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Genetics

Ability to smell androstenone is genetically determined

(twins/perception/olfaction/specific anosmia)

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ABSTRACT **Some adult humans cannot detect the odor of androstenone (5α-androst-16-en-3-one), a volatile steroid. To test for the presence of genetic variance associated with this trait, adult twins were tested for their ability to smell androstenone and another odorant, pyridine, that is readily perceived by most adults. Ascending concentration, two-sample (odor versus blank) forced choice tests were used to assess sensitivity to these odorants. Intraclass correlations for identical and fraternal twin detection thresholds to pyridine were small and not significantly different. However, intraclass correlations for thresholds to androstenone were significantly different, with the correlation for identical twins being greater than that for the fraternal twins. These data indicate a genetic component of variation in sensitivity to this odor. Investigations that use genetic variation could offer a new tool for studies of olfactory transduction mechanisms.**

The nature of olfactory receptors and the mechanisms by which the myriad of odors are discriminated and recognized remain elusive (see contributions in ref. 1). Whether the range and precision of odor recognition depends on the diversity of receptors (as in the immune system), versus combinatorial perception by a narrow range of odor receptors (analogous to color vision; ref. 2), remains unknown. The documentation of genetic control of sensitivity to specific odorants may provide a powerful tool in studies of olfactory transduction and perception.

Although some recent reports conclude that odor sensitivity is not genetically determined (3, 4), substantial individual variation, to the extent of odor blindness (specific anosmia; ref. 5), exists in sensitivity to many odorants. Many people with otherwise normal olfactory capacity have these selective olfactory deficits (5). The mechanism responsible for producing this variation remains little understood, although it has been suggested that alterations in molecular receptors on olfactory sensory cells may contribute to specific anosmias (5, 6). A significant genetic contribution to specific anosmias may imply the existence of odor-specific receptor types (6). If variation in the perception of an odor results from differences in the peripheral sensory apparatus and a major gene effect can be demonstrated, then it should be possible to find gene products related to the molecular receptor. This approach has proven to be quite successful in the study of bacterial chemoreception (7). Furthermore, studies of specific anosmias may advance our understanding of olfaction just as studies of color blindness contributed to our current knowledge of color vision (8).

Human sensory perception of androstenone, a C_{19} androgen with a distinctive odor, exhibits great individual variation. Among adults, approximately 50% report no odor, even at a high concentration (9–13). In contrast, approximately 15% detect a subtle odor, are not offended by it, and may even find it pleasant. The remaining 35% are exquisitely sensitive to androstenone, detecting less than 200 parts per trillion in air (14), and ascribe a foul odor to the steroid, usually that of stale urine or strong sweat. Our preliminary data suggested that this extreme variation in sensitivity had a significant genetic component, although this trait was not correlated with HLA haplotype (13). From further studies of identical and fraternal twins, we now conclude that the ability to perceive the odor of androstenone is genetically transmitted.

MATERIALS AND METHODS

Subjects. Twenty identical and 24 fraternal twin pairs (ages 17–25 yr) participated in a twin study conducted at the W. D. Miller General Clinical Research Center, University of Pennsylvania School of Dental Medicine. Olfactory sensitivity testing was one component of the study.

Zygocity was determined from tests of ABO, Rh, Lewis, Lutheran, Kelly, Duffy, Kidd, MNS, Wright, Cartwright, Gregory, and Junior blood markers and, in some instances, typing of HLA-A, -B, -C and -DR.

Two identical and three fraternal twin pairs were excluded from olfactory sensitivity testing because at least one twin had nasal congestion resulting from an active allergy or head cold. Data from one additional identical twin pair were excluded: it was learned that one twin lacked olfaction since childhood.

Information regarding the sex and race of participants can be extracted from Fig. 1. Smoking history was also obtained.

Stimuli. A single concentration (0.1% in odorless light white mineral oil) of phenyl ethyl alcohol (essence of rose) was used to assess general olfactory perception. This odorant was chosen because it apparently cannot be detected by people who are totally anosmic but who possess an intact trigeminal sense (15). Eight different concentrations of pyridine (odor of spoiled milk) ranging from 2.91 μM (step 1 of pyridine in Fig. 1) to 0.372 mM (step 8), each in 50 ml of mineral oil, were used. These concentrations are thought to be below threshold for trigeminal stimulation (16). Pyridine was chosen as a control odorant because few people fail to detect this compound (16).

Twelve concentrations of androstenone (Sigma), were used, ranging from 1.79 μM (step 1 of androstenone in Fig. 1) to 3.67 mM (step 12) in 10 ml of mineral oil. In general, individuals incapable of detecting the highest concentration of androstenone also fail to detect the odor of crystalline androstenone, a powerful odorant for those capable of smelling it (unpublished observations). Purity of androstenone was confirmed by combined gas chromatography/mass spectrometry.

Complete concentration series for pyridine and androstenone were obtained by binary serial dilution from the most concentrated stimulus. Each concentration step was paired with an oil blank. Polypropylene squeeze bottles (300-ml total volume) fitted with pop-top caps were used to present stimuli.

Sensory Testing. Prior to tests with pyridine and androstenone, individuals were screened for general olfactory capacity. They were instructed to squeeze the bottle, sniff, and

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identify the odor-containing bottle. They were given the blank (vehicle only) bottle first, then the odorized (phenyl ethyl alcohol) bottle.

Tests with pyridine always preceded tests with androstenone. Detection thresholds were determined with four ascending concentration series. Order of presentation (odor or blank bottle) was randomized. A series ended when a person correctly identified the odor-containing bottle in three successive odor versus blank trials, the lowest value of the three being designated the threshold (17). For each person, an average threshold was determined after discarding the high and low values of the four series.

Androstenone was tested in a similar manner, with two exceptions: (*i*) a series terminated after four, rather than three, successive correct choices (we previously determined that termination of a series after four correct choices was more reliable for estimating thresholds for odorants that could not be smelled by some people), and (*ii*) a pre-test screening determined the starting concentration of the first series. Screening began with concentration step seven and its blank. Individuals failing to correctly identify the androstenone-containing bottle in any one of three forced choice (odor versus blank) trials began threshold testing at concentration step five; those that correctly identified the odorized bottle in each of the three screening trials began threshold testing at concentration step one. In any series, individuals failing to detect androstenone at step twelve were assigned a threshold value of thirteen.

All testing occurred in the morning. Twins were tested within 3 hr of each other.

Analyses. Pyridine and androstenone threshold data were subjected to a variety of analyses. Intraclass correlations were calculated. Alternative nonparametric analyses also were conducted because the data were not normally distributed. The absolute values of pair differences were determined for each twin pair and analyzed with the Mann–Whitney test of the ranks (18).

In a second nonparametric analysis, each person was assigned to one of two categories—androstenone-sensitive (i.e., having a detection threshold less than test series step seven) or -insensitive (although we had previously found in a large sample that sensitivity to androstenone was distributed trimodally, the sample size of twins in this study was not large enough to adequately establish a trimodal distribution). Concordance rates (twin pairs in the same category) among identical and fraternal twins were subjected to χ^2 analysis.

RESULTS

Total anosmia was eliminated as a confounding factor because each person could detect pyridine. The overall mean and variance for the identical and fraternal twin sensitivity to pyridine were not significantly different (Table 1). Neither intraclass correlation (r) was significantly different from zero. Thus, the pyridine sensitivity data provide no evidence for a genetic component of variation in sensitivity to this odorant.

Some people failed to detect androstenone, even when presented with the strongest concentration (Fig. 1). Although there was a great range in individual sensitivity to the odor of androstenone, the overall mean sensitivity and variance for the identical and fraternal twins were not different (Table 1). However, identical twin pairs were more similar in androstenone sensitivity than were fraternal twin pairs (Fig. 1). The intraclass correlation for identical twins was significantly different from that of the fraternal twins ($z = 4.52$, $P < 0.0001$) and was not significantly different from 1.00 (z calculated as in ref. 3).

The Mann–Whitney comparison of identical and fraternal twin pairs also was significant ($U = 312$; $z = 5.24$, $P < 0.0000001$). In the other nonparametric analysis, 41% of identical twins and 43% of fraternal twins were classified as sensitive to androstenone. However, 100% of the identical twins were concordant, compared to only 61% of the fraternal twins ($\chi^2 = 6.08$; $P < 0.01$).

For both pyridine and androstenone, we observed no significant sex (cf. ref. 19), race, or smoker versus nonsmoker differences in mean detection thresholds.

DISCUSSION

Detection thresholds to the odor of androstenone have a significant genetic component. The intraclass correlation for identical twins approached 1.00 and identical twins had a significantly higher concordance rate than fraternal twins. The pattern of results also supports our previous suggestion (13) that extreme sensitivity to the odor of androstenone is inherited, possibly as a dominant Mendelian trait (20).

Variation in sensitivity to the musky odor of pentadecalactone also occurs. Pedigree analysis suggested that the ability to smell pentadecalactone also was inherited as a dominant trait (21). Many other odor-blindness phenotypes have been described (5), but the question of genetic transmission has yet to be addressed.

We detected no genetic component in threshold variation for pyridine, an odor that was detected by each participant. However, our testing methodology may not have been sensitive enough to detect a genetic component where variation was limited. Genes may play a role in all odor perception; but in general, sensory perception and detection threshold methods can establish a genetic component of variation only when variability is large, as in the case of androstenone. Why such a large range of individual sensitivity differences exists for some odorants is not known. We note, however, that androstenone and its related alcohol form, androstenol (5α-androst-16-en-3α-ol), have been proposed as chemical signals of sexually relevant messages (pheromones) in pigs (22–26) and humans (27–31). However, the evidence favor-

Table 1. Mean detection thresholds, variance partitions (mean square), and intraclass correlations (r) for sensitivity to pyridine and androstenone in identical and fraternal twins

	Analysis of odorant threshold data*					
	Pyridine			Androstenone		
Twin type	Mean threshold	Mean square	r	Mean threshold	Mean square	r
Identical	4.3	4.30	0.09	8.9	23.9	0.95
Among twin pairs		2.34			23.3	
Within twin pairs		1.96			0.6	
Fraternal	3.9	3.47	0.07	8.6	24.1	0.22
Among twin pairs		1.86			14.7	
Within twin pairs		1.61			9.4	

*Threshold is in concentration step units. Mean squares were obtained by analyses of variance (18).

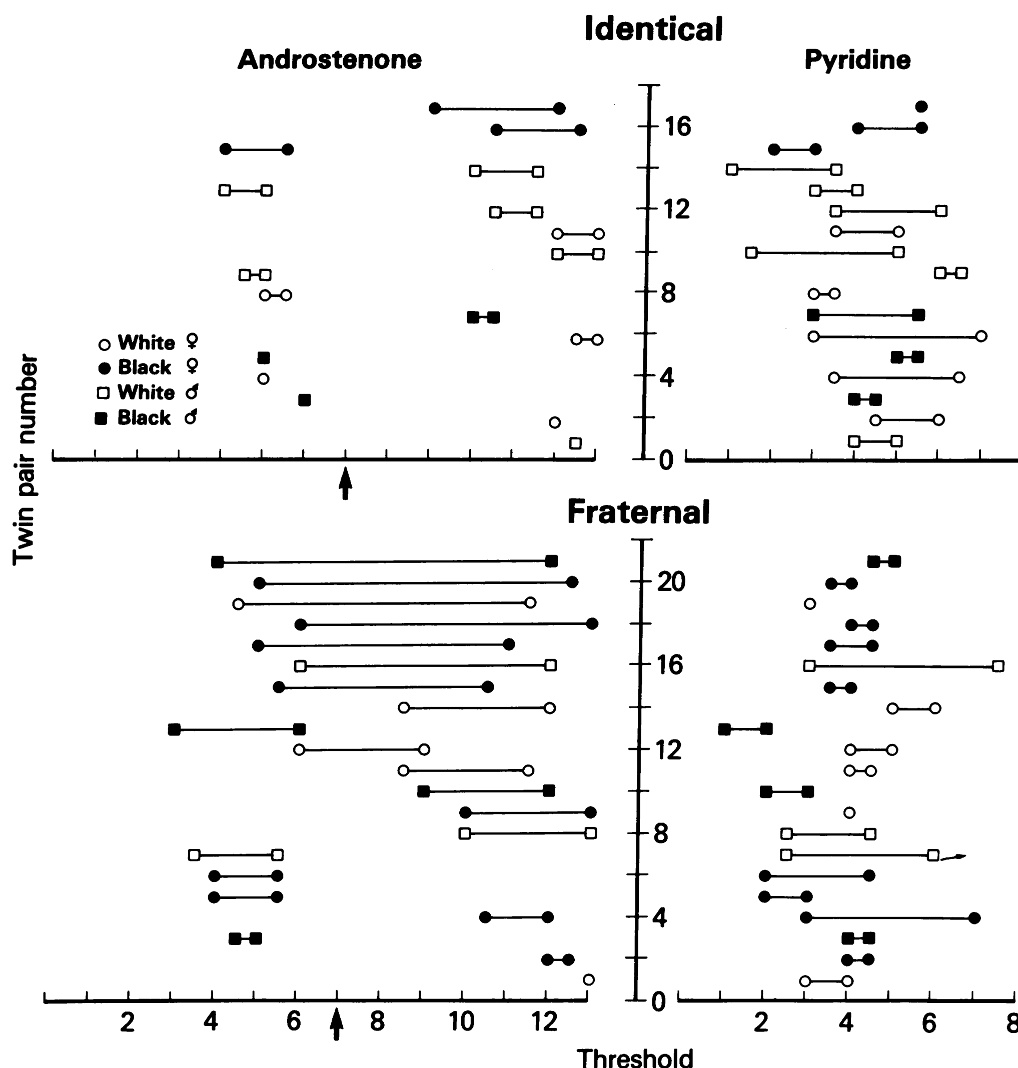

FIG. 1. Androstenone and pyridine detection thresholds for each member of 17 identical and 21 fraternal twin pairs. Pair mates are connected by horizontal lines. A single entry for a twin pair indicates identical thresholds for each. The data are plotted by decreasing threshold and increasing discordance for androstenone. The concentration for step 12 of androstenone was 3.67 mM in mineral oil and that for step 8 of pyridine was 0.372 mM. For each odorant, decreasing concentration series were prepared by serial binary dilution. The concentration of androstenone indicated by the arrow was chosen to dichotomize subjects into androstenone-sensitive and androstenone-insensitive groups.

ing androstenone or androstenol as a human pheromone is weak and the idea is controversial (32).

Unlike insects (33), no specialized receptors responsive to pheromones have yet been identified among vertebrates, although they may be found. One way to study chemical receptors is to determine whether specificity of response is genetically determined and, if so, by what process the gene(s) direct chemoreception. According to one hypothesis (34), significant genetic variation in individual differences in response to androstenone implies a specific receptor-type that is particularly sensitive to the odor. This need not be true. Specific anosmia may not be a peripherally induced phenomenon. Genes may have their effects on central processing systems. However, if genetic variants of molecular receptors for androstenone can be isolated, then these may provide a powerful tool for dissecting olfactory transduction mechanisms.

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