tion by the detection of a common disease haplotype segregating in pedigrees of the Cuban kindred, with concordance for a single allele observed at loci in close proximity to SCA2 (R. Allotey, R. Twells, C. Cemal, B. Schleich Norte, J. Weissenbach, M. Poole, R. Williamson, and S. Chamberlain, unpublished data). The observation that two alleles at the HASH1 locus cosegregate with SCA2 in these pedigrees indicates the occurrence of an additional ancestral recombination event, and this provides independent evidence for the exclusion of the HASH1 gene as a candidate for SCA2. We conclude that the neuronal NOS gene and the HASH1 gene have been definitively excluded as candidate loci for mutation in spinal cerebellar ataxia 2.

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Nonsyndromic Cleft Lip and Palate: Evidence of Linkage to a Microsatellite Marker on 6p23

To the Editor:

Nonsyndromic cleft lip with or without secondary clefting of the palate (CL±P) is one of the most common birth defects, affecting 1/700–1/1,000 Caucasians (Fraser 1970; Bonaiti-Pellie et al. 1982). Approximately 20% of patients have a positive family history of CL±P, and thus genetic factors are thought to be important in etiology (Chenevix-Trench et al. 1992). The nature of the genetic contribution to the etiology of nonsyndromic familiar CL±P remains controversial. Moreover, complex segregation analysis of several populations provides divergent models of inheritance: in some, a multifactorial threshold model is indicated (Carter 1976; Mitchell and Rish 1980), whereas in others, either a mixed model or an autosomal major locus best fits the data (Marazita et al. 1984, 1986; Chung et al. 1986; Temple et al. 1989; Hecht et al. 1991*b*). A previous linkage study concerning CL±P and cleft palate (CP) families indicated chromosome 6p, near F13A locus, as a possible region for the presence of a clefting gene (Eiberg et al. 1987). More recently, another linkage study performed on a sample of 12 families with nonsyndromic CL±P seemed to exclude this association (Hecht et al. 1993).

To test the hypothesis on the possible presence of a major gene on chromosome 6p, we carried out a study on a large sample of CL±P families. Twenty-one families, characterized by the presence of at least two affected CL±P individuals and arising from northeastern Italy, were enrolled in this study. DNA, prepared from blood (Higuchi 1989), was analyzed with five highly informative PCR markers close to the putative CL±P locus: one VNTR factor 13A (F13A) (Polymeropoulos et al. 1991) and four dinucleotide repeats at loci EDN1, D6S89, D6S109, and D6S105 (Litt and Luty 1990; Ranum et al. 1991; Weber et al. 1991; Pages 1993). PCR products were resolved by PAGE and were visualized by silver staining. Linkage was determined using MLINK/ILINK/LINKMAP from the LINKAGE computer package, by assuming a dominant mode of inheritance of a CL±P allele with a penetrance of .32 in males and .24 in females and with .001 allele frequency (Hecht et al. 1991*a*). The five markers, spanning ~35 cM, showed different informativeness. Indeed, 15 families were informative for F13A, 19 for EDN1, 20 for D6S89, 17 for D6S109, and 18 for D6S105. As shown in table 1, low positive LOD scores were found for all markers, at recombination fractions (θ) between .2 and .3. Moreover, to verify the homogeneity of the pedigrees, the results obtained from linkage analysis were further ana-

Table 1

LOD Scores for CL ± P versus Chromosome 6 Markers in 21 Families and in the 14 Linked Families

Marker	Family	LOD Score at θ = .001	.010	0.50	.100	.200	.300	.400
F13A	21	−6.37	−2.45	−.04	.68	.93	.72	.37
	14	.13	1.07	1.48	1.43	1.04	.61	.25
EDN1	21	−12.94	−6.03	−1.54	.02	.95	.93	.56
	14	−.71	1.20	2.21	2.31	1.90	1.27	.61
D6S89	21	−12.97	−6.09	−1.73	−.28	.53	.55	.32
	14	4.48	4.38	3.94	3.40	2.36	1.43	.64
D6S109	21	−9.20	−4.28	−1.15	−.11	.49	.51	.31
	14	−2.73	−.80	.31	.58	.58	.42	.21
D6S105	21	−16.77	−7.90	−2.18	−.20	1.01	1.08	.69
	14	−3.60	−.69	1.00	1.41	1.32	.92	.46

lyzed, using the HOMOG computer program. This test indicated significant heterogeneity only for D6S89, P value = .0417, while .40 was the proportion of linked families (α) at θ = .001. Since the conditional probability of linkage (W_i) is a useful value to identify families of linked type, we reasoned that applying $W_i > .5$ results in 43% of the families with a LOD score of 4.32 at θ = .001 and that applying $W_i > \alpha$ results in 62% of the families with a LOD score of 4.55 at θ = .001. However, considering that in our sample 6 of 20 families who were informative for D6S89 had a very low conditional probability of linkage ($W_i < .004$), the other class of 14 families was selected. On this basis, linkage data obtained on this homogeneous group showed the presence, for the D6S89 marker, of a significant LOD score of 4.48 at θ = .001 (table 1 and fig. 1). It is worth noting that the three alternative classification rules gave similar linkage results, mainly because the families with W_i value close to α are the least informative ones. In addition, when the conventional LOD score for exclusion (≤ -2.0) was used for the six remaining families, linkage to D6S89 at distance of 18 cM was excluded. Moreover, four of these six families were recombinants with all the informative markers.

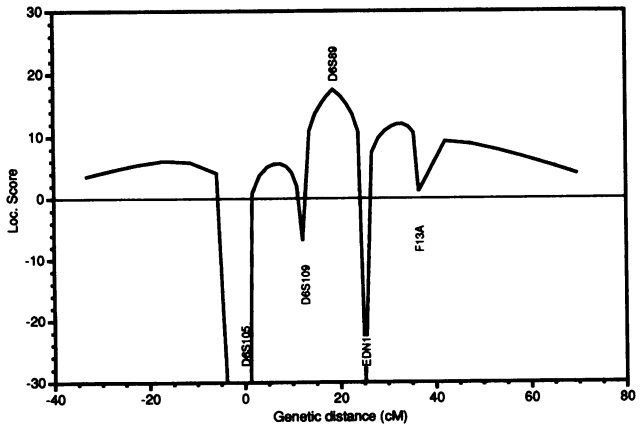

Figure 1 Multipoint analysis of five chromosome 6p markers, showing the maximum location score for the D6S89 marker.

Interestingly, analyzing the data of a previous paper (Eiberg et al. 1987) reporting a study on both CL±P and CP families, we can infer that for the F13A marker the combined LOD score for the CL±P families only was ~2.08, at θ = .01 for males and θ = .30 for females. For the same marker in our 14 CL±P linked families, a combined LOD score of 2.03, at θ = .001 for males and θ = .19 for females, was obtained. Notwithstanding that the two studies used different approaches, the results for the F13A marker are quite similar.

In conclusion, our investigation can be summarized as follows: (i) CL±P disease appears to be heterogeneous; (ii) ~66% of the pedigrees showed an autosomal dominant inheritance with incomplete penetrance; and (iii) CL±P locus maps on 6p23 very close to or at the microsatellite marker D6S89. To verify whether the D6S89 is the closest marker to the CL±P locus, additional examinations with new markers are underway.

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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-