

Oral Clefts, Maternal Smoking, and TGFA: A Meta-Analysis of Gene-Environment Interaction

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Objective: A meta-analysis was performed to examine the association among maternal cigarette smoking, infant genotype at the *Taq1* site in the transforming growth factor α (TGFA) locus, and risk of nonsyndromic oral clefts, both cleft palate (CP) and cleft lip with or without cleft palate (CL/P).

Design: Five published case-control studies were included in the meta-analysis. Pooled Mantel-Haenszel odds ratios (OR) and 95% confidence intervals (CIs) were computed. Gene-environment interaction was also assessed by using the pooled data in a case-only analysis and polytomous logistic regression.

Results: Among nonsmoking mothers, there was no evidence of any increased risk for CP if the infant carried the TGFA *Taq1* C2 allele. If the mother reported smoking, however, there was an overall increased risk for CP if the infant carried the C2 allele (OR_{smokers} = 1.95; 95% CI = 1.22 to 3.10). TGFA genotype did not increase risk to CL/P, regardless of maternal smoking status. Polytomous logistic regression revealed a significant overall smoking effect for CL/P (OR = 1.64, 95% CI = 1.33 to 2.02) and CP (OR = 1.42, 95% CI = 1.06 to 1.90).

Conclusions: While maternal smoking was a consistent risk factor for both CL/P and CP across all studies, the suggestive evidence for gene-environment interaction between the infant's genotype at the *Taq1* marker in TGFA and maternal smoking was limited to CP. Furthermore, evidence for such gene-environment interaction was strongest in a case-control study drawn from a birth defect registry where infants with non-cleft defects served as controls.

KEY WORDS: CL/P, CP, epidemiology, meta-analysis, oral clefts, TGFA

Oral clefts, including cleft lip (CL), cleft palate (CP), and cleft lip and palate (CLP), collectively constitute a heterogeneous group of nonfatal birth defects known to be multifactorial in origin, in that both genes and environmental factors contribute to their etiology. Due to both epidemiologic and embryologic similarities, CL and CLP are often grouped together as cleft lip with or without cleft palate (CL/P).

Several studies have examined the effect of maternal smoking on risk for oral clefts. As early as 1979, Ericson et al. found an association between smoking and oral clefts. Khoury et al. (1987) reported a positive relationship between smoking and CL/P (OR [odds ratio] = 3.33, 95% CI [confidence interval] = 1.3 to 8.4), although the multicenter study of Werler et al. (1990) could not replicate this finding. Using a Swedish registry, Kallen (1997) performed a large case-control analysis with a total of 1834 oral cleft cases and found a statistically significant association between maternal smoking and both

CL/P (OR = 1.16, 95% CI = 1.02 to 1.32) and CP (OR = 1.29, 95% CI = 1.08 to 1.54). The attributable risk for either CP or CL/P was estimated at 6%. Wyszynski et al. (1997) performed a meta-analysis utilizing the results from 11 published studies and found an overall OR of 1.29 (95% CI = 1.18 to 1.42) for any oral cleft in children of women who smoked. Their attributable risk of 11% suggests that smoking is a general risk factor for all oral clefts. More recently, in a multicenter case control study, Lieff et al. (1999) found a positive dose response relationship between CL/P and the quantity of smoking (light smokers: OR = 1.09, 95% CI = 0.6 to 1.9; moderate smokers: OR = 1.84, 95% CI = 1.2 to 2.9; heavy smokers: OR = 1.85, 95% CI = 1.0 to 3.5). Chung et al. (2000) performed the largest study to date, testing for an association between cigarette smoking and all oral clefts; the adjusted OR for smokers versus nonsmokers was 1.34 (95% CI = 1.16 to 1.54) from this study.

The transforming growth factor α (TGFA) locus is the most widely studied candidate gene associated with risk to oral clefts. However, results of a number of case-control studies have been ambiguous. Using the case-control design, Ardinger et al. (1989) found an increased risk for CL/P among cases carrying the *Taq1* C2 allele of the TGFA gene (OR = 3.18,

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TABLE 1 Description of the Five Published Case-Control Studies Testing for an Association Between Maternal Smoking, Transforming Growth Factor α (TGFA) *TaqI* Polymorphism and Oral Clefts Included in the Meta-Analysis

| Study | Region | Years of Data Collection | Study Information | Sample Sizes | | |
|---------------------------|-------------------|--------------------------|---|--------------|-----|----------|
| | | | | CL/P | CP* | Controls |
| Hwang et al. (1995) | Maryland/U.S.A. | 1984–1992 | Population based birth defects registry Controls: live born infants with non-cleft birth defect | 69 | 114 | 284 |
| Shaw et al. (1996) | California/U.S.A. | 1987–1989 | Population based Medical record review Controls: live born infants without a birth defect | 348 | 141 | 734 |
| Christensen et al. (1999) | Denmark | 1991–1994 | Nationwide study Controls: live born infants without a birth defect | 233 | 83 | 604 |
| Romitti et al. (1999) | Iowa/U.S.A. | 1987–1994 | Population based Direct examination of cases Controls: live born infants without a birth defect | 161 | 64 | 393 |
| Beaty et al. (2001) | Maryland/U.S.A. | 1992–1998 | Clinic based Direct examination of cases Controls: live born infants without a birth defect | 111 | 60 | 182 |

* CP = cleft palate; CL/P = cleft lip with or without cleft palate.

95% CI = 1.26 to 8.22). These results later were confirmed by two other small case-control studies: Holder et al. (1992) and Sassani et al. (1993) found an association between CL/P and the *TaqI* C2 allele (chi-square = 15.04, 1 *df*, $p = .001$ and chi-square = 5.08, $p = .02$, respectively). Mitchell (1997) performed a meta-analysis utilizing results from all available studies through 1997 and found a statistically significant overall association between CL/P and TGFA genotype (OR = 1.43, 95% CI = 1.12 to 1.80). Romitti et al. (1999) did not find evidence that the C2 allele at TGFA increased risk for either CL/P (OR = 0.9, 95% CI = 0.5 to 1.5) or CP (OR = 1.0, 95% CI = 0.4 to 2.0) in a case-control study from Iowa. Christensen et al. (1999) also found no evidence that this TGFA marker was associated with CP (OR = 0.99, 95% CI = 0.52 to 1.88) or CL/P (OR = 0.96, 95% CI = 0.63 to 1.45) in a large Danish study.

Gene-environment interaction could account for some of the ambiguity among these different studies. Hwang et al. (1995) studied the association between CP, CL/P, and TGFA. In that sample, there was an increase in the number of infants with CP who carried the rarer 2 allele at the *TaqI* site in the TGFA gene compared with controls with an isolated, non-cleft birth defect. This difference was greatly increased among infants of mothers who smoked, which suggests a gene-environment interaction. Shaw et al. (1996) confirmed these findings in a population based case-control study from California where the control infants had no birth defect. However, Romitti et al. (1999) found no evidence for gene-environment interaction between the rare 2 allele and maternal smoking during pregnancy (CL/P: OR = 0.5, 95% CI = 0.1 to 3.3; CP: OR = 3.4, 95% CI = 0.4 to 20.2). Like Romitti et al. (1999), Christensen et al. (1999) did not find evidence of a gene-smoking interaction. Beaty et al. (2001) also failed to find significant evidence of

gene-environment interaction in a smaller case-control study from Maryland.

These conflicting results from separate epidemiologic studies may arise, in part, from a lack of power due to small sample sizes, and in part due to true heterogeneity across populations. Presented here is a meta-analysis of five published case-control studies that tested for gene-environment interaction between TGFA and maternal smoking and risk to oral clefts.

METHODS

A literature review using Medline was performed to locate published articles that met the following criteria: (1) a case-control study using infants with isolated oral clefts as cases (human subjects only); (2) data on maternal smoking during the first trimester of pregnancy was collected; (3) genotype at the TGFA *TaqI* site on both case and control infants was available; and (4) count data were reported in such a manner that it could be abstracted and included in a meta-analysis. Search terms included “oral clefts,” “clefts,” “smoking,” “TGFA,” and “gene-environment interaction.” The major reasons for exclusion were: family based study design either for linkage or case-parent trio studies for association, information on TGFA genotype was not available, and maternal smoking was not recorded.

The Medline search yielded 43 studies. Only five of these studies satisfied the inclusion criteria and were used in the analyses (Hwang et al., 1995; Shaw et al., 1996; Christensen et al., 1999; Romitti et al., 1999; Beaty et al., 2001) (Table 1).

From these published studies, counts of maternal smoking and infant genotype were compiled. A table was created with these counts to display TGFA *TaqI* genotype of case and control infants with nonsmoker mothers and another with smoker

TABLE 2 Data from Five Published Studies Examining the Effect of Maternal Smoking, Infant TGFA Genotype, and Oral Clefts

| Genotype | Non-Smokers | | Smokers | |
|--------------------------|-------------|------|---------|------|
| | C1C1 | C2C- | C1C1 | C2C- |
| Hwang et al., 1995 | | | | |
| Controls | 167 | 34 | 69 | 11 |
| CP cases | 36 | 7 | 13 | 13 |
| CL/P cases | 60 | 12 | 31 | 9 |
| Shaw et al., 1996 | | | | |
| Controls | 226 | 43 | 94 | 15 |
| CP cases | 42 | 8 | 26 | 11 |
| CL/P cases | 94 | 14 | 69 | 13 |
| Christensen et al., 1999 | | | | |
| Controls | 193 | 66 | 139 | 46 |
| CP cases | 29 | 11 | 19 | 5 |
| CL/P cases | 67 | 27 | 75 | 19 |
| Romitti et al., 1999 | | | | |
| Controls | 100 | 51 | 45 | 9 |
| CP cases | 26 | 6 | 15 | 4 |
| CL/P cases | 69 | 21 | 27 | 1 |
| Beatty et al., 2001 | | | | |
| Controls | 126 | 19 | 17 | 5 |
| CP cases | 39 | 6 | 15 | 4 |
| CL/P cases | 77 | 8 | 28 | 4 |

* CP = cleft palate; CL/P = cleft lip with or without cleft palate.

mothers; CP and CL/P were analyzed separately (Tables 2 and 3). In all of these analyses, smoking was examined as a dichotomous variable. Romitti et al. (1999) and Shaw et al. (1996) grouped smoking into three categories: nonsmokers, light smokers, and heavy smokers. We combined the two separate smoking groups into a single “smoker” group. The Metan routine in Stata 6.0 (Stata Corp., College Station, TX) was used to compute pooled Mantel-Haenszel ORs and 95% CIs to determine the effect of the TGFA genotype in the presence of exposure (i.e., mothers reported smoking during pregnancy) and in the absence of exposure (i.e., mothers did not report smoking during the pregnancy) for CP and CL/P infants separately. Tests for heterogeneity were also conducted. Publication bias was assessed with funnel plots, and Begg and Egger tests (Begg and Mazumdar, 1994) were carried out using the Metabias routine in Stata (Stata Corp.). Gene-environment interaction was also assessed using the pooled data in a case-only analysis.

Botto and Khoury (2001) suggested presenting gene-environment interaction results in a 2×4 table, where three ORs are calculated, one for the genotype alone, one for the environmental risk factor alone, and one for their joint effect (which would reveal gene-environment interaction). We used polytomous logistic regression to estimate these three ORs based on the total counts from Table 2. A single logistic regression model was fit to estimate the three ORs (genotype, smoking, and gene-smoking interaction) for the two case groups (CL/P and CP) compared with the controls and simultaneously adjusting for study.

TABLE 3 Results from Polytomous Logistic Regression Examining the Effect of Smoking and TGFA *Taq1* Genotype on Oral Clefts*

| | Odds Ratio | 95% CI |
|----------------------|------------|-----------|
| CP† | | |
| Smoking only | 1.42 | 1.06–1.90 |
| Genotype only | 1.04 | 0.70–1.53 |
| Smoking and Genotype | 1.77 | 0.98–3.22 |
| CL/P | | |
| Smoking only | 1.64 | 1.33–2.02 |
| Genotype only | 1.00 | 0.75–1.32 |
| Smoking and Genotype | 0.86 | 0.53–1.40 |

* Adjusted for study.

† CP = cleft palate; CL/P = cleft lip with or without cleft palate.

RESULTS

Table 2 shows the counts for the number of cases, controls, genotype, and maternal smoking. The pooled sample size consisted of: 726 CL/P cases, 335 CP cases, and 1565 controls.

Cleft Palate

Among nonsmoking mothers, there was no evidence of any increased risk for CP if the infant carried the TGFA *Taq1* C2 allele ($OR_{\text{nonsmokers}} = 1.00$; 95% CI = 0.68 to 1.46) and there was no evidence of heterogeneity across studies (Fig. 1). However, if the mother reported smoking, there was an overall increase in risk for CP if the infant carried the C2 allele ($OR_{\text{smokers}} = 1.95$; 95% CI = 1.22 to 3.10). There was also evidence of heterogeneity across studies (heterogeneity $\chi^2=10.04$ with 4 *df*, $p=0.04$). As seen in Figure 1, the first two studies showed a much larger OR than the subsequent three. Begg’s test for publication bias was not significant for either CP group.

The joint effect of maternal smoking and infant genotype reached marginal statistical significance in the polytomous logistic regression ($OR = 1.77$, 95% CI = 0.98 to 3.22), and there was a significant smoking effect ($OR = 1.42$, 95% CI = 1.06 to 1.90) (Table 3). Case-only analysis revealed significant gene-smoking interaction ($OR = 1.90$, 95% CI = 1.09 to 3.31) under a multiplicative model.

Cleft Lip ± Palate

Results of this meta-analysis showed that infant TGFA genotype did not increase risk to CL/P, regardless of maternal smoking status ($OR_{\text{nonsmokers}} = 0.98$, 95% CI = 0.74 to 1.30; $OR_{\text{smokers}} = 0.87$, 95% CI = 0.58 to 1.30) (Fig. 2). There was no evidence of heterogeneity across studies and the test for publication bias was not statistically significant. Polytomous logistic regression revealed a significant overall smoking effect ($OR = 1.64$, 95% CI = 1.33 to 2.02). The joint effect of smoking and genotype was not statistically significant, however ($OR = 0.86$, 95% CI = 0.53 to 1.40) (Table 3). Case-only analysis also showed no evidence of gene-environment interaction ($OR = 0.89$, 95% CI = 0.59 to 1.35).

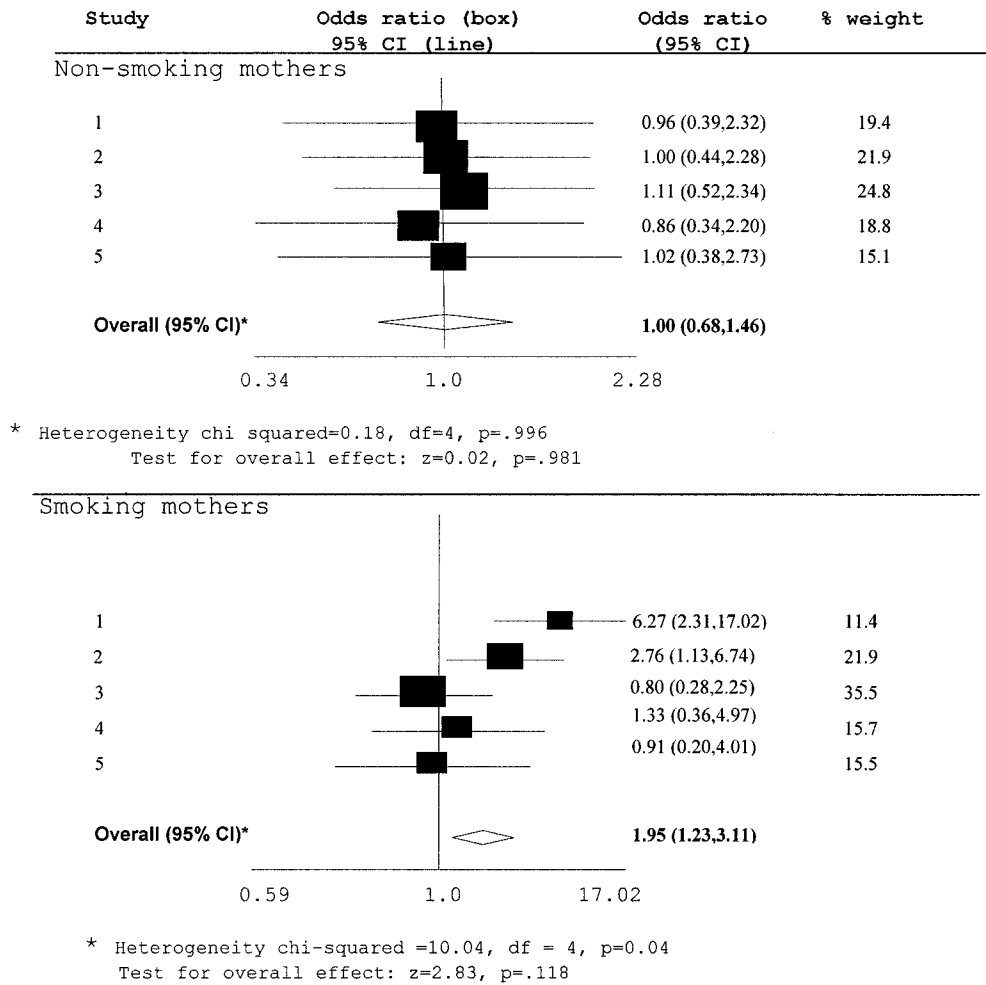


FIGURE 1 Effect of maternal smoking on risk of CP among infants carrying TGFA *TaqI* C2 allele. Studies: (1) Hwang et al. (1995); (2) Shaw et al. (1996); (3) Christensen et al. (1999); (4) Romitti et al. (1999); and (5) Beaty et al. (2001).

Figures 1 and 2 show box plots for estimated ORs for each type of oral cleft. Separate estimates are shown for smoker and nonsmoker mothers; in addition to the overall Mantel-Haenszel estimate, ORs are given for each study individually.

DISCUSSION

This meta-analysis of five published studies showed a significant interaction between maternal smoking, infant TGFA genotype, and CP. No evidence of such interaction was observed for CL/P, although there was a significant smoking effect regardless of genotype in this subgroup of oral clefts.

It should be noted that the Hwang et al. (1995) study differed from the other four studies in two important ways. First, the control group comprised infants with an isolated birth defect other than a cleft. Second, the association between CP, maternal smoking, and infant genotype was far stronger in this study than any of the others (Fig. 1). When the Hwang et al. (1995) data were removed, the pooled OR dropped dramatically and was no longer statistically significant ($OR_{\text{nonsmokers}} = 1.00$, 95% CI = 0.65 to 1.54; $OR_{\text{smokers}} = 1.40$, 95% CI = 0.81 to 2.41). Additionally, when the Hwang data were not

included in the polytomous logistic regression, the OR for the gene-smoking interaction was no longer significant (OR = 1.34, 95% CI = 0.69 to 2.64). Although the overall weight assigned to this study was less than the other four studies, dropping each other study, one at a time, did not have a large effect on the overall results. However, when comparing the polytomous logistic regression model testing for genotype, smoking, and their interaction with indicator variables for each study to a model that also included a covariate to adjust for the Hwang study, the likelihood ratio test comparing these two models was not statistically significant.

The overall smoking effect calculated from the polytomous logistic regression for CL/P (OR = 1.55, 95% CI = 1.27 to 1.91) and CP (OR = 1.42, 95% CI = 1.06 to 1.89) is similar to that estimated by Wyszynski et al. (1997). In this meta-analysis, based on 11 published studies, the estimated OR for CL/P was 1.29 (95% CI = 1.18 to 1.42) and 1.32 (95% CI = 1.10 to 1.62) for CP.

This meta-analysis could not take into account the potential effect of heavy smoking. Hwang et al. (1995) found an OR of 6.16 (95% CI = 1.09–34.7) for CP among infants carrying the C2 allele whose mothers smoked ≤ 10 cigarettes per day;

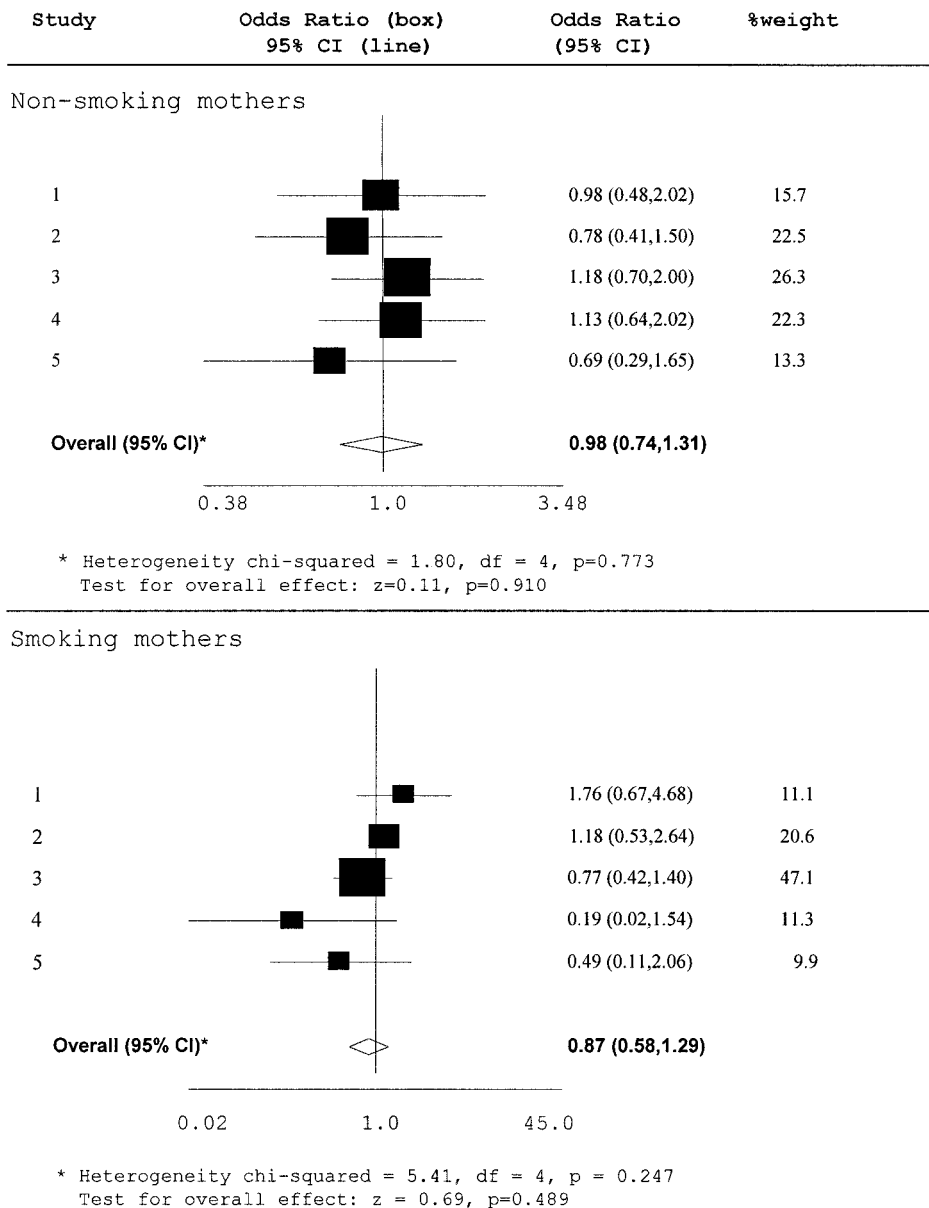


FIGURE 2 Effect of maternal smoking on risk of CL/P among infants carrying TGFA *TaqI* C2 allele. Studies: (1) Hwang et al. (1995); (2) Shaw et al. (1996); (3) Christensen et al. (1999); (4) Romitti et al. (1999); and (5) Beaty et al. (2001).

among mothers who smoked ≥ 10 cigarettes per day, this risk was further increased to an OR of 8.6 (95% CI = 1.57 to 47.8). Shaw et al. (1996) found a sixfold and ninefold increased risk for CL/P and CP, respectively, among infants carrying the C2 alleles and whose mothers reported smoking ≥ 20 cigarettes per day (heavy smokers). Romitti et al. (1999) found an increased risk for CP among heavy smoking mothers (≥ 20 cigarettes per day) regardless of genotype. However, Romitti et al. did not find any increased risk among infants carrying the C2 allele, perhaps due to extremely small numbers of infants who carried the C2 allele and had a heavy smoker mother.

Testing for gene-environment interaction requires large numbers of cases and controls, and meta-analysis may be one way to untangle the disparity among several smaller studies.

More than 1000 case-control pairs would be needed to detect an $OR_{\text{interaction}}$ of 2.0 with 80% power, a number that is virtually unachievable in any single study of birth defects (Gauderman, 2002). Even combining these five studies in this meta-analysis yielded far less than this predicted number, with just over 700 CL/P cases, 300 CP cases, and 1500 controls. Smaller studies do provide important clues as to whether a potential association exists, but consistency across populations remains a critical confirming step.

The biologic mechanisms of how maternal smoking and TGFA genotype interact in the etiology of oral clefts remain unknown. TGFA contributes to facial development; it is expressed at the medial edge epithelium of fusing palatal shelves (Miettinen et al., 1999). The teratogenic effects of smoking have been well documented (Pollack et al., 2000; Kallen,

2001; Mortensen et al., 2001). Philipp et al. (1984) postulated that women who smoked during pregnancy had compromised uteroplacental blood flow that could result in poor fetal development. Carbon monoxide affects oxygen transfer to the placenta, and nicotine constricts the uterine wall resulting in hypoxia (Philipp et al., 1984).

Other genetic markers have been associated with clefts, both alone and in the presence of environmental factors. The possibility of genetic heterogeneity, or even gene-gene interaction, could also account for some of the ambiguity observed among the five studies. The retinoic acid receptor alpha (RARA) (Chenevix-Trench et al., 1992) and BCL3 (Gaspar et al., 2002) have shown positive associations with oral clefts. Lidral et al. (1998) utilized both the case-control study design and the case-parent trio design to examine associations between several additional markers and oral clefts. They found a significant association between CL/P and variants at both MSX1 and TGFB3, and between CP and the MSX1 marker (Lidral et al. 1998). Romitti et al. (1999) showed evidence of association between allelic variants at TGFB3 and MSX1 and cleft palate; this association was increased if the mother smoked or consumed alcohol during the pregnancy. Mitchell et al. (2001) found an association between CP and TGFB3 in a Danish population.

It is very likely that several genes are involved in the etiology of nonsyndromic oral clefts and that these may interact with one another or with environmental risk factors, such as maternal smoking. It will be important to consider such possible interactions to fully understand causal relationships among genes, environmental risk factors, and risk for oral clefts. The statistical approaches of meta-analysis or organized collaborative studies of oral clefts may be necessary to more fully detect and confirm the etiologic effect of genes (Mitchell et al., 2001).

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