unpleasant than the *RT/RT* group (Fig. 4a, b and Supplementary Fig. 10), such that the proportion of *RT/WM* subjects rating the high concentration of androstadienone as "extremely unpleasant" was less than half of that of *RT/RT* subjects (Supplementary Fig. 10). None of the other 64 odours or the solvents showed a statistically significant difference between the genotypes (Fig. 4a), and the effect was statistically significant for both steroidal odours in both males and females (Supplementary Fig. 7). The group of subjects carrying the impaired *RT/P79L* variant showed a trend to rate both androstenone and androstadienone as less unpleasant than *RT/RT* controls (Fig. 4c), whereas the opposite was found in the *RT/S84N* group carrying a more sensitive variant of *OR7D4* (Fig. 4c); however these differences were not statistically significant.

We asked subjects to assess androstenone odour quality by profiling this odour with a standard set of 146 semantic descriptors (see Supplementary Methods)[10,16]. All descriptors used by more than 10% of the subjects were analysed and descriptor usage in individuals with different genotypes was compared. Of the 74 descriptors used for androstenone, pentadecalactone, vanillin and the solvent propylene glycol, only five differed significantly by genotype (see Supplementary Methods). *OR7D4 RT/WM* subjects were more likely to rate vanillin as smelling "honey", "sweet" and "vanilla" (Fig. 4d, e),

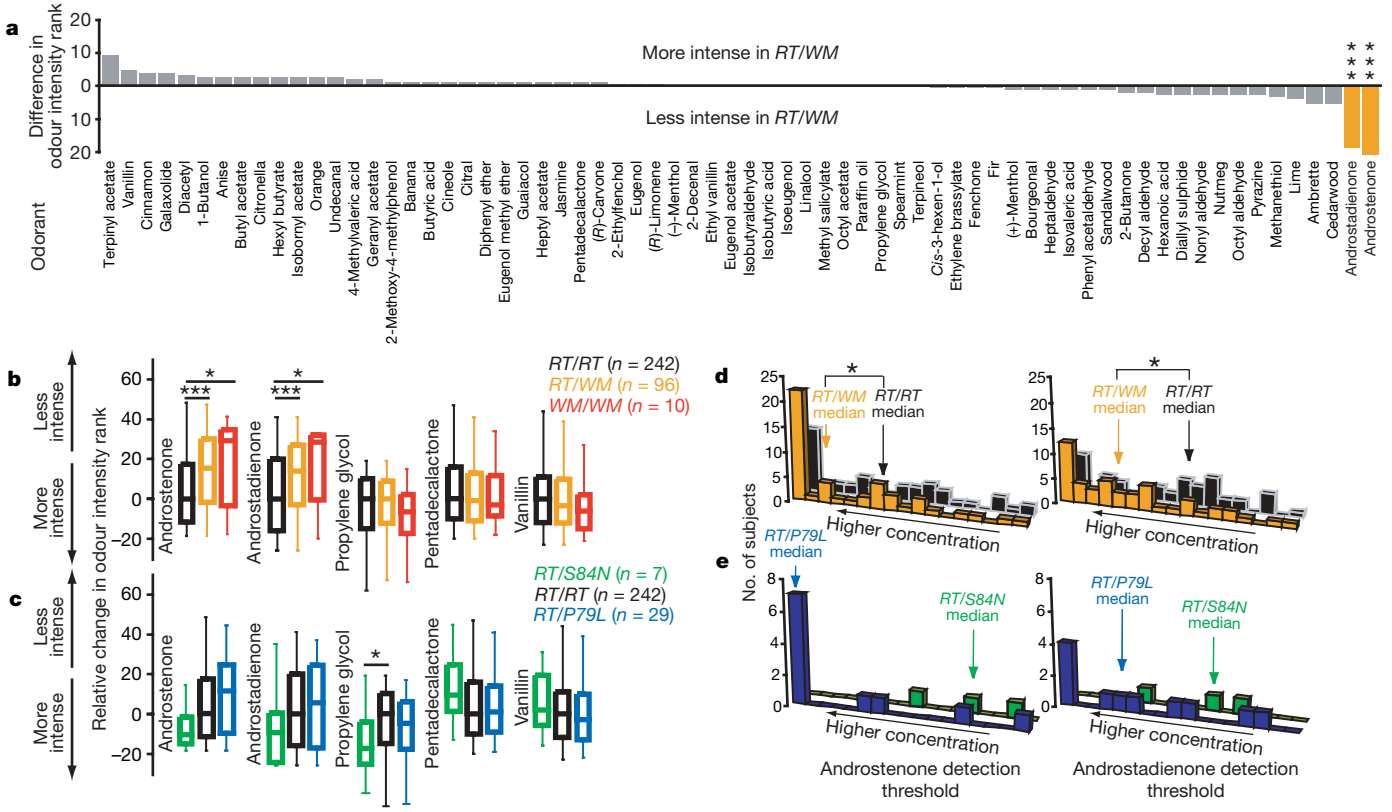

Figure 3 | *OR7D4* variation affects androstenone and androstadienone intensity perception. **a**, Differences in median odour intensity ranking of 66 odours and 2 solvents between *OR7D4 RT/WM* and *RT/RT* groups. Data for two different odour concentrations were pooled. **b**, Change in odour intensity ranking relative to solvent of four odours for the *RT/RT*, *RT/WM* and *WM/WM* groups (**b**) and for the *RT/RT*, *RT/P79L* and *RT/S84N* groups (**c**). The whisker plots show the median rank (normalized to the median rank of the *RT/RT* group), the first and third quartile and the upper and lower limits. Significance was assessed in **a**–**c** with a Mann–Whitney *U*-test with a Bonferroni correction. Before correction: asterisk, $P < 0.00073$; two asterisks, $P < 0.00014$; three asterisks, $P < 1.47 \times 10^{-5}$. After correction: asterisk, $P < 0.05$; two asterisks, $P < 0.01$; three asterisks, $P < 0.001$. **d**, **e**, Detection thresholds measured in *RT/RT* ($n = 47$) and *RT/WM* subjects ($n = 49$) (**d**) and in *RT/P79L* ($n = 12$) and *RT/S84N* ($n = 3$) subjects (**e**) plotted as the number of subjects detecting the odour at a given binary dilution (*x*-axis concentrations are binned from left to right: the first bar represents binary dilution 6, the subsequent 15 bars represent binary dilutions 7–21, and the last bar represents dilutions 22–27). Significance was assessed with a Mann–Whitney *U*-test (asterisk, $P < 0.05$).

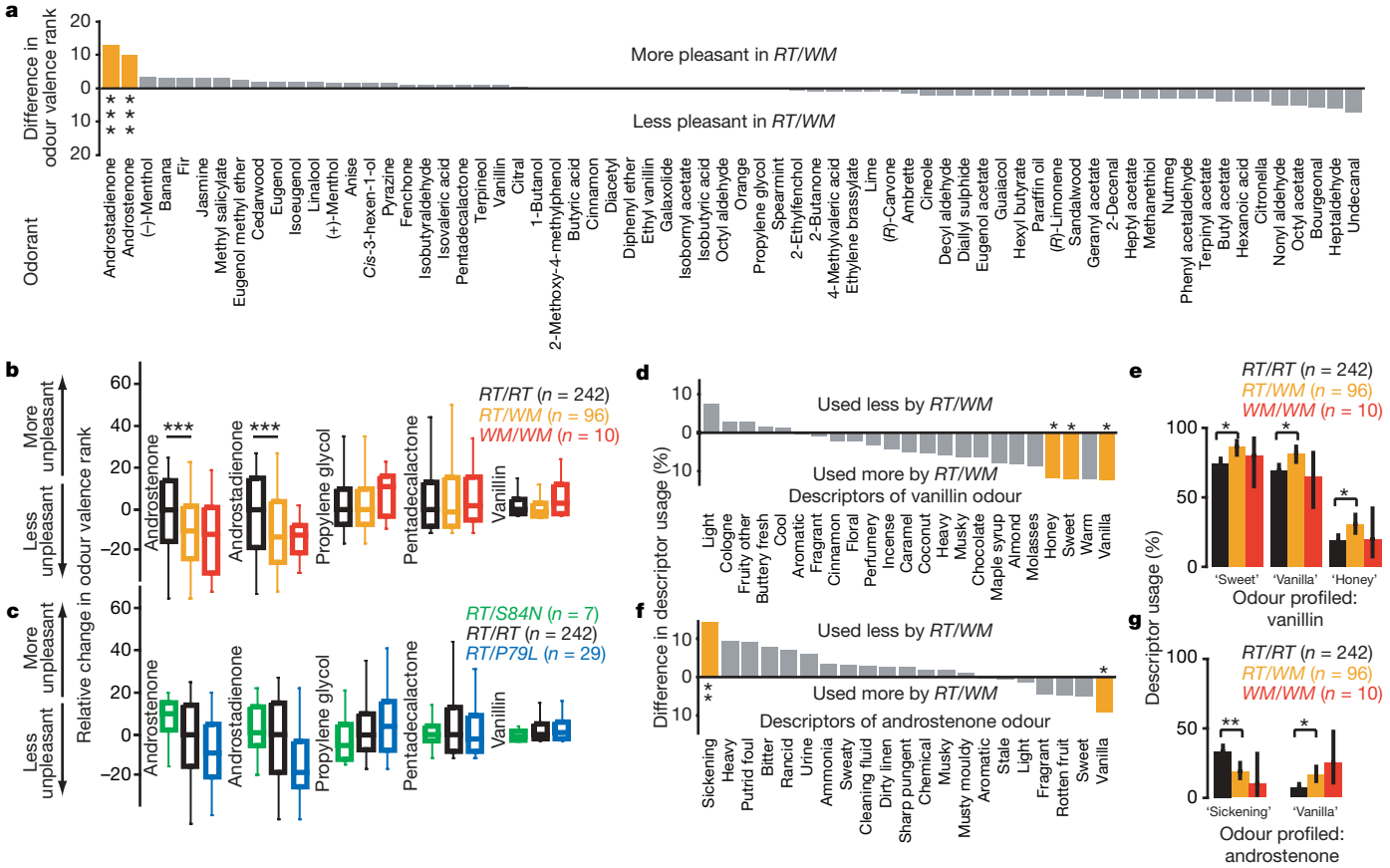

Figure 4 | *OR7D4* variation affects androstenone and androstadienone quality perception. **a**, Differences in median odour valence ranking for the same odours and genotypes as in Fig. 3a. **b**, **c**, Change in odour valence ranking for the same odours and genotypes as in Fig. 3b, c. Significance in **a**–**c** was assessed with a Mann–Whitney *U*-test with Bonferroni correction. Before correction: asterisk, $P < 0.00073$; two asterisks, $P < 0.00014$; three asterisks, $P < 1.47 \times 10^{-5}$. After correction: asterisk, $P < 0.05$; two asterisks, $P < 0.01$; three asterisks, $P < 0.001$. The whisker plots show the median rank (normalized to the median rank of the *RT*/*RT* group), the first and third quartile and the upper and lower limits. **d**, **e**, Odour profiling of vanillin by *RT*/*RT* ($n = 242$), *RT*/*WM* ($n = 96$) and *WM*/*WM* ($n = 10$) subjects. Plotted are the differences in descriptor usage by genotype of the 23 descriptors used for vanillin in more than 10% of sessions (**d**) and the percentage of sessions (with 95% confidence intervals) in which the three descriptors that showed significant differences were used (**e**). **f**, **g**, Odour profiling of androstenone for the same genotypes as in **d** and **e**; 21 descriptors were used in more than 10% of all sessions, and two descriptors showed significant differences. Significance was assessed in **d**–**g** with a χ^2 test with Bonferroni correction. Before correction: asterisk, $P < 0.0022$ (vanillin) and $P < 0.0024$ (androstenone); two asterisks, $P < 0.0004$ (vanillin) and $P < 0.0005$ (androstenone). After correction: asterisk, $P < 0.05$; two asterisks, $P < 0.01$.

42% less likely to consider androstenone "sickening" and 129% more likely to rate it as smelling like "vanilla" than *RT*/*RT* subjects (Fig. 4f, g). There was no effect of *OR7D4* genotype on odour profiling of pentadecalactone or propylene glycol (data not shown).

Last, we performed a non-parametric regression analysis[17] to estimate the fraction of the phenotypic variance in steroid odour perception attributable to *OR7D4*. We found that *OR7D4* genotype in our population explained 19% and 39% of the variance in the valence and intensity ratings of the steroid odours, respectively ($P < 0.0001$; see Supplementary Methods).

We identify *OR7D4* as a significant heritable factor influencing androstenone and androstadienone perception, thus providing the first reported link between genetic polymorphisms in an odorant receptor gene and altered perception of the ligands that activate this receptor. As predicted by the theory of combinatorial coding[18], we find that polymorphisms in the *OR7D4* protein-coding sequence alone do not fully account for specific anosmia to androstenone and androstadienone. We think it likely that additional human odorant receptors sensitive to androstenone and androstadienone remain to be discovered.

Previous work indicated that sensitivity to androstenone[19,20], and to several other odours[21], is modulated by non-genetic effects such as central processing[22] or peripheral sensitization[23] that might obscure any underlying genetic influences on androstenone perception. About half of the subjects initially unable to detect androstenone were able to smell this compound after being exposed to androstenone daily for six weeks[19]. Although it has been assumed that olfactory induction is a phenomenon that occurs only in individuals with specific anosmia to androstenone, more recent studies have found that induction of enhanced olfactory sensitivity seems to be a general phenomenon affecting several odorants[21]. The role of *OR7D4* in this sensitization can now be tested in subjects chronically exposed to androstenone.

In this study we investigated only the olfactory percept reported when odorous steroids were sniffed, but olfactory exposure to androstenone and androstadienone has also been shown to induce several physiological responses in both men and women[24,25]. The identification of an odorant receptor gene that is strongly correlated with the perception of these odours will permit future analysis of olfactory-induced autonomic responses in humans.

METHODS SUMMARY

Heterologous expression of human odorant receptors. A total of 423 human odorant receptors, including 335 predicted functional receptors, were cloned. The chimpanzee *OR7D4* orthologue was cloned from chimpanzee genomic DNA (Coriell Cell Repositories). Odorant receptors containing the first 20 amino acids of human rhodopsin[26] in pCI (Promega) were expressed in the Hana3A cell line together with a short form of *mRTP1* called *RTP1S* (M37 to

the carboxy-terminal end), which enhances functional expression of the odorant receptors[12]. For immunocytochemistry, cells were fixed, permeabilized and incubated with monoclonal anti-rhodopsin antibody (4D2; ref. 27), followed by Cy3-conjugated donkey anti-mouse IgG (Jackson Immunologicals). For fluorescence-activated cell sorting analysis, this antibody was conjugated with phycoerythrin.

Human odorant receptor genotyping and sequencing. Venous blood was collected from all subjects, and genomic DNA was prepared with the Qiagen PAXgene blood DNA kit. Polymorphisms in *OR7D4* were assayed by sequencing and allele-specific polymerase chain reaction. Polymorphisms in the other odorant receptors in the same odorant receptor gene cluster as *OR7D4* were assayed by sequencing only.

Human olfactory psychophysics. All procedures involving human subjects were approved by the Rockefeller University Institutional Review Board. All subjects completed two replicates of the test separated by at least 4 days. Odours were presented in bar-coded amber vials to ensure that subjects were blind to the identity of all odours[28]. The intensity and valence of 66 odours at two concentrations ('high' and 'low') and two solvents was rated on a seven-point scale. Thresholds were calculated by using the single-staircase method with seven reversals[14,15]. Threshold tests included both steroids as binary dilutions from 1:64 (binary dilution 6) to 1:134,217,728 (binary dilution 27). Odour profiling used a previously established method[16].

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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Author Contributions H.Z. and H.M. screened for androstenone receptors, identified polymorphisms, performed functional expression of receptor variants, and genotyped the human subjects with assistance from Q.C. A.K. and L.B.V. devised the human olfactory psychophysics study, for which A.K. supervised data collection and analysis.

Author Information The sequences of the human OR7D4 variants are deposited in Genbank under accession numbers EU049291–EU049294. Reprints and permissions information is available at www.nature.com/reprints. The authors declare competing financial interests: details accompany the paper on www.nature.com/nature. Correspondence and requests for materials should be addressed to H.M. (hiroaki.matsunami@duke.edu) and L.B.V. (leslie@mail.rockefeller.edu).

METHODS

Heterologous expression of human odorant receptors. For untagged odorant receptor experiments, OR7D4 RT and S84N variants without the Rho tag were cloned into pCI. Luciferase assays were performed as described[9]. Only a single variant of each receptor was used in the functional screen, and it is known that many human odorant receptor genes are highly polymorphic[7]. Because of this and because we do not know whether each odorant receptor is capable of functional expression in the cell line used, we cannot exclude the possibility that additional human odorant receptors respond to androstenone as strongly as OR7D4. For immunocytochemistry, cells were fixed, permeabilized and incubated with monoclonal anti-rhodopsin antibody, 4D2 (ref. 27), followed by Cy3-conjugated donkey anti-mouse IgG (Jackson Immunologicals). Western blot analysis was performed in accordance with the Mini-Protean 2 Cell (Bio-Rad) protocol. Enhanced chemiluminescence (ECL; Amersham) was used for detecting proteins on membranes. After the initial exposure, the membrane was incubated with stripping buffer (25 mM glycine-HCl pH 2, 1% SDS, 25 mM glycine, 0.036 M HCl, 1% SDS) and incubated with rabbit anti-GFP (Invitrogen). See Supplementary Methods for detailed information.

Odours. All odours were supplied by Sigma-Aldrich, with these exceptions: androstadienone (gift from Human Pheromone Sciences, Inc.), banana (Bell Flavors and Fragrances), bourgeonal (Biomol), galaxolide (gift from International Flavors and Fragrances) and (*R*)-carvone (Research Chemical Ltd). The same batch and lot of each odour was used for both cell-based analysis and human olfactory psychophysics. Detailed information on odours, odour concentrations and perceived odour quality is provided in Supplementary Tables 4–6.

Genotyping and sequencing of human odorant receptors. For sequencing, human genomic DNAs were amplified, purified and sequenced with a 3100 or 3730 Genetic Analyser (ABI Biosystems) or by GeneWiz. Detailed methods are given in Supplementary Methods.

Human olfactory psychophysics. All human subjects gave informed consent to participate in this study and were tested in a well-ventilated room of the Rockefeller University Hospital Outpatient Unit. Normal human subjects were pre-screened to exclude pregnant women and those with medical conditions causing general impairment of the sense of smell. Of the 412 subjects who completed the study, 21 were excluded because of general anosmia (see Supplementary Methods). The remaining 391 subjects (210 female, 181 male; median age 34 years, age range 19–75 years) were included in the evaluation. Detailed methods are given in Supplementary Information. Our smell tests were purposely conducted under conditions that would not be expected to induce odour sensitivity in our subjects. A given subject sniffed androstenone in only two sessions, rather than the dozens of sessions spread over six to eight weeks required for sensitization in previous studies[19]. A comparative analysis of androstenone responses in the first and second visits does not suggest that subjects became more sensitive through these brief, non-chronic exposures (data not shown).