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Segregation Analysis of Cleft Lip with or without Cleft Palate: A Comparison of Danish and Japanese Data

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SUMMARY

The genetic basis of cleft lip with or without cleft palate [CL(P)] remains unresolved. The controversy on the role of a major gene is confounded with possible population differences. This study examines the issue of population differences by comparing two contrasting populations: Caucasians and Japanese. Japanese are known to have higher population incidence of CL(P) and yet lower recurrence risks among relatives. The study subjects consist of 2,998 nuclear families of the Danish population and 627 families of the Japanese population. The uniformly coded data were subjected to complex segregation analysis based on the mixed model.

The analysis has revealed that the Danish data can be best explained by a combination of major gene action and multifactorial inheritance. The best-fitting model is characterized by recessive gene with displacement effect (t) of 2.7 in the standardized unit and gene frequency of .035. The heritability is estimated as .97. The transmission probability of $Aa \rightarrow a$ for the major gene is consistent with ½. On the contrary, the Japanese data can be best accounted for only by multifactorial inheritance with the heritability estimate of .77. No major heterogeneity could be detected between subsets of the data within the populations as grouped by types of ascertainment or mating. It is thus concluded that the observed inconsistency between the two populations is explained by a significant role of major gene in the Caucasian population, but not in the Japanese population.

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INTRODUCTION

Cleft lip and cleft palate are classified into cleft lip with or without cleft palate [CL(P)] and isolated cleft palate (CP) on the basis of genetic and embryological grounds. Our study deals with the former.

Although familial occurrence of CL(P) is well recognized, beginning with the pioneering work of Fogh-Andersen [1], clear delineation of the mode of inheritance for the condition still remains to be resolved. In general, the evolution of genetic explanations for CL(P) has followed that of the development of analytic methods. It began with a simple hypothesis that CL(P) is a recessive or dominant trait with greatly reduced penetrance [1]. Subsequently, an attempt was made to explain reduced penetrance in terms of molecular biology, introducing the concept of allelic restriction [2]. The concept presumes a molecular mechanism such that one of the two alleles important in the normal development in the organ is inactivated leading to an abnormal structure.

Following development of the theoretical groundwork [3, 4], multifactorial inheritance received serious attention as an alternative genetic basis for explaining many human diseases. Thus, some investigators proposed that CL(P) could be accounted for by multifactorial inheritance [5, 6].

With advance in the methodology in complex segregation analysis [7], the two alternative genetic hypotheses, major gene vs. multifactorial inheritance, were examined together, but under two separate models with the result of no clear discrimination between the two models [8, 9]. However, the mixed model in which the two modes of inheritance were subsumed into a single model [10–12] has provided a fresh opportunity for critical tests of alternative genetic hypotheses.

Use of the mixed model has not, however, led to complete resolution of the issue. On one hand, some investigators were not able to find a new indication for favoring one or the other genetic mechanism under the model when two diverse populations of Hawaii [13] and France [14] were studied. On the other hand, another group of investigators observed a major gene effect in a study involving a large sample of the Danish population [15]. This same group also favored involvement of a recessive gene in a study of a smaller sample of the Chinese population based on an analysis without the benefit of a mixed model [16].

Thus, it seems that we have now reached an important juncture where the underlying basis of the reported inconsistency must be examined critically. The issue of major genetic significance here is whether there is true population differences in the etiology of CL(P). Clearly relevant to this question is the puzzling observation that Japanese have a much higher population incidence of CL(P) and yet lower recurrence risks among the relatives of CL(P) cases in relation to Caucasian populations [17, 18].

Our study attempts to examine critically the issue of possible population differences in the genetic etiology of CL(P) and is based on uniformly coded data from two contrasting populations of Denmark and Japan using a unified method of complex segregation analysis based on the mixed model [12].

TABLE 1

DISTRIBUTION OF NUCLEAR FAMILIES BY AFFECTION STATUS OF PARENTS

	♂ × ♀	♂ × ♀	♂ × ♀	♂ × ♀	Total
Denmark	2,669	187	135	7	2,998
Japan	619	5	2	1	627

MATERIALS AND METHODS

The Danish sample has been described in detail [19, 20]. The sample consists of more than 3,000 families in Denmark in which at least one person was born with CL(P) between 1941 and 1971. Essentially, all oral cleft patients born during this period were operated on by a coauthor of this study (Dr. P. Fogh-Andersen). These operated cases are regarded as probands for the purpose of this study. Questionnaires were sent to families of these probands to ascertain CL(P) status of siblings of probands and other relatives. Individuals stated to be affected on the questionnaire were verified by checking with the hospital surgical record and at the State Speech Institute where all cleft newborns were assigned for speech treatment. All CL(P) cases with known syndrome or suspected clefting syndrome were excluded from this study. Such cases made up about 1% of the total number of cases. Thus, the present analysis includes 2,998 nuclear families whose distribution of mating types is shown in table 1. The Japanese sample studied has also been described in detail [18]. This sample was made up of families of CL(P) cases seen at the Clinic of Dentistry and Oral Surgery, Kyushu University, during the period of 8.5 years from January 1953 to June 1961. Questionnaires were sent to the parents of patients, requesting information on CL(P) status of siblings and other relatives of the affected cases. As with the Danish sample, those cases seen for treatment at the Clinic were considered probands for the present study. There were 627 nuclear families, which are broken down by mating type in table 1. Syndrome and suspected syndrome cases were also excluded from the Japanese data using the same criteria applied to the Danish sample.

The method of analysis was complex segregation analysis under the mixed model with consideration given to the presence of pointers in pedigree [11], which had undergone a further refinement with incorporation of the concept of transmission probability [21]. The method is now referred to as a unified model [12] and is programmed into POINTER, which was made available to the present study by Dr. N. Morton of the Population Genetics Laboratory of the University of Hawaii. Unlike the mixed models used in previous segregation analyses, the POINTER program makes it possible to utilize information on nuclear families without probands in them.

Briefly, the model has five major parameters of interest pertinent to this study. The hypothesis of a major gene effect was tested through three parameters: d = degree of dominance, t = major gene effect as measured by the distance between two homozygotes in the standardized unit, and q = gene frequency of the condition. An additional test on the major gene hypothesis was made through the transmission probability (τ_2), which represents the chance that genotype Aa transmits the A allele to offspring. Multifactorial inheritance was tested through the parameter heritability (h^2), which measures the additive component due to polygenes. The goodness of fit of an hypothesis was tested by comparing the $-2\ln L$ value obtained under a specified hypothesis with that of the generalized mixed model, where L is the likelihood. Thus, the fit of an hypothesis on k parameters is tested by $\chi^2 = -2\ln L_2 - (-2\ln L_1)$ with k degrees of freedom, where L_1 is the likelihood when p parameters are fitted under the generalized model and L_2 the likelihood when $p - k$ parameters are estimated.

In the analysis of both samples, allowance was made for sex difference in the liability

TABLE 2

SEGREGATION ANALYSIS OF DANISH DATA OF CL(P)

Hypothesis	−2lnL	d	t	q	h^2	τ_2
General mixed model	5,345.92	0*	2.715	.035	.967	.482
No family resemblance ($d = t = q = h^2 = 0$)	7,138.06	...	...	...	...	...
No major gene ($d = t = q = 0$)	5,399.40	...	...	...	.999	...
No polygenic inheritance ($h^2 = 0$)	5,364.14	0*	3.085	.050	...	...

* Reached the boundary value.

for CL(P), which was measured by the incidence. Based on the general population incidence of .0013 and the sex ratio of .67 for CL(P) cases [20], the sex-specific general incidences were estimated as .00176 and .00084 for males and females, respectively, for the Danish population. Likewise, the sex-specific incidences of the Japanese population were estimated as .00243 and .00183 based on the data from one of the comprehensive studies made on the Japanese population in Japan [22].

RESULTS

Prior to complex segregation analysis of the data, ascertainment probabilities (π) were calculated separately for the two sources of data. They were estimated from three different models, all based on the distribution of probands among affected cases: constant ascertainment, variable ascertainment under the Skellam distribution, and constant ascertainment among multiplex probands [23]. The estimates of π from these models were pooled to obtain a final estimate of π, which was used in the segregation analysis. Thus, the values used were .830 and .387 for the Danish and Japanese samples, respectively.

Table 2 presents the result of segregation analysis of the Danish data. The hypothesis of no family resemblance of CL(P) occurrence ($d = t = q = h^2 = 0$) was rejected outright ($\chi^2_4 = 1{,}792.14$, $P < .001$). The hypothesis that there is no major gene effect ($d = t = q = 0$) was also clearly rejected ($\chi^2_3 = 53.48$, $P < .001$). Likewise, the hypothesis of no multifactorial inheritance ($h^2 = 0$) could not be accepted ($\chi^2_1 = 18.22$, $P < .001$). Therefore, the best-fitting model must include both major gene and multifactorial inheritance components. The best estimates of the parameters under the general mixed model were $d = 0$, $t = 2.715$, $q = .035$, and $h^2 = .967$, indicating that both components are important and the major gene involved acts as recessive as seen from the parameter estimate of degree of dominance ($d = 0$). The important role of major gene is further corroborated by the observation that the best estimate of τ_2 was $.482 \pm .018$, consistent with the hypothesis of $\tau_2 = ½$.

The Danish sample was further examined for possible heterogeneity between subsets of the data with respect to the four parameters of interest. The result is shown in table 3. No differences were detected between families with pointer and those without pointer (heterogeneity $\chi^2_4 = 3.21$, $P > .05$) and between

TABLE 3

TEST OF HETEROGENEITY OF SUBSETS OF DANISH CL(P) DATA UNDER THE MIXED MODEL

	−2lnL	d	t	q	h^2
Pointer families	1,851.89	0	2.969	.037	.904
Nonpointer families	3,490.82	0	2.637	.032	.974
Total	5,342.71	...	...	...	...
Common	5,345.92	0	2.715	.035	.967
Difference (χ^2_4)	3.21	N.S.*			
Incomplete selection	5,298.31	0	2.740	.034	.965
Complete selection	45.02	0	2.465	.045	.988
Total	5,343.33	...	...	...	...
Common	5,345.92	0	2.715	.035	.967
Difference (χ^2_4)	2.59	N.S.			

* N.S. = not significant.

families ascertained under complete selection (through parents) and those ascertained under incomplete selection (through children) ($\chi^2_4 = 2.59$, $P > .05$).

Table 4 shows the results of the corresponding segregation analysis of the Japanese sample. Like the Danish data, the hypothesis of no familial occurrence of CL(P) ($d = t = q = h^2 = 0$) was rejected unequivocally ($\chi^2_4 = 137.49$, $P < .001$). However, unlike the Danish data, the hypothesis of no major gene effect ($d = t = q = 0$) could not be rejected ($\chi^2_3 = 1.74$, $P > .05$), whereas the hypothesis of no multifactorial inheritance ($h^2 = 0$) was clearly rejected ($\chi^2_1 = 12.91$, $P < .001$). Thus, the Japanese data can be explained adequately by multifactorial inheritance alone without invoking the active role of major gene. Under the parsimonious model involving only multifactorial inheritance, the heritability was estimated as .772 ± .075. Under this model, the subsets of families with or without pointer exhibited slight heterogeneity in heritability ($\chi^2_1 = 5.37$, $P < .05$) as shown in table 5. However, there was too little

TABLE 4

SEGREGATION ANALYSIS OF JAPANESE DATA OF CL(P)

Hypothesis	−2lnL	d	t	q	h^2
General mixed model	653.00	0	0	.001	.734
No family resemblance ($d = t = q = h^2 = 0$)	790.49	...	...	...	...
No major gene ($d = t = q = 0$)	654.74	...	...	...	.772
No polygenic inheritance ($h^2 = 0$)	665.91	0.748	3.971	.018	...

TABLE 5

TEST OF HETEROGENEITY OF SUBSETS OF JAPANESE CL(P) DATA UNDER THE MODEL OF POLYGENIC INHERITANCE

	−2lnL	h^2
Pointer families	77.75	.971
Nonpointer families	571.62	.692
Total	649.37	...
Common	654.74	.772
Difference (χ^2_1)	5.37*	...

* $P < .05$.

information in the data to test heterogeneity between types of ascertainment in the Japanese sample.

Having found an apparent difference in the genetic basis of family resemblance, a further test of consistency was made by examining the fit of the best parameter estimates from the Danish data to the Japanese sample and vice versa. The hypotheses being tested here are that the two populations are homogeneous with respect to the genetic parameters. The respective results of the heterogeneity test are shown in tables 6 and 7. Clearly, the values of $d = 0$, $t = 2.715$, $q = .035$, and $h^2 = .967$ estimated from the Danish data did not fit the Japanese sample ($\chi^2_1 = 49.79$, $P < .001$). The χ^2 value is tested with 1 d.f. because $-2\text{lnL} = 654.74$ resulted from estimation of one parameter (h^2). Likewise, the heritability estimate of .772 from the Japanese data had an equally poor fit to the Danish data ($\chi^2_4 = 196.64$, $P < .001$). Thus, this test lends further support to the earlier observation that the two sources of data appear to reflect a difference in the underlying genetic etiology.

DISCUSSION

The results of the present study are highly revealing. Using the same method of complex segregation analysis based on the mixed model, for the first time we have obtained a clear indication in possible difference in the genetic basis of CL(P) between the populations of Denmark and Japan on data that were stan-

TABLE 6

TEST OF FIT OF THE DANISH PARAMETERS UNDER MIXED MODEL TO JAPANESE DATA OF CL(P)

Parameters	−2lnL	d	t	q	h^2
Danish, mixed	704.53	0	2.715	.035	.967
Japanese, polygene	654.74	...	...	...	.772
Difference (χ^2_1)	49.79*				

* $P < .001$.

TABLE 7

TEST OF FIT OF THE JAPANESE PARAMETER UNDER MULTIFACTORIAL INHERITANCE TO DANISH DATA OF CL(P)

Parameters	−2lnL	d	t	q	h^2
Japanese, polygene	5,542.56	...	...	...	.772
Danish, mixed	5,345.92	0	2.715	.0351	.967
Difference (χ^2_4)	196.64*				

* $P < .001$.

dardized with respect to exclusion of syndrome or suspect syndrome cases and ascertainment of cases. In general, the findings of this study are in agreement with those of Marazita et al. [15] and Koguchi [18]. Marazita et al. noted the presence of major gene effect in the Danish population using complex segregation analysis, whereas Koguchi concluded that multifactorial inheritance accounted for CL(P) risk without considering alternative hypotheses. However, no direct comparison was possible between the two studies because of differences in the methods used in analyzing their data. It should be noted that the conclusion on the major gene effect is unchanged even though the sex-specific incidence used by Marazita et al. were lower than ours.

Our data show a strong suggestion of genetic heterogeneity between the two populations. However, we must examine possible effects of other factors leading to such a heterogeneity. One such factor is ascertainment. It is recalled that the Danish population had a higher value of π than that of the Japanese population. In order to check empirically possible effects of the difference in π on the segregation analysis, we used $\pi = .830$, obtained from the Danish sample, in analyzing the Japanese data, with no significant change in the conclusion. It is also assuring to note that the major source of information for segregation analysis was proband families (nonpointer families) in both populations, minimizing a possible difference in responses of the informants about distant relatives with CL(P) between the two studies.

The findings in the present study appear to explain the puzzling observation that the Japanese population with higher general incidence of CL(P) has lower recurrence risks relative to Caucasian populations. The general incidences were estimated as .0013 and .0021 for Denmark [20] and Japan [22], respectively, whereas the recurrence risks for siblings of probands with no parent affected were estimated as .0514 for Denmark [20] and .0184 for Japan [18]. The combination of lower population incidence and higher recurrence rate is not limited to the Danish population, but is general for many Caucasian populations. The average CL(P) incidence in U.S. whites is estimated as .0013 [13], while a summary of numerous published data quotes recurrence risks predominantly in the range of .03–.04 for Caucasians and .01–.02 for Japanese [18]. Thus, these apparent inconsistencies can now be explained on the basis of a significant role played by major gene in Caucasian populations, as shown by the

present study. A study is currently in progress to investigate this issue with the populations of Caucasians and Japanese living in Hawaii.

It is not clear to what extent a general morphological difference underlies the observed difference between the two groups of populations. It is conceivable that the unique morphological (inferentially developmental) characteristics of Japanese provide more opportunity for a stronger role of polygenes and environmental factors [24]. The normal development of the palate depends on successful fusion of the embryonic shelves at the midline of the palate to be formed. One can assume that the more distance that these lateral masses have to travel toward the midline, the more opportunity there may be for failure of the fusion process resulting in CL(P). A previous study showed that the average relative lateral measurements of Japanese was greater than that of Caucasians [24].

Finally, it should be noted that despite the indication of the major gene effect in the Danish sample we should not infer that the etiology of a given CL(P) case can be predicted with certainty. Our study has shown that even in the Danish population the risk of developing CL(P) is determined not only by the action of a major gene, but that it is also modified by additional influences of polygenic and environmental factors. It is estimated that among CL(P) homozygotes only 29% of females and 39% of males are expected to have CL(P) phenotype in the Danish population. Conversely, among CL(P) cases, it is estimated that only 42% of females and 27% of males are expected to be CL(P) homozygotes for the major gene. Thus, about one-third of CL(P) cases may be accounted for by a major gene in this population.

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