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Lack of Linkage Disequilibrium Between Transforming Growth Factor Alpha Taq I Polymorphism and Cleft Lip With or Without Cleft Palate in Families From Northeastern Italy

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Cleft lip with or without cleft palate (CL ± P) is the most frequent craniofacial malformation in different human populations and its cause is largely unknown. Several studies based on population associations have suggested that an allele mapping in the transforming growth factor alpha locus could be responsible, as a risk factor, for the development of the defect. Our investigation of the Taq I polymorphism at the transforming growth factor alpha locus, performed in 40 CL ± P families, did not find evidence for linkage disequilibrium with particular alleles. Moreover, tight linkage was excluded with the traditional LOD score method. Am. J. Med. Genet. 75:203–206, 1998.

KEY WORDS: cleft lip with or without cleft palate (CL ± P); linkage disequilibrium; transmission disequilibrium test (TDT); transforming growth factor alpha (TGFA); complex disease

INTRODUCTION

Cleft lip with or without cleft palate (CL ± P) is one of the most common malformations in humans. Since 20% of the patients have a family history of CL ± P, causal genetic factors are thought to be important. The mode of inheritance is still a matter of discussion. Some authors have proposed a major gene effect [Marazita, 1986; Marazita et al., 1984, 1992; Chung et al., 1986; Temple et al., 1989; De Paepe, 1989; Hecht et al., 1991a; Ray et al., 1993], while others have suggested multifactorial, or at least oligogenic inheritance [Farrall and Holder, 1992; Mitchell and Risch, 1992]. In an attempt to identify the putative locus for CL ± P, Ardinger et al. [1989] described an association between restriction fragment length polymorphisms (RFLPs) at the transforming growth factor alpha (TGFA) locus, which maps in chromosome region 2p13, and CL ± P in a patient control study. The association with the specific C2 allele of the TGFA locus Taq I polymorphism was confirmed by some authors [Chenevix-Trench et al., 1992; Holder et al., 1992; Sassani et al., 1993], but not by others [Stoll et al., 1993; Jara et al., 1995]. Jara et al. [1995] found association with a Bam HI allele. Since population association can occur even for unlinked loci, a demonstration of linkage could support the role of TGFA in the origin of CL ± P. Three different studies did not find linkage by using the LOD score method of analysis [Hecht et al., 1991b; Vintiner et al., 1992; Field et al., 1994]. However, since genetic heterogeneity was detected for CL ± P [Stein et al., 1995; Scapoli et al., 1997], failure to detect linkage to TGFA could be due to the small number of families analyzed [Farrall et al., 1993], although Feng et al. [1994] in a family-based association study found significantly positive linkage disequilibrium with the C2 allele.

In this study, we tested whether linkage, or linkage disequilibrium between CL ± P and a Taq I allele at TGFA locus could be detected. In this investigation we employed only the non-syndromic CL ± P cases that belong to our family groups previously studied [Carinci et al., 1995; Scapoli et al., 1997].

MATERIALS AND METHODS

Families

Forty families from Northeastern Italy, in which at least two individuals were affected with isolated CL ± P,

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participated in this study [Carinci et al., 1995; Scapoli et al., 1997]. Two out of 40 families were not informative for LOD score analysis because they were composed by one affected individual and his/her two parents only. The patients and their relatives were asked specific questions about the presence in the family of other somatic or neurological disorders and the use of clefting drugs such as phenitoin, warfarin, and ethanol. The pedigrees were composed of 384 individuals, 100 with CL ± P; blood samples were obtained, after informed consent, from 82 affected individuals and 274 unaffected relatives.

DNA Analysis

DNA was extracted following the method of Higuchi [1989], as described before [Carinci et al., 1995]. The Taq I polymorphism at the TGFA locus, due to a four, base-pair insertion in the intron V of the gene, was tested by the polymerase chain reaction (PCR) method of Basart et al. [1994]. Small aliquots of PCR-amplified products were resolved by polyacrylamide gel electrophoresis and visualized by silver staining.

Statistical Analysis

The data were analyzed with the transmission/disequilibrium test (TDT), described by Spielman et al. [1993]. This test considers only the matings in which at least one parent is heterozygous for the marker; under the null hypothesis the transmission probability of the two-marker alleles to the affected offspring should be equal. The deviation from equality is tested according to the formula $X^2_{td} = (B-C)^2/(B+C)$, where B is the total number of transmissions of the C2 allele to the affected offspring, and C is the total number of transmissions of C1.

The marker data were also analyzed for linkage to CL ± P with the LOD score method by MLINK program of the LINKAGE package assuming different modes of inheritance: (1) autosomal dominant, with penetrance of .32 for males and .24 for females and with .001 allele frequency [Hecht et al., 1991b; Carinci et al., 1995]; (2) autosomal recessive with a gene frequency of .035 and penetrance of .35 [Chung et al., 1986]; (3) both dominant and recessive, by setting the maximum penetrance value to .001 [Terwillinger and Ott, 1994]. Admixture tests were performed by HOMOG computer program to verify the hypothesis of genetic heterogeneity.

RESULTS

Among the parents, 25 heterozygotes transmitted the C1 and C2 alleles to their offspring with the proportion indicated in Table I. There was no significant departure from random sharing to the affected and unaffected progeny indicating absence of meiotic segregation distortion ($\chi^2 = .08$, *P*-value = .777) and there was, moreover, no evidence for linkage disequilibrium when the transmission to the clefted offspring was analyzed ($\chi^2_{td} = .037$, *P*-value = .847). Only two affected sib pairs were informative, because they had one heterozygous parent. In these two cases, the transmitted allele to each one of the two sibs was employed in the association study. In our sample, the single affected parent, heterozygous at the marker locus, transmitted the C1 allele.

TABLE I. Comparison of Alleles C1 and C2 of TGFA Transmitted to Affected and Unaffected Offspring by Heterozygous Parents

	C1	C2	X2	Significance (*P*)
Affected	14	13	.037	.847
Unaffected	12	11		
Total	26	24		

In addition, no evidence for linkage disequilibrium was found when our cases were pooled together with the familial cases reported by Feng et al. [1994] as shown in Table II.

The results from the four linkage studies with a LOD score method, between CL ± P and TGFA Taq I polymorphism, are shown in Table III. The LOD score was <-2 at a recombination fraction < 0.01 for all the modes tested, which significantly excludes linkage without recombination. Fifteen families were informative for the analysis with the dominant mode with reduced penetrance. Of these nine gave negative LOD scores, while six showed true recombinations among affected individuals. Among the 12 informative families with the recessive mode of inheritance, seven gave indications against linkage between TGFA and CL ± P. No evidence of genetic heterogeneity was detected by analyzing the linkage results by HOMOG computer program.

DISCUSSION

The possible association between CL ± P and TGFA locus has been investigated in several population studies. Only one family-based study has reported evidence of linkage and the authors suggested the importance of confirming of this finding [Feng et al., 1994].

In the present study we investigated the Taq I polymorphism at TGFA locus in 40 Italian families affected with CL ± P and the molecular data obtained were analyzed by the TDT statistic. Our data do not support a role of TGFA in the cause of CL ± P, but it is worth noting that TDT detects linkage between disease and marker only when a population association is present. Since Taq I polymorphism alleles were found in association with CL ± P in several populations, but not in all, further family studies would be helpful to clarify whether TGFA plays a role in CL ± P. In a second step, our data were further investigated to verify the linkage hypothesis regardless of disequilibrium. To this pur-

TABLE II. TDT for Alleles C1 and C2 of TGFA on Data Obtained by Summarizing Our Cases and Those Reported by Feng et al. [1994]

	C1	C2	X2	Significance (*P*)
Our cases	14	13		
Feng cases	5	13		
Total	19	26	1.089	.297

TABLE III. LOD Scores for CL ± P Versus TGFA Taq I Polymorphism Obtained With Different Models of Inheritance

	Recombination fraction						
Model[a]	.001	.010	.050	.100	.200	.300	.400
Dom. R. P.	−4.58	−3.00	−1.31	−.54	.06	.22	.17
Dom. A. O.	−4.39	−2.97	−1.34	−.60	−.01	.16	.14
Rec. R. P.	−4.70	−3.66	−1.92	−1.01	−.21	.07	.11
Rec. A. O.	−4.05	−3.33	−1.87	−1.01	−.23	.06	.10

[a]Dom. R. P., dominant with reduced penetrance; Dom. A. O., dominant affected only; Rec. R. P., recessive with reduced penetrance; Rec. A. O., recessive affected only.

pose, the marker data were analyzed for linkage to CL ± P with the LOD score method by MLINK program of the LINKAGE package using four different modes of inheritance. The 17 informative families produced, for all the models tested, LOD scores <−2 at a recombination fraction < 0.01, which significantly excludes a linkage without recombination. The absence of tight linkage between TGFA and CL ± P reported in this study, as well as by other investigators with the traditional parametric analyses [Hecht et al., 1991b; Vintiner et al., 1992], could exclude TGFA as a major gene for the development of clefting. However, this gene may be involved as a modifier of clefting status [Murray, 1995]. Although excluding tight linkage, our results show positive LOD score at recombination fraction >.2. Interestingly, the linkage studies performed so far had similar results [Vintiner et al., 1992; Field et al., 1994]. Based on the genetic heterogeneity of the disease [Carinci et al., 1995; Stein et al., 1995; Scapoli et al., 1997] we may propose TGFA as a gene product involved in CL ± P malformation only in a fraction of the cases. Alternatively, TGFA would act either as an epistatic gene or as an additive factor. To correlate TGFA with CL ± P additional linkage studies with highly polymorphic markers and a more appropriate model would be useful.

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REFERENCES

Ardinger HH, Buetow KH, Bell GI, Bardach J, VanDemark DR, Murray JC (1989): Association of genetic variation of the transforming growth factor-alpha gene with cleft lip and palate. Am J Hum Genet 45:348–353.

Basart AM, Quian JF, May E, Murray JC (1994): A PCR method for detecting polymorphism in the TGFA gene. Hum Mol Genet 3:678.

Carinci F, Pezzetti F, Scapoli L, Padula E, Baciliero U, Curioni C, Tognon M (1995): Nonsyndromic cleft lip and palate: Evidence of linkage to microsatellite markers on 6p23. Am J Hum Genet 56:337–339.

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.

Chung CS, Bixler D, Watanabe T, Koguchi H, Fogh-Andersen P (1986): Segregation analysis of cleft lip with or without cleft palate: A comparison of Danish and Japanese data. Am J Hum Genet 39:603–611.

De Paepe (1989): Dominantly inherited cleft lip and palate. J Med Genet 26:794.

Farrall M, Hoder S (1992): Familial recurrence-pattern analysis of cleft lip with or without cleft palate. Am J Hum Genet 50: 270–277.

Farrall M, Buetow KH, Murray JC (1993): Resolving an apparent paradox concerning the role of TGFA in CL/P. Am J Hum Genet 52:434–436.

Feng H, Sassani R, Bartlett SP, Lee A, Hecht JT, Malcom S, Winter RM, Vintiner GM, Buetow KH, Gasser DL (1994): Evidence from family studies, for linkage disequilibrium between TGFA and a gene for nonsyndromic cleft lip with or without cleft palate. Am J Hum Genet 55: 932–936.

Field LL, Ray AK, Marazita ML (1994): Transforming growth factor alpha: A modifying locus for nonsyndromic cleft lip with or without cleft palate? Eur J Hum Genet 2:159–165.

Hecht JT, Yang P, Michels VV, Buetow KH (1991a): Complex segregation analysis of nonsyndromic cleft lip and palate. Am J Hum Genet 49: 674–681.

Hecht JT, Wang Y, Blanton SH, Michels VV, Daiger SP (1991b): Cleft lip and palate: No evidence of linkage to transforming growth factor alpha. Am J Hum Genet 49:682–686.

Higuchi R (1989): Simple and rapid preparation of samples for PCR. In Erlich HA (ed): "PCR Technology." New York: Stockton Press, pp 31–38.

Holder SE, Vintiner GM, Farren B, Malcolm S, Winter RM (1992): Confirmation of an association between RFLPs at the transforming growth factor-alpha locus and nonsyndromic cleft lip and palate. J Med Genet 29:390–392.

Jara L, Blanco R, Chiffelle I, Palomino H, Carreno H (1995): Associations between alleles of the transforming growth factor alpha locus and cleft lip and palate in the Chilean population. Am J Med Genet 57:548–551.

Marazita ML (1986): Major gene determination of liability to cleft lip with or without cleft palate: A multiracial view. J Craniofac Genet Dev Biol Suppl 2:89–97.

Marazita ML, Spence MA, Melnick M (1984): Genetic analysis of cleft lip with or without cleft palate in Danish kindreds. Am J Med Genet 19: 9–18.

Marazita ML, Hu D-N, Spence MA, Lyu Y-E, Melnick M (1992): Cleft lip with or without cleft palate in Shangai, China: Evidence for an autosomal major locus. Am J Hum Genet 51:648–653.

Mitchell LE, Risch N (1992): Mode of inheritance of nonsyndromic cleft lip with or without cleft palate: A reanalysis. Am J Hum Genet 51:323–332.

Murray JC (1995) Face fast: Genes, environment, and cleft. Am J Hum Genet 57:227–232.

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

Sassani R, Bartlet SP, Feng H, Goldner-Sauve A, Haq AK, Buetow KH, Gasser DL (1993): Associations between alleles of the transforming growth factor-alpha locus and the occurrence of cleft lip. Am J Med Genet 45:565–569.

Scapoli L, Pezzetti F, Carinci F, Martinelli M, Carinci P, Tognon M (1997): Genetic Heterogeneity and evidence of linkage to 6p23 in nonsyndromic cleft lip with or without cleft palate. Genomics 43:216–220.

Spielman RS, McGinnis RE, Enwest WJ (1993): Transmission test for linkage disequilibrium: The insulin gene region and insulin-dependent diabetes mellitus (IDDM). Am J Hum Genet 52:506–516.

Stein J, Mulliken JB, Stal S, Gasser DL, Malcolm S, Winter R, Blanton SH,

Amos C, Seemanova E, Hecht JT (1995): Nonsyndromic cleft lip with or without cleft palate: Evidence of linkage to BCL3 in 17 multigenerational families. Am J Hum Genet 57:257–272.

Stoll C, Quian JF, Feringold J, Sauvage P, May E (1993): Genetic variation in transforming growth factor alpha: Possible association of Bam HI polymorphism with bilateral sporadic cleft lip and palate. Hum Genet 92:81–82.

Temple K, Calvert M, Plint D, Thompson E, Pembrey M (1989): Dominantly inherited cleft lip and palate in two families. J Med Genet 26: 386–389.

Terwillinger JD, Ott J (1994): "Handbook of Human Genetic Linkage." Baltimore and London: Johns Hopkins University Press, pp 224–225.

Vintiner GM, Holder SE, Winter RM, Malcolm S (1992): No evidence of linkage between the transforming growth factor alpha gene in families with apparently autosomal dominant inheritance of cleft lip and palate. J Med Genet 29:393–397.