

Candidate Genes for Nonsyndromic Cleft Lip and Palate and Maternal Cigarette Smoking and Alcohol Consumption: Evaluation of Genotype-Environment Interactions From a Population-Based Case-Control Study of Orofacial Clefts

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ABSTRACT Previous studies suggest that the relationship between genes and nonsyndromic cleft lip \pm cleft palate (CLP) or cleft palate only (CP) may be modified by the environment. Using data from a population-based case-control study, we examined allelic variants for three genes, i.e., transforming growth factor alpha (TGFA), transforming growth factor beta 3 (TGFB3), and Msh (*Drosophila*) homeobox homolog 1 (MSX1), and their interactions with two exposures during pregnancy (maternal cigarette smoking and alcohol consumption) as risk factors for CLP and CP. For each cleft phenotype, risk estimates associated with most allelic variants tended to be near unity. Risk estimates for maternal smoking (≥ 10 cigarettes/day) were significantly elevated for CP and were most elevated among infants with allelic variants at the TGFB3 or MSX1 sites. By comparison, risk estimates for maternal alcohol consumption (≥ 4 drinks/month) were significantly elevated for CLP and were most elevated among infants with allelic variants at the MSX1 site. Our results suggest that development of CLP and CP may be influenced independently by maternal exposures but more significantly by interaction of such exposures and specific allelic variants. *Teratology* 59: 39-50, 1999. © 1999 Wiley-Liss, Inc.

gene modifiable by various teratogens; a major autosomal dominant or codominant locus; or multigenic inheritance (reviewed by Murray, '95; Wyszynski et al., '96). Efforts to investigate genes involved in nonsyndromic clefting also include evaluation of allelic variants at candidate loci. Associations have been identified between allelic variants of genes for transforming growth factor alpha (TGFA) (reviewed by Murray, '95; Wyszynski et al., '96), retinoid receptor alpha (RARA) (Chenevix-Trench et al., '92), B-cell CLL/lymphoma 3 (BCL3) (Wyszynski et al., '97a), transforming growth factor beta 3 (TGFB3) (Lidral et al., '98), Msh (*Drosophila*) homeobox homolog 1 (MSX1) (Lidral et al., '98), and CLP, and between allelic variants of TGFA (Shiang et al., '93; Hwang et al., '95), MSX1 (Lidral et al., '98), and CP.

Recent inquiries into risk factors for complex traits have examined the joint effects of candidate genes and their interaction with environmental agents. These genotype-environment interactions may follow one of several biologic models (Khoury et al., '88). Demonstrated for complex traits is the model in which disease risk is increased by the environmental agent alone but is further increased by the joint effects of each exposure (Caporaso et al., '89). With regard to nonsyndromic clefting, gestational exposure to cigarette smoke has been associated with an increased risk of CLP and/or CP in several studies (reviewed by Shaw et al., '96). Also, we (Munger et al., '96) and others (Werler et al., '91) have found an increased risk of CLP associated with gestational exposure to alcohol. Motivated by this

Nonsyndromic cleft lip \pm cleft palate (CLP) and cleft palate alone (CP) affect approximately 1 per 1,000 live births, with some variation by race and ethnic group (Vanderas, '87). Work by Fogh-Andersen ('42) provided evidence that CLP and CP were developmentally distinct entities, and since then, investigators have attempted to elucidate the causal factors for each defect. Among Caucasians, the multifactorial threshold model was suggested by observational studies to best explain inheritance of CLP and CP, although models advanced by segregation analyses include a major susceptibility

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evidence, four case-control studies (Hwang et al., '95; Shaw et al., '96; Beaty et al., '97; Christensen et al., in press) of the joint risk of infant genotype and maternal smoking on the development of CLP and CP have been conducted, and results from two (Hwang et al., '95; Shaw et al., '96) of these studies suggest that infants with the rare allele at the TGFA Taq1 site and whose mothers smoked during pregnancy are at increased risk for nonsyndromic clefting. Using data from our population-based case-control study of orofacial clefts, we attempted: 1) to confirm the previously reported association between presence of the rare allele at the TGFA Taq1 site, maternal smoking during pregnancy, and risk of CLP and CP; 2) to extend the investigation of genotype-smoking interactions to include evaluation of allelic variants for TGFB3 and MSX1; and 3) to expand our previous evaluation of the risk of maternal alcohol consumption by examining potential genotype-alcohol interactions.

MATERIALS AND METHODS

Subject selection

Cases. Live-born infants, stillbirths, and aborted fetuses diagnosed with a CLP or CP between January 1, 1987–December 31, 1994 were identified through the Iowa Birth Defects Registry. The Registry is an active population-based surveillance system, which attempts to ascertain all children born to Iowa residents and diagnosed with a birth defect in the first year of life. Diagnostic information for each case was reviewed by a nurse-genetic counselor (S.D.-H.) and a physician-geneticist (J.C.M.). When possible, a physical examination of the infant was performed (J.C.M.). Eligible cases were grouped as nonsyndromic (no additional malformations) or as syndromic (presence of additional malformations or developmental delay).

Controls. For each case family, participation was sought from one control family. Controls (live-born infants without a diagnosed birth defect) were selected from among all eligible Iowa births between January 1, 1987–December 31, 1994, using a pseudorandom number generator. Initially, five controls, matched by birth month, year, and gender, were identified for each case, and one of the five was randomly selected to be contacted. A control was replaced if the child was reported to have been diagnosed with a birth defect; if the child was reported to be deceased; or if the child's mother declined to participate or was lost to follow-up. Replacement of control children resulted in control mothers being interviewed, on average, 35 months following the birth of their child compared to 29 months for case mothers (Romitti et al., '98). To minimize this difference, beginning with pregnancies delivered in 1992, controls were frequency matched by birth year and gender to cases.

Data collection

Data collection was divided into two phases (Romitti et al., '98, and unpublished data). For pregnancies delivered from 1987–1991, biologic specimens were principally obtained by either venipuncture or finger-

prick blood collection, and environmental data were obtained by a combination of telephone interviews and a mailed questionnaire (Romitti et al., '98). For pregnancies delivered from 1992–1994, collection of biologic specimens was restricted to buccal cell samples, and collection of environmental data was restricted to a mailed questionnaire (Romitti et al., unpublished data). Signed informed consent was requested from participants prior to collection of biologic specimens.

DNA extraction and genotyping. Methods used to extract genomic DNA and conduct genotyping of marker loci are detailed elsewhere (Lidral et al., '98). Markers evaluated were TGFA Taq1; TGFB3 CA, X5.1, 5'UTR.1; and MSX1 CA, X1.1, X1.3, X2.1, X2.4. For each marker, the sample was dichotomized by the allele of interest. Infants with one or two copies of the allele were classified as "exposed," and those without the allele were classified as "unexposed."

Cigarette smoke exposure. Reports of maternal cigarette smoking were collected for the 3-month period before conception and the 9-month period (or briefer for preterm deliveries) following conception. Interview reports of preconceptional smoking were limited to use and average number of cigarettes smoked per day, whereas questionnaire reports also included which months the mothers smoked before conception (Appendix A). For either method, mothers who reported smoking following conception were asked to specify which months they smoked and the average number of cigarettes smoked per day. Reports of exposure to paternal cigarette smoking were also obtained for the preconceptional and postconceptional periods. In the telephone interview, information requested for paternal smoking paralleled that requested for maternal smoking and was generally obtained from the birth fathers (Romitti et al., '98). In the mailed questionnaire, reports of paternal smoking were provided by the birth mothers and were limited to the occurrence and months exposed.

Alcohol exposure. Reports of maternal alcohol consumption were collected for the 3-month period before conception and the 9-month period following conception. Mothers who completed the telephone interview were queried about their alcohol consumption for each time period, whereas mothers who completed the mailed questionnaire were also queried about which months they consumed alcohol (Appendix B). For either method, mothers who consumed an alcoholic drink (beer, wine, or liquor) were asked to enumerate: the number of days per month they consumed alcohol; the typical number of drinks consumed on those days; and the maximum number of drinks consumed in a 24-hr interval. Using the typical frequency and quantity data reported, the average number of drinks consumed per month was calculated. Variability in consumption was evaluated by comparing the maximum and typical number of drinks consumed per day.

Statistical analyses

The risk of CLP or CP associated with infant genotype was estimated using the odds ratio and 95%

confidence intervals (SAS Institute, Inc., '89); for markers with three or more alleles, the Bonferroni correction was applied to correct for multiple pairwise tests. Estimates of the odds ratio and 95% confidence intervals were also calculated for each level of maternal cigarette smoking (1–9 or ≥10 cigarettes/day relative to 0) and alcohol consumption (1–3 or ≥4 drinks/month relative to 0). Exposure to cigarette smoke was restricted to the 2-month postconceptual period because mothers who completed the telephone interview were not asked to specify which months before conception they smoked cigarettes. Exposure to alcohol was restricted to the 2-month postconceptual period for mailed questionnaire data only because mothers who completed the telephone interview were not asked to specify which months of pregnancy they consumed alcohol. Interview forms were manually reviewed to identify mothers who mentioned that alcohol consumption occurred only after the 2-month postconceptual period. The joint risk of maternal smoking or alcohol consumption and each infant genotype was estimated by calculating the odds ratio and 95% confidence intervals, and the Bonferroni correction was applied as stated above. To test for statistical interaction ($P < 0.20$), 2×4 contingency tables were constructed for each infant genotype and maternal exposure, and the sum of the log odds was evaluated using a χ^2 test with one degree of freedom, as described by Selvin ('96). For contingency tables with one or more entries of zero, 0.5 was added to each entry in the table.

Stratified and logistic regression analyses were used to evaluate the influence of several familial characteristics on the risk estimates obtained (SAS Institute, Inc., '89). Characteristics evaluated were infant gender and family history (first- or second-degree relative with CLP or CP); maternal age (<21, 21–30, >30 years), education (high school degree or less, some college, bachelor's degree or higher) and gravidity (1, 2, >2), as well as maternal alcohol consumption (0, 1–3 or ≥4 drinks/month) or smoking (0, 1–9, ≥10 cigarettes/day), folic acid-supplemented multivitamin use (yes/no), and paternal smoking (yes/no) during the 2-month postconceptual period. Stratum-specific risk estimates for infant genotype were calculated by infant gender and family history (familial vs. nonfamilial cases). Estimates for maternal smoking or alcohol consumption were calculated for each familial characteristic. For the joint effect of infant genotype and either maternal exposure, stratum-specific estimates were calculated by infant gender and family history. Logistic regression analyses included evaluation of each characteristic excluding family history, since only case subjects were stratified for this variable.

RESULTS

Identified for the Iowa study were 556 eligible case and 763 eligible control children. Birth mothers of 532 cases and 698 controls were located, and consent was received from 378 (71.1%) cases and 407 (58.3%) controls. Of these, 287 case and 302 control mothers

TABLE 1. Selected characteristics of case and control participants by data collection method*

	Total (%)	Telephone interview (%)	Mailed questionnaire (%)	<i>P</i> **
Case mother	(N = 366)	(N = 279)	(N = 87)	
Age (years)				
<21	10.4	10.0	11.5	ns
21–30	65.3	65.6	64.4	
>30	24.3	24.4	24.1	
Education				
High school degree or less	39.1	41.9	29.9	ns
Some college	41.3	39.8	46.0	
Bachelor's degree or higher	19.7	18.3	24.1	
Marital status				
Married	85.2	83.9	89.7	ns
Unmarried	14.8	16.1	10.3	
Gravidity***				
1	17.8	17.9	17.4	ns
2	30.7	28.0	39.5	
>2	51.5	54.1	43.0	
Control mother	(N = 393)	(N = 291)	(N = 102)	
Age (years)				
<21	7.6	8.6	4.9	ns
21–30	61.8	63.9	55.9	
>30	30.5	27.5	39.2	
Education				
High school degree or less	33.8	36.4	26.5	ns
Some college	41.7	41.6	42.2	
Bachelor's degree or higher	24.4	22.0	31.4	
Marital status				
Married	87.8	88.0	87.3	ns
Unmarried	12.2	12.0	12.7	
Gravidity****				
1	14.8	12.7	20.8	<0.05
2	32.7	30.9	37.6	
>2	52.6	56.4	41.6	

*Because of rounding, percentages may not total 100. ns, not significant ($P > 0.05$).

***P* values obtained by chi-square test.

***Number of available reports: total (n = 365), telephone interview (n = 279), and mailed questionnaire (n = 86).

****Number of available reports: total (n = 392), telephone interview (n = 291), and mailed questionnaire (n = 101).

provided data on cigarette smoking and alcohol consumption by telephone interview, and the remainder provided such data by mailed questionnaire. To account for the possibility of selection bias introduced by the different data collection methods, selected characteristics of participants were compared between each method. Distributions presented were restricted to white, non-Hispanic mothers, because of the small number of participants ($n_{\text{case}} = 12$; $n_{\text{control}} = 14$) in the other racial/ethnic categories (Table 1). Case participants who completed either a telephone interview or a mailed questionnaire differed little with regard to age, education, marital status, or gravidity. Control participants who completed a telephone interview tended to have had more pregnancies than those who completed a mailed questionnaire ($P < 0.05$). The 366 case partici-

TABLE 2. Risk estimates for nonsyndromic cleft lip ± cleft palate and nonsyndromic cleft palate only by infant genotype*

Gene/ marker	Controls			CLP				CP			
	Genotyped (%)	Genotype**	N	Genotyped (%)	Genotype**	N	OR (CI)***	Genotyped (%)	Genotype**	N	OR (CI)***
TGFA											
Taq1	295 (79.1)	1,1 1,2 or 2,2	235 60	118 (76.6)	1,1 1,2 or 2,2	96 22	Reference 0.9 (0.5, 1.5)	51 (85.0)	1,1 1,2 or 2,2	41 10	Reference 1.0 (0.4, 2.0)
TGFB3											
CA	299 (80.2)	n,n 1,n or 1,1 n,n 2,n or 2,2 n,n 3,n or 3,3	50 249 134 165 269 30	111 (72.1)	n,n 1,n or 1,1 n,n 2,n or 2,2 n,n 3,n or 3,3	27 84 49 62 90 21	Reference 0.6 (0.3, 1.2) Reference 1.0 (0.6, 1.8) Reference 2.1 (1.0, 4.4)	47 (78.3)	n,n 1,n or 1,1 n,n 2,n or 2,2 n,n 3,n or 3,3	8 39 22 25 42 5	Reference 1.0 (0.4, 2.9) Reference 0.9 (0.4, 2.0) Reference 1.1 (0.3, 3.2)
X5.1	253 (67.8)	1,1 1,2 or 2,2	220 33	110 (71.4)	1,1 1,2 or 2,2	101 9	Reference 0.6 (0.3, 2.1)	42 (70.0)	1,1 1,2 or 2,2	36 6	Reference 1.1 (0.4, 2.7)
5'UTR.1	296 (79.4)	1,1 1,2 or 2,2	262 34	111 (72.1)	1,1 1,2 or 2,2	97 14	Reference 1.1 (0.6, 2.1)	43 (71.7)	1,1 1,2 or 2,2	37 6	Reference 1.3 (0.5, 3.0)
MSX1											
CA	338 (90.6)	n,n 1,n or 1,1 n,n 2,n or 2,2 n,n 3,n or 3,3 n,n 4,n or 4,4	264 74 187 151 296 42 52 286	114 (74.0)	n,n 1,n or 1,1 n,n 2,n or 2,2 n,n 3,n or 3,3 n,n 4,n or 4,4	99 15 72 42 99 15 14 100	Reference 0.5 (0.2, 1.1) Reference 0.7 (0.4, 1.3) Reference 1.1 (0.5, 2.3) Reference 1.3 (0.6, 3.1)	46 (76.7)	n,n 1,n or 1,1 n,n 2,n or 2,2 n,n 3,n or 3,3 n,n 4,n or 4,4	36 10 25 21 45 1 7 39	Reference 1.0 (0.4, 2.4) Reference 1.0 (0.5, 2.3) Reference 0.2 (0.1, 1.0) Reference 1.0 (0.4, 3.5)
X1.1	192 (51.5)	n,n 1,n or 1,1 n,n 2,n or 2,2 n,n 3,n or 3,3	171 21 7 185 138 54	92 (59.7)	n,n 1,n or 1,1 n,n 2,n or 2,2 n,n 3,n or 3,3	81 11 3 89 69 23	Reference 1.1 (0.4, 2.8) Reference 1.1 (0.2, 8.0) Reference 0.9 (0.4, 1.7)	39 (65.0)	n,n 1,n or 1,1 n,n 2,n or 2,2 n,n 3,n or 3,3	32 7 3 36 27 12	Reference 1.8 (0.5, 5.3) Reference 0.5 (0.1, 3.3) Reference 1.1 (0.4, 2.8)
X1.3	205 (55.0)	1,1 1,2 or 2,2	0 205	109 (70.8)	1,1 1,2 or 2,2	0 109	Reference nc	47 (78.3)	1,1 1,2 or 2,2	0 47	Reference nc
X2.1	232 (62.2)	n,n 1,n or 1,1 n,n 2,n or 2,2 n,n 3,n or 3,3	228 4 13 219 146 86	94 (61.0)	n,n 1,n or 1,1 n,n 2,n or 2,2 n,n 3,n or 3,3	90 4 6 88 63 31	Reference 2.5 (0.4, 15.6) Reference 0.9 (0.3, 3.3) Reference 0.8 (0.4, 1.5)	44 (73.3)	n,n 1,n or 1,1 n,n 2,n or 2,2 n,n 3,n or 3,3	42 2 4 40 26 18	Reference 2.7 (0.2, 21.0) Reference 0.6 (0.2, 3.1) Reference 1.2 (0.5, 2.6)
X2.4	166 (44.5)	1,1 1,2 or 2,2	13 153	90 (58.4)	1,1 1,2 or 2,2	8 82	Reference 0.9 (0.4, 2.3)	41 (68.3)	1,1 1,2 or 2,2	3 38	Reference 1.1 (0.3, 4.9)

*CLP, cleft lip ± cleft palate; CP, cleft palate only; OR, odds ratio; CI, confidence interval; nc, not calculated.

**n = 1, 2, or 3 for markers TGFB3 CA, MSX1 X1.1, and MSX1 X1.3, and n = 1, 2, 3, or 4 for marker MSX1 CA.

***Bonferroni correction used for multiple pairwise comparisons.

pants included 161 infants with CLP, 64 infants with CP, and 141 with associated malformations. Dysmorphic classification was based at minimum on record review; 100 of the 321 (31.2%) living cases were evaluated by physical examination. We observed a male excess in CLP (64.0%), but not in CP (45.3%), and similar proportions of CLP (14.3%) and CP (12.5%) infants had a reported family history of clefting.

Infant genotype

Biologic specimens were obtained from 373 (94.9%) control infants, 154 (95.7%) CLP infants, and 60 (93.8%) CP infants. Similar proportions of cases and controls were successfully genotyped for the TGFA and TGFB3 markers, whereas totals for the MSX1 markers were less comparable between groups (Table 2). Notable among the markers examined was that infants with the rare 2 allele at the TGFA Taq1 site did not have an elevated risk for CLP or CP. By comparison, those with

the rare 3 allele at the TGFB3 CA site were at increased risk for CLP, but less so for CP. For each of these markers, risk for CLP was further, but not significantly, elevated among infants homozygous for the allele of interest (data not shown).

Stratified analyses suggested that females compared to males were at increased risk for CLP or CP if they carried the rare 2 allele for TGFA Taq1, and were at lower risk for CLP if they carried the rare 3 allele for TGFB3 CA; however, not one of the differences was statistically significant. For each phenotype, logistic regression analyses confirmed the nonsignificant risk differences. Stratification by family history revealed that familial but not nonfamilial cases had an elevated risk of CLP associated with the rare 2 allele for TGFA Taq1 (odds ratio (OR)_{Familial} = 3.5; confidence interval (CI) = 1.3, 9.0 vs. OR_{Nonfamilial} = 0.6; CI = 0.3, 1.1) or the rare 3 allele for TGFB3 CA (OR_{Familial} = 6.7; CI = 1.7, 24.5 vs. OR_{Nonfamilial} = 1.5; CI = 0.6, 3.3). Stratification

TABLE 3. Risk estimates for nonsyndromic cleft lip ± cleft palate and nonsyndromic cleft palate only by level of maternal cigarette smoking during the 2-month postconceptional period*

	Maternal cigarette smoking					
	1–9 cigarettes/day			≥10 cigarettes/day		
	Yes	No	OR (95% CI)	Yes	No	OR (95% CI)
Controls	40	312	Reference	41	312	Reference
CLP	18	122	1.2 (0.6, 2.1)	21	122	1.3 (0.7, 2.3)
CP	8	43	1.5 (0.6, 3.3)	13	43	2.3 (1.1, 4.6)

*OR, odds ratio; CI, confidence interval; CLP, cleft lip ± cleft palate; CP, cleft palate only.

by family history modestly but not significantly influenced the risk estimates for CP.

Maternal exposure

Maternal cigarette smoking during the 3-month period before conception and/or the 9-month period following conception was reported by 70 (31.3%) case and 102 (26.0%) control mothers. Of these, 68 (97.1%) cases and 99 (97.1%) controls maintained or reduced their frequency of smoking postconceptionally compared to preconceptionally. Among mothers who smoked during the 2-month postconceptional period, risk for delivering an infant with CLP was similar for each level of smoking evaluated (Table 3). In contrast, risk for delivering an infant with CP increased with increasing number of cigarettes smoked, and the associated confidence interval for mothers who smoked ≥10 cigarettes/day excluded the null value. Inclusion of mothers who smoked only during the 3-month period before conception (interview data) or the month before conception (questionnaire data) did not materially alter the risk estimates (data not shown).

Maternal alcohol consumption, pre- and/or postconception, was reported by 142 (64.0%) case and 238 (60.6%) control mothers. For 136 (95.8%) drinking cases and 230 (96.6%) drinking controls, postconception consumption was equal to or less than preconception consumption, and, of these, the maximum and typical number of drinks consumed per day did not vary for 118 (86.8%) cases and 200 (87.0%) controls. As shown in Table 4, risk estimates for each phenotype increased with increasing level of consumption, and the estimate for CLP was significantly elevated when mothers consumed ≥4 drinks/month. Risk estimates were relatively unchanged with inclusion of mothers who reported consumption during the preconceptional period only (data not shown).

Results of gender-stratified analyses revealed that risk estimates associated with maternal smoking tended to be higher for male infants than female infants, with the largest difference found for CP when mothers smoked ≥10 cigarettes/day. Evaluation by family history marginally influenced the estimates for each phenotype. Risk for CLP tended to decrease with increas-

TABLE 4. Risk estimates for nonsyndromic cleft lip ± cleft palate and nonsyndromic cleft palate only by level of maternal alcohol consumption during pregnancy*

	Maternal alcohol consumption					
	1–3 drinks/month			≥4 drinks/month		
	Yes	No	OR (95% CI)	Yes	No	OR (95% CI)
Controls	97	285	Reference	11	285	Reference
CLP**	46	102	1.3 (0.9, 2.0)	11	102	2.8 (1.2, 6.6)
CP	16	45	1.1 (0.6, 1.9)	3	45	1.7 (0.5, 6.4)

*OR, odds ratio; CI, confidence interval; CLP, cleft lip ± cleft palate; CP, cleft palate only.

**Information on quantity and frequency of alcohol consumption missing for two mothers.

ing maternal age, with the opposite pattern found for CP. Estimates for each phenotype tended to decrease with increasing level of maternal education and number of pregnancies, as well as with maternal alcohol consumption, multivitamin use, or paternal smoking. For maternal alcohol consumption, analyses by infant gender or family history did not alter the risk estimates for CLP or CP, although estimates for each phenotype did tend to increase with increasing maternal age and level of education. In contrast, estimates tended to decrease with increasing number of pregnancies, maternal smoking, multivitamin use, or paternal smoking. Single variable control for each covariate (excluding family history) using logistic regression analysis corroborated the results of stratified analyses.

Infant genotype-maternal exposure interaction

The joint effect of infant genotype and maternal cigarette smoking or alcohol consumption was examined for each locus. Infants with one or two copies of the common 1 allele and whose mothers smoked ≥10 cigarettes/day were at increased risk for CLP and CP (Table 5). For TGFB3, infants homozygous for the common 1 allele at the X5.1 site and whose mothers smoked ≥10 cigarettes/day had a significantly increased risk for CP. Those homozygous for the common 1 allele at the 5'UTR.1 site and whose mothers smoked ≥10 cigarettes/day had a significantly increased risk for either phenotype. For MSX1, elevated but nonsignificant risks for CLP were found for infants with one or two copies of the 2 allele at the CA, X1.1, X1.3, X2.1, or X2.4 sites and whose mothers smoked ≥10 cigarettes/day. A similar pattern was observed for CP, except that the confidence intervals for estimates at loci X1.3 and X2.4 excluded the null value. Examination of the influence of maternal smoking (≥10 cigarettes/day) among homozygotes for the 2 allele at each MSX1 locus revealed increased risks for CLP (OR_{X1.1} = 2.7, CI = 0.8, 9.4; OR_{X2.1} = 3.4, CI = 1.0, 12.1; and OR_{X2.4} = 2.9, CI = 1.0, 8.6) and CP (OR_{X1.1} = 5.3, CI = 1.2, 23.0; OR_{X1.3} = 2.9, CI = 1.2, 6.8; OR_{X2.1} = 6.5, CI = 1.4, 28.3; and OR_{X2.4} = 6.0, CI = 1.8, 20.5). Evidence for statistical

TABLE 5. Risk estimates for nonsyndromic cleft lip ± cleft palate and nonsyndromic cleft palate only by maternal cigarette smoking during the 2-month postconceptional period, and infant genotype*

Gene/ marker	Genotype**	Group	Maternal cigarette smoking						
			1–9 cigarettes/day			≥10 cigarettes/day			
			Yes	No	OR (CI)***	Yes	No	OR (CI)***	
TGFA Taq1	1,1	Controls	20	190	Reference	25	190	Reference	
		CLP	11	69	1.5 (0.7, 3.3)	16	69	1.8 (0.9, 3.5)	
		CP	4	26	1.5 (0.4, 4.2)	11	26	3.2 (1.4, 7.2)	
	1,2 or 2,2	Controls	5	51	Reference	4	51	Reference	
		CLP	1	21	0.5 (0.1, 3.3)	0	21	nc	
		CP	2	6	3.4 (0.4, 20.2)	2	6	4.3 (0.5, 27.3)	
TGFB3 CA	1,n or 1,1	Controls	21	204	Reference	24	204	Reference	
		CLP	8	66	1.2 (0.4, 3.2)	10	66	1.3 (0.5, 3.2)	
		CP	6	25	2.3 (0.6, 7.4)	8	25	2.7 (0.8, 7.8)	
	2,n or 2,2	Controls	17	131	Reference	17	131	Reference	
		CLP	5	45	0.9 (0.2, 2.8)	12	45	2.1 (0.7, 5.5)	
		CP	2	17	0.9 (0.1, 4.6)	6	17	2.7 (0.7, 9.5)	
	3,n or 3,3	Controls	2	25	Reference	3	25	Reference	
		CLP	2	15	1.7 (0.1, 26.6)	4	15	2.2 (0.3, 19.4)	
		CP	3	2	18.8 (1.3, 469.5)	0	2	nc	
	X5.1	1,1	Controls	20	180	Reference	20	180	Reference
			CLP	11	79	1.3 (0.6, 2.7)	11	79	1.3 (0.6, 2.7)
			CP	4	23	1.6 (0.4, 4.6)	9	23	3.5 (1.4, 8.5)
1,2 or 2,2		Controls	1	29	Reference	3	29	Reference	
		CLP	1	6	4.8 (0.2, 134.5)	2	6	3.2 (0.4, 24.0)	
		CP	1	5	5.8 (0.2, 164.2)	0	5	nc	
5'UTR.1	1,1	Controls	24	217	Reference	21	217	Reference	
		CLP	10	71	1.3 (0.6, 2.7)	16	71	2.3 (1.1, 4.7)	
		CP	5	24	1.9 (0.6, 5.1)	8	24	3.4 (1.3, 8.4)	
	1,2 or 2,2	Controls	2	23	Reference	9	23	Reference	
		CLP	1	12	1.0 (0.1, 11.0)	1	12	0.2 (0.1, 1.4)	
		CP	0	6	nc	0	6	nc	
MSX1 CA	1,n or 1,1	Controls	7	60	Reference	7	60	Reference	
		CLP	2	12	1.4 (0.1, 10.3)	1	12	0.7 (0.1, 7.2)	
		CP	0	8	nc	2	8	2.1 (0.1, 16.8)	
	2,n or 2,2	Controls	10	128	Reference	13	128	Reference	
		CLP	2	33	0.8 (0.1, 4.4)	7	33	2.1 (0.5, 7.2)	
		CP	2	14	1.8 (0.1, 11.4)	5	14	3.5 (0.7, 14.9)	
	3,n or 3,3	Controls	4	33	Reference	5	33	Reference	
		CLP	1	12	0.7 (0.1, 8.9)	2	12	1.1 (0.1, 9.3)	
		CP	0	1	nc	0	1	nc	
	4,n or 4,4	Controls	24	233	Reference	29	233	Reference	
		CLP	9	75	1.2 (0.4, 3.1)	16	75	1.7 (0.7, 3.9)	
		CP	4	28	1.4 (0.3, 5.1)	7	28	2.0 (0.6, 6.0)	
	X1.1	1,n or 1,1	Controls	2	14	Reference	5	14	Reference
			CLP	3	6	3.5 (0.3, 57.9)	2	6	0.9 (0.1, 8.8)
			CP	1	5	1.4 (0.1, 33.5)	1	5	0.6 (0.1, 7.5)
		2,n or 2,2	Controls	17	148	Reference	20	148	Reference
			CLP	7	67	0.9 (0.3, 2.7)	15	67	1.7 (0.7, 4.0)
			CP	3	25	1.0 (0.2, 4.3)	8	25	2.4 (0.7, 7.1)
3,n or 3,3	Controls	2	44	Reference	8	44	Reference		
	CLP	3	16	4.1 (0.4, 59.2)	4	16	1.4 (0.2, 6.7)		
	CP	1	10	2.2 (0.1, 45.8)	1	10	0.6 (0.1, 5.1)		
X1.3	1,1	Controls	0	0	Reference	0	0	Reference	
		CLP	0	0	nc	0	0	nc	
		CP	0	0	nc	0	0	nc	
	1,2 or 2,2	Controls	15	170	Reference	20	170	Reference	
		CLP	9	83	1.2 (0.5, 2.9)	17	83	1.7 (0.9, 3.5)	
		CP	6	31	2.2 (0.7, 5.9)	10	31	2.7 (1.1, 6.3)	
X2.1	1,n or 1,1	Controls	1	3	Reference	0	3	Reference	
		CLP	0	3	nc	1	3	nc	
		CP	0	2	nc	0	2	nc	
	2,n or 2,2	Controls	19	180	Reference	20	180	Reference	
		CLP	7	66	1.0 (0.3, 2.9)	15	66	2.0 (0.8, 5.0)	
		CP	5	27	1.8 (0.4, 5.9)	8	27	2.7 (0.8, 7.9)	
	3,n or 3,3	Controls	6	67	Reference	13	67	Reference	
		CLP	2	23	1.0 (0.1, 6.3)	6	23	1.3 (0.3, 4.8)	
		CP	2	14	1.6 (0.1, 11.0)	2	14	0.7 (0.1, 4.1)	

TABLE 5. Risk estimates for nonsyndromic cleft lip ± cleft palate and nonsyndromic cleft palate only by maternal cigarette smoking during the 2-month postconceptional period, and infant genotype* (continued)

Gene/ marker	Genotype**	Group	Maternal cigarette smoking					
			1–9 cigarettes/day			≥10 cigarettes/day		
			Yes	No	OR (CI)***	Yes	No	OR (CI)***
X2.4	1,1	Controls	1	10	Reference	2	10	Reference
		CLP	1	6	1.7 (0.1, 47.7)	1	6	0.8 (0.1, 10.7)
		CP	1	2	5.0 (0.2, 173.4)	0	2	nc
	1,2 or 2,2	Controls	13	124	Reference	16	124	Reference
		CLP	8	60	1.3 (0.5, 3.2)	14	60	1.8 (0.8, 4.0)
		CP	4	25	1.5 (0.4, 4.7)	9	25	2.8 (1.1, 6.9)

*OR, odds ratio; CI, confidence interval; CLP, cleft lip ± cleft palate; CP, cleft palate only; nc, not calculated.

**n = 1, 2, or 3 for markers TGFB3 CA, MSX1 X1.1, and MSX1 X1.3, and n = 1, 2, 3, or 4 for marker MSX1 CA.

***Bonferroni correction used for multiple pairwise comparisons.

interaction was found for CLP ($\chi^2_{5'UTR.1} = 4.2$, $P < 0.05$; $\chi^2_{X2.4} = 2.5$, $P < 0.20$) and CP ($\chi^2_{5'UTR.1} = 2.5$, $P < 0.10$; $\chi^2_{X1.1} = 4.1$, $P < 0.05$; $\chi^2_{X2.1} = 12.0$, $P < 0.001$; and $\chi^2_{X2.4} = 4.2$, $P < 0.05$).

Maternal alcohol consumption (≥ 4 drinks/month) significantly influenced the risk for CLP among infants with the common 1 allele at the 5'UTR.1 site (Table 6). Likewise, maternal consumption of ≥ 4 drinks/month significantly elevated the risk for CLP among infants with the common 4 allele at the MSX1 CA site or the common 2 allele at the MSX1 X1.3 site. Statistical interaction was observed between maternal alcohol consumption (≥ 4 drinks/month) and infants with the common 4 allele for MSX1 CA ($\chi^2 = 3.9$; $P < 0.05$).

For CP risk associated with the joint risk of infant genotype maternal smoking, stratification by gender revealed that estimates for male infants were higher than those for female infants, and logistic regression analyses showed that the difference in risk between genders at the TGFB3 X5.1 site was statistically significant (data not shown). No consistent trend between genders was identified for risk of CLP or CP associated with the joint risk of infant genotype and maternal alcohol consumption. For each phenotype, stratification by family history did not influence the risk estimates associated with infant genotype and either exposure.

DISCUSSION

Our results for TGFA and CLP conflict with several previous studies (Ardinger et al., '89; Chenevix-Trench et al., '91, '92; Holder et al., '92; Sassani et al., '93), owing perhaps to differences in case ascertainment. In each previous study, ascertainment was nonrandom, and in all but one (Ardinger et al., '89; Chenevix-Trench et al., '91, '92; Holder et al., '92), resulted in a high proportion of familial cases (43%, 51%, 59%, and 33%, respectively). These proportions contrast sharply with that (14.3%) in the current study. Examination of risk among familial cases in the current study revealed a significant association between the rare 2 allele and CLP, and use of the transmission disequilibrium test (Spielman et al., '93) showed that the rare allele was more likely to be transmitted among familial ($\chi^2 = 6.40$,

$P < 0.05$) than nonfamilial ($\chi^2 = 1.14$, $P > 0.25$) cases (unpublished data). Indirect support for these results comes from work by Stoll et al. ('93), who reported no association between the rare allele and CLP among nonfamilial cases. The accumulated evidence that the rare allele may be more common among familial cases suggests that TGFA could be a major gene for CLP, but linkage studies of the Taq1 polymorphism (Hecht et al., '91; Vintiner et al., '92) and an additional allelic variant (Wyszynski et al., '97b) did not confirm this. Failure to identify linkage may imply an alternative explanation, i.e., inheritance of CLP in these families is multigenic, and TGFA constitutes only one contribution to genetic susceptibility. If so, many more pedigrees than were used in the previously cited studies would be required to show linkage for genes that do not have major effects (Farrall et al., '93).

In the current population, evidence that inheritance of CLP may be multigenic is suggested by the increased risk associated with the rare allele at the TGFB3 CA site. Additional evidence is provided by the finding that risk associated with this locus was most elevated among familial cases. Further evidence is revealed by the analysis of the joint risk of infants with at least one copy of the rare 2 allele for TGFA Taq1 and also the rare 3 allele for TGFB3 CA. Compared to infants without either rare allele, familial cases with at least one copy of each rare allele had a 13-fold increase in risk for CLP (unpublished data).

Our results for TGFA and CP also conflict with previously published studies (Shiang et al., '93; Hwang et al., '95). This discrepancy may be explained by the proportion of familial cases in each previous study. CP cases studied by Shiang et al. ('93) included infants with a positive family history identified from an earlier study (Ardinger et al., '89). Diagnoses of those studied by Hwang et al. ('95) were unable to be verified and may have included infants with associated syndromes. Familial cases may be more likely to occur among individuals with syndromes, and those studied by Hwang et al. ('95) were significantly more likely than the malformed controls to have a reported family history of either orofacial clefts or another isolated birth defect. In the

TABLE 6. Risk estimates for nonsyndromic cleft lip ± cleft palate and nonsyndromic cleft palate only by maternal alcohol consumption during pregnancy and infant genotype*

Gene/ marker	Genotype**	Group	Maternal alcohol consumption						
			1-3 drinks/month			≥4 drinks/month			
			Yes	No	OR (CI)***	Yes	No	OR (CI)***	
TGFA Taq1	1,1	Controls	69	162	Reference	4	162	Reference	
		CLP	30	57	1.2 (0.7, 2.1)	7	57	5.0 (1.4, 19.6)	
		CP	9	29	0.7 (0.3, 1.6)	3	29	4.2 (0.8, 20.0)	
	1,2 or 2,2	Controls	14	46	Reference	0	46	Reference	
		CLP	7	12	1.9 (0.6, 5.8)	3	12	nc	
		CP	3	7	1.4 (0.3, 5.8)	0	7	nc	
TGFB3 CA	1,n or 1,1	Controls	70	174	Reference	5	174	Reference	
		CLP	28	51	1.4 (0.7, 2.6)	3	51	2.0 (0.3, 12.0)	
		CP	11	26	1.0 (0.4, 2.6)	2	26	2.7 (0.2, 18.6)	
	2,n or 2,2	Controls	42	120	Reference	3	120	Reference	
		CLP	20	35	1.6 (0.7, 3.6)	6	35	6.9 (1.3, 51.4)	
		CP	6	18	1.0 (0.3, 3.0)	1	18	2.2 (0.1, 29.5)	
	3,n or 3,3	Controls	5	25	Reference	0	25	Reference	
		CLP	6	11	2.7 (0.5, 16.0)	3	11	nc	
		CP	0	5	nc	0	5	nc	
	X5.1	1,1	Controls	53	163	Reference	4	163	Reference
			CLP	31	60	1.6 (0.9, 2.7)	8	60	5.4 (1.6, 21.0)
			CP	8	27	0.9 (0.4, 2.0)	1	27	1.5 (0.1, 10.7)
1,2 or 2,2		Controls	7	26	Reference	0	26	Reference	
		CLP	1	8	0.5 (0.1, 3.2)	0	8	nc	
		CP	3	2	5.6 (0.8, 49.2)	1	2	nc	
5'UTR.1	1,1	Controls	66	189	Reference	7	189	Reference	
		CLP	29	56	1.5 (0.9, 2.5)	10	56	4.8 (1.8, 13.8)	
		CP	8	28	0.8 (0.3, 1.8)	1	28	1.0 (0.1, 5.7)	
	1,2 or 2,2	Controls	9	24	Reference	1	24	Reference	
		CLP	6	8	2.0 (0.5, 7.5)	0	8	nc	
		CP	1	4	0.7 (0.1, 5.3)	1	4	6.0 (0.2, 175.0)	
MSX1 CA	1,n or 1,1	Controls	22	52	Reference	0	52	Reference	
		CLP	4	10	0.9 (0.2, 4.4)	1	10	nc	
		CP	5	4	3.0 (0.5, 20.3)	1	4	nc	
	2,n or 2,2	Controls	44	105	Reference	2	105	Reference	
		CLP	16	19	2.0 (0.8, 5.3)	7	19	19.3 (2.9, 274.7)	
		CP	5	16	0.7 (0.2, 2.6)	0	16	nc	
	3,n or 3,3	Controls	14	28	Reference	0	28	Reference	
		CLP	0	12	nc	2	12	nc	
		CP	0	1	nc	0	1	nc	
	4,n or 4,4	Controls	76	205	Reference	5	205	Reference	
		CLP	31	57	1.5 (0.8, 2.8)	10	57	7.2 (1.8, 34.7)	
		CP	7	30	0.6 (0.2, 1.7)	2	30	2.7 (0.2, 20.5)	
	X1.1	1,n or 1,1	Controls	4	16	Reference	1	16	Reference
			CLP	4	6	2.7 (0.3, 22.6)	0	6	nc
			CP	0	7	nc	0	7	nc
		2,n or 2,2	Controls	52	130	Reference	3	130	Reference
			CLP	27	53	1.3 (0.6, 2.5)	7	53	5.7 (1.1, 41.4)
			CP	9	25	0.9 (0.3, 2.4)	2	25	3.5 (0.3, 34.2)
3,n or 3,3	Controls	13	40	Reference	1	40	Reference		
	CLP	10	10	3.1 (0.8, 11.8)	3	10	12.0 (0.9, 690.4)		
	CP	2	9	0.7 (0.1, 4.2)	1	9	4.4 (0.1, 324.0)		
X1.3	1,1	Controls	0	0	Reference	0	0	Reference	
		CLP	0	0	nc	0	0	nc	
		CP	0	0	nc	0	0	nc	
	1,2 or 2,2	Controls	50	150	Reference	5	150	Reference	
		CLP	35	62	1.7 (1.0, 2.9)	10	62	4.8 (1.6, 16.1)	
		CP	11	34	1.0 (0.4, 2.0)	2	34	1.8 (0.2, 8.6)	
X2.1	1,n or 1,1	Controls	1	3	Reference	0	3	Reference	
		CLP	0	4	nc	0	4	nc	
		CP	0	2	nc	0	2	nc	
	2,n or 2,2	Controls	58	157	Reference	4	157	Reference	
		CLP	25	52	1.3 (0.6, 2.6)	9	52	6.8 (1.6, 36.5)	
		CP	11	26	1.1 (0.4, 2.8)	3	26	4.5 (0.6, 31.4)	
	3,n or 3,3	Controls	22	61	Reference	3	61	Reference	
		CLP	12	14	2.4 (0.8, 7.3)	4	14	5.8 (0.8, 50.0)	
		CP	4	12	0.9 (0.2, 3.8)	2	12	3.4 (0.2, 35.6)	

TABLE 6. Risk estimates for nonsyndromic cleft lip \pm cleft palate and nonsyndromic cleft palate only by maternal alcohol consumption during pregnancy and infant genotype* (continued)

Gene/ marker	Genotype**	Group	Maternal alcohol consumption					
			1–3 drinks/month			≥ 4 drinks/month		
			Yes	No	OR (CI)***	Yes	No	OR (CI)***
X2.4	1,1	Controls	1	11	Reference	1	11	Reference
		CLP	4	3	14.7 (1.5, 355.5)	0	3	nc
		CP	0	3	nc	0	3	nc
	1,2 or 2,2	Controls	36	114	Reference	3	114	Reference
		CLP	28	46	1.9 (1.1, 3.5)	7	46	5.8 (1.5, 27.7)
		CP	11	25	1.4 (0.6, 3.1)	2	25	3.0 (0.4, 19.3)

*OR, odds ratio; CI, confidence interval; CLP, cleft lip \pm cleft palate; CP, cleft palate only; nc, not calculated.

**n = 1, 2, or 3 for markers TGFB3 CA, MSX1 X1.1, and MSX1 X1.3, and n = 1, 2, 3, or 4 for marker MSX1 CA.

***Bonferroni correction used for multiple pairwise comparisons.

current study, familial CP cases comprised a small proportion (12.5%) of the overall sample.

The finding of increased risks of CLP and CP associated with maternal cigarette smoking concurred with several, but not all previous reports (reviewed by Shaw et al., '96). Similarly, identification of increased risk of CLP associated with maternal alcohol consumption confirmed the findings of one earlier report (Werler et al., '91) but not another (Niebly et al., '85). As reviewed elsewhere (Shaw et al., '96; Munger et al., '96), methodologic limitations in many of these studies preclude interpretation of the findings for each exposure. Absent from most studies of maternal smoking was analysis of infant gender as a potential confounder. In the current study, stratified analyses showed that risks for CLP and CP associated with maternal smoking were higher among males than females, although the differences were not statistically significant. Maternal smoking during pregnancy has been associated with pronounced deficits in male birth weight (Wertelecki et al., '87; Abell et al., '91), fetal length (Wertelecki et al., '87; Bremme et al., '90; Spinillo et al., '94), fetal distress (Umaphathysivam et al., '90), and birth defects (Alderman et al., '91). Although some of these liabilities are not exclusive to males (Miller and Jekel, '89), studies have shown that alpha-fetoprotein level (Wajner et al., '86), intrauterine hormone profile (Bremme et al., '90), and enzyme activity (Bannon et al., '86) differ markedly between smoking and nonsmoking pregnant women, and that hormonal changes among smoking women may vary by fetal sex (Bremme et al., '90). The impact of these changes in the intrauterine environment for smoking women and its relationship to the development of CLP or CP remains to be explored.

Risk estimates obtained for the joint effect of the rare 2 allele at the TGFA Taq1 site and maternal cigarette smoking failed to support the conclusions of two previous studies (Hwang et al., '95; Shaw et al., '96). Hwang et al. ('95) found that infants with the rare allele at the Taq1 site and whose mothers smoked during pregnancy (≤ 10 or >10 cigarettes/day) had an increased risk of CP. In the current study, maternal smoking was associated with an increased risk of CP for infants with either

the common or rare allele. Shaw et al. ('96) reported increased risks for CLP and CP among infants with the rare allele at the Taq1 site and whose mothers smoked ≥ 20 cigarettes/day. These associations could not be verified in the current study, as no case with the rare allele had a mother who smoked ≥ 20 cigarettes/day.

We did identify elevated risks for CLP and CP associated with the joint effects of allelic variants for TGFB3 or MSX1 and maternal smoking as well as for CLP associated with the joint effects of allelic variants for each gene and alcohol consumption. The finding of interactions between TGFB3 and maternal cigarette smoking is consistent with previously published case-parental control analyses (Maestri et al., '97), whereas interactions found between MSX1 and either exposure are the first to be reported. In addition, identification of interaction between multiple variants for MSX1 is consistent with previous haplotype analyses. Using an overlapping population with the current study, Lidral et al. ('98) found that the 4–2–2–2–2 haplotype for MSX1 CA-X1.1-X1.3-X2.1-X2.4 was most frequently transmitted among both CLP and CP cases.

Animal studies can facilitate our understanding of the biologic mechanisms which may underlie identified statistical associations. In vivo and in vitro studies have shown that TGFA (Dixon et al., '91) and TGFB3 (Brunet et al., '95) are expressed at high levels in the epithelial tissue of the medial edge of the palatal shelves at the time of shelf fusion. Although TGFA null mice have normal craniofacial development (Luetke et al., '93; Mann et al., '93), TGFB3 null mice (Kartinen et al., '95; Proetzel et al., '95) produce murine models that present with cleft palate only. The phenotype for TGFB3 null mice contrasts with that produced by MSX1 null mice, which can include additional craniofacial anomalies (Satokata and Maas, '94); thus, the role that this gene contributes to palatogenesis is thought to differ from that of growth factors. The observation that the palatal shelves of MSX1 null mice elevate but do not fuse suggests a deficiency of palatal mesenchymal cells due to a problem with neural crest cell migration or the disruption of the dental papilla and follicle mesen-

chyme (Satokata and Maas, '94). Experimental studies have also identified potential mechanisms to explain the teratogenic insults of cigarette smoke. Central to several of these mechanisms is the assumption that maternal hypoxia induces malformations in the fetus (Johnston, '81). In murine models, this oxygen deficit has been attributed to carbon monoxide interference with hemoglobin function (Bailey et al., '95), disruption of the electron transport chain through substitution of an analogue of the nicotinamide in NADH dehydrogenase (Trasler et al., '78), or the effects of nicotine (Saad et al., '90-'91). Occurrence of CLP that resulted from maternal hypoxia was also discovered in genetically susceptible CL/Fr mice (Bronsky et al., '86), suggesting the potential interaction between an antecedent genetic predisposition and this environmental teratogen. Additionally, gestational exposure to cadmium, another component in cigarette smoke, has been shown to produce cleft palate in golden hamsters (Ferm, '71) and rats (Chernoff, '73). With regard to the teratogenic potential of ethanol exposure, chick (Cartwright and Smith, '95) and mouse (Kotch and Sulik, '92) models suggest that craniofacial malformations are caused by aberrations in the production and development of premigratory neural crest cells. This ethanol-induced sensitivity has been attributed to changes in membrane fluidity (Chen et al., '96) and to reduced activity of selected antioxidant enzymes, particularly superoxide dismutase, catalase, and glutathione peroxidase (Chen and Sulik, '96).

No published studies have explored the biologic interrelationship between TGFB3, MSX1, and either cigarette smoke or alcohol exposure. Kaji et al. ('94), however, observed that pretreatment of cultured bovine endothelial cells with TGFB1 reduced the cadmium cytotoxicity of the cultures, suggesting that it may serve as a protective factor. Since the mature regions of TGFB1 and TGFB3 share approximately 80% sequence homology (Massagué, '90), and since embryonic endothelial cells such as mesenchymal cells are derived from the mesoderm (Sadler, '95), it is possible that a mutation in the TGFB3 gene, which reduces the tolerance of mesenchymal cells to cadmium cytotoxicity, combined with high blood levels of unbound cadmium from cigarette smoke, may disrupt formation of the mesenchymal shelf that separates the oral and nasal cavities.

Findings from this study need to be interpreted cautiously. As discussed, nearly equal proportions of case and control infants were typed for the TGFA and TGFB3 markers, but not for the MSX1 markers. Among infants whose samples were either not available or not genotyped due to technical reasons, maternal smoking and alcohol status for CP cases and controls paralleled the overall proportions, whereas that for CLP cases was lower than in the overall proportion. This suggests that risk estimates for CLP may have been overestimated. Also, information on maternal smoking and alcohol consumption was obtained 2 years or more following

delivery of the child (Romitti et al., '98, and unpublished data). Retrospective collection of prenatal exposure data may be subject to differential recall between case and control mothers (Swan et al., '92); however, comparing prospective and retrospective reports of prenatal cigarette and alcohol use, Verkerk et al. ('94) found that the level of use provided by each type of report tended to be similar. In addition, the genotype-environment interactions identified tended to suggest that a mutation for clefting may be in disequilibrium with the common allele for a given marker. It is intuitive that a mutation would be more likely to occur on a common rather than a rare genetic background; thus, increased risks associated with maternal exposures would be expected among infants with the common allele. The highest risks will eventually be shown when the disease-specific mutations are identified.

In summary, a population-based case-control study was conducted to evaluate potential genetic and environmental factors important in the development of CLP and CP. Development of CLP was influenced by maternal alcohol consumption, but less so by maternal cigarette smoking, whereas the converse was found for CP. For both phenotypes, the combined effect of infant genotype and maternal exposures produced the greatest influence on development for either phenotype. These results provide additional evidence that CLP and CP are complex disorders, and continued investigation of the combined effects of infant genotype and environmental teratogens is urged.

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APPENDIX A. Interview and questionnaire items for maternal cigarette smoking

Telephone interview

44. Did you smoke any cigarettes during the three-month period *before* you became pregnant with (child's name)?
0 No (Go to question 46)
1 Yes
9 Unknown (Go to question 46)
46. Did you smoke at all *during* your pregnancy with (child's name)?
0 No (Go to question 49)
1 Yes
9 Unknown (Go to question 49)
47. During which months of your pregnancy did you smoke? (Circle all that apply.)
1 2 3 4 5 6 7 8 9 ALL

Mailed questionnaire

23. Did you smoke cigarettes during the three months immediately *before* your pregnancy?
 No Skip to question 26
 Yes
24. During which months *before* your pregnancy, did you smoke cigarettes? (Mark all that apply.)
③②① months before pregnancy
28. Did you smoke cigarettes *during* your pregnancy?
 No Skip to question 31
 Yes
29. During which months of pregnancy did you smoke? (Mark all that apply.)
①②③④⑤⑥⑦⑧⑨ months of pregnancy

APPENDIX B. Interview and questionnaire items for maternal alcohol consumption

Telephone interview

51. During the three-month period *before* you became pregnant with (child's name), did you drink any alcohol?
0 No (Go to question 55)
1 Yes
9 Unknown or Refused
55. During your pregnancy, did you drink any alcohol?
0 No (Go to question 61)
1 Yes
9 Unknown or Refused

Mailed questionnaire

33. Did you drink alcohol—beer, wine or liquor—during the three months immediately *before* your pregnancy?
 No Skip to question 38
 Yes
34. During which months *before* your pregnancy, did you drink alcohol? (Mark all that apply.)
③②① months before pregnancy
38. Did you drink alcohol *during* your pregnancy?
 No Skip to question 43
 Yes
39. During which months of pregnancy did you drink alcohol? (Mark all that apply.)
①②③④⑤⑥⑦⑧⑨ months of pregnancy