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A Twin Study of Odor Identification and Olfactory Sensitivity

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Abstract. Interindividual variation in odor identification and olfactory sensitivity has been explained primarily with reference to age, sex and/or experiential factors. A twin study of olfaction can, therefore, substantially contribute to current research in this area. Thirty-nine monozygotic and twenty dizygotic twin pairs have completed the University of Pennsylvania Smell Identification Test (UPSIT), an olfactory preference questionnaire, and two odor detection threshold tests (phenyl ethyl alcohol and butanol). A genetic influence on odor identification, as assessed by the UPSIT, has been demonstrated. Future plans and directions for this research program are discussed.

Key words: Olfactory sensitivity, Odor detection threshold test

INTRODUCTION

Individual differences in odor identification and olfactory sensitivity have been of research interest for some time. Attention has primarily focused on age, sex and various experiential factors as possible contributors to individual variation in olfactory function [7, 9, 15, 17, 25, 32]. An association between nostril asymmetry and handedness has also been reported [18]. In contrast, relatively few analyses concerned with genetic influences on olfaction are available [40].

Sensitivity thresholds for acetic acid, isobutyric acid and 2-sec-butyl-cyclohexanone were compared between 51 monozygotic (MZ) male twin pairs and 46 dizygotic (DZ) male twin pairs [21,22]. Genetic effects on odor sensitivity were not detected for these substances, nor was evidence of twin concordance for specific anosmias provided. Relatively reduced sensitivity to isobutyric acid and to 2-sec-butyl-cyclohexanone was shown by twins who smoked, or who were light in weight. Reduced sensitivity to isobutyric acid was also associated with infrequent consumption of alcoholic beverages. These measures were, however, able to account for only a small portion of the variance. Sensitivity to

acetic acid was unrelated to smoking, body weight, consumption of alcoholic beverages, and several other participant characteristics.

Twin resemblance for sensitivity to androstenone and pyridine was examined in a sample of 17 MZ twin pairs and 21 DZ twin pairs [39]. Sensitivity to the odor of androstenone (which has been described as urinous, sweaty, musky, like sandalwood, odorless) shows substantial variability among the adult population, while the odor of pyridine (which smells like spoiled milk) is detected by the majority of individuals [39,40]. An ascending concentration, two-sample (odorant vs. blank) forced-choice procedure was administered. Concordance for sensitivity to androstenone was observed among all MZ twin pairs. In contrast, only 61% of the DZ twin pairs were concordant for sensitivity to androstenone. The greater MZ than DZ twin resemblance demonstrated a genetic influence on androstenone sensitivity. There was, however, an absence of a genetic influence on pyridine sensitivity. It was noted that the procedures used in this investigation may have been incapable of identifying a genetic effect on sensitivity to pyridine.

Twin methods clearly offer informative tools for investigating genetic and environmental influences on human behavioral and physiological characteristics [27]. Additional twinbased approaches to the study of odor identification and sensitivity can, therefore, advance understanding of the bases of normal and dysfunctional olfactory perception. An overview of human and non-human research on the genetics of olfactory sensitivity is available [34]. The present paper reports findings from an odor identification test and a sensory detection threshold test administered as part of an ongoing twin study of olfactory sensitivity at California State University, Fullerton.

MATERIALS AND METHODS

Participants

Twin participants in this study were identified by notices in newspapers (54%), personal referrals (36%) and Mothers of Twins Clubs (10%). Zygosity was primarily diagnosed by serological analysis (51%) or by a physical resemblance questionnaire (32%) developed by Nichols and Bilbro [26]. Nine opposite-sex twin pairs (15%) were classified as dizygotic on the basis of the sex difference. One twin pair was diagnosed as DZ according to the physical resemblance questionnaire, but was reclassified as MZ owing to resemblance for highly heritable traits (eg, hair color and eye color), and a high degree of similarity in general physical appearance. The present sample includes 39 monozygotic (MZ) twin pairs and 20 dizygotic (DZ) twin pairs. The DZ twins include 11 same-sex twin pairs and 9 opposite-sex twin pairs. Two MZ/DZ triplet sets were each counted as one MZ twin pair and two DZ twin pairs. Sample characteristics, including age, sex, zygosity and ethnicity, are displayed in Table 1. Data collection will continue until a sample of eighty twin pairs is obtained.

Smell or taste problems were indicated by seven twins. These difficulties were variously related to smoking, sinus conditions, allergies and a broken nose, rather than to diagnosed olfactory deficits.

Table 1 - Sample characteristics of MZ and DZ twin pairs

Zygosity	N (pairs)	Age (yr)	SD[a]	Range	% Female	% Ethnicity[c]			
						C	A	H	O
MZ	39	32.12	18.38	10.9–82.7	67	79	4	13	4
DZ[b]	20	27.63	9.53	12.7–49.8	68	67	10	20	3

[a] MZ > DZ, $F_{(77/39)} = 3.72$, $p < 0.001$ (Based on individual scores).
[b] Includes 11 same-sex pairs and 9 opposite-sex pairs.
[c] C = Caucasian A = Asian H = Hispanic O = Other

Methods

MZ and DZ twin pairs are invited to the Twin Studies Center, at California State University, in Fullerton, CA. Cotwins are administered the UPSIT (University of Pennsylvania Smell Identification Test) at the same time, by separate examiners. The UPSIT is a standardized test of odor identification that has been used to study normal and dysfunctional olfaction among a variety of samples [10,12,14]. Reliability estimates for selected portions of the test, and for the entire test range between 0.81 and 0.87 [11]. A score on the UPSIT is equal to the number of odorants correctly identified out of 40. Age and sex norms, based upon data collected from 961 females and 649 males, are available. Modification of these norms is in progress, due to the reduced numbers of individuals in some age groups [10].

The UPSIT is a multiple choice "scratch and sniff" test, consisting of four booklets which each contain ten odorants. Subjects scratch a small label which releases an odor, and select from a list of four possible items the one that most closely matches their smell experience. After each odor is identified, participants rate the odor on five different scales (intensity, pleasantness, irritation, familiarity and coolness). These dimensions have been employed in previous studies on olfaction [17,41] and are the same dimensions along which odorants were described during development of the UPSIT. A brief rest period is allowed between booklets two and three. One MZ female twin pair did not complete this test.

Two odor detection threshold tests are administered in reverse order to each cotwin, by separate examiners. One test consists of presentation of concentrations of perfume-grade phenyl ethyl alcohol (PEA) dissolved in 20 ml of propylene glycol and 20 ml of propylene glycol alone, according to the forced choice, single staircase procedure described by Doty et al [13]. Twins are asked to sniff two randomly presented samples (odorant vs. blank) and are asked to indicate which sample has the stronger odor. Initially, correct responses to a given concentration following five consecutive trials reverses the staircase. Concentrations are then decreased by half-log steps following correct identification of the stronger odor on two trials; concentrations are increased following failure to identify the stronger odor on one or both trials. Test-retest reliability for this measure is 0.64 (Doty, personal communication). Individual scores are equal to the mean of the last four of seven reversal points. Individuals who consistently detect

increasingly lower concentrations of PEA (ie, they do not display reversals), or who obtain fewer than seven reversals at the lowest concentrations, receive a score of 10.

The other odor detection threshold test consists of presentation of an aqueous dilution series of butanol, according to the method described by Cain et al [7]. Twins are provided with two squeeze bottles, each containing 60 ml of butanol and a blank containing distilled water. They are asked to squeeze each bottle, inhale the vapor, and indicate the stronger odor. Testing begins at one of the lower concentrations. Concentrations are increased until correct responses across five consecutive trials are obtained. In the event that participants are correct on all five trials of the initial concentration, they are presented with reduced concentrations. Again, correct responses across five trials determines the detection threshold for that individual. Findings are unavailable at present because fewer twin pairs have completed this test.

A brief health history questionnaire is also completed by participants, and various anthropometric characteristics (eg, height, weight and cephalic index) are measured. This information will enable studies of associations between olfactory and medical life history characteristics.

RESULTS

University of Pennsylvania Smell Identification Test (UPSIT)

The majority of twins scored within the normal range (normosmia) for their sex, as indicated by published norms for the UPSIT [10]. Four males and seven females obtained scores indicating microsmia, or slight odor insensitivity. Gender showed a modest, but significant, association with UPSIT score ($r = 0.23$, $p < 0.05$). Female twins scored significantly higher on the UPSIT than did male twins [Males: 35.89, $sd = 2.48$; Females: 37.02, $sd = 2.18$, $t\ (108) = -2.46$, $p < 0.05$], a finding consistent with previous analyses [13]. Age effects on odor identification were nonsignificant, although there was a trend for reduction in score with increasing age. Doty et al [12] found that performance was optimal during the third through fifth decades of life, but showed substantial decline after the seventh decade.

Similarity in age and sex can inflate twin resemblance in the measured phenotype [24]. Intraclass correlations were, therefore, corrected for these effects using scores from the combined sample of MZ and DZ twins. A significantly higher MZ ($r_i = 0.36$, $N = 38$ pairs) than DZ intraclass correlation ($r_i = -0.23$, $N = 20$ pairs) on the UPSIT indicates a genetic effect on odor identification. (Both same-sex and opposite-sex twin pairs are included among the DZ twins. The modest size of the DZ twin sample precludes presentation of intraclass correlations by same- and opposite-sex pairings). Age- and sex- corrected intraclass correlations for MZ twins were 0.51 for MZ males, and 0.29 for MZ females. It is interesting that the MZ female twins showed reduced within-pair resemblance, relative to the MZ male twins; given the modest sample size this difference was, however, not significant. Both adjusted and unadjusted intraclass correlations are presented in Table 2.

Doty et al [13] demonstrated a significant relationship between smoking and UPSIT score, based upon a sample size of over 1300 subjects. However, when restricting atten-

Table 2 - Means, standard deviations, intraclass correlations and 95% confidence intervals for MZ and DZ twins on the University of Pennsylvania Smell Identification Test (UPSIT)

Zygosity	N (pairs)	Mean	SD[a]	Range	Unadj.r_i[b,c]	95% CI	Adj.r_i[d,e]	95% CI
MZ	38	36.45	2.55	27-40	*0.47*	0.18–0.68	*0.36*	0.05–0.61
MZm	13	35.46	2.66	27–38	0.62	0.15–0.86	*0.51*	–0.02–0.81
MZf	25	36.96	2.36	29–40	0.31	–0.08–0.62	0.29	–0.11–0.61
DZ	20	37.08	1.58	32–40	–0.14	–0.53–0.30	–0.23	–0.68–0.35

Note: $p<0.05$ $p<0.01$ $p<0.001$

[a] MZ > DZ, $F_{(75/39)}=2.63$, $p<0.001$ (Based on individual scores)

[b] Data unadjusted for age and sex

[c] MZ > DZ, $p<0.05$

[d] Data adjusted for age and sex

[e] MZ > DZ, $p<0.05$

tion to the smoking group, a significant relationship between number of packs smoked per day and UPSIT score was not found. In the present twin study, the scores of current smokers did not differ significantly from those of nonsmokers, although ony 14% of the twins smoked. The correlation between number of cigarettes smoked per day and UPSIT score was negligible.

Phenyl ethyl Alcohol (PEA)

Neither age nor gender was significantly associated with PEA threshold. There was, however, a suggestion of increased sensitivity to this odorant among younger twins.

The age-sex adjusted intraclass correlations for PEA sensitivity showed a negligible MZ-DZ twin difference. Age- and sex-corrected intraclass correlations for MZ twins were 0.43 for MZ males and 0.11 for MZ females. Again, it is worth noting that MZ female twins showed reduced similarity, relative to MZ male twins. Sex differences in PEA thresholds were negligible, but MZ twins scored significantly lower than DZ twins. These data are summarized in Table 3. The PEA thresholds of current smokers did not differ significantly from those of non smokers. The correlation between number of cigarettes smoked per day and PEA score was nonsignificant.

The extent to which scores on the UPSIT correlated with PEA thresholds was of interest. A meaningful association between a suprathreshold odor identification measure (UPSIT) and a detection threshold would be expected only if both measured a common dimension underlying the olfactory function [13]. Performance on the UPSIT and PEA showed a modest, but significant correlation ($r=-0.24$, $p<0.05$). This result differs

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Table 3 - Means, standard deviations, intraclass correlations and 95% confidence intervals for MZ and DZ twins for phenyl ethyl alcohol (PEA) threshold

Zygosity	N (pairs)	Mean[a]	SD	Range	Unadj.r_i[b]	95% CI	Adj.r_i[c]	95% CI
MZ	39	6.86	1.47	3.88–10.00	*0.26*	−0.05–0.53	0.19	−0.13–0.47
MZm	13	6.48	1.38	3.88–10.00	*0.48*	−0.05–0.80	*0.43*	−0.12–0.78
MZf	26	7.05	1.49	4.88–10.00	0.15	−0.24–0.50	0.11	−0.28–0.42
DZ	20	7.46	1.67	4.25–10.00	0.13	−0.31–0.53	0.17	−0.28–0.65

Note: $p < 0.05$

[a] $MZ < DZ$, $t_{(116)} = -2.01$, $p < 0.05$

[b] Data unadjusted for age and sex.

[c] Data adjusted for age and sex.

considerably from the −0.79 to −0.89 correlations reported by Doty et al [13]. The difference in the magnitude of the correlations provided by the two studies probably reflects the more variable composition of the Doty et al [13] sample.

DISCUSSION

The present twin study demonstrated a significant genetic effect on odor identification. These data, thus, underline the importance of utilizing twin methods and other behavioral-genetic designs to further explore genetic influences on individual differences in olfaction. The negative DZ twin intraclass correlation is, however, difficult to interpret. The present sample size is modest, and so more definitive findings will be available following completion of data collection. In addition, some participants in the Minnesota Study of Twins Reared Apart, at the University of Minnesota [3] are completing the UPSIT and olfactory preference questionnaire. These data will help to clarify the influence of rearing status on resemblance across these measures.

The reduced similarity in odor identification observed among MZ female twins, relative to MZ male twins, is consistent with previous twin studies of physical traits, such as body weight [4,35]. Farber's [16] comment that "it may not be incorrect in pointing to different 'plasticities' for the sexes" is relevant to our findings on the UPSIT. In other words, female physical and physiological traits appear to be highly susceptible to environmental effects, thus reducing twin resemblance across these parameters.

The MZ-DZ twin difference in similarity for PEA threshold was negligible. Olfactory sensitivity thresholds of individuals have, however, been shown to vary considerably across testing sessions [36]. The reliability of these measures would be improved by implementing a repeated measures design, as compared with single-trial testing. It is also possible that, in some cases, eating shortly before testing interfered with performance

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on some of the olfactory measures, although this information is unavailable. It will be of interest to determine if an MZ-DZ twin difference in resemblance on the PEA threshold emerges as the study progresses.

Future Directions

The possibility that organisms possess "olfactory signatures" (distinctive odors produced by an individual) which enable identification and which stimulate olfactory receptors in close kin who are sensitive to such cues, has been raised [2,28,31]. Wallace [37] showed that MZ twins could be distinguished on the basis of palm odor, but that dietary differences between twins reduced the accuracy of the judges. Kalmus [23] showed that dogs could differentiate between the odors of MZ cotwins in a tracking task, but not in a retrieval task. Hepper [20] also showed that dogs could discriminate between the odors of both MZ and DZ cotwins in a matching-to-sample experiment. Dogs could not, however, distinguish between infant MZ twins living in the same home and fed identical diets.

A twin study of kin recognition based upon olfactory cues is in progress in our laboratory at CSUF. Cotton T-shirts, prewashed with Ivory soap, are worn for three consecutive nights by adolescent MZ and DZ twins, and are stored in sterile plastic bags during the day. Twins are requested to avoid the use of deodorants or other body cosmetics during this period, and to wash with the Ivory soap that has been provided. A complete diet diary is maintained during this three-day period. Judges are then asked to sniff a T-shirt (the "standard") and to identify a "relative" from among an array of three T-shirts; the three T-shirts belong to the cotwin and to two unrelated twins matched for age and sex. Findings from this study will be compared with findings concerning olfactory recognition by other relatives [29,30]. The literature on olfaction and kin recognition among twins and non-twin relatives is further summarized in Segal [33] and in Segal and Topolski [34].

In sum, MZ-DZ twin comparisons can furnish unique insights into the genetic and environmental bases of olfaction. Variants of the classic twin design, such as the longitudinal twin study and the MZ Half-Sibling study [8,19] can be additionally informative with respect to genetic and environmental influences on olfactory characteristics. Recent advances in molecular genetics enhance the probability that specific genetic factors underlying olfactory sensitivity will be revealed [1,5,27].

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