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An epidemiological and genetic study of facial clefting in France. II Segregation analysis

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SUMMARY Familial transmission of cleft lip with or without cleft palate (CL(P)) and isolated cleft palate (CP) was studied in two French samples of 458 CL(P) and 156 CP nuclear families, using the recently implemented unified model.[1] In neither case could discrimination be achieved between polygenic inheritance and monogenic inheritance with a high proportion of sporadic cases. In this type of disorder with a complex genetic basis the information furnished by such an approach, which only considers the affected status, is discussed. Future investigations on the joint familial transmission of the disease and different marker systems may help to identify the genes involved in these developmental anomalies.

The first extensive study on clefting was performed by Fogh-Andersen[2] who suggested that cleft lip with or without cleft palate (CL(P)) and isolated cleft palate (CP) were separate genetic disorders, each following a dominant monogenic pattern of inheritance with reduced penetrance and sex limitation. However, most subsequent studies led their authors, following Carter,[3] to claim multifactorial inheritance.[4–8] Melnick *et al*[9–11] and Shields *et al*,[12] analysing Danish data, found that the expectations of the multifactorial model were not completely fulfilled. They suggested heterogeneity of both malformations, a mixture of sporadic and familial cases transmitted in a manner resembling an autosomal dominant mode of inheritance with low penetrance. They proposed the allelic restriction model as a biological mechanism to explain the reduced penetrance of those vertically transmitted cases. However, all these conclusions were based on the epidemiological features of these malformations and the observed frequencies of those affected in the relatives of the probands. As pointed out by Mendell *et al*,[13] observations of different risks in different classes of relatives, even in the expected direction, do not constitute a test for a hypothesis.

Segregation analysis, which allows several transmission hypotheses to be tested, was performed on family data collected in Hawaii by Chung *et al*,[14 15] first using the method described by Morton *et al*[16] and then under the mixed model, as described by Morton and MacLean.[17] No discrimination could be made between monogenic and polygenic modes of inheritance.

This paper presents the results of segregation analyses performed separately on CL(P) and CP French family data. The epidemiological characteristics of these data and the frequencies in relatives have been published in an earlier paper.[18]

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Subjects and methods

The method of data collection has been previously described[18] and will be summarised here. Only isolated CL(P) or CP were considered; all cases associated with chromosomal aberrations, multiple malformations, or recognised syndromes were eliminated. In the present analyses, only family information collected on the sibs and parents of the probands was used. More remote relatives were not included since confirmation of the diagnosis could not always be obtained. The two samples comprise 458 CL(P) and 156 CP nuclear families ascertained through 478 CL(P) and 168 CP probands attending plastic surgery departments of three Paris hospitals. In the CL(P) data, the familial cases are distributed among 34 of the 458 nuclear families. These 34 families are distributed according to the status of their first degree relatives of the probands as follows: 15 families with normal parents and affected sibs, and 19 families with one affected parent and, in three

of them, affected sibs also. In the CP sample, there are 12 among 156 families with multiple cases. They include five families with normal parents and affected sibs and seven families with one affected parent and, in two of them, affected sibs also.

The model used for segregation analysis is the unified model recently described by Lalouel *et al.*[1] This model differs from the mixed model,[17] which allows testing of the sub-hypotheses of monogenic and multifactorial inheritance, by the introduction of the three transmission probabilities, $\tau_{AA\,A}$, $\tau_{Aa\,A}$, $\tau_{aa\,A}$, at the diallelic major locus component. These probabilities, as previously described by Elston and Stewart,[19] denote the conditional probabilities of transmitting to offspring the deleterious allele A for parental genotypes AA, Aa, and aa, respectively. This allows testing of the Mendelian transmission hypothesis in which these three parameters are respectively equal to 1, 1/2, and 0 against a more general transmission model in which these transmission probabilities can take on any value between 0 and 1. Besides these τs, the other parameters at the major locus components are q, the deleterious allele (A) frequency, t, the displacement ($t = \mu_{AA} - \mu_{aa}$, distance between the two homozygous means on the liability scale), and d, the dominance parameter ($d = (\mu_{Aa} - \mu_{aa})/(\mu_{AA} - \mu_{aa})$). The multifactorial transmissible component is expressed in terms of H, the heritability. The mode of family detection was taken into account by including the probability of ascertainment, π, estimated before the analysis from the distribution of probands among affected sibs. Furthermore, sex specific liabilities were considered here since a difference in frequency with sex was observed for these two defects: a frequency of 0·82 per 1000 for CL(P) with 66·5% of males and a frequency of 0·35 per 1000 for CP with 38·7% of males.[18]

In each case, the joint likelihood of the parents' and offspring's phenotypes and the likelihood of the offspring's phenotypes conditional on the parents' phenotypes was computed using the computer programme POINTER.[20]

Results

CL(P)

Segregation analyses of CL(P), considering joint and conditional likelihoods respectively, are presented in tables 1 and 2. Using joint likelihood, there is no evidence for the segregation of a major gene, with d = 1 or 0·5, since the mixed model (with Mendelian τs) is almost as likely as the multifactorial hypothesis. Such a comparison could not

TABLE 1 *Segregation analysis of cleft lip ± palate using joint likelihood ($\pi = 0.589$).*

	$-2\ln L$	$\tau_{AA\,A}$	$\tau_{Aa\,A}$	$\tau_{aa\,A}$	d	t	q	H
General (d = 1)	655·66*	1	†	0	1	†	→0·0	0·780
General (d = 1), H = 0	653·67	1	0·818	0	1	2·159	0·0006	0
General (d = 0·5), H = 0	652·85	1·0	0·759	0·002	0·5	4·531	0·0008	0
General (d = 0), H = 0	655·45	1·0	0·337	0·016	0	7·344	0·029	0
Mendelian (d = 1)	656·17*	1	0·5	0	1	†	→0·0	0·820
Mendelian (d = 0·5)	656·17*	1	0·5	0	0·5	†	→0·0	0·820
Mendelian (d = 1), H = 0	656·35	1	0·5	0	1	2·552	0·0006	0
Mendelian (d = 0·5), H = 0	656·36	1	0·5	0	0·5	4·737	0·0006	0
Mendelian (d = 0), H = 0	670·53	1	0·5	0	0	3·231	0·077	0
Multifactorial, d = t = q = 0	656·17	0	0	0	0	0	0	0·817

*The likelihoods are not exactly maximum since one of the estimated parameters (q) reached a bound.
†The estimates of the major locus parameters are irrelevant when q reaches its bound zero.

TABLE 2 *Segregation analysis of cleft lip ± palate using conditional likelihood ($\pi = 0.589$).*

	$-2\ln L$	$\tau_{AA\,A}$	$\tau_{Aa\,A}$	$\tau_{aa\,A}$	d	t	q	H
General (d = 1)	467·21*	1	†	0	1	†	→0·0	0·890
General (d = 0)	467·21*	1	†	0	0	†	→0·0	0·901
General (d = 1), H = 0	467·06	1·0	1·0	0·0	1	1·979	0·0006	0
General (d = 0·5), H = 0	465·76	1·0	1·0	0·023	0·5	4·778	0·003	0
General (d = 0), H = 0	465·08	1·0	0·477	0·067	0	6·943	0·031	0
Mendelian (d = 1)	467·21*	1	0·5	0	1	→0·0	†	0·892
Mendelian (d = 0·5)	467·21*	1	0·5	0	0·5	†	→0·0	0·891
Mendelian (d = 0)	467·21*	1	0·5	0	0	→0·0	†	0·892
Mendelian (d = 1), H = 0	468·68	1	0·5	0	1	2·440	0·0007	0
Mendelian (d = 0·5), H = 0	468·69	1	0·5	0	0·5	4·872	0·0007	0
Mendelian (d = 0), H = 0	469·29	1	0·5	0	0	2·673	0·035	0
Multifactorial, d = t = q = 0	467·21	0	0	0	0	0	0	0·890

*The likelihoods are not exactly maximum since one of the estimated parameters (t or q) reached a bound.
†The estimates of the major locus parameters are irrelevant when t or q reaches its bound zero.

be performed for d = 0 since the residual variance, under the mixed model, approached zero and no converged solution could be obtained. If H is set equal to zero, Mendelian segregation at one locus is rejected for d = 0, when compared to the general transmission probability model ($-2\ ln\ \lambda = 15{\cdot}08$, higher than a χ^2 with 3 df); it is not rejected for d = 1 or 0·5 ($-2\ ln\ \lambda = 2{\cdot}68$ and 3·51, respectively). Under the unified model (d = 1), the computations were lengthy and q progressively reached its bound zero. The highest observed likelihood, although not the maximum, is no different from the highest likelihoods obtained under the corresponding sub-hypotheses.

Using conditional likelihood (table 2), the likelihoods of all hypotheses are similar. It is not possible to discriminate between the monogenic hypothesis, whatever the value of d, and the multifactorial hypothesis. We can see that, under the mixed model, the major locus parameters (t or q) converge to their bound zero. Furthermore, in this analysis, Mendelian segregation at the major locus is not rejected whatever the value of d.

Thus, these two analyses show that the data fit both a multifactorial mode of transmission with high heritability and a monogenic (dominant or additive) inheritance. Under the latter hypothesis with d = 1, the penetrance of susceptible genotypes is estimated at 0·24 and 0·17 for males and females respectively, and the proportions of sporadic cases are 70% and 58% respectively.

CP

The results of the segregation analyses are shown in tables 3 and 4 and are very similar to those obtained for CL(P). The only difference concerns the parameter estimates obtained under the mixed model with d = 0 using conditional likelihood. In this case, the multifactorial component was close to zero and not the major gene frequency. It can also be noted that under the mixed model, with d = 1 or 0·5, the major locus component, although small, was not cancelled out as was the case for CL(P).

Thus, both multifactorial inheritance with high heritability and monogenic transmission are consistent with the data. In the latter hypothesis, with d = 1, the penetrance is estimated at 0·25 and 0·30 for males and females respectively, and the respective proportions of sporadic cases are 70% and 79%.

Discussion

Our analyses did not allow discrimination between polygenic inheritance and monogenic inheritance with a high proportion of sporadic cases in CL(P) as well as in CP. We can note that in both data sets, under the monogenic hypothesis, recessivity was rejected using joint likelihood, whereas it was equally

TABLE 3 *Segregation analysis of cleft palate using joint likelihood ($\pi = 0{\cdot}438$).*

	$-2ln\,L$	$\tau_{AA\,A}$	$\tau_{Aa\,A}$	$\tau_{aa\,A}$	d	t	q	H
General (d = 1)	159·88	1	0·436	0	1	3·333	0·00002	0·785
General (d = 1), H = 0	160·94	1	0·631	0	1	2·667	0·00015	0
General (d = 0·5), H = 0	160·94	1	0·631	0	0·5	5·333	0·00015	0
General (d = 0), H = 0	161·87	1·0	0·225	0·0	0	4·369	0·026	0
Mendelian (d = 1)	159·92	1	0·5	0	1	3·265	0·00002	0·791
Mendelian (d = 0·5)	159·92	1	0·5	0	0·5	6·535	0·00002	0·791
Mendelian (d = 0)	160·54	1	0·5	0	0	1·282	0·059	0·927
Mendelian (d = 1), H = 0	161·17	1	0·5	0	1	2·784	0·00015	0
Mendelian (d = 0·5), H = 0	161·17	1	0·5	0	0·5	5·567	0·00015	0
Mendelian (d = 0), H = 0	172·69	1	0·5	0	0	3·068	0·047	0
Multifactorial, d = t = q = 0	160·66	0	0	0	0	0	0	0·933

TABLE 4 *Segregation analysis of cleft palate using conditional likelihood ($\pi = 0{\cdot}438$)*

	$-2ln\,L$	$\tau_{AA\,A}$	$\tau_{Aa\,A}$	$\tau_{aa\,A}$	d	t	q	H
General (d = 1)	91·92	1	0·322	0	1	3·601	0·00007	0·773
General (d = 0)	91·63	1	0·351	0	0	3·884	0·016	0·007
General (d = 1), H = 0	93·59	1·0	0·425	0·0	1	3·032	0·00015	0
General (d = 0·5), H = 0	91·88	1·0	0·246	0·012	0·5	12·590	0·00019	0
General (d = 0), H = 0	91·64	1·0	0·352	0·0	0	3·889	0·016	0
Mendelian (d = 1)	92·40	1	0·5	0	1	3·042	0·00005	0·755
Mendelian (d = 0·5)	92·40	1	0·5	0	0·5	6·090	0·00005	0·757
Mendelian (d = 0)	92·34	1	0·5	0	0	3·173	0·015	0·006
Mendelian (d = 1), H = 0	93·61	1	0·5	0	1	2·877	0·00015	0
Mendelian (d = 0·5), H = 0	93·61	1	0·5	0	0·5	5·737	0·00015	0
Mendelian (d = 0), H = 0	92·35	1	0·5	0	0	3·178	0·015	0
Multifactorial, d = t = q = 0	93·62	0	0	0	0	0	0	0·968

likely as dominance using conditional likelihood. A similar discrepancy in the conclusions according to the approach used (joint or conditional likelihood) has already been observed in our analysis of congenital glaucoma.[21] In the absence of any undetected bias, this may be due to the greater power of tests using joint than conditional likelihood.

In the analysis of Hawaiian data, Chung *et al*[15] attributed the absence of discrimination between polygenic and monogenic inheritance partly to the small size of their sample (217 CL(P) families and 113 CP families). In spite of the relatively large size of our CL(P) sample, it was not possible to discriminate either. How large should the data set be to lead to a definitive conclusion? In such disorders with few familial cases, this type of analysis, which uses only information on the affected status, seems to require such a huge amount of data that its value appears questionable with respect to consumption of time and money.

Family data on congenital malformations are usually ascertained through children. The study of offspring of affected persons is expected to provide additional information. Two large and interesting series of cleft probands over two[22] or three[23 24] generations led the authors to suggest the multifactorial mode of inheritance for CL(P) and genetic heterogeneity for CP, a mixture of sporadic and some dominantly inherited cases. However, no definite conclusion could be drawn from these studies.

When the mode of inheritance of a disease cannot be resolved by the analysis of its familial transmission, another way to identify genes is to discover whether marker systems and the disease in question do not segregate independently. Mice with the histocompatibility H-2^a haplotype were found to have a significant susceptibility to cortisone induced cleft palate.[25] Thus, a possible role of the HLA complex in the development of these anomalies was investigated. Up till now, no such role could be demonstrated in case control series[26] or in family studies.[27 28] Investigation of other markers, especially the recently described restriction fragment length polymorphisms, may one day prove helpful in identifying the genes implicated in these developmental disorders.

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