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SHORT COMMUNICATION

FAILURE TO DETECT ASSOCIATION OF ISOLATED CLEFT PALATE WITH HLA ANTIGENS

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HLA antigen frequencies have been determined in 282 controls and in 33 individuals with isolated cleft palate or the related birth defect velopharyngeal incompetence. No association of particular HLA antigens with this birth defect was found. A previously reported association of HLA A2 with cleft palate, in males, was not confirmed.

Glucorticoid-induced cleft palate (CP) in mice has been a popular model for this common birth defect (Fraser & Fainstat, 1951). With the discovery that variation at the major histocompatibility complex of mice, (*H-2*), contributes to variation in susceptibility to this teratogen (Bonner & Slavkin, 1975; Erickson *et al.*, 1979), a search for an effect of *HLA* on human cleft palate was performed, with mostly negative results, by several groups (Bonner *et al.*, 1978; Rappaport *et al.*, 1979; Van Dyke *et al.*, 1980). Many of the patients in these studies, however, had cleft lip with or without cleft palate (CL[P]) which is considered to be aetiologically distinct from isolated cleft palate. Aetiological heterogeneity may result in an overall lack of association with *HLA* in CP and CL(P) combined even if a significant association existed with isolated CP. In one study, 19 patients with isolated cleft palate were included (Bonner *et al.*, 1978). A suggestive HLA A2 excess in males, but not females, with isolated cleft palate was reported. We previously, briefly, noted the apparent lack of *HLA* association with cleft palate or cystic fibrosis in a smaller sample of 159 individuals (Erickson *et al.*, 1985). However, 111 of the subjects were collected in family groups, thus biasing antigen frequencies, and there was no examination of the potential effect of sex. We now report HLA antigen frequencies in 315 unrelated individuals—282 controls and 33 individuals with isolated cleft palate or velopharyngeal incompetence (a minor form of incomplete development of the secondary palate: the sample is further described in the footnote of Table 1) and do not find an association between antigen frequency and cleft palate status within or across sex.

The antigen frequencies of the three groups are presented in Table 1. The distribution of HLA A locus antigen types were tested for differences in their frequency distribution between the affected group and the two control groups in a 3 × 8 contingency table. The

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TABLE 1.

HLA Antigen	Control group 1 ($n = 185$)	Control group 2 ($n = 97$)	Affected ($n = 33$)
A			
1	53	23	10
2	85	52	17
3	23	22	7
9	18	24	7
10	17	13	4
11	19	13	6
19	26	12	4
28	6	8	1
B			
5	24	13	5
7	26	22	3
8	37	11	8
12	36	25	10
13	8	3	2
14	12	3	1
15	15	11	0
16	7	7	1
17	15	12	3
18	7	7	1
21	5	6	2
22	4	6	2
27	19	10	6
35	20	21	4
40	20	13	6

The antigen frequencies are from an expanded data set which consists of 315 unrelated Caucasian individuals (unaffected control groups of 185 unrelated individuals from the current study and 97 unrelated individuals previously collected in Ann Arbor and 33 individuals affected with cleft palate or velopharyngeal incompetence). All individuals were typed for HLA A antigens 1, 2, 3, 9, 10, 19 and 28, and HLA B antigens 5, 7, 8, 12, 13, 14, 15, 16, 17, 18, 21, 22, 27, 35, 40, 41 and 42 (see Erickson *et al.*, 1985 for details of antigen typing). HLA B 41 and 42 were dropped from the analysis due to a small expected number of individuals with the antigen in the experimental group. All persons typed as having two different alleles at either locus were counted in each allele class. Those with only one positive typing were not counted twice in that allele class, but were scored as having one known allele and one unknown allele.

HLA B locus antigens were similarly tested in a 3×15 contingency table. Neither of the ratio chi-squared tests were statistically significant at the 0·10 level. Separate comparisons of each control group with the cleft palate group were not statistically significant.

When 2×2 tables were constructed (see Erickson *et al.*, 1985 for further statistical details) with the larger control group compared to the affected patients for each antigen versus all other antigens pooled (with HLA A and B being considered separately), only HLA A2 and 11 and HLA B12, 27 and 40 gave P values $<0{\cdot}05$ for the individual tests.

These *P* values are not statistically significant when adjusted for the fact that 23 separate comparisons were done.

To examine the possibility of a sex effect, the above 2 × 2 tables were constructed separately for males (81 normal, 7 with isolated CP) and females (74 normal, 26 with isolated CP). Information on gender was not available for the sample of 97 normal subjects. Because of diminished sample sizes in these contrasts we employed Fisher's exact test. The only individual contrast to have a *P* value <0·05 was HLA A10 in males, where 3/7 CP versus 7/81 normal subjects were carriers. Again, given the number of contrasts, this outcome cannot be considered statistically significant. The *A2* allele, previously reported in excess in affected males (Bonner *et al.*, 1978), was found in 4/7 males and 13/26 females with CP (57·14% and 50·00%, respectively). This difference is not statistically significant. In normal subjects 45/81 males and 40/74 females (55·56% and 54·05%, respectively) carried HLA A2. These proportions are not significantly different from the frequency in the affected subjects.

We found no evidence of *HLA* association with isolated CP in males or females in our sample. The difference in HLA A2 results between this study and that of Bonner and colleagues (1978) may be due to differences in the patient populations, although both were Caucasian samples. It is also possible, particularly given the large number of contrasts made in the Bonner study, that the original result of males with cleft palate being more likely to carry HLA A2 than females, may be spurious. Bonner and co-workers (1978) did not report whether their sample had a significant difference between the frequency of HLA A2 in normal versus cleft palate male or female subjects.

It has recently become possible to identify single genes involved in human disease variants that were previously considered to be multifactorial. For instance, major gene effects on blood cholesterol levels have been found (Moll *et al.*, 1984; Sing & Davignon, 1985). Since *HLA* has been found to be associated with a number of diseases with multifactorial aetiology, the search for an association with isolated cleft palate was encouraged by the finding that the mouse equivalent, *H-2*, was a component of susceptibility to glucocorticoid-induced cleft palate. This association in mice, however, could only be detected in congenic lines in which the effect of a single gene is isolated from the rest of the genome. The association was not found in recombinant inbred lines where many genetic differences are present in addition to the gene of interest (Vekemans *et al.*, 1981; Liu & Erickson, 1985). Thus, the inability to find an *HLA* association with isolated cleft palate in man may reflect such a minor effect of the major histocompatibility locus, with respect to other environmental or genetic factors, that it is undetectable in a sample of this size. Alternatively, there may well be a difference between the aetiology of spontaneous and glucocorticoid-induced cleft palate in both mouse and man.

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