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Mode of Inheritance of Nonsyndromic Cleft Lip with or without Cleft Palate: A Reanalysis

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Summary

Nonsyndromic cleft lip with or without cleft palate (CL ± P) is traditionally recognized as a multifactorial threshold trait (MFT). Recently, however, evidence for the involvement of a major gene in the etiology of CL ± P has been reported. To assess the potential for major-gene involvement in the etiology of this trait, familial recurrence patterns from several family studies of CL ± P were reanalyzed. The recurrence patterns in first-degree relatives of CL ± P probands were found to be compatible with the expectations for either an MFT or a generalized single-major-locus (gSML) trait. The use of multiple thresholds based on proband sex, defect bilaterality, or palatal involvement did not help to discriminate between these models. However, the pattern of recurrence among MZ twins and more remote relatives of CL ± P probands is not consistent with gSML inheritance but is compatible with either an MFT model or a model specifying multiple interacting loci. For such a model, no single locus can account for more than a sixfold increase in risk to first-degree relatives. These findings have important implications with regard to the feasibility of detecting linkage to loci conferring susceptibility to CL ± P.

Introduction

In the late 1960s Carter (1969) suggested that the familial aggregation patterns demonstrated by nonsyndromic cleft lip with or without secondary clefting of the palate (CL ± P) could be explained by the multifactorial threshold (MFT) inheritance model developed by Falconer (1965). This model is currently considered by many to be the most appropriate model of inheritance for CL ± P. The appropriateness of this model has, however, been challenged by Melnick et al. (1980), who concluded that the overall body of data on CL ± P does not provide strong evidence in favor of MFT inheritance. In addition, the results of several segregation analyses have been interpreted as providing strong evidence in favor of a major-gene effect in the etiology of nonsyndromic CL ± P (Marazita et al. 1984, 1986*a*, 1986*b*; Chung et al. 1986, 1989; Hecht et al. 1991*b*).

Motivated by the possibility of major-gene involvement in the etiology of CL ± P, at least two linkage analyses involving nonsyndromic CL ± P families have been undertaken (Eiberg et al. 1987; Hecht et al. 1991*a*). In addition, Ardinger et al. (1989) attempted to identify a gene for CL ± P via association studies with five candidate gene loci. These authors reported a significant association between the occurrence of CL ± P and two RFLPs at the locus for transforming growth factor alpha (TGFA). Chenevix-Trench et al. (1991) also found evidence for an association between CL ± P and TGFA. However, Hecht et al. (1991*a*) found no evidence of an association or linkage between CL ± P and TGFA in 12 multiplex CL ± P pedigrees.

Undoubtedly, given the rapidity with which polymorphic DNA markers are being added to the human gene map, additional studies seeking to link CL ± P to the effects of single genetic loci will be forthcoming. However, the application of genetic linkage analysis to complex, non-Mendelian traits such as CL ± P is not

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straightforward. Interpretation of linkage evidence is difficult in the absence of strong evidence for a major locus and characterization of its effect. In addition, determination of the power to detect linkage by using robust methods, which do not require specification of mode of inheritance, is partially determined by the number of disease-susceptibility loci involved in determining a trait and by the nature of the interactions between these loci. Thus, it is important to have at least a general understanding of the number and nature of the loci which determine a complex trait, prior to undertaking linkage analysis.

The present analyses were undertaken in an attempt to obtain such an understanding for nonsyndromic CL ± P. To this end, four characteristics of familial aggregation, which can be useful in discriminating between MFT and generalized single-major-locus (gSML) inheritance, were analyzed. These characteristics include the relationship between proband sex and risk to relatives, the relationship between severity of the proband's defect and risk to relatives, the increase in risk with number of affected relatives, and the pattern of risk associated with decreasing relatedness to the proband.

Material and Methods

Data from five large family studies of CL ± P are included in the present study. The published data of Woolf (1971) and Carter et al. (1982), data analyzed by Bear (1976) and Welch and Hunter (1980), and the Danish series of CL ± P families (Bixler et al. 1971; Shields et al. 1979; Melnick et al. 1980) were included in these analyses (table 1).

The risk to siblings of probands in these studies was estimated by the singles method (Davie 1979). For all other types of relatives, risk was estimated as the proportion of relatives of type R who are also affected. Correlations in liability between relatives (r) were calculated according to the modification of Falconer's formula, which allows for reduced variance of liability in relatives of affected individuals, suggested by Reich et al. (1972).

The relationship between the sex ratio of CL ± P in the general population and the risk ratio for relatives of female versus male probands (RR_{sex}), expected under the MFT model, was quantitated in the manner set forth by Ottman (1987). The normal deviates $X_{sib,M}$ and $X_{sib,F}$, corresponding to the expected prevalence of CL ± P in the siblings of male and female probands, respectively, were calculated from a modification of the formula presented by Reich et al. (1972), where

$$x_{sib,i} = (x - a_i r) / [1 - (r^2 a_i (a_i - x_i)]^{1/2}, \quad (1)$$

i is the sex of the proband, x is the normal deviate corresponding to the prevalence of CL ± P in the general population, x_i is the normal deviate corresponding to the prevalence of the trait in the general population of type i individuals, a_i is the mean liability's deviation (of affected individuals of type i) from the mean liability in the general population of type i individuals, and r is the correlation in liability between the proband and relatives of type R. The expected value of RR_{sex}

Table 1

Summary of Data Analyzed in the Present Study

	Study Populations[a]				
	USA	ENG	DEN	CAN	LON
Population	Phoenix, UT	Northern England	Denmark	Manitoba	London
Period of ascertainment	Undefined	1970–74	1941–71	1964–77	1920–39
Source of cases	Surgical cases provided by M.D.'s	Consecutive series of clinic patients	Surgical cases	Multiple sources	Surgical cases
Prevalence of CL ± P	.0012	.0010	.0014	.0010	.0010
Heritability[b]	.86	.52	.91	.79	.78
Proband characteristics:					
Sex ratio (M:F)	1.82	1.60	1.99	1.52	1.90
% With bilateral defect	68	28	. . .	22	20
% With cleft palate	. . .	64	58	78	62

[a] References for the study populations: USA—Woolf et al. (1963) and Woolf (1971); ENG—Bear (1976); DEN—Bixler et al. (1971) and Melnick et al. (1980); CAN—Welch and Hunter (1980); and LON—Carter et al. (1982).

[b] Calculated by using population prevalence of CL ± P and risk to siblings of all CL ± P probands.

under the MFT model of inheritance is estimated by the ratio of the expected prevalence of CL ± P in the siblings of female probands, compared with siblings of male probands.

In an analogous manner, the relationship between the general population's proportion of affected individuals with a severe form of cleft (either bilateral clefting or cleft lip with cleft palate) and the risk ratio for relatives of severely affected probands versus all probands (RR_{sev}), expected under the MFT model, was quantitated. The normal deviate $X_{sib,sev}$, corresponding to the expected prevalence of CL ± P in the siblings of severely affected probands, was calculated using equation (1). Since r is estimated from the risk to all siblings of all probands, the value of $X_{sib,all}$ corresponding to the expected prevalence of CL ± P in the siblings of all probands is simply the normal deviate corresponding to the observed risk of CL ± P in the siblings of all probands.

This method for quantitating the relationship, expected under the MFT model, between severity of the probands' defect and risk to relatives differs from the approach presented by Ottman (1987), who suggested quantitating the relationship between the ratio of severe to mildly affected individuals in the general population and the risk ratio for relatives of severely versus mildly affected probands. However, mildly affected individuals do not fall within the tail of the liability distribution but, rather, occupy in the distribution a space which is defined by an upper and lower threshold, corresponding to the general population prevalence of the severe and mild forms of the trait, respectively. Thus, x_i and a_i, where i is defect severity, cannot be estimated from the prevalence of the mild form of the trait in the general population. If x_i and a_i are estimated from the prevalence of the mild form of the trait in the general population, then the expected risk to relatives will be overestimated and the ratio of risk to relatives of severely versus mildly affected probands will be underestimated.

Figure 1 illustrates the relationship between the proportion of affected individuals with the severe form of a trait and RR_{sev} for a trait with a prevalence of 0.1%, at five values of r (.10, .20, .30, .40, and .50). In first-degree relatives these values correspond to heritabilities of 20%, 40%, 60%, 80%, and 100%, respectively, if the environmental covariance between relatives is assumed to be negligible.

The USA, ENG, and LON data were pooled with data from two additional studies (Bonaiti et al. 1982; Czeizel and Tusnady 1984) which also included both

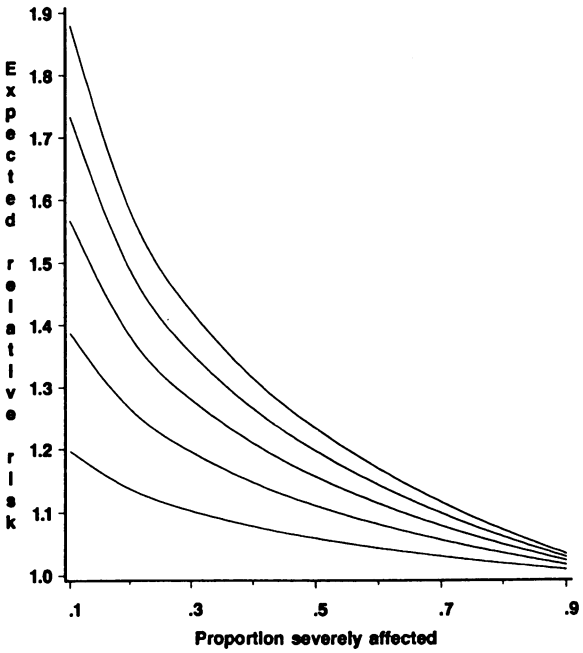

Figure 1 Relationship between the proportion of affected individuals with the severe form of a trait and the ratio of risk to first-degree relatives of severely affected probands, compared with first-degree relatives of all probands, for a trait with a frequency of 0.1%. From top to bottom, lines correspond to r values of .5, .4, .3, .2, and .1, respectively.

information on the prevalence of CL ± P in the general population and recurrence risks for first-, second-, and third-degree relatives of CL ± P probands (table 2), to obtain estimates of λ_R, the ratio of the risk to type R relatives compared with the population prevalence. The DEN data also included this information. However, family history information was specifically sought only for affected relatives (D. Bixler, personal communication), and extended family information was more likely to be provided when there was a positive family history of CL ± P (Marazita et al. 1984). Since both of these factors are likely to artificially inflate the observed risk to relatives, the DEN data were excluded from this analysis. The risk to siblings of probands in the DEN data is not thought to be biased by these factors, since sibling information was available from existing medical records (D. Bixler, personal communication).

Twin data from three sources (Metrakos et al. 1958; Hay and Wehrung 1970; Shields et al. 1979) were used to estimate λ_R for MZ twins of CL ± P probands. Each of these twin series estimated pairwise twin concordance, which provides a minimum esti-

Table 2

Risk to First-, Second-, and Third-Degree Relatives of CL ± P Probands

Population	Prevalence	First-Degree Relatives		Second-Degree Relatives	Third-Degree Relatives
		Siblings	Offspring		
USA	.0012	.0391 (60/1,534)	.0427 (7/164)	.0065 (31/4,747)	.0037 (43/11,640)
ENG	.0010	.0107 (6/562)		.0054 (11/2,022)	.0038 (12/3,185)
France[a]	.0010	.0302 (28/927)		.0037 (13/3,508)	.0037 (18/4,858)
LON	.0010	.0273 (28/1,027)	.0319 (32/1,003)	.0055 (27/4,888)	.0027 (13/4,744)
Hungary[b]	.0010	.0485 (21/433)		.0072 (15/2,072)	.0032 (7/2,203)
Total		.0319 (143/4,483)	.0334 (39/1,167)	.0056 (97/17,237)	.0035 (93/26,630)
		.0322 (182/5,650)			

[a] Bonaiti et al. (1982).
[b] Czeizel and Tusnady (1984).

mate of risk. Maximum estimates of the risk to MZ co-twins were obtained by assuming that all affected twins are probands and by estimating risk as the proportion of all MZ co-twins of CL ± P probands who are also affected.

Previously derived gSML, MFT, and mixed models of inheritance were evaluated for goodness of fit to the data from which they were derived. These models were derived by complex segregation analysis of the LON (Marazita et al. 1986*a*) and DEN (Chung et al. 1986) data. Goodness of fit was assessed by χ^2 analysis, comparing observed with predicted risks to relatives. The expected risks to relatives of probands were calculated in the following manner:

1. The parameter estimates for the mixed, gSML, or MFT model of inheritance that were obtained by Chung et al. (1986) or Marazita et al. (1986*a*) were input into the computer program POINTER (Lalouel and Morton 1981), without iteration statements. When this is done, POINTER calculates $-2\ln(L)$, where L is the likelihood of an individual pedigree, for each family.
2. The odds of a particular relative being affected were calculated as odds = $e^{[((-2\ln[L,F2])-(-2\ln[L,F1]))]/2}$, where F1 is a family in which the relative of interest is of unknown status with respect to CL ± P and where F2 is a family with the same structure as F1, except that the relative of interest is designated as being affected. In both F1 and F2, all relatives except the proband and the relative of interest are of unknown status with respect to CL ± P.
3. The risk to relatives of CL ± P probands was calculated as risk = 1/(1 + odds). The accuracy of risks estimated in this manner was confirmed by hand calculation of the appropriate probabilities (i.e., *P*[type R relative is affected|proband and model parameters]).

Results

The observed relationships between proband's sex and defect severity and the risk to siblings of probands were compared with their MFT expectations. Since these relationships were relatively homogeneous across the study populations (Mitchell 1991), only the analyses based on the combined data are presented. Data on male and female siblings were combined for these analyses, since sibling sex is independent of proband sex and severity of the proband's defect. These relationships were not evaluated in offspring, because only one study (Carter et al. 1982) provided sufficient information on offspring for these relationships to be assessed. These relationships also were not assessed in parents of probands, because the risk in this group of first-degree relatives appears to be underestimated; the combined risk to parents in these studies (2.34%, 95% confidence interval [CI] 1.99%–2.69%) is significantly less than the combined risk to siblings (4.02%, 95% C.I. 3.61%–4.43%). It is likely that the relatively low risk to parents is attributable to selection against affected individuals rather than to a major dominance component for CL ± P, since the risk to offspring and siblings of CL ± P probands tends to be similar (Woolf et al. 1963; Fujino et al. 1967; Bixler et al. 1971; Koguchi 1975; Carter et al. 1982).

1. *Relationship between Proband's Sex and Defect Severity and the Risk to Relatives*

The sex ratio among probands, the proportion of probands with unilateral defects, and the proportion with clefting of the palate are summarized in table 1.

Information on bilaterality of the proband's defect and information on palatal involvement of the proband's defect are not available in the DEN and USA data, respectively. In each study there is a predominance of affected males. There is also a predominance of probands with clefting of the palate. Unilateral defects predominate among the probands in three studies, but in the USA data they are less frequent than bilateral defects.

The proportion of probands with bilateral defects is significantly greater in the USA data than in the other studies. However, approximately 39% of the probands in the USA data could not be classified by this criterion. The relatively high proportion of bilateral cases may, therefore, reflect differences in the proportion of unilateral and bilateral cases which could be classified, rather than true differences between the USA data and the other populations. Thus, the USA data were excluded from the analysis of the relationship between laterality of the proband's defect and the risk to relatives.

The observed values of RR_{sex} and RR_{sev} are consistent with their expected values under the MFT model of inheritance (table 3). These values are, however, also consistent with several gSML models of inheritance. Hence, these relationships cannot be used to discriminate between MFT and gSML models of inheritance for CL ± P.

2. *Association between Risk and Number of Affected Relatives*

For the combined data from all five studies, the risk to a proband's sibling, when at least one additional first-degree relative is also affected, is 15.15% (95% CI 12.60%–17.07%). This risk is approximately 3.8 times greater than the risk to siblings of all CL ± P probands. The MFT model predicts that this risk should be 3.6–4.1 times greater than the risk to siblings of all probands (Kruger 1973, fig. 16), for a trait with a prevalence of 0.1% and a heritability of 50%–90%. Thus, the observed value of 3.8 is in relatively good agreement with its MFT expectation.

Simple gSML models with reduced penetrance and no phenocopies are incompatible with the observed risk increase associated with the presence of at least one affected first-degree relative in addition to the proband (Kruger 1973). Generalized SML models which allow for phenocopies could, however, account for the observed value of this ratio. Thus, the relative risk increase associated with the presence of more than one affected first-degree relative is also unable to discriminate between MFT and gSML models of inheritance for CL ± P.

3. *Familial Recurrence Patterns*

The two studies providing information on risk to siblings and offspring (Woolf et al. 1963; Carter et al. 1982) provide no evidence for a dominance variance component in CL ± P, since the risk to siblings of probands is less than the risk to offspring of probands (table 2). Therefore, the data on sibling and offspring were pooled and, under the assumption that the population prevalence is 0.1%, were used to estimate λ_1. Under the formula presented by Risch (1990*b*), this value of λ_1 was used to predict λ_R for MZ twins (λ_{MZ}), and for second- (λ_2) and third-degree (λ_3) relatives of CL ± P probands, under a single-locus model, a model with infinite loci of small effect, and a number of multiplicative models of inheritance.

The estimated risk to MZ twins is 25.32% by the

Table 3

Relationship between Proband's Sex and Defect Severity and the Risk to Relatives

Populations	Expected RR_{sex}	% Risk to Siblings of		Observed RR_{sex} (95% CI)[a]
		Male Probands	Female Probands	
USA, ENG, DEN, CAN, and LON: proband sex ratio = 1.88	1.19[b]	4.32 (247/5,714)	4.24 (129/3,040)	.98 (.80–1.21)
ENG, CAN, and LON: probands with bilateral clefts = 23%	1.39[c]	4.79 (19/397)	2.25 (44/1,957)	2.13 (1.27–3.58)
ENG, DEN, CAN, and LON: probands with CLP = 61%	1.16[d]	4.90 (217/4,430)	4.39 (316/7,199)	1.12 (.94–1.32)

[a] $(RR_{sex})\exp[1 \pm (1.96/\sqrt{X_1^2})]$.
[b] Calculated for a trait with a prevalence of .0013 and $r = .42$.
[c] Calculated for a trait with a prevalence of .0010 and $r = .35$.
[d] Calculated for a trait with a prevalence of .0013 and $r = .42$.

pairwise method and 40.40% by the probandwise methods. The corresponding rates in DZ twins are 2.98% and 5.63% and are in relatively good agreement with the pooled estimate of risk to siblings (3.19%, 95% CI 2.68%–3.70%) that is used in this analysis. This suggests that the range of values used for MZ twins is also likely to encompass the true MZ twin risk.

The predictions for a gSML model with λ_1 fixed at 32.2 are given in table 4. Under this model, the MZ twin ratio is dramatically underestimated and the ratios for second- and third-degree relatives are severely overestimated. The predicted ratio for second-degree relatives is consistent with the observed value under a model of infinite loci with small effect. However, the MZ ratio is overestimated and the ratio for third-degree relatives is underestimated by this model (table 4). Comparison of the observed values of λ_R for all three relative types with the predictions of various multiplicative models suggests that no single gene is likely to have an effect of more than $\lambda_1 = 6$ and that, at most, two or three loci of relatively small effect ($\lambda_1 \leq 3$) are involved in the etiology of CL ± P.

The formula of Reich et al. (1972) was used to calculate the expected risk to MZ twins and to more remote relatives, under the traditional MFT model. A heritability of .82, estimated from the sibling recurrence rate of 3.22% and a population prevalence of 0.1%, was used in these calculations. This model predicts relative recurrence risks of 308.5, 3.0, and 1.8 for MZ twins, second-degree relatives, and third-degree relatives, respectively. The predictions of the MFT model are, therefore, in relatively good agreement with the observed data, although this model does tend to underestimate the risk to second- and third-degree relatives.

Tables 5 and 6 summarize, respectively, Marazita et al.'s (1986*a*) and Chung et al.'s (1986) results for the fit of the mixed, gSML, and MFT models to the data from which these models were derived. Chung et al. (1986) and Marazita et al. (1986*a*) both concluded that a mixed model of inheritance, including a major locus as well as a multifactorial component, provided the best fit to their data.

The segregation analysis of the data of Carter et al. (1982), performed by Marazita et al. (1986*a*), was based solely on the first-degree relatives of CL ± P probands. From table 5 it can be seen that both the MFT model and the gSML model provide a better fit to the observed risks to first-degree relatives of CL ± P probands than does the mixed model. However, the gSML model severely overpredicts the observed risk to the second- and third-degree relatives. Thus, the data of Carter et al. (1982) appear to be most consistent with the predictions of the MFT model of inheritance.

Information on extended family members was included in the segregation analysis of the DEN data (Chung et al. 1986). However, because of previously discussed concerns about overestimation of the risk to

Table 4

Genetic Models for CL ± P

	λ_1	λ_{MZ}	λ_2	λ_3
Observed	32.2	253.2–404.0[a]	5.6	3.5
Model:[b]				
SML		63.4	16.6	8.8
Infinite loci		1,036.8	5.7	2.4
$\lambda_{11} = 2$		777.6	6.0	2.5
$\lambda_{11} = 3$		572.4	6.5	2.7
$\lambda_{11} = 4$		453.6	7.1	2.9
$\lambda_{11} = 5$		373.3	7.6	3.2
$\lambda_{11} = 6$		317.2	8.1	3.4
$\lambda_{11} = 7$		275.1	8.6	3.7
$\lambda_{11} = \lambda_{21} = 2$		583.2	6.4	2.6
$\lambda_{11} = \lambda_{21} = 3$		320.4	7.6	3.1
$\lambda_{11} = \lambda_{21} = \lambda_{31} = 2$		436.3	6.8	2.8
$\lambda_{11} = \lambda_{21} = \lambda_{31} = 3$		177.0	8.7	3.5

[a] Represents the pooled values for the minimum and maximum estimates of risk to MZ co-twins of CL ± P probands.

[b] λ_{iR} = relative increase in risk to relatives of type R, which is attributable to the effects at the ith locus.

Table 5

Comparison of the Observed Risks in the Data of Carter et al. (1982) versus the Predictions Based on the Models[a] of Marazita et al. (1986a)

Relationship	Observed Risk	Expected Risk (X^2 value) under		
		Mixed Model	MFT Model	gSML Model
Father-son	3.06 (10/327)	5.14 (2.91)	4.56 (1.70)	3.22 (.03)
Father-daughter	2.29 (7/306)	3.72 (1.75)	2.77 (.27)	1.87 (.29)
Mother-son	6.77 (13/192)	7.12 (.03)	5.54 (.58)	3.74 (4.90)
Mother-daughter	1.12 (2/178)	4.30 (4.37)	3.42 (2.84)	2.17 (.92)
Brother-brother	3.04 (11/362)	6.16 (6.10)	4.56 (1.93)	3.70 (.45)
Brother-sister	2.62 (9/343)	3.82 (1.33)	2.77 (.03)	2.34 (.12)
Sister-brother	2.40 (4/164)	7.63 (6.27)	5.54 (3.02)	4.76 (1.95)
Sister-sister	2.96 (5/169)	4.84 (1.30)	3.42 (.36)	3.28 (.05)
Sum of X^2 for first-degree relatives		(24.06)	(10.73)	(8.71)
Two affected sibs	18.18 (10/55)	19.94 (.10)	15.74 (.25)	12.89 (1.37)
Affected sib and affected parent	7.14 (1/14)	18.29 (1.16)	13.35 (.46)	5.84 (.08)
Second-degree relative	.55 (27/4,888)	1.05 (11.66)	.85 (4.97)	1.43 (27.32)
Third-degree relative	.27 (13/4,744)	.37 (1.31)	32 (.31)	.77 (15.28)

[a] Obtained by using conditional likelihoods and sex-specific prevalence rates of .00134 and .00067 in males and females, respectively. Model parameters were as follows: mixed model—d = .28, t = 2.58, q = .006, h^2 = .99, and $-2\ln(L)$ = 597.88; MFT—h^2 = .90, and $-2\ln(L)$ = 629.20; gSML—d = .28, t = 6.84, q = .006, and $-2\ln(L)$ = 638.64.

CL ± P probands' relatives other than siblings, it was only possible to assess the fit of Chung et al.'s (1986) models to the observed risk to siblings of CL ± P probands. On the basis of results presented in table 6, the MFT model also provides the best fit to the observed data from the Danish population.

Discussion

Melnick et al. (1980) argued that the theory of MFT inheritance of CL ± P was not strongly supported by the available family data. This argument was largely based on the observation that risk to first-degree relatives of CL ± P probands is independent of proband sex and defect severity.

The present series of analyses demonstrates that the observed relationships of proband sex and defect severity to the risk to relatives or probands are not inconsistent with MFT inheritance of CL ± P. In fact, unless the sex ratio is severely distorted (Ottman 1987) or the proportion of severely affected individuals is quite low (fig. 1), the expected difference in risk to relatives

Table 6

Comparison of the Observed Risks in the Danish Data versus the Predictions Based on the Models[a] of Chung et al. (1986)

Relationship	Observed Risk	Expected Risk (X^2 value) under		
		Mixed Model	MFT Model	gSML Model
Brother-brother	6.26 (133/2,124)	8.39 (12.52)	6.68 (.60)	8.26 (11.19)
Brother-sister	3.36 (56/1,669)	5.46 (14.32)	3.96 (1.59)	5.20 (11.52)
Sister-brother	7.35 (67/911)	11.43 (14.95)	8.32 (1.11)	10.90 (11.80)
Sister-sister	4.08 (35/858)	7.96 (17.63)	5.07 (1.75)	6.87 (10.45)
Sum of X^2 for first-degree relatives		(59.42)	(5.05)	(44.96)
Two affected sibs	15.58 (48/308)	11.77 (4.32)	18.30 (1.52)	11.65 (4.63)
Affected sib and affected parent	15.76 (38/241)	18.87 (1.59)	18.30 (1.03)	19.83 (1.03)

[a] Obtained by using sex-specific prevalence rates of .00176 and .00084 in males and females, respectively. Model parameters were as follows: mixed model— d = 0, t = 2.715, q = .035, h^2 = .967, and $-2\ln(L)$ = 5345.92; MFT—h^2 = .999, and $-2\ln(L)$ = 5,399.40; gSML—d = 0, t = 3.085, q = .050, and $-2\ln(L)$ = 5,364.14.

of different proband types is negligible under the MFT model. The relationship between these characteristics and risk to relatives is, therefore, insufficient for discriminating between alternate models of inheritance for CL ± P, and, from a genetic standpoint, CL ± P may be considered a single-threshold trait.

For single-threshold traits, mode of inheritance can be difficult to establish from nuclear-family data (Smith 1971). The pattern of decline in risk with decreasing relatedness to the proband can, however, provide some clues regarding mode of inheritance of complex, single-threshold traits (Risch 1990*a*). For CL ± P, the observed decline in risk with decreasing relatedness to the proband is incompatible with any gSML model of inheritance and is suggestive of multilocus inheritance, although not necessarily MFT inheritance. The existence of sporadic (nongenetic) cases, or genetic heterogeneity due to several distinct alleles independently causing CL ± P, would not alter this conclusion, since the predictions of the gSML model would apply in either case (Risch 1990*a*). Patterns of decline in risk that are similar to those observed for CL ± P could, however, be attributable either to underreporting of affected second- and third-degree relatives, or to the involvement of environmental determinants of risk.

The potential for biased ascertainment of affected second- and third-degree relatives could not be evaluated in these data. Both the observed decline in risk to second- and third-degree relatives and the difference in risk to MZ and DZ co-twins of CL ± P probands are, however, consistent with CL ± P being determined by multiple loci. In addition, the involvement of environmental factors in the etiology of this condition would have little impact on the conclusions concerning the role of a major locus in the etiology of CL ± P. If such a familial environmental effect exists, then the effect of genes on familial aggregation must be correspondingly reduced. Hence, any single locus can have only a minor effect on familial aggregation (Risch 1990*a*). Therefore, in view of the limitations of the available data, the family recurrence patterns exhibited by CL ± P are compatible with either the MFT model of inheritance or a model which includes multiple, interacting loci.

Since the submission of the present paper, Farrall and Holder (1992) have also reported a reanalysis of the familial recurrence patterns for CL ± P. Their conclusions are largely consistent with those reported here, in that they reject a single-locus model for CL ± P. These authors also found the familial recurrence patterns to be equally compatible with either the MFT model of inheritance or a model which includes multiple, interacting loci. Examining five data sets (four of which were also included in our analyses) from four countries, Farrall and Holder (1992) found that the maximum effect of any single locus (λ) ranged from approximately 5 to 12. This is in relatively good agreement with our estimate of $\lambda = 6$.

On the basis of complex segregation analysis, Chung et al. (1986) and Marazita et al. (1986*a*) both concluded that a mixed model, including a major-locus effect in addition to an MFT component, provided the best fit to CL ± P. Although it is possible that these analyses had sufficient power to detect a gene with an effect of $\lambda_1 = 6$ or less, a number of factors suggest that the results of these analyses should be cautiously interpreted. The relatively poor fit of the mixed and gSML models' predictions to the observed risks in these populations is one such factor. Another factor is the different conclusions drawn from the analyses by Chung et al. (1986) and Marazita et al. (1986*a*) regarding the action of alleles at the major locus.

Marazita et al. (1986*a*) concluded that the allele conferring high risk of CL ± P acts in a dominant fashion, whereas Chung et al. (1986) concluded that this allele acts as an autosomal recessive. This would suggest that different genetic loci are involved in the determination of CL ± P in these populations. However, in view of the interpopulation similarity in the familial aggregation patterns demonstrated by CL ± P (Mitchell 1991), this seems unlikely. Furthermore, autosomal recessive inheritance of CL ± P is quite unlikely, since the observed CL ± P risk to siblings does not exceed the risk to offspring (Woolf et al. 1963; Fujino et al. 1967; Bixler et al. 1971; Koguchi 1975; Carter et al. 1982).

The different conclusions concerning mode of inheritance, drawn from the analysis of the LON and DEN data, are likely to be attributable to differences in the types of families analyzed, rather than to etiologic heterogeneity of CL ± P. Discrimination between autosomal recessive and autosomal dominant inheritance requires information on the risk to both siblings and offspring (or parents) of probands. When information on one of these types of relatives is missing or contributes relatively little to the overall likelihood of the data, discrimination between these models will be difficult, and misleading conclusions may be reached. Over 50% (424/785) of the pedigrees analyzed by Marazita et al. (1986*a*) included proband parents, whereas in the DEN data only 11% (329/2,998) of

the pedigrees included an affected parent, an unspecified number of which were probands. Thus, it is likely that the data analyzed by Chung et al. (1986) did not provide the information required to discriminate between autosomal recessive and autosomal dominant inheritance.

The results of complex segregation analysis of 79 CL ± P families ascertained in southeast Minnesota have recently been reported and have been interpreted as providing strong evidence that CL ± P is inherited as an autosomal dominant or codominant trait with reduced penetrance (Hecht et al. 1991*b*). These data were analyzed by using both POINTER (Lalouel and Morton 1981) and regressive models (Bonney 1986). The autosomal dominant and codominant models with reduced penetrance were the most parsimonious regressive models, as judged by Akaike's information criteria (AIC). These models, as estimated by POINTER, also provide a good fit to the data, relative to the fit of the general model. However, on the basis of AIC, the MFT, which is not evaluated in the regressive approach, was the most parsimonious model. It is not possible to assess the fit of these models to the data from which they were derived, since risk figures were not provided for the relatives of probands in this study. The likelihood distribution for nine of the multiplex families in these data, under the competing hypotheses evaluated by POINTER (Hecht et al. 1991*b*, fig. 2), does, however, indicate that the likelihood of each of these families is similar under the MFT model and the autosomal dominant and codominant models.

The results obtained from the POINTER analysis of these 79 families are, therefore, consistent with our conclusions concerning the mode of inheritance of CL ± P. The regressive results cannot be used as evidence against our conclusion that CL ± P is determined by multiple loci, since complex models of inheritance were not evaluated.

Additional claims for single-gene inheritance of CL ± P have come from studies which suggested an association between CL ± P and TGFA (Ardinger et al. 1989; Chenevix-Trench et al. 1991). On the basis of combined data from these studies, λ_R for TGFA in offspring and siblings of affected individuals is only 1.21 and 1.23 (calculated as in Risch 1987), respectively. It is not surprising that, given the small values of λ_R, Hecht et al. (1991*a*) found no evidence of linkage between TGFA and CL ± P. Thus, neither TGFA nor any locus in strong linkage disequilibrium with TGFA appears to significantly influence the risk of nonsyndromic CL ± P.

In conclusion, the available data are consistent with CL ± P being determined by either MFT inheritance or multiple, interacting loci, with a maximum effect of $\lambda_1 = 6$ for any single locus. However, these results should be interpreted somewhat cautiously, because of limitations of the available data. Of particular concern are the lack of precise estimates of the risk to MZ twins of CL ± P probands and the potential for biased reporting of second-degree relatives of CL ± P probands, since λ_{MZ} and λ_2 provide (*a*) critical information for determining the number and magnitude of effect (i.e., λ) of individual genetic loci and (*b*) the power to detect linkage using affected relative pairs depends only on λ (Risch 1990*c*). Prior to undertaking linkage analyses of CL ± P, it would, therefore, seem prudent to obtain more reliable estimates of λ_{MZ} and λ_2 and to delineate more precisely the maximum effect of any single locus on the risk of CL ± P.

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