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Analysis of the Recurrence Patterns for Nonsyndromic Cleft Lip With or Without Cleft Palate in the Families of 3,073 Danish Probands

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The identification of several putative susceptibility loci for nonsyndromic cleft lip with or without cleft palate (CL ± P) has sparked a renewed interest in the genetics of this condition. However, prior to undertaking linkage studies for complex traits such as CL ± P it is desirable to have some understanding of the number and nature of the loci involved in disease susceptibility. The ability to obtain valid estimates of these parameters is contingent on the availability of family data which are unbiased by factors that distort the true familial recurrence pattern. In an effort to obtain such data, 2 centralized data repositories (the Danish Central Person Registry and the Danish Facial Cleft Database), were linked and used to estimate the risks to first, second, and third-degree relatives of 3,073 CL ± P probands born in Denmark from 1952 to 1987. Analyses of these data excluded single locus and additive multilocus inheritance of CL ± P, and provided evidence that CL ± P is most likely determined by the effects of multiple interacting loci. Under a multiplicative model, no single locus can account for more than a threefold increase in the risk to first-degree relatives of CL ± P probands. These data provide further evidence that nonparametric linkage methods (ex. affected relative pair studies) are likely to represent a more realistic approach for identifying CL ± P susceptibility loci, than are traditional pedigree-based methods. However, at least 100 and more realistically several hundred (300–500) affected sib pairs are likely to be required to detect linkage to CL ± P susceptibility loci. © 1996 Wiley-Liss, Inc.

KEY WORDS: cleft lip, genetics, etiology, familial recurrence

INTRODUCTION

Nonsyndromic cleft lip with or without cleft palate (CL ± P) is one of the most common, serious malformations of the human structure. The prevalence of CL ± P typically ranges from 1 to 2 per 1,000 livebirths in the general population, but is significantly higher among the relatives of affected individuals. Compared to the risk in the general population, it has been estimated that the risks to first, second, and third-degree relatives of CL ± P probands are increased by approximately 32, 6, and 4-fold, respectively [Mitchell and Risch, 1992].

The work reported by Fogh-Andersen [1942] appears to represent the first rigorous analysis of the familial aggregation patterns demonstrated by CL ± P. Based on this work, Fogh-Andersen concluded that CL ± P is etiologically distinct from isolated cleft palate (CP), and suggested that CL ± P is inherited as a "conditional dominant" with sex limitation to males. Fogh-Andersen's conclusion regarding the etiological distinction between CL ± P and isolated CP has been supported by several subsequent studies [Woolf et al., 1963; Welch and Hunter, 1980; Carter et al., 1982], and is still generally regarded to be true. However, the mode of inheritance for CL ± P has been, and continues to be, a much debated topic [Melnick et al., 1980; Melnick, 1992; Mitchell and Risch, 1992].

Although mode of inheritance questions are difficult to answer definitively, Risch [1990a] has described methods for analyzing familial recurrence patterns that can offer some clues. These methods have recently been used to reanalyze the existing family data for CL ± P [Farrall and Holder, 1992; Mitchell and Risch, 1992] and indicate that the familial recurrence pattern for CL ± P is not compatible with the effects of a single

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major locus, but is compatible with either the multifactorial threshold (MFT) model of inheritance or a model involving multiple interacting loci (MIL). However, it has been noted that these results should be interpreted somewhat cautiously, due to several limitations of the data which were available for these reanalyses [Mitchell and Risch, 1992]. These limitations included the lack of a precise estimate of the risk to monozygotic (MZ) cotwins of affected individuals, as well as the potential for biased reporting of second and third-degree relatives of CL ± P probands. The latter is of concern because all of the family data used in the reanalyses were obtained directly from the proband (or a relative of the proband), and are thus subject to systematic reporting errors that could distort the true pattern of familial aggregation. The possibility that the proband's (or proband's family's) willingness to participate was related to the presence of additional affected relatives also represents a potential source of bias (e.g., selection bias) in the recurrence risks obtained in these studies. In addition, many of these studies were subject to selection bias resulting from the ascertainment of probands through surgical records.

Therefore, the goals of the present analyses were to i) obtain estimates of the risks to first, second, and third-degree relatives of CL ± P probands that are unlikely to be influenced by systematic reporting errors or selection bias and ii) use this information to delineate more precisely the mode of inheritance of CL ± P using the methods developed by Risch [1990a]. To this end, recurrence risks for first, second, and third-degree relatives of 3,073 Danish CL ± P probands were obtained by linking records from 2 centralized data repositories: The Danish Central Person Registry (CPR) and The Danish Facial Cleft Database (DFCD).

MATERIALS AND METHODS

The Danish CPR was established in 1968 and has assigned a unique, 10-digit personal identification number (PIN) to all individuals residing in Denmark on, or after April 1, 1968. Parental PINs are also recorded for the individuals in this registry, thus allowing for the construction of sibships (by matching individuals with identical maternal and/or paternal PINs) which can be linked to form more complex pedigrees. However, the availability of parental PINs is strongly related to an individual's year of birth. Maternal PINs are available on less than 10% of individuals born prior to 1952, 43% of individuals born in 1952, 96% of individuals born in 1959, and approximately 100% of later born individuals. A similar trend is also apparent for paternal PINs, although their availability tends to be slightly less than that of maternal PINs. In addition, it should be noted that the parental PINs for adopted individuals refer to adoptive rather than biologic parents.

The DFCD ascertains liveborn infants with facial clefts from multiple sources and includes information on 7,290 cases born from 1936 to 1987. Within the DFCD, a case is designated as having an associated malformation or malformation syndrome, if the presence of such was documented in one or more of the ascertainment sources. Malformations such as neural tube defects, monogenic traits, syndromes, and sequences are designated as major anomalies in the DFCD, whereas conditions such as polydactyly and clubfoot are considered to represent minor anomalies.

It has been shown that the DFCD identifies nearly all liveborn individuals with CL ± P without associated anomalies [Christensen et al., 1992]. PINs are available on 89% of cases in this registry. Six percent of the individuals registered in the DFCD died prior to the introduction of PINs in 1968 (39% of these were born prior to 1952), and 5% could not be identified beyond name and date of birth (over 50% of these were born prior to 1952).

The present analyses are restricted to 3,073 non-twin individuals with CL ± P and no other malformation, who were born in Denmark from 1952 to 1987 and had a PIN listed in the DFCD. Individuals born prior to 1952 were excluded from these analyses, since their CPR records were unlikely to include parental PINs. In addition, individuals born prior to 1952, on whom parental PINs are available in the CPR, may not represent a random sample of all cases (e.g., parental PINs may preferentially be available for individuals who remained in their parents' home throughout adulthood as the result of physical and/or mental disabilities).

The 3,073 CL ± P probands were linked to the CPR by their PINs, and information on the total number and number of affected parents, sibs, offspring, maternal half-sibs, nieces/nephews, aunts/uncles, grandparents, cousins and half-aunts/uncles of these probands was obtained. For example, aunts and uncles of the probands were identified in the following manner: i) identify the parental PINs for the proband, ii) use the parental PINs to identify the individual CPR records for the probands' parents, iii) obtain the PINs for the parents of the probands' parents, e.g., the grandparents of the probands, iv) identify all other individuals in the CPR who also have these parental PINs, e.g., the aunts/uncles of the proband, and finally, v) link these individuals, by their PINs, to the DFCD to identify affected aunts/uncles. With the exception of grandparents, only relatives born in Denmark from 1952 to 1987 were included in these analyses. In order to obtain a reasonable sample size, grandparents born between 1936 and 1987 were included if the intervening parent was born from 1952 to 1987. Hence, all family links were established through individuals born from 1952 to 1987.

The process described above led to the identification of several relatives through more than one proband. For example, an individual with 2 sibs, each of whom had a child with CL ± P, would be included twice in the list of aunts and uncles. However, when calculating recurrence risks, such individuals were included only once in the denominator and, if applicable, once in the numerator. The risk to full sibs of CL ± P probands was estimated by the singles method [Davie, 1979], and by the proportion of affected individuals among later born sibs. Sibs who were members of a twin pair were not included in these estimates. The risks to all other types of relatives were estimated as the proportion of relatives of type R (e.g., R = parents, offspring, etc.) who were also affected. Although twins were not excluded from

these calculations, none of the affected relatives had an affected cotwin.

The pattern of familial recurrence for CL ± P was assessed by comparing the observed drop-off of $\lambda_R - 1$, where λ_R is the risk to a relative R compared to the population prevalence, with that predicted by the single major locus, additive multilocus, and multiplicative multilocus models of inheritance described by Risch [1990a], and with the MFT model of inheritance [Falconer, 1965]. Since the decline in $\lambda_R - 1$ between MZ cotwins and first-degree relatives provides information critical to the assessment of familial recurrence patterns, the risk to MZ cotwins of CL ± P probands born in Denmark from 1970 to 1990 [Christensen and Fogh-Andersen, 1993] was used to estimate λ_{MZ}. The zygosity of these twins has been assigned on the basis of 6–17 blood, serum, and enzyme types, and the probability of having misclassified a dizygotic pair as MZ is estimated to be less than 2% [Christensen and Fogh-Andersen, 1993]. These data are likely to represent the most precise estimate of the risk to MZ cotwins of CL ± P probands that is currently available.

RESULTS

The distribution of the 3,073 CL ± P probands by sex and defect severity is summarized in Table I. The characteristics of the probands in this series were similar to those reported in previous studies of Danish probands [Bixler et al., 1971; Melnick et al., 1980] and in studies from other, predominantly Caucasian, populations [Woolf et al., 1963; Bear, 1976; Carter et al., 1982; Welch and Hunter, 1980]. Specifically, there was a predominance of males (67%); unilateral clefts of the lip (77%)—of which most (68%) were left; and malformations with associated clefts of the palate (56%).

The overall sex ratio among the probands in this study was 2.1 and was relatively constant from 1952 to 1987 (Table II). However, the sex ratio among affected individuals was influenced by the specific characteristics of the malformations (Table I), with higher male to female ratios occurring among individuals with the severest malformations. Compared to affected females, affected males were slightly more likely to have bilateral lip defects (OR = 1.16; 95% CI, 0.96–1.41) and significantly more likely to have an associated cleft of the palate (OR = 1.27; 95% CI, 1.09–1.48).

TABLE I. Distribution of 3,073 CL ± P Probands Born in Denmark, 1952–1987, by Sex and Defect Severity

Defect	Total (%)	Males	Females	Sex ratio (M:F)
Cleft lip				
Unilateral				
Right	388 (13)	254	134	1.9
Left	831 (27)	531	300	1.8
Bilateral	126 (4)	85	41	2.1
Unknown	48 (2)	28	20	1.4
Total	1,393 (45)	898	495	1.8
Cleft lip + palate				
Unilateral				
Right	378 (12)	258	120	2.2
Left	768 (25)	534	234	2.3
Bilateral	503 (16)	355	148	2.4
Unknown	31 (1)	25	6	4.2
Total	1,680 (55)	1,172	508	2.3
Total	3,073	2,070	1,003	2.1

TABLE II. Distribution of 3,073 CL ± P Probands Born in Denmark, 1952–1987, by Sex and Year of Birth

Year of birth	Total (%)	Males	Females	Sex ratio (M:F)
1952–1959	680 (22)	446	234	1.9
1960–1964	503 (16)	351	152	2.3
1965–1969	473 (15)	316	157	2.0
1970–1974	440 (14)	301	139	2.2
1975–1979	435 (14)	291	144	2.0
1980–1987	542 (18)	365	177	2.1
Total	3,073	2,070	1,003	2.1

The risk to sibs of these CL ± P probands was similar when estimated by the singles method (3.14%) and when estimated as the proportion of affected individuals among all later born sibs (3.10%). In addition, the risks to sibs and offspring of these CL ± P probands were quite comparable and provided no evidence for a dominance variance component in CL ± P (Table III). However, the risk to parents was relatively (although not significantly) lower than the risks to either of these 2 groups of first-degree relatives. This is most likely attributable to selection against affected individuals, since there was no evidence of a dominance variance component for CL ± P in these data or data from several previous studies [Woolf et al., 1963; Fujino et al., 1967; Bixler et al., 1971; Koguchi, 1975; Carter et al., 1982]. Hence, for the analysis of the familial recurrence patterns, data on all sibs and offspring, but not parents, were pooled and used to estimate λ_1.

Risks were estimated for 4 types of second-degree relatives: maternal half-sibs, nieces/nephews, aunts/uncles, and grandparents. The risks for these 4 subgroups were all lower than the observed risks to first-degree relatives, and were quite similar to each other. Consequently, data for these relatives were pooled and used to estimate λ_2.

Risks were estimated for 2 types of third-degree relatives: first cousins and half-aunts/uncles. The risks for both types of third-degree relatives are higher than the observed risks for second-degree relatives. Given the small number of affected relatives, the observed increase in risk between second and third-degree relatives is likely to reflect the effects of random error. Nonetheless, this pattern is inconsistent with any genetic model of inheritance and limits the usefulness of third-degree relatives in the analysis of familial recurrence patterns. Finally, the probandwise concordance rate (0.60, 95% CI, 0.38–0.82) among MZ twins born in Denmark from 1970 to 1990 [Christensen and Fogh-Andersen, 1993] was used to estimate λ_{MZ}.

All estimates of λ_R were calculated assuming a population prevalence of CL ± P of 0.0013. This estimate is based on the total number of livebirths (N = 2,523,023) in Denmark from 1952 to 1987 and the total number of CL ± P cases without associated malformations (N =

TABLE III. Risk for First, Second, and Third-Degree Relatives of 3,073 CL ± P Probands Born in Denmark, 1952–1987

Relative	Total no.	No. affected	Risk (95% C.I.)[a]	
First-degree				
Parents	1,149	28	0.0244 (0.0162–0.0342)	
Offspring	2,882	95	0.0330 (0.0267–0.0399)	0.0321
Sibs[b]	3,435	108	0.0314 (0.0258–0.0377)	(0.0279–0.0367)
Second-degree				
Half-sibs	348	2	0.0057 (0.0005–0.0165)	
Nieces/nephews	1,144	6	0.0052 (0.0019–0.0103)	0.0055
Aunts/uncles	913	5	0.0055 (0.0017–0.0113)	(0.0031–0.0086)
Grandparents	333	2	0.0060 (0.0006–0.0170)	
Third-Degree				
Cousins	720	6	0.0083 (0.0030–0.0163)	0.0099
Half-aunts/uncles	89	2	0.0225 (0.0021–0.0644)	(0.0042–0.0179)

[a] 95% confidence interval = $\frac{\left[\sqrt{a} \pm \left(\frac{1}{2}\right)Z_{\alpha/2}\right]^2}{n}$, where a is the number of affected relatives of type R, n is the total number of relatives of type R, and $\alpha = 0.05$.
[b] Sib risk estimated by the singles method [Davie, 1979].

3,317) identified in the DFCD as having been born in Denmark during this period. Using the formulas developed by Risch [1990a], and fixing λ_1 at 24.7 (0.0321/0.0013), the expected values of λ_{MZ}, λ_2, and λ_3 were estimated under a single-locus model (which also provides a good approximation for the expectations under any additive multilocus model), a model with infinite loci of small effect, and a number of multiplicative models of inheritance. In addition, these parameters were also estimated under the MFT model of inheritance using the formula of Reich et al. [1972]. A heritability of 79%, estimated from the combined risk to sibs and offspring and a population prevalence of 0.0013, was used in these calculations.

The predications for a single-locus model with λ_1 fixed at 24.7 are given in Table IV. This model clearly underestimated the risk to MZ cotwins and overestimated the risk to second-degree relatives. Although less dramatic, the risk to MZ cotwins is also underestimated by the MFT model. In contrast, several multiplicative models of inheritance, including the model with infinite loci of small effect, provided relatively good fits to the data. Comparison of the observed risk ratios with the predictions of various multiplicative models suggests that no single gene is likely to increase the risk to first-degree relatives by more than 3-fold ($\lambda_{1(max)} = 3$), and that at most 2 or 3 loci of moderate ($\lambda_1 \sim 1.5$–2.0) effect are involved in the etiology of CL ± P.

TABLE IV. Genetic Models for CL ± P Based on Data From the Families of 3,073 CL ± P Probands Born in Denmark, 1952–1987

	λ_1	λ_{MZ}	λ_2	λ_3
Observed values	24.7	461 (292–631)[a]	4.2 (2.4–6.6)	7.6 (3.2–13.8)
Predicted values, assuming:				
Single major locus		48	12.8	6.9
MFT[b]		201	6.2	3.5
$\lambda_{11} = 1.25$[c]		586	5.0	2.2
$\lambda_{11} = 1.5$		542	5.1	2.3
$\lambda_{11} = 2.0$		457	5.3	2.3
$\lambda_{11} = 3.0$		339	5.7	2.5
$\lambda_{11} = \lambda_{21} = 1.25$		562	5.0	2.2
$\lambda_{11} = \lambda_{21} = 1.5$		482	5.2	2.3
$\lambda_{11} = \lambda_{21} = 2.0$		343	5.6	2.5
$\lambda_{11} = \lambda_{21} = \lambda_{31} = 1.25$		539	5.1	2.3
$\lambda_{11} = \lambda_{21} = \lambda_{31} = 1.5$		428	5.3	2.3
$\lambda_{11} = \lambda_{21} = 1.5, \lambda_{31} = 2.0$		361	5.5	2.4
$\lambda_{11} = \lambda_{21} = \lambda_{31} = \lambda_{41} = \lambda_{51} = \lambda_{61} = 1.25$		477	5.2	2.3
$\lambda_{11} = \lambda_{21} = \lambda_{31} = \lambda_{41} = \lambda_{51} = \lambda_{61} = 1.5$		300	5.6	2.5
Infinite loci		610	5.0	2.2

[a] Range based on 95% confidence interval for point estimate of the recurrence risk to a relative of type R.
[b] Heritability = 79%.
[c] λ_{iR} = relative increase in risk to relatives of type R that is attributable to the ith locus.

DISCUSSION

Denmark has provided and continues to offer a unique setting for genetic studies of nonsyndromic CL ± P. The Danish surgical files have provided the foundation for several family studies of nonsyndromic CL ± P [Fogh-Andersen, 1942; Bixler et al., 1971; Melnick et al., 1980], which have contributed greatly to our current understanding of the familial nature of this condition. However, these studies, like most family studies of CL ± P, were subject to several sources of bias (e.g., recall and selection bias) that may have distorted the true familial recurrence pattern for CL ± P.

The introduction of PINs and the development of the DFCD have provided the opportunity to estimate recurrence risks for CL ± P that are unlikely to be influenced by recall or selection biases. However, 2 factors may have resulted in a slight downward bias in the recurrence risks estimated from these sources: i) The lack of PINs for a small proportion (~5%) of individuals in the DFCD with birth dates from 1952 to 1987 and ii) the inclusion of adoptive relatives (who could not be distinguished from biologic relative in the CPR) in estimates of risk. Neither of these factors is likely to have had a major impact on the estimates of recurrence obtained in this study. In particular, the impact of the latter is expected to have been negligible, since only a small proportion (~2%) of individuals born from 1952 to 1987 were adopted. Furthermore, a relatively large proportion of these individuals (45% after 1972) were born outside of Denmark and would have been excluded from this study.

Comparison of recurrence risks obtained in this and earlier family studies of Danish CL ± P probands indicated that, relative to the present study, the earlier studies may have overestimated the risk to first-degree relatives (Table V). In particular, the estimates of sib recurrence obtained in the earlier studies are higher—significantly so in the 2 largest [Fogh-Andersen, 1942; Melnick et al., 1980]—than the estimate obtained in the present study. However, relative to the present study, the earlier studies exhibit no clear pattern of over- or underestimation of the risk to more remote relatives of the CL ± P probands.

The results of this study are, in general, consistent with the 2 previous studies which used similar approaches to evaluate the familial recurrence patterns exhibited by CL ± P [Farrall and Holder, 1992; Mitchell and Risch, 1992]. All 3 studies clearly excluded single-locus and additive multilocus inheritance of CL ± P, and provided evidence that CL ± P is most likely determined by the effects of multiple loci acting in a multiplicative fashion. However, in contrast to the findings of Mitchell and Risch [1992], the present data provided relatively little evidence in favor of MFT inheritance of this condition.

The present study indicated that, under a multiplicative model, no single locus can account for more than a 3-fold increase in the risk to first-degree relatives of CL ± P probands ($\lambda_{1(max)} = 3$). This estimate is considerably lower than that obtained from the studies of Mitchell and Risch [1992] and Farrall and Holder [1992]. This is at least partially attributable to the relatively high population prevalence of CL ± P (0.0013) that was used in the present analyses. For example, using estimates of the risk to first (0.0322) and second (0.0056)-degree relatives that were virtually identical to those used in the present analyses, but assuming a population prevalence of only 0.0010 for CL ± P, Mitchell and Risch [1992] estimated $\lambda_{1(max)} = 6$.

It is of interest to note that the multiplicative models of inheritance tended to overestimate the observed value of λ_2. A similar tendency was also observed in the analyses of Mitchell and Risch [1992], and could indicate that the effects of at least some CL ± P susceptibility loci are synergistic. However, such a trend is also compatible with the involvement of environmental determinants of CL ± P.

The relatively low value of $\lambda_{1(max)}$ obtained from these data provides further evidence that nonparametric linkage methods, such as affected relative pair studies, are likely to represent a more realistic approach for identifying CL ± P susceptibility loci, than are traditional pedigree-based methods [Mitchell and Risch, 1992; Farrall et al., 1993]. However, optimistically, at least 100 affected sib pairs are likely to be required in order to detect linkage to even the strongest CL ± P susceptibility loci (assuming: $\lambda_1 = 3$, $\theta = 0$ and a fully informative marker), and more realistically several hundred such pairs (300–500) will be required. Clearly then, the identification of specific CL ± P susceptibility loci is likely to require multicenter collaborations.

TABLE V. Estimates of the Recurrence Risks Among Relatives of Danish CL ± P Probands

Relative	Fogh-Andersen [1942]	Bixler et al. [1971]	Melnick et al. [1980]	Present study
Parents	0.0171		0.0260	0.0244 (0.0162–0.0342)
Sibs	0.0491	0.0387	0.0514	0.0314 (0.0258–0.0377)
Offspring	0.0195	0.0389	0.0500	0.0330 (0.0267–0.0399)
Aunts/uncles	0.0080			0.0055 (0.0017–0.0113)
Grandparents	0.0020			0.0060 (0.0006–0.0170)
Cousins	0.0025			0.0083 (0.0030–0.0163)

In conclusion, these data are relatively consistent with CL ± P being determined by a multiplicative, multilocus model of inheritance. Under such a model, no single locus is likely to account for more than a 3-fold increase in the risk to first-degree relatives of CL ± P probands. These conclusions hold regardless of the true values of other genetic parameters (i.e., the number of alleles at a given locus, gene frequencies, or penetrances), since the power to detect linkage using affected sib pairs depends only on the risk ratios λ_R [Risch, 1990b]. Moreover, these results are consistent with the estimated effects of putative CL ± P susceptibility loci, including transforming growth factor alpha and the retinoic acid receptor [Chenevix-Trench et al., 1992], and further emphasize the need for appropriately designed, large scale linkage studies of CL ± P.

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