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Evidence for an Association between RFLPs at the Transforming Growth Factor Alpha (Locus) and Nonsyndromic Cleft Lip/Palate in a South American Population

To the Editor:

Ardinger et al. (1989) reported a significant association between nonsyndromic cleft lip with or without cleft palate (CL(P)) and two RFLPs (*Bam*HI and *Taq*I) of the transforming growth-factor alpha locus (TGFA) in 80 American Caucasian CL(P) patients and 102 controls. This association has subsequently been analyzed in other studies. Chenevix-Trench et al. (1991) genotyped the *Taq*I RFLP in 96 unrelated Australian Caucasians with CL(P) and in 100 unrelated controls. These authors observed an elevated frequency of the *Taq*I C2 allele in CL(P) individuals, compared with controls. They concluded that, while neither the *Taq*I polymorphism itself nor any polymorphism in tight linkage disequilibrium with it is responsible for the disorder, either TGFA or a linked gene does contribute to the development of clefting in some individuals.

Holder et al. (1992) studied three RFLPs at the TGFA locus in 60 unrelated British Caucasians with CL(P) and in 60 controls. They found a highly significant association between the *Taq*I RFLP and clefting and no association with the two other RFLPs (*Bam*HI and *Rsa*I).

Stoll et al. (1992) detected a significant association with *Bam*HI RFLP and not with *Taq*I RFLP in a sample of 67 Alsacian Caucasians with CL(P) and in 99 controls. They concluded that TGFA may be a modifier gene, not a major gene, that may play a role in the development of bilateral clefting in some individuals. Chenevix-Trench et al. (1992) published an extension of their original study that included two other TGFA RFLPs and seven other RFLPs at five new candidate genes. Significant associations with the TGFA *Taq*I and *Bam*HI RFLPs were confirmed.

Recently, Sassani et al. (1993) published the results of a study in a sample of CL(P) individuals, consisting of 83 of Caucasian ethnicity, 6 of Asian ancestry, 11 African Americans, and 84 controls. They found among the Caucasians (83 CL(P) and 84 controls) a significant association for CL(P) and C2 *Taq*I allele. When the data for Cauca-

Table 1

***Bam*HI RFLPs in Chilean CL(P) Patients and in Controls**

Subject Category (*N*)	Allele Frequency[a]		Genotype Frequency		
	A1	A2	A1/A1	A1/A2	A2/A2
CL(P) (65)	3 (.023)	127 (.977)	1 (1.5%)	1 (1.5%)	63 (96.9%)
Controls (100)[b]	22 (.11)	178 (.89)	5 (5.0%)	12 (12.0%)	83 (83.0%)
Sporadic CL(P) (45)[c]	3 (.033)	87 (.97)	1 (2.2%)	1 (2.2%)	43 (95.6%)
Familial CL(P) (20)[d]	0 (.00)	40 (1.00)	0 (.0%)	0 (.0%)	20 (100.0%)

[a] TGFA/*Bam*HI: A1 = 7.0 kbp; and A2 = 4.0 kbp.
[b] Results of comparison of allele frequencies of CL(P) vs. controls were significant ($P = .004$; $\chi^2 = 8.50$).
[c] Results of comparison of allele frequencies of sporadic CL(P) vs. controls were significnat ($P = .03$; $\chi^2 = 4.63$).
[d] Results of comparison of allele frequencies of familial CL(P) vs. controls were significant (Fisher exact test; $P = .01$).

sians, African Americans, and Asians were examined jointly, the significance of the difference was even greater.

We have genotyped in the Chilean population the *Bam*HI RFLP in 65 unrelated patients with nonsyndromic CL(P) and in 100 controls. The CL(P) sample was obtained from patients attending the Cleft Lip/Palate Clinic of the Faculty of Dentistry of the University of Chile and/or the Dr. Alfredo Gantz Mann Foundation (a private clinic for the cleft patient). Clinical examination for dysmorphic features (such as lip pits) was undertaken by dysmorphologists and medical geneticists who work as staff members of these two institutions. Cases were excluded from the study if there was evidence of prenatal exposure to any prescribed medication (such as phenytoin) or if other dysmorphic features were present. Each proband included in the present study has a detailed family history (extended pedigrees). The CL(P) sample consisted of 37 males (57.0%) and 28 females (43.0%). A positive family history of clefting was observed in 20 CL(P) probands (30.8%). There were 45 (69%) with unilateral CL(P) and 20 (31%) with bilateral CL(P). The controls were obtained from blood donors (without a family history of clefting) of the blood bank of J. J. Aguirre University Hospital of the Faculty of Medicine of the University of Chile. This sample was randomly obtained after a genetic inquiry was performed on those donors to be included in the control sample. The genetic inquiry included specific questions, in order to rule out the possibility of a family history of clefting. The samples of blood donors and of CL(P) patients correspond to Chilean urban middle-low and low socioeconomic strata (Valenzuela 1988).

The contemporary Chilean population stems from the admixture of the Native Amerindians with the Spaniards (Rothhammer et al. 1968). The incidence rates of CL(P) in the urban mixed Chilean population show, on average, intermediate values when compared with those reported for Asians or Caucasians (Blanco et al. 1988). The relationship between ethnicity, Amerindian admixture, genetic markers, and socioeconomic strata in public and private health care systems in Chile has been extensively studied (Valenzuela and Harb 1977; Valenzuela 1984, 1988; Valenzuela et al. 1987; Palomino et al. 1990, 1991). These reports demonstrated that the degree of Amerindian admixture can be determined by the frequencies of the alleles of the ABO and Rh loci. The Chilean socioeconomic strata present a gradient of Amerindian admixture, with the highest values in lower socioeconomic strata. Moreover, a strong correlation between Amerindian admixture and the incidence of CL(P) has been reported in Santiago, Chile (Palomino et al. 1990). In the present study, cases and controls belong to middle-low and low socioeconomic strata. Therefore, their ethnic composition should be very similar.

The aforementioned description of the Chilean population allows one to state that it is ethnically different from those where associations have been previously reported between TGFA RFLPs and CL(P). The results of the genotyping for the *Bam*HI TGFA RFLP, using phTGF1-10-3350 (Murray et al. 1986), are shown in table 1. The frequency of the *Bam*HI A2 allele in the Chilean CL(P) sample is the highest reported in the literature. The excess in the frequency of the *Bam*HI A2 allele in CL(P) individuals provides additional support to the reports of Ardinger et al. (1992), Chenevix-Trench et al. (1992), and Stoll et al. (1992). In the comparison of sporadic versus familial cases, no significant differences were detected in the frequency of the *Bam*HI alleles. Nevertheless, the frequency of the A2 allele in familial cases is 1.0. No significant differences were observed between the control population of the present study and those of Ardinger et al. (1989), Holder et al. (1992), and Stoll et al. (1992). Also, in the comparison of the *Bam*HI RFLP by type of cleft (unilateral or bilateral), no significant differences were detected. Our data do not support the hypothesis that TGFA is a major gene in the etiology of CL(P), but, rather, it seems to be either a predisposing gene involved in the susceptibility to clefting in some individuals or a gene interacting with those involved in its etiology.

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Heterogeneity of the Autosomal Dominant Split Hand/Split Foot Malformation

To the Editor:

I read with interest the three articles relating to the split hand/split foot (SHSF) malformation in the July issue of the Journal (Palmer et al. 1994; Roberts and Tabin 1994; Scherer et al. 1994). While one gene for the autosomal dominant SHSF malformation is mapped to the chromosome 7q21-22 region, there is at least one other gene responsible for the malformation.

A similar heterogeneity of the nonsyndromal autosomal dominant SHSF malformation was demonstrated from the clinical analysis of 55 families from the literature (Zlotogora 1994). It appears that there are *at least* two types of autosomal dominant SHSF: one in which the malformation is isolated (type I; McKusick 183600), the other in which at least one individual in the pedigree had other limb defects, in particular tibial hypoplasia (type II; McKusick 119100). While variability is evident in both types, the penetrance among the obligatory carriers of the descendant of the first affected individual is high in type I (96%) and low in type II (66%). In type II, within one family SHSF may be isolated in some individuals and associated with other limb defects in others. Genuardi et al. (1993) reported on a family with SHSF malformation type II that segregated with a translocation t(2,7). In this family the penetrance was low; isolated SHSF was the malformation in all affected individuals but one infant who had also left tibial hypoplasia. Since this translocation was one of the chromosomal aberrations used for the fine mapping of the gene for SHSF on chromosome 7 (Scherer et al. 1994), it may be concluded that this gene is the one associated with SHSF malformation type II.

In many of the families with SHSF type II, the obligatory carriers of the gene are unaffected sometimes for generations before the birth of the first affected individual (Zlotogora 1994). This phenomenon, which is not observed in SHSF type I, was referred to as *premutation* by Spranger and Shapera (1988). This suggests that SHSF type II may be causally related to the expansion of a trinucleotide repeat sequence as it was demonstrated in other diseases with anticipation. This trinucleotide repeat sequence may be lo-