

# Epidemiological and genetic study in 207 cases of oral clefts in Alsace, north-eastern France

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## Abstract

The epidemiology of oral clefts was studied in the geographical area covered by our registry of congenital malformations. For each of the 207 new cases studied during the period 1979 to 1987, more than 50 factors were compared in probands and controls. The incidence of oral clefts was 1.75 per 1000, with cleft lip/palate (CL(P)) 0.98 and cleft palate only (CP) 0.77 per 1000. A total of 8.2% of cleft cases were stillbirths and 5.3% were induced abortions. The more common types of associated malformations in the 76 affected cases (36.7%) with at least one anomaly other than oral cleft were neural tube defects and skeletal malformations. At birth, infants with oral clefts and other malformations were smaller, weighed less, and their head circumference was lower than in controls. Placental weight was also lower than in controls. Pregnancies with oral clefts were more often complicated by threatened abortion, polyhydramnios, and arterial hypertension. There was a significant association between clefting and consanguinity; heritability of CL(P) was 81% and first degree relatives of probands had more than three times the prevalence of non-cleft malformations as controls. These results are of relevance to genetic counselling.

The epidemiology and genetics of cleft lip with or without cleft palate (CL(P)) and isolated cleft palate (CP) have been studied in various countries by many investigators.<sup>1-12</sup> Despite the large mass of data available, the mode of inheritance and the role of environmental factors are not yet entirely clear. These malformations may be part of genetic syndromes with Mendelian inheritance, of other recognised syndromes, or of unclassified multiple malformations. After removal of these syndromic cases, which represent a

small proportion of clefts, there remain the isolated cases, thought to be the consequence of multifactorial inheritance.<sup>10</sup> It is generally accepted that CL(P) and CP are developmentally and genetically different and that hereditary factors are somewhat more important in CP than in CL(P).<sup>1 13-15</sup> However, Chung *et al*<sup>16</sup> and Marazita *et al*,<sup>17</sup> using complex segregation analysis, could not discriminate between single locus and polygenic inheritance.

We present the results of a study undertaken to assess the epidemiology and the recurrence rates of clefts in families of affected children.

## Material and methods

The material for this study came from 118 265 consecutive births of known outcome, including 814 stillborn babies, registered in our registry of congenital malformations, which covers 11 maternity hospitals, for the period 1 January 1979 to 31 December 1987. The region of investigation was the city of Strasbourg, France (an urban area) and the area defined by the 'Département du Bas-Rhin' in which Strasbourg is situated (a rural area). All newborn babies were registered within the first eight days after birth, as were all fetuses with a minimum age of 20 weeks. No delivery took place at home.<sup>18</sup> When a suspected or a confirmed case was notified, the requested information was checked by a doctor from available records, including prenatal consultation records, maternity files, neonatal unit files, necropsy reports, outpatient clinic files, and paediatric and surgical files.

For each infant with a cleft a complete description was available, including photographs, karyotype, and radiographs for children with syndromes and multiple malformations. Clefts were subdivided into two groups: 'isolated' when only CL(P) or CP were present and 'associated' when additional malformations were found. Infants with Pierre-Robin sequence were classified in the group CP with additional malformations.

For each case more than 50 factors contained in the registration forms were studied, including parity and previous pregnancies, parental age, area of residence, education, ethnic origin, length, head circumference, and weight at birth, genetic factors (consanguinity of parents, inheritance, cytogenetics, occurrence in

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twins), environmental factors, seasonality, and pregnancy. Follow up information was obtained through the paediatric surgeon and the physician in charge of the infant's care.

The control group comprised normal children born after each cleft case in the same maternity hospital and matched for sex. For seasonality studies, the monthly occurrences of all normal births from 1979 to 1987 were chosen as control rates. To determine sex ratios the normal newborn population was used as a control group.

Statistical analysis was performed using the Statistical Analysis System (SAS) procedure software package.<sup>19</sup> Comparison of frequencies was by the  $\chi^2$  test with Yates's correction where appropriate. When numbers were very small Fisher's exact test was used and *t* tests were used to compare means. Multiple linear regression and the spectra procedure<sup>19 20</sup> were used for seasonality studies. For detection of time clusters, observed/expected ratio<sup>21</sup> and scan techniques<sup>22</sup> were used. For monitoring, the cumulative sum technique<sup>23 24</sup> was used. Odds ratio values were calculated according to the SAS procedure.<sup>19</sup>

## Results

During the nine year study period, 207 cases of CL(P) and CP were detected; 179 were livebirths (86.5%), 17 (8.2%) were stillbirths or late spontaneous abortions, and in 11 cases (5.3%) pregnancies had been terminated after prenatal diagnosis of associated malformations.

The incidence of clefts and associated malformations and the distribution of patients by type and side of cleft are shown in tables 1 to 3.

Table 4 shows the distribution of malformations associated with clefts, excluding chromosomal abnormalities. The expected number of non-cleft malformations in cleft children with another malformation of a specific type was calculated from the frequency of that specific malformation in multi-malformed infants without clefts registered during the period 1979 to 1987.

The male:female ratio for all infants with clefts was 113:94=1.20 (controls 1.07, not significant (NS)). For CL(P) and for CP the male:female ratios were

Table 2 Distribution of patients by type of cleft.

	Males	Females	Total No	%
CL	32	14	46	22.2
CLP	44	26	70	33.8
CP	37	54	91	44.0
Total	113	94	207	100.0

Table 3 Distribution of CL(P) cases by side and sex.

	Sex	No	%
Unilateral Right	M	25	21.6
	F	16	13.8
Left	M	33	28.4
	F	25	21.6
Bilateral	M	10	8.6
	F	7	6.0
Total		116	100.0

respectively 76:40=1.90 ( $p<0.05$ ) and 37:54=0.69 (NS). No statistically significant difference between cleft and control cases was found for maternal age, paternal age, parity, previous pregnancy, area of residence, education, or ethnic origin.

Analysis of weight, length, and head circumference at birth and weight of placenta was carried out after standardisation for sex and gestational age for all infants with clefts (infants with isolated clefts and infants with associated clefts). No statistically significant difference was shown between either of the first two groups and controls. By contrast these factors were significantly decreased in the associated group compared to the controls ( $p<0.01$ ).

## FACTORS ASSOCIATED WITH CLEFTS

### Genetic factors

*Consanguinity of parents.* There were eight cases of parental consanguinity (3.9%), two second cousins once removed, four first cousins once removed, and two first cousins (controls 0.9%,  $p<0.05$ ).

*Occurrence in twins.* Seven pairs of twins and one set of triplets were observed. Only one twin sib was affected in a pair of dizygotic twins.

Table 1 Incidence of clefts and associated malformations.

	No (%)			Incidence per 10 000 births		
	CL(P)	CP	Total	CL(P)	CP	Total
Isolated	83 (40.1)	48 (23.2)	131 (63.3)	7.0	4.1	11.1
Associated	33 (15.9)	43 (20.8)	76 (36.7)	2.8	3.6	6.4
Chromosomal abnormalities	10	4	14 (6.8)	0.8	0.3	1.2
Non-chromosomal recognised syndromes	8	19	27 (13.0)	0.6	1.6	2.3
Multiply malformed	15	20	35 (9.6)	1.2	1.7	2.9
Total	116	91	207	9.8	7.7	17.5

Table 4 Associated malformations observed in infants with clefts\* and expected number of non-cleft malformations by standardisation.

Anomalies	Observed				Expected by standardisation (%)	
	CL(P)		CP		CL(P)	CP
	No	%	No	%		
<i>Neural tube defects</i>	16	31.4	2	3.3		
Hydrocephalus	9		2		1.6	3.1
Encephalocele	4				0.2	0.3
Anencephaly	1				0.3	0.6
Other	2					
<i>Skeletal</i>	12	23.5	15	25.0		
Club foot	5		3		3.6	2.8
Polydactyly	3				1.5	2.2
Syndactyly	1		3		1.3	2.4
Reduction limb defects	1		6		0.8	6.1
Other	1		3			
<i>Cardiac</i>	2	3.9	9	15.0		
Ventricular septal defect	1		3		3.3	6.3
Atrial septal defect			4		1.8	3.4
Other	1		2			
<i>Gastrointestinal system</i>	2	3.9	4	6.7	1.8	3.5
Common mesenter	1		3			
Other	2		1			
<i>Urinary system</i>	9	17.6	3	5.0		1.5
Polycystic kidney	3		1		1.9	
Other	6		2			
<i>Genital</i>	1	2.0	2	3.3		
<i>Diaphragmatic hernia</i>			2	3.3	1.5	3.6
<i>Head and face</i>	7	13.7	22	36.7		
Microcephaly	1		2		0.8	1.6
Microphthalmia	3		3		0.6	1.0
Facial dysmorphism	3		2		5.2	3.0
Micrognathia			15			19.0
<i>Ear anomalies</i>	2	3.9	1	1.7	2.1	1.3
Total	51		60			

\*Some patients had malformations in more than one additional system.

*Other relatives.* Nine mothers, seven fathers, and 11 sibs had the same non-Mendelian cleft anomaly as the proband, eight second degree relatives had a cleft (five on the maternal side and three on the paternal side), while 10 mothers, two fathers, and 11 other first degree relatives had non-cleft congenital malformations (11.1%) (controls 3.3%,  $p < 0.01$ ).

*Inheritance.* An inherited Mendelian condition was present in seven cases: three Meckel's syndrome, two COFS syndrome, one TAR syndrome, and one orofacioidigital syndrome.

*Recognised non-chromosomal syndromes.* Four infants had non-chromosomal syndromes: one CHARGE association, one VATER association, one fetal alcoholism syndrome, and one Seckel's syndrome.

*Cytogenetics.* Karyotypes were obtained in 95 of the cases (45.9%), of which all but 14 were 46,XX or XY. Ten of the abnormal karyotypes were in the CL(P) infants and included five trisomy 13, two trisomy 18, one inversion with deletion 5q, one inversion with deletion 4q, and one Klinefelter's syndrome. The remaining four abnormal karyotypes were in CP

children and included one trisomy 13, one Turner's syndrome, one extra small unidentified metacentric chromosome, and one duplication 18q.

#### *Environmental factors*

Fifty two mothers of cleft infants were smokers (25.1%) (controls 21.2%, NS). The number of mothers not exposed to industrial agents (unemployed, housewife, clerk, professional, and managerial) was 165 (79.7%). The number of mothers exposed to industrial agents (unskilled, semiskilled, and skilled workers) was 42 (20.3%) (controls: not exposed 74.9%, exposed 25.1% (NS)). A total of 107 (51.7%) of the fathers was exposed to industrial agents during work (controls 52.6%, NS).

*Seasonality.* No seasonal variation in birth incidence could be shown by comparison with normal newborn babies in the area under study during the same period of time.

*Pregnancy.* Twenty-two mothers (10.6%) took contraceptive pills during the three months before pregnancy

and four had used an IUD (controls 11.1% (NS) and four respectively).

During pregnancy 10.1% of the women had had threatened abortions, 10.6% hydramnios, and 2.9% oligohydramnios (controls 4.3% ( $p=0.01$ ), 3.8% ( $p=0.006$ ), and 1.3% (NS) respectively); 3.3% of the mothers of the cleft cases were diabetic (IDDM), 2.9% were epileptic, and 8.7% had arterial hypertension (controls 2.4% (NS), 0.9% (NS), and 3.4% ( $p=0.01$ ) respectively); 4.3% had had x rays, 7.7% fever, and 7.7% influenza (controls 3.4%, 8.2%, and 6.7% (NS) respectively); 42.5% took medication (controls 38.6% (NS)). Five mothers out of 131 infants with isolated CL(P) and CP were epileptic (controls 1 out of 131). Only one mother out of 76 children with associated CL(P) or CP was epileptic (controls 1 out of 76). In 11 (5.3%) of mothers prenatal diagnosis was performed after discovery of fetal malformations associated with CL(P) or CP and was followed by termination of pregnancy.

Risk factors were studied for total clefts and for CL(P) and CP separately. Odds ratios were not significant for diabetes, radiographs, epilepsy, fever, influenza, medication, cigarette smoking, occupational exposure of mother or father, maternal age, and paternal age.

**Heritability in sibs.** The 83 isolated CL(P) cases had a total of 51 sibs. Two of these sibs had CL(P) (3.9%). The general population incidence used for calculating the heritability of CL(P) was  $q=0.0701\%$ .<sup>12</sup> Assuming multifactorial inheritance, the estimated heritability from first degree relatives is  $0.81 (\pm 0.16)$  according to the method of Falconer.<sup>25</sup> Only one affected sib was found among CP cases.

## Discussion

The population incidence of CL(P) and CP in this series is comparable to that in a previous French study,<sup>10</sup> which estimated the frequencies of CL(P) and isolated CP as 0.082% and 0.035% respectively, after exclusion of malformation syndromes. The prevalence of all clefts in our population (1.75 per 1000) is comparable to that in Denmark (1.89 per 1000) and in the other Scandinavian countries,<sup>11</sup> higher than in Emilia Romagna, Italy (1.33 per 1000 or 0.075% for CL(P) and 0.058% for CP)<sup>12</sup> where a similar population was under study, and higher than in most of the other registries.<sup>26 27</sup> Thirty-six percent (76/207) of facial cleft malformations were associated with at least one other major malformation compared with 33% in the study of Calzolari *et al*,<sup>12</sup> 22% in Denmark,<sup>28</sup> and 63.4% in New York.<sup>29</sup> These associated anomalies were more common in CP (47.2%) than in CL(P) (28.4%) as in the studies of Welch and Hunter<sup>9</sup> and of Calzolari *et al*.<sup>12</sup>

The distribution of the total sample of our 207 patients within the three main types of clefts was

22.2% for CL, 33.8% for CL(P), and 44.0% for CP, which is higher for CP and lower for CL than in the Danish population.<sup>11</sup>

No significant trend in cleft incidence was detected over time and there were no time clusters and no urban-rural differences.

The karyotype was abnormal in 14 cases (6.7%) which is twice that reported by Calzolari *et al*.<sup>12</sup> The more common abnormalities were trisomies 13 and 18 and structural chromosomal anomalies. The higher frequency of chromosomal anomalies in our study compared to that of Calzolari *et al*<sup>12</sup> may be explained by the fact that karyotypes were obtained in 45.9% of our cases and in only 21.5% of their cases. All our cleft cases with chromosomal anomalies had additional malformations. Therefore, in our opinion, the karyotype should be studied in the patients with associated clefts unless a recognised non-chromosomal syndrome is present.

Several specific malformations appeared to be more common among our multiply malformed infants with CL(P) than among such infants without CL(P) (table 4), especially neural tube defects and microphthalmia, whereas other malformations, such as ventricular septal defect, were less common.

Infants with clefts and additional malformations were of lower birth weight, lower birth length, had a smaller head circumference, and lower placental weight than controls. It seems unlikely that clefts are directly responsible for poor intrauterine growth as our study showed that patients with isolated clefts have no intrauterine growth retardation. Lower birth weight of infants with clefts was also found by Calzolari *et al*<sup>12</sup> and Saxen.<sup>30</sup>

Pregnancies of mothers with infants with clefts were more often complicated by threatened abortions, hydramnios, and arterial hypertension. Saxen<sup>30</sup> found an increase of pregnancies complicated by threatened abortion in her series. Hydramnios and arterial hypertension may be also considered to be risk factors. As in the study of Bonaiti *et al*,<sup>10</sup> there was an increased frequency of epileptic mothers of isolated CL(P) and CP in our cases compared to the control group, but we have too few cases to draw a definitive conclusion.

As in the other series,<sup>9 12 31 32</sup> this study found a significant predominance of males with CL(P) and of females with CP.

Contrary to the previous studies which found no significant association between consanguinity and clefts,<sup>7 9 12</sup> we found a significant association between these two factors. This cannot be explained by ascertainment bias owing to inherited syndromes as the parents of the cases with the syndromes were not related. A more reliable conclusion is that, as for urinary tract malformations for instance, a multifactorial aetiology must be involved depending upon genetic predisposition with a recessive component and

upon environmental factors. The risk of recurrence for CL(P) in first degree relatives was 3.9% and heritability from first degree relatives was 0.81. These results are in accordance with those of Welch and Hunter,<sup>9</sup> Tolarova,<sup>33</sup> Tenconi *et al.*,<sup>3</sup> and Calzolari *et al.*<sup>12</sup> Theoretical recurrence risks for CL(P) were estimated by Tenconi *et al.*<sup>3</sup> Non-cleft congenital malformations were present in 11.1% of first degree relatives of our cases, which is three times more than in the controls. Therefore, for genetic counselling, not only the recurrence risk of CL(P) has to be taken into consideration but also the risk of non-cleft congenital malformations in first degree relatives. If parents of children with CL(P) want to have other children they have to be aware that the risk of congenital malformations is higher than in the general population. Ultrasound examination for the detection of non-cleft malformations should be offered in subsequent pregnancies.

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