COMPLEX SEGREGATION ANALYSIS OF NONSYNDROMIC CLEFT LIP/PALATE IN AMERINDIAN/CAUCASIAN ADMIXTURE POPULATIONS FROM COLOMBIA

ANÁLISIS DE SEGREGACIÓN COMPLEJA DE LABIO/PALADAR HENDIDO NO SINDRÓMICO EN UNA POBLACIÓN MIXTA AMERINDIA/CAUCASOIDE DE COLOMBIA

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Abstract

The present study was undertaken to examine the pattern of inheritance of cleft lip and palate (CLP) in pedigrees ascertained from localities belonging to Cundiboyacense altiplano, Colombia. This population has long been known to be the result of the admixture between Amerindian/Spaniard communities five centuries ago. Ninety-eight extended and multigenerational pedigrees, constituted by 125 nuclear components and 651 records were analyzed. Nine hypothetical models were contrasted using likelihood ratio tests. The hypotheses of no familial transmission, multifactorial component compared against that of the existence of a major gene only, the existence of a recessive major gene, that of non major component in the mixed model and that of the non transmission of major effect (t1 = t2 = t3) were rejected. In contrast, hypotheses postulating a major locus (dominant, codominant) and that of no polygenic component in the mixed model could not be rejected. The major gene model without restrictions in *d* was the most parsimonious. Thus far, the most parsimonious model is that of a major gene (dominant-codominant) without multifactorial effects. Thus, it must be emphasized that, at least in the Colombian communities, environmental components in predisposing to CLP, as has been pointed out in those hypothesis involving fungicides and some kind of avitaminosis, must be discarded and the genetic mendelian factors must be the focus of research.

Key words: Colombia, South-America, cleft lip and palate, genetics, major gene, Antioquia, racial admixture, ethnic, complex segregation analysis, CLP.

Resumen

El presente estudio fue realizado para evaluar el patrón hereditario de labio/paladar hendido (LPH) en genealogías analizadas en localidades pertenecientes al altiplano cundiboyacense colombiano. Esta población se originó hace cinco siglos como resultado de la mezcla entre las comunidades Amerindia e Hispánica. Se analizaron 98 genealogías multigeneracionales extendidas, constituidas por 125 componentes nucleares y 651 individuos. Se contrastaron nueve modelos hipotéticos mediante el test de verosimilitud. Las hipótesis de no-transmisión familiar y el componente multifactorial, comparadas contra la hipótesis de existencia de un gen mayor y de un gen mayor recesivo, y la no-existencia del componente mayor en el modelo mixto y no-transmisión de efectos mayores (t1 = t2 = t3), fueron refutadas. En contraste, no pudieron refutarse las hipótesis postulando un locus mayor (dominante, codominante) y un componente no poligénico en el modelo mixto. El modelo de gen mayor (dominante, codominante) sin efectos multifactoriales fue el más parsimonioso. En conclusión, debe enfatizarse que, por lo menos en las comunidades colombianas, deben descartarse las hipótesis que involucren componentes ambientales, tales como fungicidas y algunos tipos de avitaminosis como factores que predisponen al desarrollo de LPH, y enfocar las investigaciones en la determinación de dichos factores mendelianos.

Palabras clave: Colombia, Sudamérica, labio y paladar hendido, genética, gen mayor, Antioquia, mezcla racial, étnico, análisis de segregación compleja, CLP.

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INTRODUCTION

Although the genetic component in the etiology of nonsyndromic cleft lip with or without cleft palate (CLP) has been recognized, the inheritance model explaining available data remains controversial. The most widely accepted model for CLP during the 70's and early 80's was that of a multifactorial inheritance (Fogh-Andersen, 1942; Carter, 1969; Fraser, 1970; Carter, 1976; Carter *et al.*, 1982). More recently, the complex segregation analysis based on the mixed model (Lalouel and Morton, 1981; Lalouel *et al.*, 1983) has suggested the existence of a mendelian component. Marazita *et al.* (1986) reexamined the data of Carter *et al.* (1982) and found that the multifactorial threshold model, as an unique explanation of the familial clustering of CLP, could be rejected in favor of a mixed model (single major locus plus multifactorial components). Similar results were previously reported by Demenais *et al.* (1984) in France. In this way, in the last ten years, considerable work has been done on different populations, performed to elucidate the genetic model underlying susceptibility to CLP, have been suggestive of the existence of major genes. For instance, Melnick *et al.* in China (1986), Chung *et al.* (1986) on the Danish data, Bixler *et al.* (1971), Melnick *et al.* (1980), Hecht *et al.* (1991) on US families, Marazita *et al.* (1992) in China, Nemana *et al.* (1992) in India, Ray *et al.* (1993) in West Bengal, Palomino *et al.* (1997) and Blanco *et al.* (1998a) on the Chilean population, all suggested the possibility of a major gene for clefting.

The fundamental problem with the major gene component is that dominance appears to be heterogeneous among different populations. Melnick *et al.* (1986), Marazita *et al.* (1992) and Chung *et al.* (1986) have found compatibility with the recessive model of inheritance, while De Paepe (1989), Temple *et al.* (1989), Hecht (1990), Hecht *et al.* (1991a), Nemana *et al.* (1992), Ray *et al.* (1993), Clementi *et al.* (1995), Palomino *et al.* (1997) and Blanco *et al.* (1998b) with that of the codominant-dominant model. In all cases, penetrance has been found to be incomplete. Indeed, several analysis have postulated the existence of at least two major epistatically interacting loci (Farral and Holder, 1992; Mitchell and Risch, 1992; Clementi *et al.*, 1995).

In accordance with the hypothesis of a major gene, several studies have been carried out where various candidate genes have been analyzed in different populations, either through association or linkage studies (Ardinger *et al.*, 1989; Hecht *et al.*, 1991b; Chenevix-Trench *et al.*, 1991; 1992; Holder *et al.*, 1992; Stoll *et al.*, 1992; Vintiner *et al.*, 1992, 1993; Sassani *et al.*, 1993; Hecht *et al.*, 1993; Davies *et al.*, 1995; *Carinci et al.*, 1995; Stein *et al.*, 1995; Mitchell *et al.*, 1995; Jara *et al.*, 1995; Lidral *et al.*, 1997, 1998; Blanco *et al.*, 1998b). The results of these investigations have also shown inconsistent results, which seem to reflect genetic heterogeneity. Ethnical background influence and a great inter-population variability suggest that more than one susceptibility locus is involved in the etiology of CLP.

Latin-American tropical countries offer an interesting situation for studying the genetics of diseases because the mixed ethnical background which originated, about ten-twenty generations ago among Amerindians, Europeans and Negroids. This would allow the use of linkage disequilibrium methods such as Mapping by Admixture Linkage Disequilibrium (MALD) as a powerful strategy to find out major genes predisposing the development of complex disease such as CLP (Chakraborty and Weiss, 1988; Blanco and Rosales, 1988; Blanco *et al.*, 1998a; Arcos-Burgos *et al.*, 1999.

The human population in Colombia stems mostly from the admixture of Europeans (Caucasoid), Africans (Negroid) and Amerindians (Mongoloids). The population inhabiting Cundiboyacense's altiplano (geographically located in the Eastern Colombian Andean Mountains, Santafé de Bogotá 4° 35' N and 74° 00' W, Duitama 5° 49' N and 73° 02' W) is

ethno-culturally formed by an admixture among Amerindian populations (mainly Chibcha in nature) and Spaniard population; the relation between these two groups and the degree of mixture within them allows to differenciate this region from other ethnocultural Colombian regions. Thus far, this community appears to be very interesting for the study of familial aggregation of CLP and for the linkage analysis on extended pedigrees and new mapping strategies, such as MALD, in order to locate major genes involved in susceptibility to CLP.

In two previous studies carried out in the Paisa community from Antioquia, Colombia, a group with a high Caucasian component (85%) we have determined genetic factors predisposing to CLP. Because of ethnic heterogeneity that characterizes the Colombian population, the results of these studies cannot be generalized to the remaining Colombian community. The present study was undertaken to examine the pattern of inheritance of CLP in a community with a high component of Amerindian admixture. We tried to characterize the multifactorial and mendelian factors underlying the predisposition to CLP and to compare these results with those observed in communities with high Caucasian admixture. Here, we present evidence that a dominant major locus without multifactorial effects is responsible for the familial aggregation observed in the pedigrees ascertained from the community of high Amerindian component.

METHODS

Sample

Probands affected with CLP were randomly selected according to a sequential sampling strategy among individuals seeking the medical care at the Operation Smile Organization program on CLP in Santafé de Bogotá and Duitama, Colombia. Affected individuals were admitted to surgical treatment. Colombian and North-American plastic surgeons, orthodontists and geneticists evaluated each one of the probands and a complete clinical and phenotypic record was obtained. Phenotypic classification was defined according to lip and/or palate compromise and unilateral or bilateral affection. Records of facial, maxilla, hands and feet were taken in order to compare these probands with other individuals ascertained world over. Pedigree data and a complete family history of CLP were collected using a specially devised semi-structured questionnaire, which was filled out the same day in which interviews and the surgical procedures were carried out at Military Hospital and Duitama¢s Hospital. Affected relatives pointed out by the probands were clinically analyzed and their phenotypic status was defined.

Ascertainment and complex segregation analysis

The ascertainment probability (π) was estimated separately from the segregation analysis according to the equation $\Sigma a(a - 1)/\Sigma a(r - 1)$ where a is number of probands and r is total number of affected (Simpson, 1983). Complex segregation analysis was carried out according to the unified model of Lalouel *et al.* (1983), implemented in POINTER computer program (Lalouel and Morton, 1981). The model partitions the total variation in the underlying liability to CLP into three independent components: a diallelic single major locus component, a polygenic background, and a random environmental component. Model parameters are: q, the frequency of the high-risk allele A; t, the displacement at the single major locus; d, degree of dominance at the major locus, such that $d = 0$ corresponds to a recessive gene, $d = 1$ corresponds to a dominant gene, $0 < d < 1$ corresponds to some degree of additivity and $d = 0.5$ is referred to as codominant; H, is the polygenic heritability in the offspring; Z, the ratio of adult to childhood heritability; and $t1$, $t2$ and $t3$, the respective probabilities that genotypes AA, Aa, and aa transmit the allele A (better known as "Elston" probabilities). For example, if the single major locus is mendelian, then $t1 = 1$, $t2 = .5$, and $t3 = 0$, whereas the t's are equal if there is no transmission of a major effect.

The analysis using pointers only accepts nuclear families as input. Therefore, extended pedigrees were analyzed by dividing them into their component nuclear families. Those nuclear families not containing affected probands but containing affected relatives of the "pointer" (nominal proband) were codified in each sibship considering that the ascertainment probability value $\pi = 1$. Only nuclear families ascertained through pointers with at least one affected individual were included. This last approach was chosen because simulations and empirical results have shown similar results either including or not-including families with unaffected members (Marazita *et al.*, 1992). In addition, as nonsyndromic CLP is a disorder with sex dependent liability, and our preliminary results in the Colombian population have confirmed this assumption (Bravo *et al.*, 1998), two classes of susceptibility were defined, males (0.58 per 500 live births) and females (0.42 per 500 live births). Conditional likelihood was used when maximizing the different models based on the sex incidences. Neither mortality nor marriage differential risk was taken into account. When the heritability parameter was iterated, different numbers of quadrature points (between five and twenty) were used to reach stable values of likelihood.

RESULTS

A total number of 98 extended multigenerational pedigrees were analyzed, constituted by 125 nuclear components and 651 records. The number of probands was 90 and the total number affected was 130 (75 males and 55 females) ($\pi = 0.42$). Family distribution of sibship size was as follows: 1 sib (39 sibships), 2(29), 3(21), 4(14), 5(8), 6(2), 7(3), 8(4), 9(3), 10(1), 11(0), 12(1).

Table 1 presents the results of complex segregation analysis of the data. Nine hypothetical models were contrasted using likelihood ratio tests. -2 log likelihood values for each comparison were examined using χ^2 tests. Parameter estimates corresponding to maximum likelihood models under each set of constraints are shown for each examined model.

The hypothesis of non-familial transmission for CLP in these families (cohort effect) (comparison between models 1 and 2, and between models 1 and 7) was rejected ($\chi^2 1$ df = 63.06, $P < 0.0001$ and $\chi^2 3$ df = 105.87, $P < 0.0001$). The hypothesis of a multifactorial component was rejected when it was compared against that of the existence of a major gene only (comparison between model 3 and model 7) ($\chi^2 1$ df = 42.81, $P < 0.0001$). Contrast of the multifactorial hypothesis evaluating the *Z* parameter (comparison between model 2 and 3) did not show significant differences, meaning that there are not intergenerational differences in the heritability. Among the models postulating a major locus (dominant, codominant or recessive), only the recessive model could be rejected (comparison between model 6 and 7) ($\chi^2 1$ df = 45.67, $P <$ 0.0001). On the contrary, codominant (comparison between models 5 and 7) and dominant models (comparison between models 4 and 7) could not be rejected ($\chi^2 1$ df = 0.00, $P >$ 0.05 and $\chi^2 1$ df = 0.08, $P > 0.05$, respectively). The model postulating a non-polygenic component in the mixed model (comparison of models 7 and 8) could not be rejected ($\chi^2 1$ df = 1.82, $P > 0.05$), while the model of no major component in the mixed model was rejected (comparison of models 2 and 8) ($\chi^2 3$ df = 40.99, $P < 0.0001$). Finally, the model of non-transmission of major effect ($t1 = t2 = t3$) (comparison of models 7 and 9) was rejected ($\chi^2 3$ df = 105.87, $P < 0.0001$).

The estimations of penetrance (affection status | genotype) and risk (genotype | affection status) values according to the two susceptibility classes are shown in table 2. It is worth mentioning that the probability to be affected when an individual is heterozygote (those individuals with a greater risk) is greater for males, reaching a value close to 50% (45.23).

Table 1. Results of complex segregation analysis

	Parameters								
Hypothesis	**d**	**t**	**q**	**H**	**Z**	**t1**	**t2**	**t3**	**-2ln(L) + C**
1. No trasmission (q = H = O). Sporadic	(0)	(0)	(0)	(0)	(0)	...	...	...	471.40
Multifactorial									
2. No cohort effect	(0)	(0)	(0)	0.99	(1.0)	...	...	...	408.34
3. Cohort effect	(0)	(0)	(0)	0.68	0.14	...	...	...	408.34
Major locus									
4. Dominant	(1)	4.38	.0048	(0)	(1.0)	(1.0)	(0.5)	(0)	365.61
5. Codominant	(0.5)	8.42	.0048	(0)	(1.0)	(1.0)	(0.5)	(0)	365.53
6. Recessive	(0)	4.61	.0088	(0)	(1.0)	(1.0)	(0.5)	(0)	411.20
7. Unrestricted d	(0.83)	8.92	.0048	(0)	(1.0)	(1.0)	(0.5)	(0)	365.53
Mixed model									
9. General model	1.0	7.42	.0042	0.50	(1.0)	(1.0)	(0.5)	(0)	367.35
10. No transmission of major effect (t's equal)	0.83	8.92	.0048	(0)	(1.0)	0.99518	0.99518	0.99518	471.40

d = dominance; t = standard desviations among homozygotes; q = gene frequency; H = heritability; Z = intergenerational ratio among heritabilities; t1, t2, t3 = Elston probabilities.

DISCUSSION

Thus far, the most parsimonious model of inheritance for CLP is that of a major gene (dominant-codominant) without multifactorial effects. From the general unrestricted model (model 9), it is deduced that the gene frequency of this major gene is 0.0048 with a high penetrance.

Although the results of the present analysis are embedded in the results of many other studies, they could be important in several ways:

First, they are compatible with other reports on the mode of inheritance of CLP in other Latin-American communities (Chilean population) (Palomino *et al.*, 1997; Blanco *et al.*, 1998b). The hypotheses of ethnic heterogeneity in the CLP risk has been tested in other studies, such as those of Chung *et al.* (1989), Amidei *et al.* (1994), Blanco and Rosales (1988), but the results were not conclusive. The results of this analysis could reflect a homogenous behavior of the major genes which account for the susceptibility to develop CLP in the Latin-American admixtured populations.

Second, the use of linkage analysis for CLP makes the results of this segregation analysis important since an incorrectly specified model leads to difficulties. Moreover, misprediction of allele frequencies, especially in the presence of sporadic cases of CLP, will greatly influence the power of any linkage study.

Table 2. The estimations of penetrance and risk values

P (affection/genotype)					
	Incidence	**Threshold**	***AA***	***Aa***	***aa***
Males	0.00230	3.84948	0.80324	0.45233	0.00003
Females	0.00160	4.19194	0.69078	0.31741	0.00001
P (genotype/aff. status)					
	Liability = males				
	P(G/A)		0.00220	0.98477	0.01303
	P(G/N)		0.00000	0.00275	0.99725
	Liability = females				
	P(G/A)		0.00272	0.99336	0.00000
	P(G/N)		0.00000	0.00342	0.99657
	Over classes				
	P(G/A)		0.00241	0.98829	0.00929
	P(G/N)		0.00000	0.00309	0.99691
	P(G/U)		0.00001	0.00501	0.99499

Third, empiric risks can be used in clinical practice for counseling. One objective of segregation analysis is to define a more accurate estimate of risk than could be obtained through empiric calculations, which ignore the etiology of the disorder (Houlston *et al.*, 1991).

Fourth, as has been pointed out by Lalouel *et al.* (1983), dominance at the major locus leads to greater correlation between sibs than between parent and offspring. The later observation may, however, result from a variety of other factors such as a common sibling environment, trends of variance components with age, or deviations from assumptions about linearity and additivity effects. More generally, multifactorial transmission may concern environmental as well as genetic effects. One approach can allow for intergenerational differences in the multifactorial variance components, as well as general transmission through the parameter r (the parent-offspring correlation, conditional on the major genotypes and random residuals). The data can be adjusted so that V is the same in each generation; if C_A and C_K denote variance components due to multifactorial transmission in adults and young, respectively. We may define childhood heritability as $H = C_K/V$, adult heritability as $HZ = C_A/V$, where $Z = C_A/C_K$, and parent-offspring and sib correlations as $rHZ^{1/2}$ and $2r^2H$, respectively. In

our case, when the Z parameter was iterated we observed a significant ($p < 0.0001$) increment of the $-2\ln(L) + C$ (model 3) to expenses of a dramatic fall of Z which can be explained by saying that there are no differences in the multifactorial variance.

It must be emphasized that an environmental component in the predisposition to CLP, as has been pointed out in those hypotheses involving fungicides and some kind of avitaminosis, must be discarded, at least in the Colombian communities, and the genetic mendelian factors must be considered.

REFERENCES

Amidei RL, Hamman RF, Kassebaum DK, Marshall JA. 1994. Birth prevalence of cleft lip and palate in Colorado by sex distribution, seasonality, race/ethnicity, and geographic variation. *Spec Care Dentist* 14(6):233-40.

Arcos-Burgos M, Palacio G, Sánchez JL, Londoño AC, Uribe CS, Jiménez M, Villa A, Anaya JM, Bravo ML, Jaramillo N, Espinal C, Builes JJ, Moreno M, Jiménez I. 1999. Multiple Sclerosis: association to HLA DQα in a tropical population. *Exp Clin Immunogenet* 16:131-138.

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 and palate. *Am J Hum Genet* 45:348-353.

Bixler D, Fogh-Andersen P, Conneally P.M. 1971. Incidence of cleft lip and palate in the offspring of cleft lip parents. *Clin Genet* 2:155-159.

Blanco R, Rosales C. 1988. Diferencias étnicas y dimorfismo sexual de la fisura labiopalatina. *Rev Med Chile* 24:216-225.

Blanco R, Arcos-Burgos M, Paredes M, Palomino H, Jara L, Carreño H, Obreque V, Muñoz MA. 1998a. Complex segregation analysis of nonsyndromic cleft lip/palate in a Chilean population. *Genet Mol Biol* 21(1):139-144.

Blanco R, Jara L, Villaseca C, Palomino H, Carreno H. 1998b. Genetic variation of MSX1 has a sexual dimorphism in non syndromic cleft palate in the Chilean population. *Rev Med Chile* 126(7):781-7.

Bravo ML, Jailler G, Valencia C, Villegas LF, López O, Moreno L, Blanco R, Arcos-Burgos M. 1998. Evidencias de un gen mayor autosómico en la etiología de labio hendido con o sin paladar hendido y asociación al grupo sanguíneo Kidd en genealogías estudiadas en el departamento de Antioquia, Colombia. *Actual Biol* 20(68): 37-42.

Carinci F, Pezzetti F, Scapoli L, Padula E, Baciliero U, Curioni C, Tognon M. 1995. Nonsyndromic cleft lip and palate: evidence of linkage to a microsatellite marker on 6p23. *Am J Hum Genet* 56:337-339.

Carter CO. 1969. Genetics of common disorders. *Br Med Bull* 25:52-57.

Carter CO. 1976. Genetics of common single malformations. *Br Med Bull* 32(1):21-6.

Carter CO, Evans K, Coffrey R, Roberts JA, Buck A, Roberts MF. 1982. A three generation family study of cleft lip with or without cleft palate. *J Med Genet* 19:246-261.

Chakraborty R, Weiss KM. 1988. Admixture as a tool for finding linked genes and detecting that difference from allelic association between loci. *Proc Natl Acad Sci USA* 85:9.119-9.123.

Chenevix-Trench G, Jones K, Geen A, Martin N. 1991. Further evidence for an association between genetic variation in transforming growth factor alpha and cleft lip and palate. *Am J Hum Genet* 48:1.012-1.013.

Chenevix-Trench G, Jones K, Green AC, Duffly 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* 51:1.377-1.385.

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.

Chung CS, Beechert AM, Lew RE. 1989. Test of genetic heterogeneity of cleft lip with or without cleft palate as related to race and severity. *Genet Epidemiol* 6 (5):625-31.

Clementi M, Tenconi R, Collins A, Calzolari E, Milan M. 1995. Complex segregation analysis in a sample of consecutive newborns with cleft lip with or without cleft palate in Italy. *Hum Hered* 45: 157-164.

Davies A, Stephens J, Olavesen M, Heather L, Dixon M, Magee A, Flinter F, Ragoussis J. 1995. Evidence of a locus for orofacial clefting on human chromosome 6p24 and STS content map of the region. *Hum Mol Genet* 4:121-128.

Demenais F, Bonaiti-Pelie, Briard MI, Feingold J. 1984. An epidemiological and genetic study of facial clefting in France. II Segregation analysis. *J Med Genet* 121:436-440.

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

Farral M, Holders S. 1992. Familial recurrence-pattern analysis of cleft lip with or without cleft palate. Am *J Hum Genet* 50:270-277.

Fogh-Andersen P. 1942. Inheritance of harelip and cleft palate. A Busck, Copenhagen.

Fraser FC. 1970. The genetics of cleft lip and palate. *Am J Hum Genet* 22:33-352.

Hecht J. 1990. Dominant CLP families. *J Med Genet* 27:597.

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 YP, Blanton SH, Michels VV, Daiger SP. 1991b. Cleft lip and palate: no evidence of linkage to transforming growth factor alpha [see comments]. *Am J Hum Genet* 49(3):682-686.

Hecht JT, Wang Y, Connor B, Blanton SH, Daiger SP. 1993. Nonsyndromic cleft lip and palate: no evidence of linkage to HLA or factor 13A. *Am J Hum Genet* 52:1.230-1.233.

Holder SE, Vintiner GM, Farren S, Winter RM. 1992. Confirmation of an association between RFLP's at the transforming growth factor-alpha locus andnon-syndromic cleft lip and palate. *J Med Genet* 29:390-392.

Houlston RS, Collins A, Slack J, Campbell S, Collins WP, Whitehead MI, Morton NE. 1991. Genetic epidemiology of ovarian cancer: segregation analysis. *Ann Hum Genet* 55 (Pt 4):291-299.

Jara L, Blanco R, Chiffelle I, Palomino H, Carreño H. 1995. Association 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.

Lalouel JM, Morton NE. 1981. Complex segregation analysis with pointers. *Hum Hered* 31:312-321.

Lalouel JM, Rao DC, Morton NE, Elston RC. 1983. A unified model for complex segregation analysis. *Am J Hum Genet* 35:816-826.

Lidral AC, Murray JC, Buetow KH, Basart AM, Schearer H, Shiang R, Naval A, Layda E, Magee K, Magee W. 1997. Studies of the candidate genes TGFB2, MSX1, TGFA, and TGFB3 in the etiology of cleft lip and palate in the Philippines. *Cleft Palate Craniofac J* 34(1):1-6.

Lidral AC, Romitti PA, Basart AM, Leysens NJ, Daack-Hirsch S, Munger R, Semina EV, Millholin L, Machida J, Burds A, Wise M, Parnell T, Murray JC. 1998. Association of MSX1 and TGFB3 with nonsyndromic clefting in humans. *Am J Hum Genet* 63(2):557-568.

Marazita ML, Goldstein AM, Smalley SL, Spence MA. 1986. Cleft lip with or without cleft palate: re-analysis of a three generation family study from England. *Genet Epidemiol* 3:335-342.

Marazita ML, Hu DN, Spence MA, Liu YE, Melnick M. 1992. Cleft lip with or without cleft palate in Shanghai, China: evidence for an autosomal major locus [see comments]. *Am J Hum Genet* 51(3):648-653.

Melnick M, Bixler D, Fogh Andersen P, Conneally PM. 1980. Cleft lip cleft lip and /or cleft palate: an overview of the literature and an analysis of Danish cases born between 1941 and 1968. *Am J Med Genet* 6:83-87.

Melnick M, Marazita ML, Hu DN. 1986. Genetic analysis of cleft lip with or without cleft palate in Chinese kindreds. *Am J Med Genet* 21(Suppl.2):183-190.

Mitchell LE, Rish N. 1992. Mode of inheritance of nonsyndromic cleft lip with or without cleft palate: a reanalysis. *Am J Hum Genet* 51:323-332.

Mitchell LE, Risch N. 1993. Correlates of genetic risk for non-syndromic cleft lip with or without cleft palate. *Clin Genet* 43(5):255-260.

Mitchell L, Healey C, Chenevix-Trench G. 1995. Evidence for an association between nonsyndromic cleft lip with or without cleft palate and a gene located on the long arm of chromosome 4. *Am J Hum Genet* 57:1.130-1.136.

Nemana LJ, Marazita ML, Melnick M. 1992. A genetic analysis of cleft lip with or without cleft palate in Madras, India. *Am J Med Genet* 42:5-10.

Palomino H, Cerda-Flores RM, Blanco R, Palomino HM, Barton SA, De Andrade M, Chakraborty R. 1997. Complex segregation analysis of facial clefting in Chile. *J Craniofac Genet Develop Biol* 17(2):57-64.

Ray AK, Leigh L, Marazita LM. 1993. Nonsyndromic cleft lip with or without cleft palate in West Bengal, India: evidence for an autosomal major Locus. *Am J Hum Genet* 52:1.006-1.011.

Sassani R, Bartlett SP, Hongshu F, Goldner-Sauve A, Hag AK, Buetow KH, Gasser DL. 1993. Association between alleles of the trasnforming growth factor-alpha locus and the occurrence of cleft lip. *J Med Genet* 45:565-569.

Simpson SP. 1983. Estimating the ascertainment probability from the number of ascertainments per proband. *Hum Hered* 33:103-108.

Stein J, Mulliken J, Stal S, Gasser D, Malcom S, Winter R, Blanton S, Amos C, Seemanova E, Hecht J. 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, Qian JF, Feingold J, Sauvage P, May E. 1992. Genetic variation in trasnforming growth factor alpha: possible association of Bam HI polymorphism with bilateral sporadic cleft lip and palate. *Am J Hum Genet* 50:870-871.

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.

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(6):393-397.

Vintiner GM, Lo KK, Holder SE, Winter RM, Malcolm S. 1993. Exclusion of candidate genes from a role in cleft lip with or without cleft palate: linkage and association studies [see comments]. *J Med Genet* 30(9):773-778.