Cleft lip with or without cleft palate: identification of sporadic cases with a high level of genetic predisposition

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SUMMARY Previous studies have suggested that asymmetry for certain bilaterally represented features may be an indicator of genetic predisposition to cleft lip with or without cleft palate and may therefore be of value in the individual assessment of recurrence risk, particularly for sporadic cases. An asymmetry score has been devised that may be of use in identifying those with a high level of genetic predisposition. Stepwise logistic regression selected nine variables that together correctly classified 85% of familial cleft patients and unrelated non-cleft controls. Applying the same regression equation to sporadic cases, 26% fell into the range occupied by the majority of familial patients, suggesting that these had a high level of genetic predisposition.

Cleft lip with or without cleft palate (CL±P) has a variety of aetiologies based on a spectrum of genetic predisposition. At the upper extreme are single gene disorders such as the popliteal pterygium, ectrodactyly-ectodermal dysplasia-clefting, and Van der Woude syndromes.1 Towards the lower extreme, environmental teratogens such as cigarette smoke, 2-4 anticonvulsant drugs, 5-10 and perhaps viral infections 11 predominate. Superimposed on this spectrum of general predisposition there may be major genes conferring susceptibility to particular teratogenic agents.12 Furthermore, predisposition may not depend solely on the genotype of the fetus but also on that of the mother13 and on the interaction between fetal and maternal genotypes.14 15 Thus, different combinations of genetic and environmental factors may be responsible for the malformation in different subjects. The level of genetic predisposition applying to a given patient, and consequently the risk of recurrence, is therefore often difficult to assess.

It is generally agreed that the overall risk for familial cases is higher than for sporadic cases and that the recurrence rate varies with the number and relationship of affected persons in the family.8 16-19 Some aspects of this consensus have, however, been challenged.20 The risk also appears to be higher for relatives of severe cases than for relatives of mild cases.8 9 17 19 Furthermore, the malformation is more frequent in males, the favoured explanation being that a higher level of predisposition is required for females to express the abnormality,9 21-24 with a consequently higher risk of recurrence among the relatives of affected females.8 9 17 19 For sporadic cases, an empirical recurrence risk of around 4% is usually used.8 16 18 25 26 However, sporadic cases include not only those with primarily environmental aetiologies, but also those with a high level of genetic predisposition which, by chance, has not yet resulted in other affected persons being born into the family. The observed overall risk of 4% may therefore be composed of near zero risk for the majority and a high risk for an unidentified minority of sporadic cases.

Previous workers have reported that groups of familial CL±P cases and groups of their non-cleft relatives showed abnormally high levels of asymmetry for certain bilaterally represented features. The variables used were the buccolingual diameter of the lower first molar and three dermatoglyphic characteristics: the aid angle and a-b ridge count (features of the palm print) and fingerprint pattern. By contrast, groups of sporadic CL±P cases and groups of their relatives showed more normal levels of asymmetry.27-29 These observations suggest that asymmetry can be used as an indicator of genetic predisposition to the malformation.

The present study was undertaken to devise an...
asymmetry score, based on a larger number of dental and dermatoglyphic variables than used previously, that might be of value in identifying those with a high level of genetic predisposition, excluding those with known single gene disorders. The score would therefore have to provide maximum discrimination between familial CL±P cases (presumed to have the highest predisposition) and controls (having the lowest), with asymmetry levels for non-cleft relatives of familial cases, sporadic cases, and relatives of sporadic cases ranged in this order between the two extremes.

Materials and methods

The sample

The sample comprised 102 families of 107 CL±P probands aged 15 to 29 years (296 subjects) and 33 families of 33 control probands (91 subjects) in the same age range, having similar sex and social class distributions and coming from the indigenous (non-immigrant) Scottish population. The recruitment of subjects, identified through hospital discharge listings, was very difficult and resulted in a sample of only about half that anticipated. This was largely because of the length of time that had elapsed since the last admission to hospital. Thus, records were often not traced or not up to date, so that only 19% of listed cases could be contacted. Furthermore, presumably because of the demands of the study, only rather less than half of those contacted consented to participate.

Cleft families were divided into two categories, those in which only the proband was affected (sporadic) and those where the malformation was reported to have occurred in first, second, or third degree relatives (familial). The numbers of subjects recruited to the study are shown in table 1. One cleft family was found to have Van der Woude syndrome and was therefore excluded, reducing the total number of subjects in the analysis to 384. None of the other cases appeared to fit with any clearly defined Mendelian syndrome.

Collection and measurement of material

Families either attended a hospital clinic or were visited at home for a full family history and for taking dental impressions and finger, palm, and toe prints from all participating family members. The family history included pregnancy histories for the proband and his or her participating sibs. An effort was made to validate this information, usually supplied by the mother, through general practitioner records but, since so many years had elapsed, very few GPs still had these records and so the attempt was abandoned.

Individually rolled fingerprints were made on standard forms using pre-inked paper strips (Ozalid, Essex, England). Palm prints were taken with fully abducted fingers31 using a 7 cm diameter roller. The palm was rolled evenly over the roller, first with a pre-inked strip and then a plain form attached to the roller's surface.32 Toe prints were made by brushing powdered graphite onto the surface of each toe, gently applying a white self-adhesive label, removing the label carefully, and sticking it permanently to a transparent acetate sheet.

Each fingerprint and toe print was examined under a binocular dissecting microscope (×10) and print pattern and ridge count33 34 were recorded. Fingers and toes were numbered from 1 to 5, with 1 referring to the thumb or big toe. Two measurements were made from palm prints, the atd angle and a-b ridge count.34 The atd angle was corrected to the long axis of the palm,35 the corrected angle being given as 2T where tanT = sinatd/2 sindat. sinatd.

Upper and lower dental impressions were taken using a dimensionally stable silicone material (Bisico S3, Nuclined, Lancashire, England). The impressions were cast in dental stone on return to the laboratory. Mesiodistal and buccolingual diameters of all teeth present, except third molars, were measured according to established procedures.36 37 Teeth that were grossly malformed or had dental caries or restorations that disrupted the natural contour were excluded. All measurements were made by the same operator (FCC) using electronic calipers connected to a BBC Model B microcomputer via a Unilab interface that converted the variable voltage input from the calipers to digital values in the range 0 to 255, each unit increase corresponding to an increase of 0.1 mm in the calipers. The measurements were displayed on a monitor, saved on discs, and later transferred to a mainframe computer for analysis. Duplicate measurements were taken on two separate occasions from the right side of 20 randomly selected subjects to assess repeatability of the technique. Dental measurements for each side were coded as upper or lower (U or L), mesiodistal or buccolingual (MD or BL), and numbered from central incisor to second molar (1 to 7). Thus, for example, UMD4 refers to the mesiodistal diameter of the upper first premolar.

Analysis

For each subject on each side of the body there were therefore ten variables for finger and toe ridge counts, one each for the atd angle and a-b ridge count, and 28 for the dental measurements, a total of 40 count or measurement variables. In addition, there were the 10 finger or toe print pattern
variables. All variables, together with coded information from the family history, were analysed using standard statistical programmes from SPSSx\(^{38}\) and BMDP.\(^{39}\)

The family history data were analysed for effects of sex, birth rank, parental age, frequency of miscarried sibs, pregnancy history, and characteristics of the malformation itself. Repeatability for dental dimensions was assessed by estimating the percentage contribution of measurement error to the observed variation between subjects (the between-subject variance based on single measurements for each variable in each subject). This estimate was:

\[
\frac{100\Sigma (F-S)^2}{2n \cdot \text{var}((F+S)/2)+\Sigma (F-S)^2/2}
\]

where F and S are first and second measurements and n is the number of subjects.

The 384 subjects were divided into five groups: all control subjects, familial cases (familial probands and affected parents), non-cleft relatives of familial cases, sporadic probands, and relatives of sporadic probands. Asymmetry for each of the 40 count and measurement variables was expressed as the squared difference between values on the two sides, adjusted for group mean as appropriate. Asymmetry for finger and toe print pattern was expressed by a single additional variable as the proportion of right-left pairs discordant in pattern type over all pairs of fingers and toes scored.

Stepwise logistic regression (BMDPLR) was then used to select a set of variables that discriminated between familial cases and controls, the two groups representing extremes of genetic predisposition. The programme first identifies the best discriminating variable and then adds (or removes) a variable at each subsequent step to maximise the improvement in overall discrimination between the groups. The logistic regression equation can be given in the form:

\[
g = e^y/(1 + e^y)
\]

where g is the probability of an individual subject belonging to the familial group as opposed to the control group. The value of g can therefore be taken to represent the level of genetic predisposition. Also:

\[
y = a + bx_1 + bx_2 + \ldots + b_nx_n.
\]

The constant a and the coefficients b\(_1\) to b\(_n\) are calculated by the programme and x\(_1\) to x\(_n\) are the observed values for the different selected variables in each subject. Variables were considered for inclusion if they showed greater asymmetry in familial cases than in controls. Highly correlated variables, inducing negative coefficients in the regression equation, were eliminated. Once the constant and coefficients had been determined in relation to these two groups, the equation was applied to the other three.

Results

FAMILY HISTORY

Sex ratio and affected relatives

There were more males than females among all cleft probands (table 1), but the difference in sex ratio from unity just failed to reach statistical significance (males/females = 64/43 or 1.49, \(\chi^2 = 3.74, p = 0.053\)). However, there was a significant difference for sex ratio between sporadic and familial probands (\(\chi^2 = 4.31, p = 0.038\)) and between sporadic probands only and an expected ratio of unity (\(\chi^2 = 7.62, p = 0.006\)). The proportion of affected relatives of female probands was significantly higher than that of male probands for second degree but not first or third degree relatives. Among second degree relatives of female probands 15/178 or 8% were affected, while among those of male probands only 1/120 or 1% was affected (\(\chi^2 = 6.71, p = 0.01\)).

Birth rank, parental age, and miscarried sibs

There was no statistically significant difference for birth rank (by Mann-Whitney U test) or for maternal or paternal age (by t test) between cleft and control probands, sporadic and control probands, familial and control probands, or sporadic and familial probands. Control probands together had six miscarried sibs out of a total of 74 miscarried and liveborn sibs (8%), sporadic probands had 21/197 (11%), and familial probands had 13/74 (18%), but none of the differences between groups was statistically significant.

| TABLE 1 Distribution of the 387 subjects recruited to the study. |
|------------------|------------------|------------------|
|                  | Non-cleft controls | Families of sporadic cases | Families of familial cases |
| Male probands    | 20                | 54                | 10                |
| Female probands  | 13                | 28                | 15                |
| Brothers         | 10                | 33                | 5                 |
| Sisters          | 14                | 32                | 6                 |
| Fathers          | 14                | 37                | 4 (1 CL±P)        |
| Mothers          | 20                | 60                | 12 (2 CL±P)       |
| Total            | 91                | 244               | 52                |
Pregnancy history

Two comparisons were made for the reporting of various environmental factors during pregnancy: (a) between sporadic and familial probands, and (b) between cleft probands, non-cleft sibs of cleft probands, and control probands and sibs. The environmental factors were divided into three categories: smoking, drugs, and illnesses (table 2). For smoking, a 'yes' response indicates regular smoking of any number of cigarettes a day. For drugs, there were different positive responses: Epanutin, phenobarbitone, Mysoline/phenobarbitone, an unspecified anticonvulsant, Debendox, an unspecified antiemetic, 'headache tablets', and two unspecified drugs. For illnesses, one mother reported folic acid deficiency, one an unspecified illness, and 10 rubella or contact with rubella. There was a higher proportion of positive responses for sporadic than familial cases for drugs, illnesses, and any factor (table 2a), and for cleft probands compared with the other two groups for all three categories and for any factor (table 2b), but the differences were statistically significant only for illnesses ($\chi^2 = 16.01, p = 0.0003$) and any factor ($\chi^2 = 9.31, p = 0.01$) in table 2b.

The cleft

There was no statistically significant difference, either between sporadic and familial cases or between the sexes, for the relative prevalence of cleft lip (CL) as opposed to cleft lip and palate (CLP), unilateral as opposed to bilateral clefts, incomplete as opposed to complete clefts, or left sided as opposed to right sided unilateral clefts. There was also no difference between left sided and right sided unilateral clefts for the prevalence of CL as opposed to CLP or incomplete as opposed to complete clefts.

Unilateral clefting occurred predominantly on the left side (left/right = 51/24 or 2.13, $\chi^2 = 9.01$, $p = 0.003$).

Repeatability of dental measurements

No systematic difference between first and second measurements was found (by $t$ test) with either of the two diameters for any of the 14 tooth types (28 comparisons). The contribution of measurement error to the observed variation between subjects was estimated as $\leq 5\%$ for 13 comparisons, 6 to $10\%$ for eight comparisons, and $>10\%$ for seven comparisons.

Group means

Analysis of variance in terms of $(R+L)/2$ for the count and measurement variables, where $R$ and $L$ are corresponding values on the right and left sides, showed that the variance of $(R+L)/2$ between groups was significantly greater than that within groups for seven of the 40 variables. Four of the seven were finger ridge counts (fingers 1 to 4) with controls always showing the lowest count (table 3). There was no such consistency about the other three (toe 4, LMD5, UBL3).

Discrimination between groups

Over all 384 subjects, there were missing values in

| Table 2 | Environmental factors reported for the first three months of pregnancy. |
|---------|------------------|------------------|------------------|------------------|
|         | Smoking          | Drugs            | Illness          | Any factor       |
|         | Yes  | No  | Yes  | No  | Yes  | No  | Yes  | No  |
| (a) Sporadic probands | 20  | 62  | 9    | 73  | 12   | 70  | 35   | 47  |
| Familial probands | 7    | 62  | 9    | 33  | 0    | 23  | 0    | 16  |
| (b) All cleft probands | 27  | 78  | 9    | 96  | 12*  | 93  | 42*  | 63  |
| Non-cleft sibs of cleft probands | 13  | 63  | 4    | 72  | 0    | 76  | 17   | 59  |
| Control probands+sibs | 12  | 45  | 0    | 57  | 0    | 57  | 12   | 45  |

*p<0.01.

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Mean finger ridge count (group mean of (R+L)/2).</th>
</tr>
</thead>
<tbody>
<tr>
<td>Finger</td>
<td>1</td>
</tr>
<tr>
<td>Controls</td>
<td>12.78</td>
</tr>
<tr>
<td>Familial cases</td>
<td>14.96</td>
</tr>
<tr>
<td>Non-cleft relatives of familial cases</td>
<td>16.32*</td>
</tr>
<tr>
<td>Sporadic probands</td>
<td>15.88*</td>
</tr>
<tr>
<td>Relatives of sporadic probands</td>
<td>16.13*</td>
</tr>
<tr>
<td>PAOV</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

*Denotes a significant difference from controls and † denotes a significant difference from relatives of sporadic probands (p<0.05). PAOV indicates significance of the difference for between-group versus within-group variances.
2.9 to 6.2% of subjects for finger or palm asymmetry variables, 9.6 to 38.4% for toe, 44.8 to 64.8% for incisor to first molar, and 66.4 to 85.7% for second molar asymmetry variables. BMDPLR requires complete data sets, so when there was a missing value the mean for the subject's group was substituted. Four asymmetry variables for which at least one group mean was based on fewer than five subjects were excluded (UMD2, UMD7, UBL2, UBL7).

For finger ridge counts, an adjustment was made to the asymmetry variables through multiplying by \( (m_c/m_r)^2 \), where \( m_c \) is the control group mean and \( m_r \) the mean of the subject's group. In this way, differences of asymmetry and of mean between groups could be considered independently, total finger ridge count (the sum of counts on all ten fingers) being added as a possible discriminator.

Stepwise logistic regression applied to familial cases and controls showed that finger and toe ridge count and \( \delta \) angle asymmetries provided only poor discrimination between groups, and that mesiodistal tooth diameter asymmetries were generally better discriminators than buccolingual asymmetries. A set of the nine best discriminators with their coefficients and constant is shown in Table 4. Using \( g=0.3 \) as the point demarcating predicted group membership, 99/117 or 85% of subjects from these two groups were classified correctly. The distributions of \( g \) for all groups are summarised in Table 5. Differences in the distribution of \( g \) between groups were highly significant for eight of the ten possible pairwise combinations of the five groups (two tailed \( p<0.001 \) by Mann-Whitney U test), the exceptions being controls versus non-cleft relatives of familial cases and sporadic probands versus their relatives. Using \( g=0.5 \), there was no difference in the proportion of subjects above this point between controls, non-cleft relatives of familial cases, and relatives of sporadic probands (\( \chi^2=0.06, \ p=0.971 \)), but there were differences between familial cases and controls (\( \chi^2=27.4, \ p<0.0001 \)), sporadic probands and controls (\( \chi^2=5.71, \ p=0.017 \)), and sporadic probands and familial cases (\( \chi^2=8.75, \ p=0.003 \)).

The same coefficients and constant were then used to make comparisons for the distribution of \( g \) within each of five pairs of subgroups of sporadic probands: (1) those for whom no environmental factor had been reported versus those for whom any of the factors listed in Table 2 had been reported, (2) CL versus CLP, (3) unilateral versus bilateral clefts, (4) incomplete versus complete clefts, and (5) males versus females. None of the differences was statistically significant.

### Discussion

The high frequency of males among cleft probands was expected. However, this preponderance was not found for familial probands alone, nor was there any relationship between sex and different levels of severity of the malformation, contrary to indications from other studies.

Evidence for a higher proportion of affected subjects among the relatives of female as opposed to male probands was in keeping with previous findings. The higher frequency of left sided clefting in unilateral cases is well known.

Absence of a demonstrable birth order or parental age effect was consistent with a number of earlier studies but not with others. The increase in the proportion of miscarried sibs from control, through sporadic, to familial probands, despite the lack of statistical significance, may reflect increasing genetic predisposition, although a reduction in abortion frequency has been reported among the sibs of cleft cases.

The lack of evidence for a difference in severity of the malformation between sporadic and familial probands was contrary to expectation.

Possible environmental teratogens were reported more frequently for cleft probands than for their
non-cleft sibs and for control probands and sibs, and there was a suggestion of a higher rate of reporting for sporadic compared with familial probands (table 2). The findings are generally consistent with previous studies.3 8 9 40 50 However, the overwhelming reference to rubella made by mothers of cleft probands is likely to be spurious since there is little epidemiological evidence to implicate this virus in the aetiology of CL±P.9 51

Discrimination between groups was hampered by small sample size and a high proportion of missing values for some of the variables. Nevertheless, four of the five groups were ordered as expected on the scale of g, with sporadic probands significantly lower than familial cases, and relatives of sporadic cases very little different from controls. It might be argued that this result could be related directly to the severity or presence/absence of the cleft, through local disturbances in formation of the upper teeth. However, this is unlikely (1) because of the lack of evidence for a difference of severity between sporadic and familial cases in the sample, and (2) because removal of the three upper jaw dental asymmetries from the logistic regression resulted in a similar pattern of relationships between groups. Contrary to expectation, non-cleft relatives of familial cases were closer to controls than to familial cases. However, non-cleft relatives of familial cases made up the smallest group, so that sampling variation may have contributed to their apparently anomalous position.

The absence of any evidence for an association between g and severity of malformation, either between familial cases and sporadic probands or among sporadic probands, is not in keeping with a number of previous observations or the predictions of the multifactorial threshold model.8 9 19 22 42 43 although the applicability of the simple threshold model to CL±P has been questioned.20 45 52 53 Furthermore, the level of g in sporadic cases for whom possible environmental aetiologies had been reported was not significantly lower than that for other sporadic cases. Thus g may not be a good enough measure of general genetic predisposition to have practical value, even though the intermediate position of sporadic probands (with the same severity of malformation as familial cases) suggests that it did provide some indication of the inherited component of liability to the malformation.

If g were to be regarded as an acceptable measure of predisposition then, bearing in mind the overall empirical recurrence risk of around 4% for sporadic cases, and assuming that those falling within the control range for g had a near zero risk of recurrence, the average risk for the 26% of sporadic patients having g values in the familial range can be estimated as approaching 4/26 or 15%. This is roughly equivalent to the empirical risk among sibs of affected probands when there is already one other affected sib or an affected parent.17 18 However, the ultimate test of validity for any indicator of genetic predisposition would be a prospective study of recurrence among relatives of sporadic CL±P cases. In view of the low overall recurrence risk for sporadic cases, a larger sample than that used in the present investigation would be required for such a study.

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