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Genetic Analysis of Candidate Loci in Non-Syndromic Cleft Lip Families From Antioquia-Colombia and Ohio

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Non-syndromic cleft lip with or without cleft palate (CL/P) is a genetically complex birth defect, with a prevalence from 1/500 to 1/1,000 live births. Evidence from linkage and linkage disequilibrium studies is contradictory suggesting that heterogeneity between study populations may exist. A recent report of a genome widescan in 92 sib pairs from the United Kingdom revealed suggestive linkage to 10 loci [Prescott et al., 2000]. The purpose of this study is to replicate those results and evaluate additional candidate genes in 49 Colombian and 13 Ohio families. Genotypes were obtained for STRPs at 1p36, 2p13 (TGFA), 4p16 (MSX1), 6p23-25, 6q25-27, 8q23-24, 11p12-q13, 12q13, 14q24 (TGFB3), 16q22-24, 17q12-21 (RARA), and Xcen-q21. Linkage was performed using parametric (dominant and recessive models) and non-parametric (GenehunterNPL and SimIBD) analyses. In addition, heterogeneity was analyzed using GenehunterHLOD, and association determined by the TDT. The Colombian families showed significant SimIBD results for 11p12-q13 ($P = 0.034$), 12q13 ($P = 0.015$), 16q22-24 (0.01), and 17q12-21 (0.009), while the Ohio families showed significant SimIBD results for 1p36 ($P = 0.02$), TGFA ($P = 0.005$), 6p23 ($P = 0.004$), 11p12-q13 ($P = 0.048$) and significant NPL results for TGFA (NPL = 3.01, $P = 0.009$), 4p16 (MNPL = 2.07, $P = 0.03$) and 12q13 (SNPL = 3.55, $P = 0.007$). Significant association results were obtained only for the Colombian families in the regions 1p36 ($P = 0.046$), 6p23-25 ($P = 0.020$), and 12q13 ($P = 0.046$). In addition several families yielded LOD scores ranging from 1.09 to 1.73, for loci at 4p16, 6p23-25, 16q22-24, and 17q13. These results confirm previous reports for these loci. However, the differences between the two populations suggest that population specific locus heterogeneity exists. This article contains supplementary material, which may be viewed at the American Journal of Medical Genetics website at http://www.interscience.wiley.com/jpages/0148–7299/suppmat/index.html.

KEY WORDS: candidate loci; complex trait; linkage; linkage disequilibrium

INTRODUCTION

Non-syndromic cleft lip with or without cleft palate (CL/P) is a common birth defect, with a birth prevalence

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ranging from 1/500 to 1/1,000 live births among the different ethnic groups. African-Americans exhibit the lowest birth prevalence (0.3/1,000) followed by Caucasians with an intermediate prevalence (1/1,000) and populations from Asian and native American with the highest prevalence (2.1/1,000 and 3.6/10,000, respectively) [reviewed in Wyszynski et al., 1996]. Evidence from twin studies confirms a genetic role in the cause of non-syndromic CL/P, showing a monozygotic concordance rate of 25–50% and a dizygotic rate of 3–6% [Mitchell and Risch, 1992]. However, the mode of transmission has not yet been clearly defined. Some studies support the hypothesis of a major gene [Marazita et al., 1992] with either autosomal dominant [Hecht et al., 1991; Palomino et al., 1997] or recessive modes of inheritance [Marazita et al., 1986]. Other studies support an oligogenic model in which 2–20 genes may play a role [Farrall and Holder, 1992; Mitchell and Risch, 1992; Clementi et al., 1995]. Furthermore, environmental factors such as smoking, alcohol consumption, maternal nutrition, and uptake of vitamins are also thought to modulate the genetic susceptibility [Maestri et al., 1997; Wyszynski et al., 1997; Romitti et al., 1999]. Thus, CL/P is an example of a complex genetic trait.

Genes and loci that have been considered for a role in clefting include *TGFA* (transforming growth factor alpha), *TGFB3* (transforming growth factor beta 3), *MSX1* (muscle segment homeobox *Drosophila* homolog 1), *BCL3* (B-cell CLL/lymphoma 3), RARA (retinoic acid receptor alpha), *PVRL1* (nectin-1), various genes involved in folic acid metabolism such as *MTHFR* (methylenetetrahydrofolate reductase), *GABRB3* (β3 subunit of the γ-aminobutyric acid receptor), and the chromosomal region 6p23-25. Results from previous studies using linkage and linkage disequilibrium methods have yielded contradictory results, partly because of incomplete penetrance, genetic heterogeneity, different study designs, and limited sample sizes [Mitchell et al., 2002]. To date, two genome-wide scans for NSCL/P have been reported. The first, involving 92 sib pairs from the United Kingdom, revealed suggestive evidence of linkage to 9 loci (1p36, 2p13, 6p23-25, 8q23-24, 11cen, 12q13, 16p22-24, and Xcen-q) [Prescott et al., 2000]. The second one, studying 36 multiplex families from Shanghai, reported positive multipoint linkage results and/or NPL, $P \leq 0.05$ in seven loci (90–110 cM in chromosome 1, 220–250 cM in chromosome 2, 130–150 cM in chromosome 3, 140–170 cM in chromosome 4, 70–100 cM in chromosome 6, 110 cM in chromosome 18, and 30–50 cM in chromosome 21). The most significant multipoint linkage results were for loci on 3q and 4q. Positive association results were also found for loci on chromosomes 3, 5, 6, 7, 9, 11, 12, 16, 20, and 21, with the most significant result found for D16S769 [Marazita et al., 2002].

In spite of the many studies done in different populations, no mutations with a clear functional effect for CL/P have been identified. The purpose of this study was to determine the role of a variety of candidate loci in the cause of cleft lip in two different populations. The first population is from the province of Antioquia, which is in Northwest Colombia. This population could be particularly useful for complex trait mapping because it is derived from the admixture of Amerinds, Europeans, and Africans during the 16th century. Since then this population has extended in relative isolation until the late 19th century [Carvajal-Carmona et al., 2000; Mesa et al., 2000; Arcos-Burgos and Muenke, 2002]. The second population (Southeast Ohio) is part of the Appalachian region and is characterized by its unique culture and heritage [Marger and Obermiller, 1987].

METHODS

Subjects

The study group consisted of 49 non-syndromic multiplex families recruited from the Clinica Noel in Medellín, Colombia and 8 non-syndromic multiplex families recruited from the Children's Hospital in Columbus, Ohio. Inclusion criteria were the diagnosis of non-syndromic cleft lip with or without cleft palate in two or more family members. A total of 123 affected and 198 unaffected subjects from Colombia as well as 26 affected and 39 unaffected subjects from Ohio were recruited, including, when possible, all linking relatives between affected individuals. Subjects were clinically examined and interviewed using a clinical survey to gather information regarding medical history, family history, and gestational environmental exposures to rule out any syndromes or phenocopies. This study was approved for the use of human subjects by the internal review board at the Ohio State University, the University of Iowa and the scientific committee at the dental school of the University of Antioquia-Colombia.

Molecular Analysis

After obtaining written consent, blood samples were obtained and DNA extraction was performed using a commercial kit (Puragene, Gentra Systems, Minneapolis). Tetra, tri, and dinucleotide, simple tandem repeat polymorphisms (STRP's) (Appendix Table A (http://www.interscience.wiley.com/jpages/0148–7299/suppmat/index.html)) were amplified using the polymerase chain reaction (PCR) under the following conditions: 0.24 μM each primer, 200 μM dNTPs, 50 mM KCl, 10 mM Tris Cl, 1.5 mM $MgCl_2$, 0.01% gelatin, 0.0225 U Taq polymerase, and 20 ng DNA in an 8 μl reaction volume. Thermocycling was carried out by means of two different 3-step PCR touchdown protocols [Morin et al., 1998]. The first protocol was as follows: 5 min at 95°C followed by 16 cycles of 30 s at 95°C, 30 s at 66°C (−1°C per cycle), 30 s at 72°C, and then 19 cycles at which the annealing temperature was held constant at 50°C. The second protocol had an annealing temperature starting at 60°C and ending at 44°C. Amplification products were separated in PAGE gels and visualized by a silver staining protocol [Budowle et al., 1991]. Genotypes were scored by two different observers and Mendelian inheritance errors were identified using the computer program Pedcheck [O'Connell and Weeks, 1998]. Inconsistencies found were resolved by repeat genotyping as necessary.

Statistical Analysis

Population allele frequencies for each marker were calculated in the Colombian and in the Ohio study families using founder individuals. Genotyping results were analyzed using parametric linkage analysis under dominant and recessive models (30 and 80% penetrance, respectively and a gene frequency of 0.001 and 0.01, respectively) and also using model-free (GenehunterNPL and SimIBD) linkage analysis [Davis et al., 1996; Kruglyak et al., 1996]. Single-point and multi-point LOD scores were calculated using the LINKAGE program and Genehunter, respectively [Elston and Stewart, 1971; Kruglyak et al., 1996]. In addition, association analyses (TDT) [Spielman et al., 1993] calculated by FBAT [Laird et al., 2000] were also performed. Heterogeneity in the LOD scores was assessed by calculating HLOD's using the Kruglyak algorithm implemented in Genehunter [Kruglyak et al., 1996].

In order to calculate the mean maximum LOD score expected in each family in the presence or absence of linkage families were evaluated by computer simulations using the software Simlink [Ploughman and Boehnke, 1989]. Simulated markers have the same number of alleles and the same frequencies as the ones that were genotyped. Allele frequencies were calculated for each marker allele using founder controls. Both recessive (allele frequency of 0.01 and penetrance of 80%) and dominant (allele frequency of 0.05 and penetrance of 30%) models were simulated. Simultaneously, the probability of finding linkage when unlinked was estimated.

RESULTS

Parametric Linkage Analysis

Results obtained from the parametric linkage analysis are displayed in Table I and Appendix Table B. Evidence against linkage can be seen for all the Colombian data set assuming linkage homogeneity. For the Ohio data set, suggestive evidence for linkage to the 4p16 region was found at D4S2366 (recessive LOD score of 1.53 at $\theta = 0$). HLOD analysis also did not show significant evidence of linkage for the Colombian data set, and did not provide any evidence of heterogeneity in the Ohio data set.

Model-Free Linkage Analysis

In the Colombian data set, significant SimIBD results were obtained for markers in the regions 11p12-q13 (D11S2371, $P = 0.034$), 12q13 (D12S1056, $P = 0.015$), 16q22-24 (D16S750, $P = 0.01$), 17q12-21(D17S1293, $P = 0.009$) (Table I and Appendix Table B (http://www.interscience.wiley.com/jpages/0148–7299/suppmat/index.html)). In addition, two positive NPL results, although not significant, were obtained on 16q22-24 (D16S539, NPL = 1.39, $P = 0.084$) and 17q12-21(D1S1293, NPL = 1.48, $P = 0.07$). For the Ohio data set, significant SimIBD results were found for 1p36 (D1S1597, $P = 0.02$), TGFA (D2S1342, $P = 0.005$), 6p23-25 (D6S1019, $P = 0.004$), and 11p12-q13 (D11S2002, $P = 0.048$). Furthermore, significant NPL results were observed for the TGFA region (D2S1342, NPL = 3.01, $P = 0.009$), for 4p16 (D4S2366, NPL = 0 2.07, $P = 0.03$), and for 12q13 (NPL = 3.55, $P = 0.007$) in the Ohio data set.

Association Analysis

For the Colombian data set, significant results were obtained for the region 1p36 (D1S1635, $P = 0.046$), 6p23-25 (D6S1574, $P = 0.020$; D6S1029, $P = 0.036$) and 12q13 (D12S398, $P = 0.046$) (Table I and Appendix Table B (http://www.interscience.wiley.com/jpages/0148–7299/suppmat/index.html)). In addition, a suggestive although non-significant result was obtained for the region 17q12-21 (D17S1293, $P = 0.057$). For the Ohio data set, no significant results were obtained for any of the markers; although suggestive results were observed for the TGFA region (D2S1342, $P = 0.063$) and 4p16 (MSX1CA, $P = 0.07$).

Positive Families

Eight families were observed to have LOD scores greater than 1.0 at one or more loci (Table II). The loci involved included TGFA, MSX1, 6p23-25, 12q13, 16q22-24, and 17q12-21. Simulations using Simlink [Ploughman and Boehnke, 1989] indicated that several of these families had yielded near maximum LOD scores. The maximum probability of finding a maximum LOD score>1 when unlinked in these families was 0.032 (Table II).

DISCUSSION

Markers for 12 chromosomal regions harboring candidate loci for clefting were genotyped in 49 Colombian and 8 Ohio families. Results obtained from parametric and model-free linkage analysis as well as from association methods were in agreement with previous studies suggesting the presence of susceptibility loci for clefting in 1p36, 6p23-25, 11p12-q13, 12q13, 16q22-24, 17q12-21 in the Colombian data set and 1p36, TGFA, MSX1, 6p23-25, 11p12-q13 in the Ohio data set (Table III). It is important to mention, however, that the criteria utilized in this study for positive results were loci with either $P < 0.05$ or LOD scores >1.0 in at least one of the three types of statistical analysis used and that no correction for multiple testing was performed. This strategy allows the identification of candidate loci, including those with moderate effects on the phenotype, which need to be replicated and confirmed in future studies under more stringent criteria [Marazita et al., 2002]. The regions for which no significant results were found in either of the data sets in this study were 6q25, 8q23-24, TGFB3, and Xcen-q21. Although there are biological and genetic evidence to consider TGFB3 a good candidate gene [Proetzel et al., 1995; Kaartinen et al., 1995, 1997; Lidral et al., 1998], no significant evidence of linkage was found for this gene in the UK nor the Shanghai studies [Prescott et al., 2000; Marazita et al., 2002], perhaps

TABLE I. Linkage and Association Results Summary for Colombian and Ohio Data Sets

Locus	Position in the Marshfield map	Type of analysis	Colombian data set	Ohio data set
1p36	D1S2132-D1S3669, 10.78–37.05 cM	Parametric linkage analysis assuming homogeneity	Exclusion of linkage	Exclusion of linkage
		Analysis of heterogeneity	NS	[D1S1597, 29.97 cM, Dom HLOD = 0.74, $\alpha = 0.53$]**
		Genehunter, NPL	NS	[D1S1597, 29.97 cM, NPL = 1.20, $P = 0.11$]**
		SimIBD	NS	[D1S1635, 23.35 cM, $P = 0.07$, D1S1597, 29.97 cM, $P = 0.02$]*
		TDT	[D1S1635, 23.35 cM, multiallelic $P = 0.046$]*	NS
2p13 TGFA	D2S1364-D2S1777, 79.97–99.41 cM	Parametric linkage analysis assuming homogeneity	Exclusion of linkage	No evidence of linkage
		Analysis of heterogeneity	[D2S443, 88.15 cM, Rec HLOD = 0.68, $\alpha = 0.25$]**	[D2S1342, 82.80 cM, Rec HLOD = 0.65, $\alpha = 1$]**
		Genehunter NPL	NS	[D2S1342, 82.80 cM, NPL = 3.01, $P = 0.009$]*
		SimIBD	[D2S1394, 90.82 cM, $P = 0.08$]**	[D2S1342, 82.80 cM, $P = 0.005$]*
		TDT	NS	[D2S1342, 82.80 cM, multiallelic $P = 0.063$]**
4p16 MSX1	D4S3023-D4S2366, 8.24–12.93 cM	Parametric linkage analysis assuming homogeneity	Exclusion of linkage	[D4S2366, 12.93 cM, Rec LOD = 1.53, $\theta = 0$]*
		Analysis of heterogeneity	NS	[D4S2366, 12.93 cM, Rec HLOD = 1.6, $\alpha = 1.0$]*
		Genehunter NPL	NS	[D4S2366, 12.93 cM, NPL = 2.07, $P = 0.03$]*
		SimIBD	[$P = 0.07$]**	NS
		TDT	NS	[MSX1 CA, 8.30 cM, multiallelic $P = 0.07$]**
6p23-25	F13A-D6S1019, 7.43–53.81 cM	Parametric linkage analysis assuming homogeneity	Exclusion of linkage	No evidence of linkage
		Analysis of heterogeneity	NS	NS
		Genehunter NPL	NS	NS
		SimIBD	[D6S1029, 39.20 cM, $P = 0.09$]**	[D6S1019, 53.81 cM, $P = 0.004$]*
		TDT	[D6S1574, 9.18 cM, multiallelic $P = 0.020$, D6S1029, 39.20 cM, multiallelic $P = 0.036$]*	NS
6q25	D6S1277-D6S503, 173.31–184.51 cM	Parametric linkage analysis assuming homogeneity	Exclusion of linkage	Exclusion of linkage
		Analysis of heterogeneity	[D6S1277, 173.31 cM, Dom HLOD = 0.68, $\alpha = 0.22$]**	NS
		Genehunter NPL	NS	NS
		SimIBD	NS	NS
		TDT	NS	NS
8q23-24	D8S2324-D8S1132, 94.08–119.22 cM	Parametric linkage analysis assuming homogeneity	Exclusion of linkage	Exclusion of linkage
		Analysis of heterogeneity	NS	NS
		Genehunter NPL	NS	NS
		SimIBD	NS	NS
		TDT	NS	NS

11p12-q13	D11S1392-D11S2002, 43.16–85.48 cM	Parametric linkage analysis assuming homogeneity	Exclusion of linkage	No evidence of linkage
		Analysis of heterogeneity	NS	NS
		Genehunter NPL	NS	NS
		SimIBD	[D11S2371, 76.12 cM, $P = 0.03$]*	[D11S2006, 59.24 cM, $P = 0.08$, D11S2002, 85.48 cM, $P = 0.048$]*
		TDT	NS	NS
12q13	D12S301-D12S1056, 56.25–75.17 cM	Parametric linkage analysis assuming homogeneity	Exclusion of linkage	No evidence of linkage
		Analysis of heterogeneity	NS	[D12S398, 68.16 cM, Dom HLOD = 0.98, $\alpha = 0.50$]**
		Genehunter NPL	NS	[D12S398, 68.16 cM, NPL = 3.55, $P = 0.007$]*
		SimIBD	[D12S1056, 75.17 cM, $P = 0.015$]*	NS
		TDT	[D12S398, 68.16 cM, multiallelic $P = 0.046$]*	NS
14q24 TGFB3	TGFβ3CA-D14S143, 84.69–85.0 cM	Parametric linkage analysis assuming homogeneity	Exclusion of linkage	No evidence of linkage
		Analysis of heterogeneity	NS	NS
		Genehunter NPL	NS	NS
		SimIBD	NS	NS
		TDT	NS	NS
16q22-24	D16S750-D16S539, 105.17–124.73 cM	Parametric linkage analysis assuming homogeneity	Exclusion of linkage	No evidence of linkage
		Analysis of heterogeneity	[D16S750, 105.17 cM, Dom HLOD = 0.89, $\alpha = 0.5$]**	NS
		Genehunter NPL	[D16S539, 124.73 cM, NPL = 1.39, $P = 0.084$]**	NS
		SimIBD	[D16S750, 105.17 cM, $P = 0.01$]*	NS
		TDT	NS	NS
17q12-21 RARA	D17S1294-D17S1299, 50.74–62.01	Parametric linkage analysis assuming homogeneity	Exclusion of linkage	Exclusion of linkage
		Analysis of heterogeneity	[D17S1294, 50.74 cM, Rec HLOD = 0.73, $\alpha = 0.25$]**	NS
		Genehunter NPL	[D17S1293, 56.48 cM, NPL = 1.48, $P = 0.07$]**	NS
		SimIBD	[D17S1293, 56.48 cM, $P = 0.009$]*	NS
		TDT	[D17S1293, 56.48 cM, multiallelic $P = 0.057$]**	[D17S1299, 62.01 cM, multiallelic $P = 0.11$]**
Xcen-q21	GATA144D04-DXS6789, 46.54–62.52 cM	Parametric linkage analysis assuming homogeneity	Exclusion of linkage	Exclusion of linkage
		Analysis of heterogeneity	NS	NS
		Genehunter NPL	NS	NS
		SimIBD	ND	ND
		TDT	ND	ND

NS, not significant.
*Corresponds to significant results.
**Corresponds to borderline significant results.

TABLE II. Simlink Results

Locus	Population	Fam ID (n = size of the family)	Observed single or multipoint LOD	Model	Maker	Mean max location score	Max location score	Probability[a]
TGFA	Ohio	505 (n = 7)	1.03	Dom	D2S1342 (82.80 cM)	1.77	1.97	0.016
MSX1	Ohio	528 (n = 9)	1.27	Rec	D4S2366 (12.93 cM)	1.46	2.32	0.028
6p	Colombia	58 (n = 10)	1.06	Rec	F13 (7.43 cM)	1.08	1.69	0.024
		26 (n = 12)	1.28	Dom	D6S1574 (9.18 cM)	1.08	1.47	0.028
		58 (n = 10)	1.14	Rec	D6S1279 (23.29 cM)	0.86	1.69	0.016
		58 (n = 10)	1.73	Dom	D6S2434 (25.08 cM)	0.79	1.76	0.012
		58 (n = 10)	1.18	Dom	D6S1019 (53.81 cM)	1.22	1.765	0.032
		8 (n = 11)	1.23	Dom	D6S1019 (53.81 cM)	1.48	2.06	0.032
12q13	Ohio	505 (n = 7)	1.42	Dom	D12S398 (68.16 cM)	0.71	1.97	0.016
16q22-24	Colombia	114 (n = 4)	1.09 MP	Rec	D16S750 (105.17 cM)	0.4	1.18	0.000
		92 (n = 8)	1.09 MP	Rec	D16S2625 (120.59 cM)	0.93	1.72	0.008
		110 (n = 10)	1.06 MP	Dom	D16S539 (124.73 cM)	1.45	2.07	0.028
17q12-21 RARA	Colombia	58 (n = 10)	1.22	Rec	D17S1294 (50.74 cM)	0.98	1.68	0.028
		114 (n = 4)	1.15 MP	Rec	D17S1294 (50.74 cM)	0.61	1.178	0.012

MP, multipoint observed LOD.
[a]Probability of finding a maximum location score>1 when unlinked.

suggesting a minor role in the etiology of CL/P. The expression data and knockout phenotype clearly suggest a role during secondary palatogenesis.

The main purpose of this study was to evaluate candidate genes for CL/P in two sets of families from different geographical and ethnic backgrounds. The Colombian families come from the Province of Antioquia located in the Northwest part of the country. This is an interesting population from a genetic point of view. Demographic and population genetic studies using chromosome Y, mitochondrial DNA, and autosomal markers have shown that this population is derived mostly from the admixture of immigrant men from Spain (Basque, Catalan) and native American women descendents most probably from the Embera Amerindian community that still inhabits a region in the northern part of the Antioquian Province [Carvajal-Carmona et al., 2000; Mesa et al., 2000]. There is also some evidence of founder effects for various monogenic traits [Carvajal-Carmona et al., 2000] seen in this community such as early-onset Alzheimer disease [Lendon et al., 1997]. These founder effects together with historical and genetic evidence indicating this is an admixed population suggest that there may be high levels of linkage disequilibrium that can be useful for mapping complex diseases [Seielstad, 2000]. In contrast, the Ohio population belongs to the Appalachian region and it is considered heterogeneous. Immigrants from the British Isles, Germany, and France settled in Appalachia in the 17th century and there is evidence of admixture with native Americans (Cherokee or Appalache). Since then the population has become increasingly ethnically diverse [Marger and Obermiller, 1987].

The highest LOD scores obtained in this study were for the TGFA, the MSX1, and the 12q13 regions in the Ohio data set. It is important to note, however, that the positive markers are 5.35 cM distal and 4.63 cM proximal from the actual *TGFA* and *MSX1* genes, respectively. Furthermore, linkage results obtained from intragenic markers (D2S443, MSX1CA) for each of these two genes were not significant. No significant results were found for any of these two regions in the Colombian data set. When these results are compared with the ones obtained by Prescott et al. [2000] and Marazita et al. [2002] for the TGFA and MSX1 region some differences can be seen (Table III). For the UK sibpair study, the multipoint NPL obtained for a region between D2S2368-D2S1790 (85.48–103.16 cM) that starts slightly proximal to D2S1342 (82.80 cM) was 1.77, $P = 0.04$. In addition, when this region was submitted to a denser scan in the UK study the NPL did not change significantly. In the Shanghai genome-wide scan, the peaks on chromosome 2 were seen at 210, 225, and 227 cM. When the MSX1 region is compared within the three studies, only the current study showed positive results in this region. TGFA and MSX1 have been implicated in the etiology of non-syndromic clefting by genetic association studies [Ardinger et al., 1989; Chenevix-Trench et al., 1991; Holder et al., 1992; Feng et al., 1994; Mitchell, 1997; Lidral et al., 1998; Beaty et al., 2001; Blanco et al., 2001; Vierra et al., 2003], as well as biological evidence composed of expression studies and knockout mouse models

TABLE III. Comparison of Results Between the Three Studies

	Colombian data set	Ohio data set	Prescott et al., 2000	Marazita et al., 2002
1p36, D1S2132-D1S3669, 10.78–37.05 cM	D1S1635, 23.35 cM, multiallelic TDT, $P = 0.046$	D1S1597, 29.7 cM, Dom HLOD = 0.74, $\alpha = 0.53$, SimIBD, $P = 0.02$	MNPL = 2.3, $P = 0.009$	Exclusion
2p13, TGFA, D2S1364-D2S1777, 79.97–99.41 cM	D2S443, 88.25 cM, Rec HLOD = 0.68, $\alpha = 0.25$	D2S1342, 82.80 cM, SimIBD, $P = 0.005$, NPL = 3.0, $P = 0.009$	MNPL = 1.7, $P = 0.04$	Exclusion
4p16, MSX1, D4S3023-D4S2366, 8.24–12.93 cM	Exclusion	D4S2366, 12.93 cM, Rec LOD 1.53, $\theta = 0$, Rec HLOD = 1.6, $\alpha = 1.0$, NPL = 2.07, $P = 0.03$	Exclusion	Exclusion
6p23-25, F13A-D6S1019, 7.43-53.81 cM	D6S1574, 9.18 cM, multiallelic TDT, $P = 0.020$, D6S1029, 39.20 cM, multiallelic TDT, $P = 0.036$	D6S1019, 53.81 cM, SimIBD, $P = 0.004$	MLS = 1.34, MNPL = 2.35, $P = 0.009$	Exclusion
6q25, D6S1277-D6S503, 173.31–184.51 cM	D6S1277, 173.31 cM, Dom HLOD = 0.68, $\alpha = 0.22$	Exclusion	MLS = 1.50, MNPL = 2.08, $P = 0.01$	Exclusion
8q23-24, D8S2324-D8S1132, 94.08–119.22 cM	Exclusion	Exclusion	MLS = 1.51, MNPL = 2.15, $P = 0.015$	Exclusion
11p12q13, D11S1392-D11S2002, 43.16–85.48 cM	D11S2371, 76.12 cM, SimIBD, $P = 0.03$	D11S2002, 85.48 cM, SimIBD, $P = 0.048$	MNPL = 2.70, $P = 0.002$	D11S1392, 43 cM TDT, $P = 0.032$
12q13, D12S301-D12S1056, 56.25–75.17 cM	D12S1056, 75.17 cM, SimIBD, $P = 0.015$, D12S398, 68.16 cM, multiallelic TDT, $P = 0.046$	D12S398, 68.16 cM, Dom HLOD = 0.98, $\alpha = 0.50$, NPL = 3.55, $P = 0.007$	MNPL = 2.08, $P = 0.02$	D12S1294, 78 cM TDT, $P = 0.031$
14q24, TGFβ3, TGFβ3CA-D14S143, 84.69–85.0 cM	Exclusion	Exclusion	Exclusion	Exclusion
16q22-24, D16S750-D16S539, 105.17–124.73 cM	D16S750, 105.17 cM, Dom HLOD = 0.89, $\alpha = 0.5$, SimIBD, $P = 0.01$	Exclusion	MNPL = 1.97, $P = 0.02$	Exclusion
17q12-21, RARA, D17S1294-D17S1299, 50.74–62.01 cM	D17S1294, 50.74 cM, Rec HLOD = 0.73, $\alpha = 0.25$, D17S1293, 56.48 cM, SimIBD, $P = 0.009$	D17S1299, 62.01 cM, multiallelic TDT, $P = 0.11$	Exclusion	Exclusion
Xcen-q21, GATA144D04-DXS6789, 46.54–62.52 cM	Exclusion	Exclusion	MLS = 2.89, MNPL = 2.40, $P = 0.008$	Exclusion

[see reviews Schutte and Murray, 1999; Wyszynski, 2002]. Mutations in the *MSX1* gene have been found in families with hereditary tooth agenesis [Vastardis et al., 1996; Lidral and Reising, 2002] and Witkop syndrome [Jumlongras et al., 2001]. Most strikingly, a MSX1 mutation has been described in a family with hereditary tooth agenesis and orofacial clefting, including both CL/P and isolated CP [van den Boogaard et al., 2000]. This family clearly demonstrates the role of MSX1 during primary and secondary palatogenesis. It is unique that the phenotype includes both forms of clefting, suggesting a possible interaction with IRF6, which causes the van der Woude syndrome, that also includes both types of orofacial clefts [Kondo et al., 2002]. The fact that positive results were found in a Caucasian population is in agreement with the majority of the population-based studies. However, the highest LOD scores obtained in this study are some distance away from the actual genes, which may indicate that signals of other interesting candidate genes are being picked up in this analysis. Another possible explanation is that the location estimate for a linked disease locus may vary largely when the signal for a susceptibility locus is weak due to incomplete penetrance or heterogeneity [Roberts et al., 1999].

When the rest of the regions analyzed in this study are compared with both genome-wide scans, chromosomal region 11p12-q14, and 12q13, yielded positive results in both data sets in the current study and in both genome-wide scans. Although the position of the peaks obtained varied within the studies for both regions, an interesting finding is that for chromosome 12 the significant peaks are clustered inside a 10 cM region flanked by the markers D12S398 (68.16 cM) and D12S1294 (78 cM). On the other hand, the chromosomal region 11p12-q14 yielded the second highest multipoint LOD scores in the UK study (NPL = 2.70, $P = 0.002$).

Several studies have shown some evidence of the existence of a CL/P locus on the 6p23-25 region. Linkage has been found with *EDN1* and *AP2* genes and also balanced translocations and deletions in this region associated with a cleft phenotype have been reported [see reviews Schutte and Murray, 1999; Carinci et al., 2000]. In the current study, we found positive association results for the markers D6S1574 (9.18 cM) and D6S1029 (39.20 cM) in the Colombian data set and a significant SimIBD result for D6S1019 (53.81 cM) in the Ohio data set. No significant results were obtained for the region 6q25. Prescott et al. [2000] obtained positive results on their 5 cM scan for 6p23-25 and 6q25. Whereas Marazita et al. [2002] excluded linkage at 6p23-25 and obtained a significant result in the 6q region for the marker D6S1031 (89 cM), which is more proximal than the UK positive marker.

Chromosomal regions 1p36 and 17q12-21 that harbor *MTHFR* and *RARA*, genes respectively, have been considered for a role in clefting [see reviews Wyszynski et al., 1996; 2002]. However, variable association and linkage results have been observed in different populations [Chenevix-Trench et al., 1992; Shaw et al., 1993; Vintiner et al., 1993; Mitchell, 1994; Shaw et al., 1998; Mills et al., 1999; Blanton et al., 2000; Prescott et al., 2002]. For the 1p36 region, a positive TDT result was observed with the marker D1S1635 (23.35 cM) in the Colombian data set. This marker is approximately 11 cM proximal from the *MTHFR* gene. In the Ohio data set, the marker D1S1597 (29.27 cM) located 19 cM proximal to MTHFR yielded a positive SimIBD and NPL scores. This is one of the loci identified in the UK study, however, no significant results were obtained in the Shanghai study for this region. The RARA region did not yield significant results in the Ohio data set nor both genome-wide scans. However, positive results were observed in the Colombian population for a marker located 5 cM proximal to the actual *RARA* gene. In addition, one of the largest Colombian families showed a recessive LOD score of 1.15 in the marker D17S1294 (50.74 cM).

In the Colombian data set, three of the six largest multigenerational families (the number of affected people ranges from a minimum of 3 to 5 affected people per family), yielded parametric dominant LOD scores ranging from 1.23 to 1.73 in different loci on 6p23-25. The other three families gave positive LOD scores ranging from 1.06 to 1.15 under recessive and dominant models on loci in 16q22-24 and 17q12-21 regions. In addition, two families from Ohio had positive LOD scores for TGFA and MSX1. The maximum probabilities of finding a maximum LOD score >1 when unlinked (type I error) found in these families was 0.032. These families will form the basis for mutation screens of candidate genes in these loci.

Further evidence for population heterogeneity exists when comparing these results to the two companion papers [Blanton et al., 2003; Schultz et al., 2003] in which TGFA and MSX1 were positive in Philippine families and loci at 11p12-q13 and 12q13 were positive in families from Texas.

In summary, our findings corroborate the presence of susceptibility loci in the regions 6p23-25, 11p12q13, 12q13, 16q22-24, and 17q12-21 in the Colombian data set and 1p36, TGFA, 4p16, 6p23-25 11p12-13, and 12q13 for the Ohio families. In addition, the positive although not significant parametric LOD scores obtained for different loci in six chromosomal regions in several large families are in agreement with the genetic heterogeneity that characterizes this complex trait. From all these regions, 11p12q13 and 12q13 deserve special attention for follow-up as they showed positive results in the three studies that were compared.

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