Genetic Epidemiology 6:413–421 (1989)

Phenylthiocarbamide Taste Sensitivity Revisited: Complete Sorting Test Supports Residual Family Resemblance

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Phenylthiocarbamide (PTC) taste thresholds were determined in 100 nuclear families using the complete sorting test. Segregation analysis using the mixed model suggested that the variability in PTC thresholds is controlled by a major locus with incomplete dominance as well as by a multifactorial component with significant residual heritability. Such a model explained nearly 96% of the variance, leaving only 4% of the variance in thresholds arising from measurement error and other environmental factors.

Key words: **PTC, mixed model, incomplete dominance**

INTRODUCTION

A dominant major locus for ability to taste phenylthiocarbamide (PTC) has been amply demonstrated [Harris, 1955]. Later, Das [1956] and Kalmus [1958] reported evidence suggesting that the dominance of the taster gene was incomplete. More recently, Rao and Morton [1977] applied a mixed model of segregation analysis [Morton and MacLean, 1974] to search for residual family resemblance due to incomplete dominance, polygenes, and sibling common environment. While they failed to obtain statistically significant evidence for any of these residual sources based on a large Brazilian study, it was concluded that: "A larger sample, a different population, or performance of the complete sorting test might give evidence of residual family resemblance" [Rao and Morton, 1977]. No genetic analysis has yet been performed using the complete

sorting test in families. Here we present the results of a family study that has information on the full range of the sorting test.

MATERIALS AND METHODS

Phenylthiocarbamide taste thresholds were determined on 435 individuals in 100 families belonging to three caste groups (Kapu, Kurava, and Ekila) of Chittore District in the state of Andhra Pradesh, India. All three groups are distributed in geographically close hamlets within a radius of half a kilometer. The subjects were tested by using the serial dilution technique [Harris and Kalmus, 1949; Mohr, 1951]. The complete sorting test consists of using the full range of dilutions. The solution numbers of phenylthiourea represent serial dilutions by 1/2 of a solution containing 0.13 gm per 100 cc of distilled water. Thus, solution 1 has 0.13 gm per 100 cc, solution 2 has 0.065 gm per 100 cc, etc. An individual is said to have threshold k if he or she can discriminate a $1{:}2^k$ dilution, but not $1{:}2^{k+1}$ dilution. The observed thresholds ranged from < 1, 1, . . ., 13. The value < 1, corresponding to those who could not taste even the highest concentration, is taken as zero for the purposes of all quantitative analyses.

METHODS AND RESULTS

In this study, age does not have any effet on the threshold (correlation between age and threshold, $r = 0.023 \pm 0.048$), not even within the subgroup of children ($r = -0.031 \pm 0.062$). Since very young children are generally considered to give inconsistent results [Azevedo et al., 1965], we present in Table I an age by threshold distribution to further explore the reliability of such data. We failed to detect any association between the two ($\chi^2_9 = 8.95$, $P = 0.44$, see Table I). Therefore, neither was any age adjustment attempted, nor were very young children deleted from the data. Further, the threshold distributions are not heterogeneous among parents and children in any of the three caste groups (Table II). After combining parents and children, we failed to detect heterogeneity among the three castes ($\chi^2_8 = 7.25$, $P = 0.51$, see footnote to Table II). Clearly, pooling the thresholds into four groups on account of small sample sizes limits our ability to detect possible heterogeneity at the ends of the distribution. Likewise, our investigation of heterogeneity among parents and children is not strictly valid as they represent nonindependent observations. However, we believe that there is no evidence to suggest major differences. Accordingly, data on the three caste groups were pooled for further analysis. Even though the antimode corresponds to a smaller

TABLE I. Distribution of PTC Thresholds by Age*

	Age (yr)			
Threshold	0–6	6–8	8–10	≥10
≤3	7	8	10	85
4–5	3	5	7	57
6–7	10	8	6	63
≥8	5	14	13	134
Total	25	35	36	339
Heterogeneity test: $\chi^2_9 = 8.95, P = 0.44$				

*Due to small sample sizes involved, ages and thresholds were grouped into four categories each.

TABLE II. Distribution of PTC Thresholds Among Parents and Children in Each of Three Caste Groups From the State of Andhra Pradesh, India*

	Kapu caste		Kurava caste		Ekila caste		All Castes		
Threshold	Parents	Children	Parents	Children	Parents	Children	Parents	Children	Total
< 1[a]	2	4	4	5	5	10	11	19	30
1	2	4	7	7	6	3	15	14	29
2	7	6	6	4	2	6	15	16	31
3	2	7	2	1	4	4	8	12	20
4	1	6	2	10	2	6	5	22	27
5	8	9	7	7	4	10	19	26	45
6	4	4	7	11	3	6	14	21	35
7	8	18	4	10	5	7	17	35	52
8	3	11	6	11	8	9	17	31	48
9	8	7	4	10	7	13	19	30	49
10	7	2	6	6	1	5	14	13	27
11	3	3	7	5	1	2	11	10	21
12	0	0	2	1	0	0	2	1	3
13	3	3	4	3	4	1	11	7	18
Total	58	84	68	91	52	82	178	257	435
Heterogeneity χ^2 with 4 d.f[b]	2.74 $P = 0.60$		5.26 $P = 0.26$		1.76 $P = 0.78$		6.31 $P = 0.18$		

*Between caste heterogeneity, after pooling parents and children together, and after pooling thresholds below 3 and above 7 yielded $\chi^2_8 = 7.25$ ($P = 0.51$).
[a]Threshold of < 1 was taken as 0 in further analysis.
[b]Due to small frequencies, thresholds up to 3 (including) were pooled together, and thresholds beyond 7 (including) were pooled together for heterogeneity tests.

threshold value in children than in parents (see Table II), which is inconsistent with the direction of age-dependence reported by Harris and Kalmus [1949], these differences are not statistically significant ($\chi_4^2 = 6.31, P = 0.18$). Therefore, we defined a dichotomy of taster status by classifying all individuals with a threshold value of five or greater as tasters [Harris and Kalmus, 1949]. This yielded 137 nontasters and 298 tasters, with a taster frequency of A = 298/435 = 0.685. Under the assumption of dominant mode of inheritance for taste sensitivity, the taster gene frequency is calculated as

$$q = 1 - \sqrt{1 - A} = 0.439$$

which is well within the observed range for Indian populations [e.g., see Das, 1966, and the references therein].

Table III presents the means, variances, skewness, and kurtosis in the distributions of thresholds among males and females within each caste group. Even though the differences are small between sexes and caste groups, we standardized each individual's threshold by the corresponding sex-caste specific mean and variance to yield a standardized threshold (Z).

Commingling Analysis

Since skewness in the distribution of a quantitative trait can distort the evidence for and characteristics of a major locus, we applied the transformation of MacLean et al. [1976] to investigate skewness and commingling in the distribution of the standardized threshold (Z):

$$x = \frac{6}{p}\left[\left(\frac{Z}{6} + 1\right)^p - 1\right]$$

A suitable value of p is then chosen that will approximate the distribution of x, as well as possible, to that of a mixture of up to three normal distributions each with the same variance (e). The relative weights of the component distributions are assumed to be in Hardy-Weinberg proportions. When fitting a mixture of three distributions, there arise six parameters: 1) q, square root of the proportion for the third distribution (extreme right), 2) t, displacement between the two extreme distributions, 3) d, displacement of the middle distribution from the first distribution (extreme left), relative to t, 4) u, the overall mean, 5) e, the common variance for each distribution, and 6) p, the power transformation measuring skewness (p = 1 corresponds to no skewness). In this formulation, q = 0 corresponds to no commingling, d = 1 corresponds to two distribu-

TABLE III. Means, Variances, Skewness (β_1), and Kurtosis (β_2) in the Distributions of Thresholds Among Males and Females in Each Caste Group

Caste	Sex	No.	Mean	Variance	$\gamma_1 = \beta_1$	$\gamma_2 = \beta_2 - 3$
Kapu	Male	80	6.263	11.437	−0.005	−0.721
	Female	62	6.161	9.744	−0.141	−0.411
Kurava	Male	83	6.120	13.668	−0.116	−1.122
	Female	76	6.579	12.354	−0.076	−0.608
Ekila	Male	80	5.613	11.076	−0.215	−0.932
	Female	54	5.815	15.059	0.023	−0.966
All castes	Male	243	6.000	12.058	−0.099	−0.914
	Female	192	6.229	12.240	−0.070	−0.673

tions with the last two distributions superimposed on one another, and $0 < d < 1$ corresponds to three distributions. Null hypotheses on these parameters are tested using the likelihood ratio criterion. Analyses were performed using the computer program SKUMIX [MacLean et al., 1976; Morton et al., 1983].

Table IV presents the results. We confined our attention to mixtures of two and three distributions only, as would be expected under monogenic segregation with two alleles. Mixtures of distributions were fitted both with and without skewness. When a mixture of two distributions was fitted, skewness was statistically significant. ($\chi^2_1 = 20.21, P \doteq 0$), as was the case when a mixture of three distributions was fitted ($\chi^2_1 = 11.82, P < 0.001$). Although a mixture of three normal distributions fitted the data significantly better than a mixture of two normal distributions ($\chi^2_1 = 10.42, P = 0.0012$), a mixture of three skewed distributions did to fit significantly better than a mixture of two ($\chi^2_1 = 2.03, P = 0.16$). Therefore, we took a mixture of two skewed distributions as the parsimonious model, which yielded $q = 0.483$, $t = 2.003$, and $P = -0.765$; the standardized threshold values (Z) were power transformed using this P-value, and it is this variable that we defined as the quantitative trait for segregation analysis.

Segregation Analysis

We performed segregation analysis of two traits separately: 1) PTC as a qualitative variable (defining nontasters as normal, and tasters as affected), and 2) PTC as a quantitative trait (power transformed threshold). For analysis of the affection status, we assume an underlying continuous liability (not measured). The frequency of affection ($A = 0.685$) defines a threshold on the liability scale, so that individuals above the threshold are affected.

The mixed model of Morton and MacLean [1974], as reformulated by Lalouel and Morton [1981], was applied here. This model involves seven parameters:

u = mean
V = variance
H = heritability in children
HZ = heritability in adults
q = gene frequency of the major locus (for elevated levels)
t = displacement between the two homozygote means at the major locus
d = dominance at the major locus (of the allele for elevated levels)

Under the general model, all relevant parameters are estimated. For the analysis of affection status, the mean and variance of liability are arbitrary, and are therefore fixed at $u = 0$ and $V = 1$, leaving the other five free parameters to be estimated. For the analysis of quantitative traits, all seven parameters are relevant. Under specific null hypotheses, certain paramters are fixed, such as $H = 0$ or $q = t = d = 0$, and only the remaining parameters are estimated. For each hypothesis we present $-2 \ln L + c$, where $\ln L$ is the maximum value of the log-likelihood of the sample under the hypothesis and c is a constant. Null hypotheses are tested against the general model using the likelihood ratio criterion (LRT); if $-2 \ln L_1 + c$ is the value when $m + k$ parameters estimated, and $-2 \ln L_2 + c$ when only m of the $m + k$ parameters are estimated, then, $2(\ln L_1 - \ln L_2)$ provides the LRT for the null hypothesis under which the other k parameters were fixed. This LRT criterion, under the usual asymptotic

theory, is known to follow a χ^2 distribution with k df. For certain hypothesis, there is some controversy on the exact df. For instance, it is sometimes argued that in testing for the significance of a major locus effect, one need only collapse the three genotypic means into one, and therefore the LRT χ^2 should have 3 − 1 = 2 df. Others argue that, under the hypothesis of no major locus effect, three parameters are eliminated (q = t = d = 0), and therefore the LRT χ^2 should have 3 df instead. However, we prefer the conservative approach and choose 3 df for this test. Analysis was carried out using the computer program POINTER [Morton et al., 1983].

Analysis of PTC affection status. Fitting a dominant major locus yielded q = 0.472 and t = 2.202, in close agreement with the inference based on commingling analysis of standardized thresholds (see Table IV). When the parameters d and H were also estimated simultaneously, they consistently went to the boundary values of 1 and 0 respectively. We, therefore, conclude that analysis of the affecton status alone supports the pure dominant major gene hypothesis, with no evidence for any background variation. This conclusion was also supported by an analysis of the larger Brazilian family study [Rao and Morton, 1977].

Analysis of *P*-transformed thresholds (*P* = −0.765). Table V presents the results. There is profound evidence for a major locus effect ($\chi^2_3 = 100.69 - 0.00 = 100.69, P \doteq 0$). However, a pure dominant hypothesis without background variation (d = 1, H = Z = 0) is rejected ($\chi^2_3 = 28.39 - 0.00 = 28.39, P < 0.0001$). The hypothesis of no multifactorial effect (H = Z = 0) is also rejected $\chi^2_2 = 20.56 - 0.00 = 20.56, P < 0.0001$), and so is the hypothesis of complete dominance even in the presence of background multifactorial variation ($\chi^2_1 = 9.64 - 0.00 = 9.64, P = 0.002$). Finally, the effects of the multifactorial component are significantly different in parents and children ($\chi^2_1 = 9.72 - 0.00 = 9.72, P = 0.002$). Therefore, it appears that the variation in PTC thresholds is controlled by a major locus with incomplete dominance, as well as by a multifactorial component with unequal effects in children and adults. Thus, the full mixed model is invoked, which yields q = 0.521, t = 2.175, d = 0.896, H = 0.107, and Z = 3.016. Since the ability to taste a given dilution may really improve with age, the conclusion that the adult heritability (HZ = 0.323) is significantly greater than the childhood heritability (H = 0.107) may at first seem to be important. To test further whether inclusion of young children may have given rise to a spurious result, we repeated the analysis after deleting children ≤ 8 years of age. This yielded identical results, both for commingling and segregation analysis, except that the hypothesis of

TABLE IV. Comingling Analysis of Standardized Thresholds*

No. of distributions	−2 *l*nL + c	e	u	q	t	d	p
Two normal	22.24	0.396	−0.000	0.375	1.574	1	1
Two skewed	2.03	0.362	−0.155	0.483	2.003	1	−0.765
Three normal	11.82	0.231	0.002	0.461	2.392	0.588	1
Three skewed	0.00	0.235	−0.097	0.513	2.495	0.660	−0.234

*There is significant skewness in two ($\chi^2_1 = 22.24 - 2.03 = 20.21, P \doteq 0$) as well as three distributions ($\chi^2_1 = 11.82 - 0.00 = 11.82, P < .001$). Since a mixture of three skewed distributions does not fit significantly better than a mixture of two ($\chi^2_1 = 2.03 - 0.00 = 2.03, P = .16$), we take the latter as the parsimonious model.

TABLE V. Segregation Analysis of *P*-Transformed Thresholds Under Mixed Model Using the Joint-Likelihood Approach

Hypothesis	$-2/nL + c$	V	U	q	t	d	H	Z
All Data								
General[a]	0.00	1.133	−0.122	0.521	2.175	0.896	0.107	3.016
q = t = d = 0	100.69	1.152	−0.158	0	0	0	0.725	0.953
H = Z = 0, d = 1	28.39	1.158	−0.167	0.465	1.953	1	0	0
H = Z = 0	20.56	1.149	−0.160	0.482	2.297	0.802	0	0
d = 1	9.64	1.163	−0.168	0.483	1.970	1	0.170	1.419
z = 1	9.72	1.166	−0.164	0.487	2.158	0.891	0.172	1
Excluding children ≤ 8 years old								
General	0.00	1.385	−0.156	0.523	2.388	0.907	0.301	1.047
q = t = d = 0	105.05	1.182	−0.142	0	0	0	0.781	0.826
H = Z = 0, d = 1	48.26	1.181	−0.138	0.458	1.951	1	0	0
H = Z = 0	35.93	1.171	−0.130	0.488	2.420	0.755	0	0
d = 1	15.50	1.459	−0.132	0.531	2.378	1	0.272	1.077
z = 1[b]	3.09	1.436	−0.082	0.535	2.405	0.942	0.322	1

[a]This model explains nearly 97% of the variability in the thresholds of parents, but only 76% in that of the children.
[b]This model explains nearly 96% of the variability in the thresholds.

Z = 1 was now tenable (see bottom half of Table V). The best-fitting mixed model with Z = 1 yielded the following parameter estimates: q = 0.535, t = 2.405, d = 0.942, and H = 0.322. Note that the multifactorial heritability is now estimated close to the adulthood heritability when all children were included. This model explains nearly 96% of the variability in the thresholds. Thus, only 4% of the variability appears to be due to measurement error and other environmental factors. The effect of excluding children $\leq$ 8 years of age is difficult to explain in view of our earlier finding (Table I) that there are no differences between young children and older individuals. One likely explanation is that there are differences in extremes of the threshold distributions which we could not investigate due to small sample sizes.

DISCUSSION

Although this exercise illustrates the added power of quantitative traits, there is a limitation in the quantitative analyses presented here. It has been conveniently assumed that the 14-point thresholds represent a continuous trait. Clearly, the scale is not continuous, and the heavy tails violate the assumption inherent in the models. Testing the null hypothesis of d = 1 and H = 0 against the alternative hypothesis of z = 1 yields a likelihood ratio $\chi^2_2 = 45.17$ ($P \doteq 0$) (after deleting children below 8 years of age). To investigate how much of this evidence against the classic hypothesis (d = 1, H = 0) derives from the tails, we computed the contribution of each individual family to the total χ^2. Each of 16 families contributed a value ≥ 2. In only 7 of these 16 families, at least one member had an extreme threshold value (0, 1, 12, or 13). None of the other nine families involved extreme thresholds. Therefore, the evidence against the classic hypothesis did not arise primarily on account of heavy tails. In fact, reanalysis of the data after excluding the 7 families with extreme thresholds still provided strong evidence against the classic hypothesis, with nearly identical parameter estimates ($\chi^2_2 = 28.46, P \doteq 0$). Also, contrary to the findings of Olson et al. [1989], nontaster $\times$ nontaster matings with taster offspring did not contribute heavily to the evidence. There was only one such family in the entire data set which contributed a value of 2.68 to the total χ^2 (both parents had a threshold value of 2, a 14-year-old son had a value of 5, and an 11-year-old daughter had a value of 8). It is difficult to determine if the discontinuity of the scale had an effect.

The ability to taste PTC has long been recognized and accepted as a classical dominant character. Although exceptions were noted from time to time, none of the early investigations successfully countered the complete dominance theory. Based on mixed model segregation analysis of a large Brazilian family study that carried out serial dilutions in the vicinity of the antimode, but not the full range, Rao and Morton [1977] concurred with the complete dominance theory. Our findings reported here, based on a systematic analysis of the complete sorting test in a sample of 100 nuclear families, appear to suggest that the age-old complete dominance theory can be put to rest. We were able to show that dominance of the taster allele is incomplete, and that there are additional multifactorial effects influencing the extent of taste sensitivity. In comparing the two separate analyses of PTC affection status and power-transformed thresholds, we are led to speculate that perhaps the ability to taste PTC is determined by a nearly dominant major locus, while quantitative variation in the thresholds is controlled by a multifactorial component. It is unclear whether this particular mixed model

is sufficient to describe the data or whether alternative models with multiple alleles and/or multiple loci would provide a better fit. For example, assuming no multifactorial effects and complete recessivity of the nontaster allele (for lower thresholds), Olson et al. [1989] were able to show that an additional allele or an additional locus improves the fit significantly as compared to the classic theory, with the two-locus model providing the best fit. Although additional work is necessary to describe the genetics of PTC taste sensitivity accurately, it appears clear that the single locus complete dominance thoery is inadequate.

Our findings appear to have been anticipated by Harris [1955] some 30 years ago: "The variation in each group is quite extensive and leads to some overlap between the groups . . . although there seems little doubt that the dimorphism is largely genetically determined, the detailed character of the hereditary process involved still remains somewhat obscure." Hopefully, the obscurity is beginning to be clarified.

ACKNOWLEDGMENTS

This study was partly supported by NIH and NIMH grants GM28719, HL 33973, and MH31302. The authors are grateful to Professors Harry Harris and Newton Morton for their comments on an earlier version.

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Edited by Jean W. MacCluer