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## Genetic Variation in Transforming Growth Factor Alpha: Possible Association of *Bam*HI Polymorphism with Bilateral Sporadic Cleft Lip and Palate

*To the Editor:*

Ardinger et al. (1989), using the candidate-genes approach, observed a significant association between clefting and two RFLPs (*Taq*I and *Bam*HI) of transforming growth-factor alpha (TGFα) in 80 American Caucasians with nonsyndromic cleft lip with or without cleft palate (CL/P) compared with 102 controls. Chenevix-Trench et al. (1991) genotyped the *Taq*I RFLP in 96 unrelated nonsyndromic Australian Caucasians with CL/P and in 100 unrelated controls. Their results demonstrated a striking replication of the high frequency of the *Taq*I C2 allele reported by Ardinger et al. (1989). Frequencies, from the combined patient and control groups were .155 and .053, respectively for the *Taq*I C2 allele. In contrast, none of the TGFα haplotype associations reported by Ardinger et al. (1989) and by Chenevix-Trench et al. (1991) were exhibited in one study of 12 multiplex families with clefting segregating in a dominant manner (Hecht et al. 1991). The log score was −2.1 at a recombination fraction of .05, indicating that CL/P and TGFα are not tightly linked in these families.

We have genotyped the *Taq*I and the *Bam*HI RFLPs in 67 unrelated Alsacian Caucasian nonsyndromic patients with CL/P, in 38 individuals with isolated cleft palate (CP), and in 99 Alsacian Caucasian controls. All the patients were demonstrated to be sporadic cases. The distribution of cleft type was 31.3% bilateral and 68.6% unilateral CL/P; 64.1% of the cases with CL/P were male, as were 60.6% of the controls.

The probes used in this study have been described elsewhere (Qian et al., in press). They revealed the same *Taq*I RFLP alleles as published by others (Ardinger et al. 1989; Chenevix-Trench et al. 1991), whereas the *Bam*HI polymorphism detected in our study differed from that published by Ardinger et al. (1989) and by Hecht et al. (1991). We detected one 10-kbp and one 7-kbp allele instead of the 7-kbp and 4-kbp allele polymorphisms detected by those authors. However, in all of these studies, the less frequent allele is the larger one (.13 in Ardinger et al. 1989, .04 in Hecht et al. 1991, and .08 in our study).

Our results are shown in table 1. There was no high frequency of the *Taq*I C2 allele as reported by Ardinger et al. (1989) and by Chenevix-Trench et al. (1991) in CL/P patients. However, the *Bam*HI 10-kbp allele was significantly more frequent in patients with bilateral CL/P. This discrepancy is not the result of differences in the distribution of sex or of uni- or bilaterality of the clefting, since these distributions were similar in the three studies. No familial case was included in our series, while approximately half of the cases of Ardinger et al. (1989) and of Chenevix-Trench et al. (1991) were familial cases. The absence of linkage between TGFα and clefting in the 12 families analyzed by Hecht et al. (1991) suggests that TGFα plays a role in certain families or contributes to the development of sporadic CL/P (Chenevix-Trench et al. 1991). In agreement with Hecht et al. (1991),

**Table 1**

***Bam*HI and *Taq*I RFLPs in CL/P Patients and Controls**

| Group (no. of chromosomes) | No. (frequency) of Allele | | | |
|---|---|---|---|---|
| | *Bam*HI | | *Taq*I | |
| | 10.0 kbp | 7.0 kbp | C1 (3.0 kbp) | C2 (2.7 kbp) |
| Unilateral CL/P (92) ..... | 4 (.043)[a] | 88 (.956) | 86 (.934)[a] | 6 (.065) |
| Bilateral CL/P (42) ..... | 8 (.190)[b,c] | 34 (.809) | 40 (.969)[a] | 2 (.031) |
| Cleft palate (76) ..... | 9 (.118)[a] | 67 (.881) | 71 (.934)[a] | 5 (.065) |
| Controls (198) ..... | 15 (.076) | 183 (.924) | 184 (.930) | 14 (.070) |

[a] Results of comparison with controls were not statistically significant.
[b] Results of comparison with controls were significant at $P < .05$, $\chi^2 = 4.02$.
[c] Results of comparison with unilateral CL/P were significant at $P < .05$, $\chi^2 = 5.95$.

our data suggest that TGFα may be a modifier gene, not a major gene, that might play a role in the development of bilateral clefting in some individuals.

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## Identification of Heterogeneous PrP Gene Deletions in Controls by Detection of Allele-specific Heteroduplexes (DASH)

*To the Editor:*

Recently in the *Journal* Puckett et al. (1991) reported the genomic structure and partial DNA sequence of the human PrP gene, which they isolated from a genomic Hela cell library. They described in the open reading frame (ORF) of exon 2 a 24-bp deletion encompassing codons 81–88 or 82–89 and not identified in previously characterized cDNA clones (Kretzschmar et al. 1986). Since this 24-bp deletion was present in both genomic Hela and human brain cDNA libraries, they proposed that this deletion most likely represented an uncommon polymorphism, although they were not able to detect it in 30 unrelated individuals.

**Table 1**

**Classification of Control Chromosomes Used in Prion Gene Analysis**

| | Caucasian | | Black | |
|---|---|---|---|---|
| PrP Type | M | F | M | F |
| Normal..... | 64 | 49 | 1 | 0 |
| Deleted..... | 1 | 4 | 0 | 1 |

We have identified a previously uncharacterized family with familial Creutzfeldt-Jakob disease (FCJD) (Bosque et al., submitted) in which an abnormal PrP allele segregating in the family contained both a G-to-A point mutation at the first base of codon 178 and a 24-bp deletion identical to the one identified by Puckett et al. (1991). To determine whether the 24-bp deletion in the proband represented either a mutation underlying FCJD or a DNA polymorphism, we characterized PCR products representing 240 chromosomes from unrelated controls by using oligonucleotide primers and the conditions specific for amplification of the ORF in exon 2 of the PrP gene (Collinge et al. 1989). Control specimens were randomly selected from samples submitted for DNA analysis of disorders not related to FCJD and represent individuals predominately residing in middle Tennessee and surrounding areas (table 1). It is interesting that similar-appearing deletions of ~20–30 bp were detected by PAGE in 6 (2.5%) of 240 chromosomes (fig. 1). Both the 860-bp

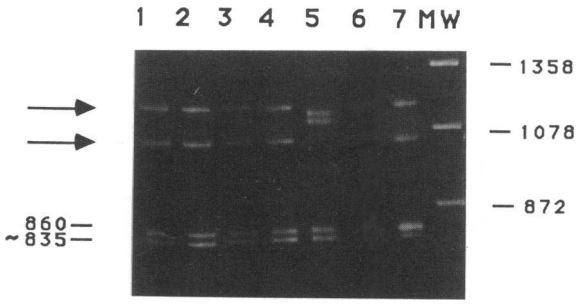


**Figure 1** Ethidium bromide–staining patterns of PCR products of PrP gene from controls with no history of FCJD (lanes 1–6) and from FCJD patient (lane 7). MW = standard molecular-weight marker. Fragments representing homoduplexes of 860-bp (normal) and ~835-bp (deleted) PrP alleles are indicated. Arrows indicate fragments representing heteroduplexes formed by the annealing of strands derived from normal and deleted alleles.