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A cohort study of recurrence patterns among more than 54,000 relatives of oral cleft cases in Denmark: support for the multifactorial threshold model of inheritance

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Abstract

Objectives—To determine if the anatomical severity of oral clefting affects familial recurrence in a large population based sample. To provide reliable recurrence risk estimates for oral cleft for first-, second-, and third-degree relatives.

Design—Population based cohort study

Setting—Denmark

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All authors had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Participants—6,776 individuals affected with an oral cleft born from 1952 to 2005 and 54,229 relatives.

Main outcome measures—Recurrence risk estimates for oral cleft for first-, second-, and third-degree relatives and stratification by severity, specificity, parent of origin effect, and family size for first-degree relatives.

Results—For cleft lip and palate probands we observed recurrence risks for first-, second-, and third degree relatives of respectively 3.5% (95% confidence interval 3.1% to 4.0%), 0.8 % (0.6% to 1.0%), and 0.6% (0.4% to 0.8%). Individuals affected by the most severe oral cleft had a significantly higher recurrence risk among both offspring and siblings, e.g. the recurrence risk for siblings of a proband with isolated bilateral cleft lip with cleft palate was 4.6% (3.2 to 6.1) versus 2.5% (1.8 to 3.2) for a proband born with a unilateral defect.

Conclusions—Anatomical severity does have an effect on recurrence in first-degree relatives and the type of cleft is predictive of the recurrence type. Highly reliable estimates of recurrence have been provided for first cousins in addition to more accurate estimates for first and second degree relatives. These results and the majority of prior data continue to support a multifactorial threshold model of inheritance.

Keywords

recurrence risk; cleft lip and palate; severity; genetics; multifactorial threshold model

INTRODUCTION

Oral clefting is one of the most frequent congenital malformations with a birth prevalence of one to two per 1,000 live births varying by ancestral origin.[1] Despite corrective surgery, being born with an oral cleft has lifelong implications for those affected and their families. [2] Therefore, there is a continuing need for a better understanding of the aetiology and the mechanism of clefting in order to improve the counselling of families at increased risk and to identify etiologic factors that may suggest improvements in therapy or prevention.

The aetiology of oral clefting is complex with both genes [1,3–12] and the environment playing important roles.[13–19]

Oral clefts are commonly subdivided into two phenotypically and etiologically distinct groups, cleft lip with or without cleft palate and the cleft palate only.[20,21] Cleft lip with or without cleft palate can be further subdivided into cleft lip only and cleft lip with cleft palate. Cleft lip and cleft lip with cleft palate may be etiologically distinct or represent a continuum of severity with cleft lip with cleft palate being the more severe form of the defect.[22] Cleft lip with or without cleft palate can be incomplete or complete depending on the involvement of the alveolus (primary palate) and the length of the cleft in the palate (sub-mucous cleft palate or cleft in the soft palate only versus cleft in both the soft and the hard palate). Either sub-phenotype can be associated with major physical or developmental anomalies and/or be a part of a recognised syndrome. In these cases the oral cleft is classified as a syndromic cleft as opposed to an isolated or non-syndromic cleft. Isolated clefts can however be associated with minor associated anomalies. A wide range of the frequency of syndromic clefts has been reported in the literature: 10–30% for cleft lip with or without cleft palate and 20–60% for cleft palate only.[23,24]

Since the early 1950s clinical practice has been to counsel parents of a child born with a cleft on the risk of having a subsequent child with an oral cleft using empiric recurrence risks consistent with the multifactorial threshold model of inheritance.[25–27] This model has been challenged by several complex segregation analysis studies but there has never

been sufficient evidence to reject the model.[28,29] Since the 1990s several studies of both recurrence patterns as well as the identification of specific loci or genes contributing to clefts have ruled out a single, major locus model and the multiplicative additive or independent loci models. This leaves us with the best fitting model of inheritance being multiple genes interacting in a multiplicative manner which agrees with the multifactorial threshold model.[5,6,30–36] A recent study using a single, well-defined population from Norway has challenged the multifactorial threshold model since they found no effect of severity on inheritance.[37] If this result can be replicated in additional and larger studies it would have substantial implications for the clinical counselling of families and the understanding of the underlying causes of clefting.[38]

This Danish study on more than 54,000 relatives provided not only the opportunity to examine this possible paradigm shift but also the opportunity for the first time to estimate the recurrence risk for first cousins (third-degree relatives) and markedly improve the accuracy of the existing recurrence risk estimates on first- and second-degree relatives to an individual with an oral cleft.

METHODS

The present study is a population based cohort study based on record linkage between three nationwide, population based registers in Denmark.

The Civil Registration System was established in April, 1968 and it registers all individuals alive and residing in Denmark since then. All individuals have a unique ten-digit personal identification number. This register also includes identifiers that link all first-degree relatives (parents and siblings). These identifiers allow construction of sibships (by matching individuals with parental personal identification numbers) which can be linked using parent sibships to form complex pedigrees. On the maternal side links have been almost complete (96%) since 1959, but for individuals born before 1952 it is considerably lower (46%). A similar pattern is apparent for the paternal personal identification numbers although the availability tends to be slightly lower (92% post-1959 and 39% pre-1952).

The Danish Facial Cleft Database now encompasses the 1936 to 2005 cohort. It includes 10,022 live born individuals born with an oral cleft of which 9,143 (91.4%) individuals are registered by a personal identification number. Two nationwide ascertainment sources are used to ascertain the Danish individuals with oral cleft. For the earlier birth cohorts (1936 to 1987) patient lists were used, maintained by Dr. Poul Fogh-Andersen from 1934 to 1986 and since then continued at the Rigshospitalet where all surgical treatment has been centralised since 1986. At the two National Institutes for Defects of Speech, where treatment other than surgical may occur, all reports from the midwives on children born with an oral cleft have been kept since 1954. Oral clefts, mainly sub-mucous cleft palate, recognized later in a child's life are also reported to the institutes. The ascertainment is very high for the complete cohort, and capture-recapture methods have indicated 99% ascertainment for the sub-phenotype isolated cleft lip with or without cleft palate in the period 1983 to 1987.[39] In the Danish Facial Cleft Database overt oral clefts are classified into three groups, i.e. cleft lip, cleft lip with cleft palate, and cleft palate. Both cleft of the lip only and cleft of the lip and the primary palate is considered a cleft lip phenotype. A distinction is not possible in this study, which is also the case with regards to completeness of the cleft lip when it occurs together with cleft palate. Cleft lip with or without cleft palate can be subdivided into unilateral and bilateral clefts, with unilateral clefts being the mildest form and the bilateral the most severe form of cleft. The cleft palate phenotype includes the range of sub-mucous cleft palate being the mildest form, to cleft in the soft palate only (the intermediate form) to the most severe form, cleft in the hard and soft palate. Bifid uvula is considered a microform

of cleft palate. Recently it has been suggested that orbicularis oris muscle defects and dental anomalies can also be considered microforms of oral cleft. Expanding the phenotypes of oral clefting will greatly improve future genetic studies, but in this study it has not been possible to take the microforms into account due to incomplete ascertainment.[40–44]

In the Danish Facial Cleft Database 876 (9.6%) of the individuals born with an oral cleft are registered as also having at least one non-cleft major anomaly or a recognized syndrome. Malformations such as neural tube defects were designated as major anomalies. Defects such as polydactyly were considered minor malformations. Minimal defects such as nevi were not considered associated anomalies. The classification of the associated anomalies into minor and major has been maintained to maintain consistency in the Danish Facial Cleft database since it has been used from the inception of the registry.[1] It is based on whether the anomaly is likely to be part of a syndrome. For the earlier birth cohorts from 1936 to 1987 the number of individuals born with either an associated major anomaly or a syndrome was likely underestimated[39] but for the later birth cohorts medical records were reviewed by Bille et al. in 2005 to obtain more complete information about associated anomalies/syndromes.[45,46] The recorded number of associated anomalies/syndromes are slightly lower in the Danish population compared to other populations,[23,47] but the pattern with more anomalies/syndromes associated with cleft palate compared to cleft lip with or without cleft palate is the same. Table 1 shows the frequency of the syndromic oral clefts according to the cleft phenotypes and the time period observed in the Danish Facial Cleft Database.

The Danish Twin Registry includes the birth cohorts from 1870 to 2004 corresponding to more than 80,000 twin pairs. The twins are all born in Denmark and they were ascertained independently of any disease. Before 1968 the ascertainment was about 90%, but since the establishment of the Civil Registration System it has been considered complete.[48] Zygosity determination on same sex twins has been validated and the misclassification rate has been found to be less than 5%.[49,50] About 75% of the twins in the registry have an assigned zygosity. In the Danish Twin Registry overall 85% of the twins are registered with a personal identification number, and since 1968 100% of the twins have a personal identification number that enables linkage to the Civil Registration System, hence linkage to relatives of an individual with an oral cleft can be established.

Study Population

For the present study the population was restricted to all live born individuals with a valid personal identification number in the Civil Registration System. The children were born in Denmark between 1952 and 2005 and were registered with an isolated cleft lip, cleft lip with cleft palate, or cleft palate only with no recognized syndrome or non-cleft major malformation. Individuals born before 1952 were excluded since their records in the Civil Registration System were unlikely to include parental links. We made an exception for the grandparents of the probands so that grandparents born from 1936 to 2005 were included but only if the intervening parent was born between 1952 and 2005.

Operationally, the probands from the Danish Facial Cleft Database were first linked to the Civil Registration System using their personal identification numbers. Because the Civil Registration System allowed the identification of the parents, full and half siblings, offspring, grandparents, full- and half-nieces/-nephews, full- and half-aunts/-uncles, and cousins for each proband we were able to count the total number of affected and unaffected relatives of each cleft type. Different sets of files were created with the proband or the parents of the proband as the index-case. Finally the Danish Twin Registry was linked to the Danish Facial Cleft Database in order to identify twin pairs of whom at least one of the twins was affected with an oral cleft. Using the described procedure, several relatives were identified more than once through one proband. For example, a woman with two siblings,

each of whom had a child with an oral cleft, could be included as an aunt twice. In our computations of the recurrence risk such individuals were only counted once.

The recurrence risk was estimated by dividing the number of affected relatives of type R (R = parents, offspring, etc.) by the total number of relatives R . The risk to full siblings is therefore equivalent to the 'singles' method described by Davie (1979)[51] under complete ascertainment. We also estimated the recurrence risk among later born siblings and for full siblings according to family size. Proband and siblings who were members of a twin pair were not included in these estimates. For all other types of relatives, twins were included as both probands and relatives in order to keep the groups as comparable as possible. We computed the relative risk (λ) for a type of relative R of affected individuals compared with the background population by dividing the recurrence risk to a relative R by the population prevalence.[5,6]

For relatives of the three groups of probands, isolated cleft lip, isolated cleft lip with cleft palate, and isolated cleft palate we have provided the estimates of the recurrence risk of all types of oral clefts. For first-degree relatives we also provided the recurrence risks for different degrees of severity, for the same or dissimilar types of isolated oral clefts, according to family size, and with respect to parent of origin effect. We graded the bilateral clefts as more severe than unilateral for both cleft lip with cleft palate and for cleft lip only. For the cleft palate cases, sub-mucous cleft palate was graded as the mildest form and involvement of both the hard and soft palate as the most severe form.

Heterogeneities between risks were computed from the Pearson's chi-squared test or from the exact test using mid- p approach when numbers were very small.[52]

A total of 3,703,337 live births were registered in Denmark during the period of 1952 to 2005. The analyses were carried out on 2,116 isolated cleft lip probands, 2,572 isolated cleft lip with cleft palate probands, and 2,088 isolated cleft palate only probands.

The Intercooled Stata 9.2 and SAS software (SAS Institute, Inc., SAS/STAT® Version 9.1, Cary, NC) were used for all computations.

RESULTS

Unless specifically noted, all of the results and discussion are for isolated oral clefts.

The population prevalence of oral clefts in Denmark for the period 1952 to 2005 including associated anomalies/syndromes was 2.1 per 1,000 live births.

Among the 9,143 individuals affected by an oral cleft registered in the Danish Facial Cleft Database from 1936 to 2005 we observed two cleft lip with or without cleft palate cases for each cleft palate only case (Table 1). Approximately 2% of cleft lip, 8% of cleft lip with cleft palate, and 18% of the cleft palate only cases were associated with one or more major anomalies or syndromes. In the youngest birth cohorts these proportions had increased to approximately 5%, 12%, and 37%, respectively. We observed a predominance of males in the cleft lip and cleft lip with cleft palate groups and an excess of females in the cleft palate group, all in accordance with previous studies.

Recurrence risk for first-, second-, and third-degree relatives

The results of the recurrence risk (absolute and relative risk (λ)) for relatives of individuals affected by a cleft lip, cleft lip with cleft palate, or cleft palate only are shown in Table 2. The recurrence risk for siblings of the cleft lip with cleft palate probands was estimated to 3.9% (95% confidence interval 3.2% to 4.7%) and it was comparable to the

estimate for the later-born siblings of 4.6% (3.5% to 5.8%). The risk of cleft lip with cleft palate for the offspring was 4.1% (3.2% to 5.1%) and also similar to the risk for the siblings. The risk to parents however was 2.5% (1.8% to 3.1%); this was significantly lower than the risk to either of the two other groups of first-degree relatives. The relative risk of cleft lip with cleft palate for all first-degree relatives was 17 (95% confidence interval 15 to 19) times higher than the risk observed in the background population.

Recurrence risk was estimated for four types of second-degree relatives: half siblings, nieces/nephews, aunts/uncles, and grandparents; they were lower than the risk to first-degree relatives and yet quite similar to each other. The risk of cleft lip with cleft palate for second-degree relatives was 4 (3 to 5) times higher than the risk observed in the background population.

Recurrence risks were estimated for three types of third-degree relatives: first cousins, half-nieces/nephews, and half-aunts/uncles. The risks were all lower than the risks to second-degree relatives and were quite similar to each other. The risks of cleft lip with cleft palate for third-degree relatives were 3 (2 to 4) times higher than the risk observed in the background population.

The same pattern was found for the other two cleft types for all three kinds of relatives.

For first cousins in particular the recurrence risk estimates for the three cleft types were indistinguishable (Table 2). The overall estimate of the recurrence risk for oral cleft for first cousins was 0.4% (95% confidence interval 0.3% to 0.6%), i.e. 2 (1.5 to 2.7) times higher than in the background population.

Recurrence risk by severity, specificity, parent of origin effect, and family size for first-degree relatives

The recurrence risk stratified by severity for siblings, e.g. from bilateral cleft lip with cleft palate to bilateral cleft lip with cleft palate of 4.6% (95% confidence interval 3.2% to 6.1%) show that the most severe cleft type for both cleft lip and cleft lip with cleft palate tends to recur. The only exception from that pattern was for cleft palate only where the moderate severity (from soft cleft palate to soft cleft palate) had the highest recurrence risk (3.9% (95% confidence interval 2.5% to 5.6%)), although not statistically different from the severest form (Table 3). We observed the same pattern for subsequent siblings (results not shown). Using data from the Norwegian study[37], re-classified in order to be comparable to our severity classification, we found a consistent pattern of repeating the most severe cleft type for all cleft types, including cleft palate (Table 4). No statistically significant heterogeneity between the recurrence risks was seen within each phenotype. The same pattern was seen for offspring (results not shown).

The recurrence risk within each subtype of oral cleft for siblings, e.g. from cleft lip with cleft palate to cleft lip with cleft palate showed a consistent pattern of recurrence specificity with the highest recurrence risk to the same subtype within all three subtypes (Table 5). The same pattern was found for subsequent siblings and offspring but with less statistical power (data not shown). For the two known distinctly different defects cleft lip with or without cleft palate and cleft palate we found a crossover risk that was significantly lower than the recurrence risk within the type but slightly higher than the risk in the background population (e.g. for cleft palate to cleft lip with cleft palate 0.2% (95% confidence interval 0.0 to 0.4%).

We estimated the recurrence risk for the offspring stratified by whether the relatives were on the maternal or paternal side of the case. For the cleft lip and cleft lip with cleft palate that is predominant in males, we found the highest recurrence risk for children when the mother

was affected and for the cleft palate with female predominance we found the highest recurrence risk for children when the father was affected (Table 6). Within each phenotype the recurrence risks were however not statistically significant.

The recurrence risk according to family size for full siblings (1,787 siblings, 44 affected) showed the same pattern of increasing risk with an increasing number of children in a family for all sub-phenotypes, e.g. for cleft lip with cleft palate the recurrence risk increased from 2.0% (95% confidence interval 1.2 to 2.9%) in a family with 2 children to 6.5% (95% confidence interval 1.2 to 16.0%) in a family with 4 children. Though not statistically significant, the direction of the point estimate is clear.

DISCUSSION

For the siblings and offspring we found that severity does have an effect on the recurrence risk for oral clefting, with the only statistically non-significant exception for moderate cleft palate severity. We found complete specificity of the recurrence risk within the distinct cleft types. As have others, we found that cleft lip and cleft lip with cleft palate occur more frequently in males than females whereas there is a female excess with cleft palate.[53] Affected mothers have the highest risk of passing on cleft lip and cleft lip with cleft palate and the affected fathers have the highest risk of passing on cleft palate.

Our recurrence risk estimates on first-, second-, and third-degree relatives are in good agreement with the recurrence risk estimates on our first report on a smaller subset of the Danish population for the isolated oral clefts born between 1952 and 1987.[5,6] The precision of the estimates has been increased markedly and has benefitted from an increase in sample size by a factor ten for numbers of phenotyped relatives. For the first time it is possible to present reliable estimates for first cousins (and other third-degree relatives) of individuals affected by a cleft lip, cleft lip with cleft palate, or cleft palate.

Due to the use of record linkage from the highly reliable Danish national registers instead of self recorded family history and the fact that the Danish population is well-defined and genetically homogeneous, our study avoids common limitations such as the grouping of all oral clefts together or incomplete ascertainment.[38]

The hallmarks for multifactorial inheritance are: 1. Most affected children have normal parents, 2. Recurrence risk increases with the number of affected children in a family, 3. Recurrence risk increases with severity of the defect, 4. Consanguinity slightly increases the risk for an affected child, 5. Risk of affected relatives falls off very quickly with the degree of relationship and, 6. When the two sexes have a different probability of being affected, the least likely sex, if affected, is the most likely sex to produce an affected offspring.[54]

In the present study we observed a higher recurrence risk among offspring and siblings compared to parents, a tendency to repeat the same cleft type in the recurrence, a strong effect on recurrence according to severity, a steep drop-off in the recurrence risk from first to second degree relatives and from second to third degree relatives, and the highest recurrence risk in the least frequently affected sex. We also observed a tendency towards an increasing recurrence risk for full siblings with an increased number of sibs but the results did not reach formal significance. All these results support the multifactorial threshold model of inheritance; hence our data do not support a shift away from the use of the multifactorial threshold model of inheritance. This model has been in use since the 1960s [25–27] and despite several challenges[28,29,37] it still appears to be the best model to explain the aetiology of oral clefting.[5,6,30–36]

The recent study from Norway challenged this model. The Norwegian analysis included stillbirths and syndromic forms of clefting with the isolated forms and pooled cleft lip only cases with cleft lip with cleft palate cases. It found no effect of severity on the recurrence risk using a detailed classification system different from the one used in Denmark for cleft lip. For cleft palate the classifications were the same.[37] When the Norwegian data were reanalysed using a similar strategy to the one in this report (i.e. with exclusion of stillbirths and associated malformations and syndromes, the distinction between cleft lip with cleft palate and cleft lip only, and the use of the same classification of severity as the one reported here), we found that the observed values of recurrence risks according to cleft severity are consistent with the expectations under the multifactorial threshold model (Table 4). We therefore find that the Norwegian results and the results presented here using a larger (three times the size) population based sample on a cohort from a neighbouring country do not contradict each other.

A few factors may, however, contribute to a slight underestimation of our recurrence risk estimates. First is the lack of personal identification numbers on the 6% that we excluded in order to be able to do the linkage to the Civil Registration System for all probands. To exclude any selection bias on this behalf we did the analysis for the 1968 to 2005 birth cohorts (results not shown) in which fewer personal identification numbers are missing (3%), and the point estimates remained virtually unchanged. Another factor that may have biased our results towards an underestimation is the fact that only legally identifiable parental links are used in the Civil Registration System so in the case of adoption or non-paternity the children cannot be identified. In the Danish Facial Cleft Database, however only individuals born in Denmark are included. According to the national Statistics Denmark adoptive children comprise a maximum of 1.5% of all the birth cohorts in the present study, and about 90% of them are born outside of Denmark, hence excluded in the Danish Facial Cleft Database.[55] Any bias from this is likely to be minimal.

In general the ascertainment is very high for oral clefts in Denmark, but the ascertainment of cleft palate is slightly lower due to the milder forms being asymptomatic until development of speech or even longer, but when diagnosed they are reported to the speech institutes. Due to the 70 year long follow-up period in the Danish Facial Cleft Database selection bias due to this late entry of the cleft palates is likely to be minimal.[39,56]

To some extent there is differential misclassification in the earlier birth cohorts in the Danish Facial Cleft Database, since individuals with undiagnosed associated anomalies or syndromes can be misclassified as individuals with isolated oral clefts. Yet this only concerns the milder forms of associated anomalies/syndromes since the most severe cases were ascertained in connection with surgery. The slight increase in the crossover risk between clefts involving the lip and those involving the palate only could be explained by the chance occurrence of syndromic clefts or by genes like *MSX1* and *IRF6* where both types of clefts may appear in the same family and with no additional phenotypic traits to result in it being assigned to a syndrome category. Since the initiation of the update of the Danish Facial Cleft Database from 1988 to 2005 the ascertainment and classification of associated anomalies/syndromes have been enhanced considerably.[45] The analyses based on truncated periods, such as the 1968 to 2005 birth cohorts, provided similar results to those of the present study so we believe that this bias is likely to be minimal.

We did not expect a higher prevalence of oral cleft among those who married persons from the oral cleft cohort. We did indeed observe very few affected spouses, 1.5 per 1,000, which is a little less than the population frequency; hence it is unlikely to influence the present results of familial recurrence risk patterns. In addition, the Danish population is in general

known to be both homogeneous and to have a low incidence of consanguinity among ethnic Danes.[57]

The recurrence risk for siblings might be biased if parents had fewer children than expected after having a first child born with oral cleft. If that is the case, the risk for all siblings would be underestimated and different from the risk for the later-born siblings. We computed both risks and the results support no such assumption.

In the present study, for each sub-phenotype of oral cleft and for each grouping of relatives of individuals affected by an isolated oral cleft, we chose to present the recurrence risks to oral cleft of any kind. These estimates were expectedly higher than the estimates of the recurrence risks to isolated oral cleft.

Although cleft lip alone and cleft lip with cleft palate have been considered the same both embryological and epidemiological since the work of Fogh-Andersen[20] in the 1940s increasing evidence, including the work reported here, suggests that important differences may be present. Earlier Harville et al[22] presented evidence of epidemiological differences in cleft lip only cases and molecular data for differences have also been recently published. [19] In the molecular case a common variant in a *TFAP2A* binding site in the enhancer regions of the *IRF6* gene has one allele that strongly predisposes families to isolated cleft lip only (odds ratio ~ 3) but has little effect on cleft lip with cleft palate. The effect acts in populations of different geographic origin and has an attributable fraction of 18% in Danish and Norwegian cases. This coupling of epidemiological and molecular findings, as well as new data on the role of sub-phenotypes such as orbicularis oris defects in clefts[43] or evolving data from genome wide associations studies of clefting[8] will enable more specific studies of aetiology as well as the ability to provide more family specific recurrence risks in the future.

In conclusion, these analyses benefit from the very high quality of the Danish population based data sources in which biases are likely to be minimal and the large sample size has allowed us to provide very reliable estimates. Our results are consistent with the majority of studies done on oral cleft recurrence which support the multifactorial threshold model as the best explanation of the inheritance of oral clefting and consistent with a recent study when the same variables are analysed.

We have substantially improved the precision of the estimate of the recurrence risk for the Danish population and for the first time we have provided estimates for first cousins. This study will improve the counselling of individuals with an oral cleft or relatives of an individual with an oral cleft. Some similarities between different populations can be shown, as in the current study between the Danish and the Norwegian population, but the Danish population also shows some significant genetic and environmental differences from other populations. Thus these results should be replicated in other populations to improve their generalisability. It also supports looking for etiologic factors based on specific cleft type and that different factors (genes or variants within the same genes) may be relatively more active in cleft lip alone versus cleft lip with cleft palate versus cleft palate alone.

Summary

Oral clefting is one of the most frequent congenital malformations. The inheritance is complex and related to both environmental and genetic factors. Recently it was questioned whether the sustained practice of recurrence counselling based on the multifactorial threshold model was valid. In particular it was suggested that severity did not affect recurrence estimates.

Anatomical severity does indeed have an effect on recurrence in first degree relatives, and the type of cleft is predictive of the recurrence type according to findings from a Danish cohort study by Grosen and colleagues of more than 54,000 relatives of an individual with oral cleft. For the first time recurrence risk estimates for first cousins have been provided. These results and the majority of prior data continue to support a multifactorial threshold model of inheritance for oral clefting. This study will improve the counselling of families at increased risk and the continued search for etiologic factors for oral clefting.

What is already known on this topic

The aetiology of oral cleft is complex with respect to both genes and environment.

The recurrence risk is increased for both first and second degree relatives but results for third-degree relatives have been inconclusive.

A recent study showed no impact of anatomical severity on the recurrence risk of oral cleft.

What this study adds

Anatomical severity does have an effect on recurrence in first-degree relatives and the type of cleft is predictive of the recurrence type.

The recurrence risk is increased for third-degree relatives by a factor 2 compared to the background population.

The results support a multifactorial threshold model of inheritance and provide important knowledge to affected family members and the persons who counsel them.

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Table 1

Frequency of individuals affected by an oral cleft according to phenotypes, time period, and sex from the Danish Facial Cleft Database (1936–2005)

	Oral clefts			CL			CLP			CP		
	Total	With major birth defects/ syndromes (%)	Sex ratio (M:F)	Total	With major birth defects/ syndromes (%)	Sex ratio (M:F)	Total	With major birth defects/ syndromes (%)	Sex ratio (M:F)	Total	With major birth defects/ syndromes (%)	Sex ratio (M:F)
1936–1951	1,524	16(1.1)	1.4	491	3(0.6)	1.8	601	4(0.7)	2.4	432	9(2.1)	0.6
1952–1961	1,268	44(3.5)	1.5	401	2(0.5)	1.7	497	20(4.0)	2.2	370	22(5.9)	0.8
1962–1971	1,541	104(6.7)	1.6	435	9(2.1)	1.8	599	33(5.5)	2.4	507	62(12.2)	1.0
1972–1981	1,455	135(9.3)	1.5	416	5(1.2)	2.0	516	41(7.9)	2.1	523	89(17.0)	1.0
1982–1991	1,289	172(13.3)	1.5	350	15(4.3)	1.7	456	42(9.2)	2.3	483	115(23.8)	0.9
1992–2001	1,548	314(20.3)	1.3	411	17(4.1)	1.6	557	93(16.7)	2.0	580	204(35.2)	0.8
2002–2005	518	91(17.6)	1.6	159	8(5.0)	2.0	201	25(12.4)	2.1	158	58(36.7)	0.9
1952–2005	7,619	860(11.3)	1.5	2,172	56(2.6)	1.8	2,826	254(9.0)	2.2	2,621	550(21.0)	0.9
1936–2005	9,143	876(9.6)	1.5	2,663	59(2.2)	1.8	3,427	258(7.5)	2.2	3,053	559(18.3)	0.8

CL: cleft lip; CLP: cleft lip with cleft palate; CP: cleft palate

Table 2
Risks of oral cleft for first, second, and third degree relatives according to the probands' three phenotypes of cleft (Denmark, 1952–2005)

	First degree relatives				Second degree relatives				Third degree relatives						
	Offspring	Siblings [†]		Parents	ALL	Half-siblings [†]	Nieces/nephews	Aunts/uncles	Grand-parents [‡]	ALL	First cousins	Half nieces/nephews	Half aunts/uncles	ALL	
		All	Subsequent												
iCL probands (n=2,116)															
Total number	1,439	2,442	1,162	1,861	5,742	810	362	2,155	2,213	1,729	6,907	3,532	544	468	4,544
No. affected*	50	60	25	47	157	8	3	20	13	4	45	11	3	1	15
Risk (%)	3.5	2.5	2.2	2.5	2.7	1.0	0.8	0.9	0.6	0.2	0.7	0.3	0.6	0.2	0.3
Relative Risk (λ) (95% confidence interval)	17 (13 to 22)	12 (9 to 15)	10 (7 to 15)	12 (9 to 16)	13 (11 to 15)	5 (2 to 9)	4 (0.8 to 10)	5 (3 to 7)	3 (2 to 5)	1 (0 to 2)	3 (2 to 4)	2 (0.8 to 3)	3 (0.5 to 7)	1 (0.0 to 4)	1.6 (0.9 to 3)
iCLP probands (n=2,572)															
Total number.	1,591	2,954	1,389	2,209	6,754	938	454	2,702	2,742	2,093	8,475	4,303	649	547	5,499
No. affected*	65	116	64	55	236	5	3	22	29	10	66	22	6	5	33
Risk (%)	4.1	3.9	4.6	2.5	3.5	0.5	0.7	0.8	1.1	0.5	0.8	0.5	0.9	0.9	0.6
Relative Risk (λ) (95% confidence interval)	20 (15 to 25)	19 (16 to 23)	22 (17 to 28)	12 (9 to 16)	17 (15 to 19)	3 (0.8 to 5)	3 (0.6 to 8)	4 (2 to 6)	5 (3 to 7)	2 (1.1 to 4)	4 (3 to 5)	2 (1.6 to 4)	4 (2 to 9)	4 (1.4 to 9)	3 (2 to 4)
iCP probands (n=2,088)															
Total number	1,211	2,379	1,171	1,820	5,410	828	396	2,002	2,175	1,550	6,555	3,344	478	521	4,343
No. affected*	51	78	39	38	167	8	5	22	12	7	49	15	2	4	21
Risk (%)	4.2	3.3	3.3	2.1	3.1	1.0	1.3	1.1	0.6	0.5	0.7	0.4	0.4	0.8	0.5
Relative Risk (λ) (95% confidence interval)	20 (15 to 26)	16 (13 to 20)	16 (12 to 22)	10 (7 to 14)	15 (13 to 17)	5 (2 to 9)	6 (2 to 13)	5 (3 to 8)	3 (1 to 4)	2 (0.9 to 4)	4 (3 to 5)	2 (1.2 to 3)	2 (0.2 to 6)	4 (1.0 to 8)	2 (1.5 to 3)

NOTES: Prevalence of oral clefts in the background population born in Denmark between 1952 and 2005: (7,619)/(3,703,337) = 0.21%

Confidence intervals are computed from $C^*(\sqrt{a} \pm \frac{1}{2}Z_{\alpha/2})^2/n$, where a = no. of affected relatives of type R, α =0.05 and C=100 for the confidence interval of the risk in percentage and C=1/prevalence in the background population) for the confidence interval of the relative risk

iCL: isolated cleft lip; iCLP: isolated cleft lip with cleft palate; iCP: isolated cleft palate

* Number of relatives affected by an oral cleft (including syndromic oral cleft and oral cleft with associated anomalies)

[‡] For computation of the recurrence risks for siblings, twins are excluded from both groups of the probands and their siblings; the numbers of iCL, iCLP and iCP probands are respectively 2,055, 2,487 and 2,044.

[‡]Grandparents of probands are born between 1936 and 2005

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Recurrence Risk for siblings of having the same phenotype of cleft as the probands according to laterality or severity of clefting (Denmark, 1952–2005)

Table 3

Phenotype	Sub-phenotype	Total number of siblings	Number	Recurrence for siblings	
				Risk (%) (95% confidence interval)	<i>p</i> (heterogeneity) *
iCL probands	Unilateral	1,977	27	1.4 (0.9 to 1.9)	0.50
	Bilateral	205	4	2.0 (0.5 to 4.3)	
iCLP probands	Unilateral	1,963	49	2.5 (1.8 to 3.2)	0.004
	Bilateral	854	39	4.6 (3.2 to 6.1)	
iCP probands	Sub-mucous CP	659	18	2.7 (1.6 to 4.1)	0.75
	Soft CP	622	24	3.9 (2.5 to 5.6)	
	Soft-Hard CP	999	26	2.6 (1.7 to 3.7)	

NOTES: 11%, 5% and 5% of siblings for respectively the iCL, the iCLP and the iCP probands are not included in these numbers because of unknown sub-phenotype of the probands
Twins are excluded from both groups of the probands and their siblings.

iCL: isolated cleft lip; iCLP: isolated cleft lip with cleft palate; iCP: isolated cleft palate

*
Pearson Chi-squared test

Recurrence risk for subsequent siblings of having the same phenotype of cleft as the probands according to laterality or severity of clefting (Norway from Sivertsen et al. 2008, BMJ)

Table 4

Phenotype	Sub-pheno Type	Total number of siblings	Recurrence for siblings		
			Number	Risk (%) (95% confidence interval)	p (heterogeneity)*
iCL probands	Unilateral	189	1	0.5 (0.0 to 2.1)	0.23
	Bilateral	24	1	4.2 (0.0 to 16.3)	
iCLP probands	Unilateral	173	4	2.3 (0.6 to 5.1)	0.18
	Bilateral	65	4	6.2 (1.6 to 13.7)	
iCP probands	Submucous CP	22	0	-	0.17
	Soft CP	84	2	2.4 (0.2 to 6.8)	
	Soft-Hard CP	71	4	5.6 (1.5 to 12.5)	

Compared to the published data (Sivertsen et al. 2008), we excluded stillbirths and minor anomalies from both groups of the probands and their subsequent siblings.

iCL: isolated cleft lip; iCLP: isolated cleft lip with cleft palate; iCP: isolated cleft palate

* Exact test using mid-p approach, specific for very small numbers

Table 5

Specificity of the recurrence risks for siblings (Denmark, 1952–2005)

Phenotype	Total number of siblings	Recurrence for siblings					
		Number		Risk(%) (95% confidence interval)			
		iCL	iCLP	iCL	iCLP	iCLP	iCP
iCL probands	2,442	35	24	0	1.4 (1.0 to 1.9)	1.0 (0.6 to 1.4)	-
iCLP probands	2,954	22	87	4	0.7 (0.5 to 1.1)	2.9 (2.4 to 3.6)	0.1 (0.0 to 0.3)
iCP probands	2,379	0	4	67	-	0.2 (0.0 to 0.4)	2.8 (2.2 to 3.5)

NOTE: Twins are excluded from both groups of the probands and their siblings.
iCL: isolated cleft lip; iCLP: isolated cleft lip with cleft palate; iCP: isolated cleft palate

Recurrence risk for offspring of having the same phenotype of cleft as the probands by the gender of the affected relative (Denmark, 1952–2005)

Table 6

Phenotype	Gender	Total number of offspring	Recurrence for offspring		
			Number	Risk (%) (95% confidence interval)	p (heterogeneity)*
iCL probands	Male	865	14	1.6 (0.9 to 2.6)	0.27
	Female	574	14	2.4 (1.3 to 3.9)	
iCLP probands	Male	993	19	1.9 (1.1 to 2.9)	0.11
	Female	598	19	3.2 (1.9 to 4.8)	
iCP probands	Male	456	15	3.3 (1.8 to 5.2)	0.35
	Female	755	18	2.4 (1.4 to 3.6)	

iCL: isolated cleft lip; iCLP: isolated cleft lip with cleft palate; iCP: isolated cleft palate

* Pearson Chi-squared test