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Short Communication

Lack of mutations in the *PVRL3* gene in North American Caucasians with non-syndromic cleft lip/palate

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Abstract

Cleft lip with or without cleft palate (CLP) is one of the most common birth defects. In about 70% of cases, CLP occurs as an isolated anomaly, denoted non-syndromic CLP (nsCLP). Genetic linkage and association studies have implicated many loci in susceptibility to nsCLP, including some members of the nectin gene family. We performed mutation screening of the *PVRL3* gene that encodes nectin-3 in 73 unrelated Caucasian nsCLP patients and 105 unrelated controls from North America. We detected no sequence variants in the *PVRL3* gene in either the nsCLP patients or the controls. These data suggest that *PVRL3* is not an important susceptibility gene for nsCLP in the North American Caucasian population.

Key words: orofacial clefts, nectins, *PVRL3*, mutation analysis.

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Cleft lip with or without cleft palate (CLP; MIM 119530) is among the most common birth defects, occurring in most populations in about 0.2 to 3 per 1000 live-born infants (Schutte and Murray, 1999; Spritz, 2001; Cobourne, 2004). In about 30% of cases, CLP occurs as part of heritable Mendelian syndromes, but in the majority of cases it occurs as an isolated, complex trait, denoted non-syndromic CLP (nsCLP). Genetic linkage and association studies have implicated many loci in the pathogenesis of nsCLP, including *PVRL1*, which encodes nectin-1, a cell adhesion molecule (Reymond *et al.*, 2000; Satoh-Horikawa *et al.*, 2000). Homozygous loss-of-function mutations in *PVRL1* result in a rare recessive CLP syndrome, CLPED1 (Suzuki *et al.*, 2000), and heterozygous *PRVL1* variants have been associated with nsCLP (Sozen *et al.*, 2001; Turhani *et al.*, 2005; Avila *et al.*, 2006; Neiswanger *et al.*, 2006; Scapoli *et al.*, 2006; Tseng *et al.*, 2006).

Two other nectin-family paralogues, *PVR* and *PVRL2*, were recently evaluated as candidate genes for nsCLP and found to show evidence of genetic association (Warrington *et al.*, 2006). Here, we describe the analysis of another member of the nectin gene family, *PVRL3*, which encodes nectin-3, as a candidate gene for nsCLP. *PVRL3* is expressed mainly in testis and placenta, and at a lower level in heart, brain, lung, liver, and kidney (Reymond *et al.*, 2000; Satoh-Horikawa *et al.*, 2000). It is located on chromosome 3q13.13, which has not been implicated in the pathogenesis of nsCL/P so far.

We screened for variants in the six coding exons of the *PVRL3* gene and adjacent intron and non-coding sequences in 73 unrelated North American Caucasian patients with nsCLP (33 from Texas, 20 from Maryland, 20 from Ohio) and 100 unrelated Caucasian unaffected controls, using the single-stranded conformation polymorphism (SSCP)/heterduplex technique (Lee *et al.*, 1995). Exon 6 was divided into two overlapping amplicons, 6A and 6B. PCR and sequencing primers were:

Exon 1, 5'-AGCGTTCGGCCAAGTGTCAG-3' / 5'-TCCAGAGAACGGCTGGCAGA-3'; Exon 2, 5'-GAAG GGAGGAGAGTGTTGAC-3' / 5'-CTTCACCACTATC ACCAAAATAC-3'; Exon 3, 5'-GCAGTTGTCCTTAAG CTTGTG-3'/5'-AGTTTGATAAACATGCTGAC-3'; Exon 4, 5'-GTAATCTGTCCTGTCATGC-3' / 5'-CTGCTCC ACACAGATTTGA-3'; Exon 5, 5'-CAGTGAATTTTGT CTGAGATGC-3' / 5'-CTCATTTTGCAAGCAGATA G-3'; Exon 6A, 5'-GCCTTTCTGTGTCTTCTCTA-3' / 5'-A CACTGTCTGGGTAAGAATC-3'; Exon 6B, 5'-CATTC CACCATCAGATATGC-3' / 5'-CTAGTGTGAATGAA CATTGTAC-3'. All genomic DNA samples were obtained with informed consent.

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We observed no sequence variations in the regions of *PVRL3* examined, in either patients or controls. It is possible that some variants were not detected by the SSCP/heteroduplex screening method used; nevertheless, only two verified SNPs have been observed in *PVRL3* exonic regions in Caucasians, rs15611 and rs34163852, both with very low minor allele frequencies (Ensembl), and neither of these was observed here. Although we cannot rule out variation outside the genomic regions evaluated, our results do not support a significant role for *PVRL3* in the pathogenesis of nsCL/P in Caucasian populations.

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Internet Resources

Ensembl, http://www.ensembl.org/Homo_sapiens/geneview?gene=ENSG00000177707.

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