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Further Evidence of a Relationship between the Retinoic Acid Receptor Alpha Locus and Nonsyndromic Cleft Lip with or without Cleft Palate (CL ± P)

To the Editor:

Chenevix-Trench et al. (1992) reported a significant difference between nonsyndromic cleft lip with or without cleft palate (CL ± P) cases and unrelated controls in the frequency of alleles at the retinoic acid receptor alpha (RARA) *Pst*I RFLP located at 17q21.1. They also observed borderline significant ($P = .055$) differences between allele frequencies in subjects with cleft lip and palate (CL + P) compared with those with cleft lip only (CL). Retinoic acid (RA) is a known teratogen capable of producing cleft palate in rodents (Abbott and Birnbaum 1990). Chenevix-Trench et al. (1992) hypothesized that variation in susceptibility to the effects of RA in humans may result from alterations at the RARA locus.

We have investigated association and linkage between CL ± P and a microsatellite marker (D17S579) located at 17q21 (Hall et al. 1992), selected for its proximity to RARA, in 14 extended multiplex families from rural West Bengal, India. The RARA locus has recently been localized to a 4-cM region flanked by D17S579 and THRA1 (Black et al. 1993; Bowcock et al. 1993); thus, D17S579 is <4 cM from RARA. We chose to type a microsatellite in order to conserve DNA and to maximize the information available for linkage. Of the 35 affected individuals, 58% were male, and 31% had CL + P. The proportion of individuals affected with CL + P was much lower than that seen in most studies of CL ± P; a low proportion (36%) were also observed in our sample of 90 extended simplex and multiplex families from rural West Bengal, which was the subject of a complex segregation analysis (Ray et al. 1993). We hypothesized that the low proportion of affected individuals with cleft palate in this part of rural India may result from decreased survival of such children because of problems with breast feeding (Ray et al. 1993).

Our control group was composed of unaffected pedigree members who were unrelated to each other (although they were often related to affected individuals). It included one unaffected person per pedigree (e.g., a sibling of the proband) and any *unrelated* unaffected individuals who married into the pedigree (e.g., uncles and aunts by marriage). The group of 41 controls thus created was utilized for the association analysis.

The D17S579 microsatellite PCR products were run on polyacrylamide gels, dried, and autoradiographed. Thirteen alleles were detected, ranging in size from 103 bp to 133 bp. The three alleles (113 bp, 123 bp, and 125 bp) with the highest frequencies were individually considered, while the remaining rarer alleles were combined for the association analysis. Table 1 shows that the D17S579 allele frequencies were similar in the control and all affected individuals ($\chi^2 = 2.68$, df = 3, $P = .44$). However, those with CL had a higher frequency of the 125-bp allele and a lower frequency of the combined rarer alleles than did those with CL + P ($\chi^2 = 8.68$, df = 3, $P = .034$; Fisher's exact test, two tailed $P = .029$).

An autosomal dominant model (based on results of segregation analyses by Ray et al. [1993]) with reduced penetrance was used for linkage analysis. No linkage of D17S579 to CL ± P was detected; linkage was significantly excluded (lod score <-2) at recombination (θ) values of $\leqslant .10$, with penetrance of either .6 or .4. Analysis under recessive models with penetrance of either .4 or .6 also significantly excluded linkage at θ's of $\leqslant .10$.

There were four affected children (two with CL and two with CL + P) resulting from three consanguineous matings, which are common in India. Although the parents of all four children had genotypes which could have produced offspring who were either heterozygous or homozygous at the marker locus (and, because of inbreeding, homozygosity may have extended to the surrounding chromosomal segment), three of the four affected children were heterozygous. Thus, there was no evidence from the affected inbred children that the effect of the RARA region locus on CL ± P acts in a recessive manner.

Our results would appear to provide independent confirmation of the findings of Chenevix-Trench et al.

Table 1

D17S579 Alleles in CL ± P Patients and Controls

Allele Size (bp)	No. (frequency) of Alleles in			
	Controls	All Affected Patients	CL Patients	CL + P Patients
113	19(.23)	22(.31)	15(.31)	7(.32)
123	19(.23)	14(.20)	9(.19)	5(.23)
125	20(.24)	20(.29)	18(.38)	2(.09)
All others	24(.29)	14(.20)	6(.13)	8(.36)
Total	82	70	48	22

(1992), indicating that genes in the *region* of RARA (and possibly genetic variation at that locus itself) are involved in the formation of CL ± P defects. The data also imply that D17S579 is closely linked to either RARA or a nearby locus influencing clefting. Unlike the RARA results of Chenevix-Trench et al. (1992), we found no significant difference in D17S579 allele frequencies between CL ± P subjects and controls; this discrepancy may be due to the unusual proportions of CL versus CL + P subjects in our study. However, our observation of a significant difference in D17S579 allele frequencies between CL versus CL + P subjects suggests that RARA (or a nearby locus) acts primarily to *modify the expression of the CL ± P trait.* In other words, it is possible that no particular genotype at the RARA region locus is *necessary* for development of the CL ± P trait but, rather, that various genotypes at this locus *alter the expression* (presence or absence of cleft palate, or severity) of the trait. This would explain why the families of the same subjects analyzed for association failed to produce positive evidence for linkage between CL ± P and the RARA region marker. Greenberg (1993) recently presented a similar argument with respect to the difficulty of detecting linkage between a marker and a "disease" locus which merely alters the *risk* of, but is not *necessary* to, the disease trait. We believe that it is unlikely that the RARA region effect reported here represents the "major" locus detected in our previous segregation analyses.

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References

Abbott BD, Birnbaum LS (1990) Retinoic acid-induced alterations in the expression of growth factors in embryonic mouse palatal shelves. Teratology 42:597–610

Black DM, Nicolai H, Borrow J, Solomon E (1993) A somatic cell hybrid map of the long arm of human chromosome 17, containing the familial breast cancer locus (BRCA1). Am J Hum Genet 52:702–710

Bowcock AM, Anderson LA, Friedman LS, Black DM, Osborne-Lawrence S, Rowell SE, Hall JM, et al (1993) THRA1 and D17S183 flank an interval of <4 cM for the breast-ovarian cancer gene (BRCA1) on chromosome 17q21. Am J Hum Genet 52:718–722

Chenevix-Trench G, Jones K, Green AC, Duffy DL, Martin NG (1992) Cleft lip with or without cleft palate: associations with transforming growth factor alpha and retinoic acid receptor loci. Am J Hum Genet 51:1377–1385

Greenberg DA (1993) Linkage analysis of "necessary" disease loci versus "susceptibility" loci. Am J Hum Genet 52:135–143

Hall JM, Friedman L, Guenther C, Lee MK, Weber JL, Black DM (1992) Closing in on a breast cancer gene on chromosome 17q. Am J Hum Genet 50:1235–1242

Ray AK, Field LL, Marazita ML (1993) Nonsyndromic cleft lip with or without cleft palate in West Bengal, India: evidence for an autosomal major locus. Am J Hum Genet 52:1006–1011

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