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Genetic Analysis of Cleft Lip With or Without Cleft Palate in Madras, India

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We performed a genetic analysis of 331 *nonsyndromic* cleft lip with or without cleft palate (CL ± P) proband families ascertained in Madras, India. Predictions of the multifactorial threshold (MF/T) model are tested; goodness-of-fit tests of the MF/T model and complex segregation analysis are also utilized to clarify the genetic etiology of CL ± P in this study population. There was little evidence for the MF/T model. The most reasonable conclusion from mixed model analysis is that of a major locus with reduced transmission probability. This is not altogether surprising if manifestation of CL ± P also depends on in utero exposure to harmful environmental agents during the critical period of facial development, as suggested by Melnick et al. [1980] and demonstrated in an animal model of CL ± P [Melnick et al., 1981]. Further the results in the Madras population are quite similar to those in other populations of Europe and Asia.

KEY WORDS: cleft lip, cleft palate, complex segregation analysis, India

INTRODUCTION

Cleft lip with or without cleft palate (CL ± P) is a major public health problem worldwide. Approximately 1 in 500–1000 newborn infants is affected with this malformation, the incidence varying by race and nationality [Melnick et al., 1980]. Asians (Chinese, Japanese, Koreans, and Filipinos) are clearly at higher risk for CL ± P than Caucasians or Blacks [Chung et al., 1974; Hu et al., 1982; Koguchi, 1975; Melnick et al., 1986; Myrianthopoulos and Chung, 1974; Tanaka et al., 1969]. These racial differences still persist in Hawaii, where the environment is relatively uniform among different races and after removal of ascertainment biases [Chung et al., 1974]. The need to perform genetic hypothesis testing with families who reside in various Asian countries, including the Indian subcontinent, remains. It is not at all certain that the same genetic etiology obtains across races or even across national groupings within races [Marazita et al., 1986a].

The present study is a genetic analysis of 331 CL ± P proband families ascertained in Madras, India. Predictions of the multifactorial threshold (MF/T) model are tested; goodness-of-fit tests of the MF/T model and complex segregation analysis are also utilized to clarify the genetic contribution to the etiology of CL ± P in this study population.

MATERIALS AND METHODS

Birth records at the Raja Sir Ramaslami Mudaliar Hospital (Madras, India) were used to determine the incidence of CL ± P among births occurring in this hospital for the years 1983–1985. Every newborn infant was examined by a physician, and apparent congenital anomalies were recorded. Over the 3-year period, 54 ***nonsyndromic*** cases of CL ± P were recorded among 34,267 newborn infants, an incidence of 1.6/1,000 births; the incidence was 2.14/1,000 in males and 1.06/1000 in females. For family studies, **nonsyndromic** CL ± P probands were ascertained through the Department of Plastic Surgery, Government Stanley Hospital (Madras, India). There were 331 **nonsyndromic** surgical probands identified during the years 1982–1987, 127 with cleft lip alone and 204 with cleft lip and cleft palate. The probands and affected first degree relatives were examined; other affected and unaffected relatives and consanguinity were noted from the interviews of the probands' parents. A total of 1,271 first-degree relatives and 1,524 second-degree relatives was reviewed; information about third-degree relatives was too uncertain to be considered.

The predictions of the MF/T model [Carter, 1976] were tested in the usual manner [Melnick et al., 1980]. The MF/T model was then investigated using the goodness-of-fit test (PGOODFIT) described by Gladstien et al. [1978].

The probands' families were also analyzed using the

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complex segregation analysis method of Morton and MacLean [1974], expanded as the "unified model" [Lalouel et al., 1983]. The purpose of this approach is to sort major gene effects from other sources of familial resemblance [Lalouel and Morton, 1981]. To do so, the unified model assumes that an individual's genotype is composed of a multifactorial component and a major gene component [Lalouel et al., 1983]. Further descriptions of the model and its underlying assumptions can be found in Morton and MacLean [1974] and Lalouel et al. [1983]. The parameters of interest for the present analysis are given in Table I. Likelihoods were calculated and maximum likelihood estimates of the parameters were obtained using the computer program POINTER [Morton et al., 1983]. For hypothesis tests, parameter estimates and likelihoods were obtained under the model with various restrictions. For example, the model with $d = t = q = 0$ corresponds to an hypothesis of no major gene. Hypothesis tests were based on the likelihood ratio criterion comparing each restricted model to the general, unrestricted model.

POINTER can only analyze nuclear families which may or may not have a "pointer"—defined to be the closest affected relative outside the nuclear family who led to the ascertainment of that family. Larger family structures can be included by breaking them into their component nuclear families and specifying the method of ascertainment for each nuclear family. The pointers facilitate this process.

The extended CL±P kindreds were therefore broken into their component nuclear families. The following types of nuclear families were formed, for a total of 345 nuclear families: (1) nuclear families composed of the probands, their parents, and sibs (multiple incomplete ascertainment, with ascertainment probability = 0.235, estimated using the model-free method in Gladstein et al. [1978]); (2) where appropriate, families composed of probands, their spouses and children (complete ascertainment); and (3) nuclear families with other (nonproband) affected relatives of the probands (truncate ascertainment through pointers). Nuclear families with no affected relatives contribute very little to a segregation analysis and therefore were not included.

TABLE I. Parameters of the Unified Model for Complex Segregation Analysis

Parameter	Description
d	Degree of dominance at the major locus
t	Displacement between homozygotes at the major locus
q	Gene frequency
τ_1	Probability that an individual of type AA will transmit A
τ_2	Probability that an individual of type Aa will transmit A
τ_3	Probability that an individual of type aa will transmit A
H	Childhood heritability, $H = CK/V$ ($V = 1$ for a qualitative trait
Z	Ratio of adult to childhood heritability ($Z = CA/CK$)
x	Proportion of sporadic cases

The probabilities of children's phenotypes were conditioned on the parental phenotypes, so including some probands twice (in one nuclear family as a parent and in another nuclear family as a child) does not bias the results.

Several probands were the result of consanguineous marriages. In those cases, only the nuclear families meeting the above descriptions (1) and (2) were included. There is no straightforward method in POINTER to specify consanguineous relationships, therefore no appropriate way to include type (3) nuclear families (i.e., with affected relatives of inbred probands). However, the (1) and (2) families for inbred probands (i.e., probands, their sibs, parents and children) are handled appropriately given that the probabilities of children's phenotypes are conditioned on the parental phenotypes.

RESULTS AND DISCUSSION

As noted in Table II, the M:F proband sex ratio of 1.24 was not significantly greater than expected; the same applies to the M:F ratios of unaffected sibs and total sibs. Since the M:F ratio of the affected sibs was significantly greater than expected, the estimated proband ascertainment probability was only 0.235, and the population estimate of the affected M:F ratio from births at the Mudaliar Hospital was greater than 2.0, one may suspect an unexplained ascertainment bias of surgical cases in favor of females. In any case, these data provide no evidence of sex-influenced inheritance or sex-biased natural prenatal selection as seen in other populations [Melnick et al., 1980, 1986].

Over the years the MF/T model has been endowed with several predictions [Carter, 1976]. The incidences of CL±P in the first- and second-degree relatives of CL±P probands were 26/1,271 (0.0205) and 7/1,524 (0.0050), respectively. As predicted, there is a marked drop in q/p (relative incidence/population incidence) as one goes from first-degree (~13) to second-degree (~3) relatives. However, this prediction is complicated by the fact that one would expect the same phenomenon if there were common familial environmental effects alone (i.e., no polygenic effect) [Smith, 1977].

Another prediction of the MF/T model is that the least affected sex could be expected to show the highest risk in its sibs as compared to the most affected sex. Since females are only marginally less affected in this sample than males, testing this prediction here may not be very meaningful. Nevertheless, it can be seen in Table III that sex of the proband was independent of the risk to sibs.

TABLE II. Sex Ratios in CL±P Proband Sibships

	Male	Female	Total	M:F ratio
Probands	183	148	331	1.24
Affected sibs	13	3	16	4.33*
Unaffected sibs	296	297	593	1.00
Total	492	448	940	1.10

*$X^2 = 5.86, P < 0.025$; all other ratios not significantly different from an expected M:F ratio of 1.04.

TABLE III. Risk to Sibs by Sex of the CL±P Proband*

Sex of proband	Total Sibs	Affected	Incidence
Male	345	10	0.0290
Female	264	6	0.0227

*$X^2 = 0.23, P > 0.5$.

A prediction with a similar theoretical basis is that the more severely affected probands could be expected to show the highest risk in first-degree relatives as compared to the less severely affected probands. This was not the case (Table IV) for either comparisons of CL and CL±P ($X^2 = 1.06, P > 0.10$), unilateral and bilateral CL+P ($X^2 = 3.73, P > 0.05$), unilateral and bilateral CL±P ($X^2 = 3.05, P > 0.05$), or bilateral CL±P and unilateral CL ($X^2 = 3.67, P > 0.05$). Comparison of unilateral and bilateral CL was not possible owing to the small sample size of bilateral CL.

In summary, then, based on tests of the stated predictions of the MF/T model, we found little evidence for it in this study population, not unlike most others worldwide [Melnick et al., 1980; Marazita et al., 1986b; Melnick et al., 1986]. To further test the MF/T model, we employed the goodness-of-fit test described by Gladstein et al. [1978]. The method incorporates the necessary corrections for ascertainment bias; the probabilities of observing the actual study data are derived for a range of ascertainment probabilities (π) and heritabilities (h^2). This analysis was done on 236 nuclear families, excluding consanguineous and single-child families.

In 15 of the 236 nuclear families there were 2 or more affected sibs. For a range of π (0.1–0.9) and h^2 (0.1–0.9) 2 probabilities (P-values) were calculated: (1) the probability of observing 15/236 or more families with 2 or more affected children under the MF/T model; (2) the probability of observing fewer than 15/236. Regarding the P-values for (1), many, but not all, of the values were less than 0.05 (Table V); regarding the P-values for (2), for each combination of π and h^2 it was equal to 1.0. The MF/T model could again be rejected, for at the best estimates of π (0.24) and h^2 (0.55) there were significantly more families with 2 or more affected children than would be expected under this model.

Since the above relatively simple tests of the MF/T hypothesis gave no evidence in favor of the MF/T model, we then performed complex segregation analysis on the data in order to test alternatives. Table VI presents the results of complex segregation analysis of the family data. The likelihood ratio criterion was used for hypothesis tests, comparing the likelihood of each restricted hypothesis to that of the general, unrestricted hypothesis (hypothesis 1a on Table VI). Note that none of the hypotheses presented in Table VI included the parameter x, proportion of sporadic cases. In every case, if x was included in a model, the parameter estimate converged to 0.0 and the likelihood was identical to that obtained if x was not included. Therefore, x is omitted from the table and this discussion.

TABLE IV. Risk to Sibs by Cleft Type of the Proband

Cleft type of proband	Total sibs	Affected sibs	Incidence
CL alone			
Unilateral	242	5	0.0210
Bilateral	25	0	0.00
CL+P			
Unilateral	244	5	0.0209
Bilateral	98	6	0.0652
CL±P			
Unilateral	486	10	0.0206
Bilateral	123	6	0.0513

TABLE V. PGOODFIT Test of the MF/T Model*

Heritability (h^2)	Ascertainment probability (π)							
	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8
0.1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.4	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.5	0.02	0.01	0.01	0.01	0.00	0.00	0.00	0.00
0.6	NS	NS	NS	NS	0.04	0.02	0.02	0.01
0.7	NS	NS	NS	NS	NS	NS	NS	NS
0.8	NS	NS	NS	NS	NS	NS	NS	NS
0.9	NS	NS	NS	NS	NS	NS	NS	NS

*Entries in the table are the probabilities of observing 15/236 or more families with two or more affected children under the MF/T model. NS means not significant, i.e., greater than the specified significance level of .05, so the MF/T model cannot be rejected at those combinations of ascertainment probability and heritability. At the other points it can be rejected.

When estimating the parameters of hypothesis 1a, 2 of the parameters converged to their boundary values. Therefore, for purposes of calculating the degrees of freedom for each hypothesis test, there were only 4 parameters actually estimated in hypothesis 1a.

The hypothesis of no familial transmission (hypothesis 2) was clearly rejected ($X_4^2 = 87.06, P \ll 0.0001$). The hypothesis of multifactorial transmission (MF/T, hypothesis 4) could also be rejected, either including a generation difference (4a, $X_2^2 = 6.28, P < 0.05$) or not (4b, $X_3^2 = 7.82, P = 0.05$).

When we estimated the parameters under an hypothesis of a major locus τ was significantly less than the Mendelian expected value of 0.5 ($X^2 = 4.66, P < 0.05$—hypothesis 3a compared to 3b). In addition, hypothesis 3b (major locus with Mendelian τs) could be rejected ($X^2 = 4.68, P < 0.05$) when compared to the general model (1a). Hypothesis 3a (major locus with non-Mendelian τ_2) and the most general hypothesis (1a) were equally likely. Since only 4 parameters were actually estimated in 1a, and 4 were estimated for 3a, there are no degrees of freedom for the hypothesis test, but the likelihoods were essentially the same.

A final hypothesis tested was 1b (major locus plus MF/T with no generational differences). Hypothesis 1a and 1b were equally likely, although as with hypothesis 3a, there were no degrees of freedom for an hypothesis test.

Since hypothesis 1a, 1b, and 3a were all equally likely, neither hypothesis 1b nor hypothesis 3a can be rejected. Furthermore, when the parameters were estimated under hypothesis 1a and 1b (major locus MF/T—with and without generation differences), H converged to 0.0. Therefore, the most reasonable conclusion from mixed model analysis of this dataset is that the best-fitting

TABLE VI. Results of Complex Segregation Analysis of CL ± CP in the Families of Surgical Probands Born Between 1982 and 1987*

Hypothesis	Parameters[a]						
	d	t	q	τ_2	H	Z	$-2 \ln L + C$[b]
(1) Mixed models							
(a) Major locus, and MF/T with generation difference	0.0[c]	1.68	0.051	0.14	0.0[c]	1.0[c]	−211.39
(b) Major locus, and MF/T no generation difference (Z = 1.0)	0.45	2.39	0.015	0.13	0.0[c]	[1.0]	−210.28
(2) No familial transmission ($q - H = 0.0$)	—	—	[0.0]	—	[0.0]	—	−124.33
(3) Major locus (H = 0.0)							
(a) General	0.28	1.83	0.040	0.09	[0.0]	—	−211.37
(b) Mendelian (τ_2 = 0.5)	0.44	3.73	0.013	[0.5]	[0.0]	—	−206.71
(4) MF/T (Multifactoral, q = 0.0)							
(a) Generation difference	—	—	[0.0]	—	0.76	.30	−205.11
(b) No generation difference (Z = 1.0)	—	—	[0.0]	—	0.77	[1.0]	−203.57

*Numbers in brackets represent parameters not estimated; set to value inside brackets.
[a]See Table I for descriptions of parameters.
[b]C, a proportionality constant.
[c]Parameter converged to a boundary value.

model is that of a major locus with a reduced transmission probability.

Simulation studies [Williams and Beutow, 1986] have shown that if there is incomplete penetrance and/or variable phenocopies present in the data, then analyses will result in non-Mendelian estimates of the transmission probabilities, and should not necessarily be interpreted as evidence against the major gene hypothesis. It is quite likely that phenocopies may be present in CL ± P (e.g., teratogen induced clefts). It is also possible that incomplete penetrance might exist (e.g., if the locus is a "susceptibility-to-the-environment" locus) or there is simply measurement error (failing to detect microforms or inaccurate reporting of family histories).

CONCLUSION

The resulting data analysis is consistent with a major locus for CL ± P in the population of Madras, India. The non-Mendelian transmission probability associated with this conclusion may be due to phenocopies or incomplete penetrance. This is not altogether surprising if manifestation of CL ± P also depends upon in utero exposure to harmful environmental agents during the critical period of facial development, as suggested by Melnick et al. [1980] and demonstrated in an animal model of CL ± P [Melnick et al., 1981]. Further, the results in the Madras population are quite similar to those in other populations of Europe and Asia [Chung et al., 1986; Marazita et al., 1984, 1986b, 1989; Melnick et al., 1980, 1986], albeit with some idiosyncratic differences in each population. It remains to be determined whether the major gene is identical among populations, whether there are allelic differences within and among populations, and what the environmental factors are.

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