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Orofacial Clefts, Parental Cigarette Smoking, and Transforming Growth Factor-Alpha Gene Variants

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Summary

Results of studies to determine whether women who smoke during early pregnancy are at increased risk of delivering infants with orofacial clefts have been mixed, and recently a gene-environment interaction between maternal smoking, transforming growth factor-alpha (TGFa), and clefting has been reported. Using a large population-based case-control study, we investigated whether parental periconceptional cigarette smoking was associated with an increased risk for having offspring with orofacial clefts. We also investigated the influence of genetic variation of the TGFa locus on the relation between smoking and clefting. Parental smoking information was obtained from telephone interviews with mothers of 731 (84.7% of eligible) orofacial cleft case infants and with mothers of 734 (78.2%) nonmalformed control infants. DNA was obtained from newborn screening blood spots and genotyped for the allelic variants of TGFa. We found that risks associated with maternal smoking were most elevated for isolated cleft lip with our without cleft palate, (odds ratio 2.1 [95% confidence interval 1.3–3.6]) and for isolated cleft palate (odds ratio 2.2 [1.1–4.5]) when mothers smoked ≥20 cigarettes/d. Analyses controlling for the potential influence of other variables did not reveal substantially different results. Clefting risks were even greater for infants with the TGFa allele previously associated with clefting whose mothers smoked ≥20 cigarettes/d. These risks for white infants ranged from 3-fold to 11-fold across phenotypic groups. Paternal smoking was not associated with clefting among the offspring of nonsmoking mothers, and passive smoke exposures were associated with at most slightly increased risks. This study offers evidence that the risk for orofacial clefting in infants may be influenced by maternal smoke exposures alone as well as in combination (gene-environment interaction) with the presence of the uncommon TGFa allele.

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Introduction

Orofacial clefts are among the more common congenital malformations, with reported prevalences of 1–2/1,000 livebirths (Shaw et al. 1991). These anomalies appear to have heterogeneous, but largely unknown etiologies (Fogh-Anderson 1967). Recognized associations include chromosomal anomalies, Mendelian disorders, and teratogens, but all are individually rare (Gorlin et al. 1990). Recently, non–population-based genetic studies have identified twofold to fivefold increased risks for cleft lip and palate among individuals with the uncommon allele for transforming growth factor-alpha (TGFa) (Ardinger et al. 1989; Chenevix-Trench et al. 1991, 1992; Holder et al. 1992; Sassani et al. 1993; Shiang et al. 1993). TGFa is a secretory protein that binds to the epidermal growth factor receptor and has been localized to palatal epithelium prior to and during palatal closure (Dixon et al. 1991).

Epidemiologic studies suggest that exogenous factors also play a role in the etiology of orofacial clefts. Several studies have investigated whether parental, primarily maternal, cigarette smoking increased the risk of having offspring with orofacial clefts (Andrews and McGarry 1972; Saxen 1974, 1975; Kelsey et al. 1978; Ericson et al. 1979; Evans et al. 1979; Hemminki et al. 1983; Czeizel and Nagy 1986; Shiono et al. 1986; Khoury et al. 1987, 1989; Malloy et al. 1989; Seidman et al. 1990; Van Den Eeden et al. 1990; Werler et al. 1990; Savitz et al. 1991; McDonald et al. 1992; Zhang et al. 1992). The interpretation of these studies, however, is problematic, owing to (i) non–population-based, or potentially incomplete, case ascertainment (Andrews and McGarry 1972; Kelsey et al. 1978; Evans et al. 1979; Shiono et al. 1986; Khoury et al. 1987; Malloy et al. 1989; Seidman et al. 1990; Van Den Eeden et al. 1990; Werler et al. 1990; Savitz et al. 1991; McDonald et al. 1992); (ii) sample sizes inadequate to classify (or classification not done) the overall group of clefts into more homogeneous phenotypic subgroups (Andrews and McGarry 1972; Kelsey et al. 1978; Ericson et al. 1979; Evans et al. 1979; Hemminki et al. 1983; Shiono et al. 1986; Malloy et al. 1989; Seidman et al. 1990; Werler et al. 1990; McDonald et al. 1992); (iii) lack of information on passive cigarette smoke exposures (Andrews and McGarry

1972; Saxen 1974, 1975; Ericson et al. 1979; Evans et al. 1979; Hemminki et al. 1983; Czeizel and Nagy 1986; Shiono et al. 1986; Khoury et al. 1987, 1989; Malloy et al. 1989; Seidman et al. 1990; Van Den Eeden et al. 1990; Werler et al. 1990; Savitz et al. 1991; McDonald et al. 1992; Zhang et al. 1992); (iv) inability to consider specific embryologically relevant exposure time periods (Khoury et al. 1987; Malloy et al. 1989; Van Den Eeden et al. 1990); (v) lack of information on paternal cigarette use (Andrews and McGarry 1972; Saxen 1974, 1975; Kelsey et al. 1978; Ericson et al. 1979; Evans et al. 1979; Hemminki et al. 1983; Czeizel and Nagy 1986; Shiono et al. 1986; Khoury et al. 1987, 1989; Malloy et al. 1989; Van Den Eeden et al. 1990; Seidman et al. 1990; Werler et al. 1990; McDonald et al. 1992); and (vi) lack of information on the influence of potential confounders (Evans et al. 1979; Czeizel and Nagy 1986; Khoury et al. 1987).

We conducted a large population-based case-control study of California infants born with an orofacial cleft, to investigate whether mothers who smoked cigarettes or were exposed passively to cigarette smoke in early pregnancy, or fathers who smoked, had an increased risk for having offspring with orofacial clefts. We also investigated the influence of genetic variation of the infant's TGFa locus on the relation between maternal smoke exposures and clefting, to explore a possible gene-environment interaction.

Material and Methods

Case Ascertainment

For this case-control study, case infants or fetuses with an orofacial cleft were ascertained by the California Birth Defects Monitoring Program by reviewing medical records at all hospitals and genetic centers in a known geographic population base (Croen et al. 1991). Eligible were infants and fetuses diagnosed with an orofacial cleft within 1 year after birth among births and fetal deaths (n = 552,601) between January 1, 1987, and December 31, 1989, to women residing in most California counties (metropolitan areas of Los Angeles and San Francisco were excluded).

Diagnostic information from medical records, autopsies, and surgical reports of all infants and fetuses with orofacial clefts or similar orofacial anomalies was reviewed in order to restrict eligibility to those infants with cleft of the palate, lip, or both. Infants with diagnoses of bifid uvula, submucous cleft palate (CP), notching of the alveolar ridge, or vermillion border of the upper lip were excluded because ascertainment was thought to be incomplete. Infants cytogenetically diagnosed with trisomy or Turner syndrome (45,X) were also excluded (n = 81). There were 891 infants/fetuses ascertained with an eligible diagnosis (93% were liveborn infants). Twelve additional cases were identified after the interview phase was completed, or participated in a prior study, and they were not contacted.

Case Classification

To create developmentally homogeneous subgroups, cases were phenotypically grouped as: "isolated" CP, "isolated" cleft lip with or without cleft palate (CL±P), "multiple" CP, "multiple" CL±P, and clefts whose etiology is "known," nearly all of which were monogenic conditions (known-etiology clefts). Cases were classified blind to maternal smoking status and infant's TGFa genotype. A medical geneticist (M.M.T.) classified each case as isolated or multiple on the basis of the nature of any accompanying congenital anomalies. CP and CL±P cases with no other anomaly or with anomalies considered minor (e.g., low-set ears) or not true anomalies (e.g., undescended testicles) were classified as isolated. CP and CL±P cases with at least one accompanying major anomaly or with a combination of phenotypic features (including minor anomalies) that were suggestive of a known syndrome were considered to be either "multiple" or of "known etiology" (for example, Robin sequence with flat face, hypotonia, and characteristic ophthalmologic findings was evidence for Stickler syndrome). A similar classification has been described by others (Emanuel et al. 1973; Hanson and Murray 1990). A description of the classification procedure used is available from the authors.

Control Selection

A control infant was eligible if (1) he or she was born alive during 1987–89; (2) his/her mother was a resident of the same counties as cases; and (3) he or she did not have a reportable birth defect (Croen et al. 1991) prior to the first birthday. A total of 972 controls were electronically selected from California vital records by using a pseudorandom number generator among all eligible infants (n = 548,844). Other than being delivered during a similar time period and within the same geographic area, controls were not matched to cases.

Maternal Interviews

The study protocol was approved by the California State Health Department Institutional Review Board. Interviews were conducted with mothers of cases and controls in English (91%) or Spanish, and nearly all were over the telephone. Women who only spoke languages other than English or Spanish (25 cases and 33 controls) and three case mothers who died prior to interview contact were excluded, yielding 863 cases and 939 controls eligible for study. Interviews were completed an average of 3.5 years for cases and 3.6 years for controls after the date of delivery. Considerable efforts were made to keep interviewers uninformed of whether the woman being interviewed was a case or a control mother until the end of the interview. On beginning the inter-

view, an interviewer assisted each woman with establishing a 4-mo time period that was referred to throughout the interview, to elicit information on exposures and events. The 4 mo included the time frame from 1 mo before to 3 mo after the date of conception, as provided by the woman. This period encompasses the embryologic timing of palate and lip formation and closure, which is complete by ~8 wk postconception. In addition to detailed inquiries about tobacco exposures, the average 40-min interview elicited information on maternal medical history, medication use, recreational drug use, and exposures in the periconceptional period associated with employment or hobbies.

Cigarette Smoke Exposure

To assess maternal smoking, women were asked how many cigarettes they smoked daily for the 4-mo period as well as for each month during the period. To assess passive smoke exposures, a woman was asked whether during the 4-mo period anyone smoked inside her home, near her at work or school, while she was commuting to work or school, or if she regularly frequented (at least once a week) a place, such as a restaurant or laundromat, where others smoked nearby. To assess paternal smoking, a woman was asked how many cigarettes per day her infant's natural father smoked in the 3 mo before through 3 mo after conception.

TGFa Genotyping

Genomic DNA was obtained from residual dried blood spots on newborn screening specimens (filter papers). These specimens are collected from all liveborn children in California. Among the 802 liveborn case and 939 control infants considered eligible, a blood specimen was identified for 678 (84.5%) cases and for 829 (88.3%) controls. Reasons for not obtaining a specimen included (1) no sample remained on filter paper, (2) filter paper could not be located, and (3) information available insufficient to match infant records. Information used for matching subjects to blood spots included infant's name, sex, and weight, hour and hospital of birth, as well as mother's name, her birth date and zip code of residence. Cases and controls not matched were somewhat more likely to be Hispanic.

DNA was extracted from filter papers by using available techniques (Schwartz et al. 1990) and was amplified using the PCR. Genotyping was done using a PCR assay to detect a 4-bp insertion/deletion polymorphism that is the basis of the *Taq*I RFLP described elsewhere (Hayward et al. 1987; Qian et al. 1993; Basart et al. 1994). For analysis, cases and controls were categorized A1 homozygous for the more common allele (A1,A1), or A2 heterozygous (A1,A2) or homozygous for the uncommon allele (A2,A2). Of the 678 case and 829 control blood specimens, 97.8% of cases and 98.1% of controls were genotyped for TGFa.

Statistical Analyses

The odds ratio along with its 95% confidence interval was used to estimate risk. Risk estimates, including those derived from logistic regression models, were computed using EGRET (1991). For each phenotypic case group, analyses were performed for maternal cigarette smoking, paternal smoking, and both parents' smoking, considering smoking as a polychotomous variable (0, 1–19, and ≥20 cigarettes/d). Analyses were also performed for maternal cigarette smoking for each phenotypic group with or without the uncommon (A2) TGFa allele. Maternal age, race/ethnicity, education, gravidity, alcohol use, diabetes, and folic acid–containing multivitamin use were considered as potential covariates. Risks associated with passive smoke exposures were explored among women who were nonsmokers in the 4-mo period.

Results

Interviewed were 731 (84.7%) of 863 eligible case mothers and 734 (78.2%) of 939 eligible control mothers. Information was unavailable from 2.3% of case and 3.4% of control mothers who refused to be interviewed, and from 13.0% of case and 18.4% of control mothers who could not be located after considerable tracing. As shown in table 1, CP case mothers were more likely to be white non-Hispanic, to have had four or more prior pregnancies, and less likely to have been employed, as compared with control mothers. CL±P case mothers were less likely to have attended or graduated college, more likely to be 39 years old, more likely to have had male infants, and more likely to have had a history of epilepsy or seizures, as compared with control mothers.

The 731 cases consisted of 348 with isolated CL±P, 141 with isolated CP, 99 with multiple CL±P, 74 with multiple CP, and 69 with "known etiology." The risk associated with maternal smoking in the period 1 mo before through 3 mo after conception for each case group is shown in table 2. Risk estimates were modestly elevated and increased with increasing number of cigarettes smoked, for nearly all groups (except those women who had offspring with multiple CP and smoked 1–19 cigarettes/d). The largest risks were observed for isolated cleft groups, the only groups whose risk estimates had associated confidence intervals that did not include 1.0.

Of the women who smoked, 97% reported they inhaled and 99% reported use of a filtered cigarette. Case mothers reported smoking in the month before conception more frequently than did control mothers (32% vs. 23%). Among those who smoked 1 mo before conception, control mothers were less likely than case mothers to continue smoking through the end of the third month postconception (64% vs. 74%, respectively). Approximately 22% of women who stated that they were non-

Table 1

Maternal and Infant Characteristics of Cases and Controls

	CP Cases (N = 215) (%)	CL±P Cases (N = 447) (%)	Controls (N = 734) (%)
Race/Ethnicity:			
Hispanic	22.8	30.1	28.1
Non-Hispanic/white	65.6	58.9	58.3
Black	4.2	3.3	3.3
Asian	5.1	3.1	5.2
Other	1.9	4.5	5.0
Age (Years):			
<20	14.4	12.7	10.4
20–24	22.3	27.9	26.7
25–29	34.9	30.6	31.1
30–34	20.9	18.3	22.2
35–39	6.5	8.5	8.3
>39	.9	1.6	.8
Education:			
Not a high school graduate	22.8	25.7	21.8
High school graduate	30.7	36.4	28.3
Some college	29.8	27.0	31.9
College graduate	16.3	10.9	17.2
Employed	50.2	55.1	57.2
Gravidity:[a]			
1	26.0	23.4	28.3
2	27.4	27.7	24.7
3	18.6	20.3	22.1
4+	27.4	26.1	21.9
Diabetes	5.6	7.8	5.6
Epilepsy/seizures	1.9	3.1	1.4
Male child	46.0	62.3	48.9

NOTE.—Percentages may not add to 100, because of rounding or missing information for some subjects. This table excludes 69 cases with known etiology.

[a] No. of previous pregnancies.

smokers in the 4-mo period of interest reported that they had smoked sometime earlier in their lives. These women did not have an increased risk of having a proband infant with an orofacial cleft.

Analyses were performed controlling for the influence of maternal race/ethnicity (white/non-Hispanic, black, Hispanic, Asian, other), education (more than a high school graduate, high school graduate, some college, college graduate), alcohol use (none, some, weekly during 4-mo period), use of a multivitamin containing folic acid (yes vs. no, from 1 mo before through 2 mo after conception), diabetes (yes/no), gravidity (0, 1, 2, 3, 4+ previous pregnancies), age (19, 20–24, 25–29, 30–34, 35–39, 40 years), and infant's sex. For isolated CL±P, simultaneous adjustment revealed odds ratios of 1.5 (1.0–2.2) and 1.7 (0.96–3.2) for maternal smoking of 1–19 and ≥20 cigarettes/d, respectively. Data were too sparse to perform similar simultaneous variable adjustment for other case groups. Thus, single variable control was done. For the other four cleft groups, control for maternal race/ethnicity, education, diabetes, gravidity, or age did not produce risk estimates that were sufficiently different to change the interpretation revealed by the crude estimate (for nearly all, the risk changed 10%). Control for multivitamin use resulted in a lowering of risks from maternal smoking for multiple CP and known-etiology clefts, but not for the two other case groups. Control for alcohol use did not substantially alter maternal smoking risks for isolated CP, multiple CP, or known-etiology clefts, but stratification on the basis of alcohol use revealed heterogeneity in maternal smoking risk for multiple CL±P cases. The increased risk for maternal smoking was only observed among women who reported alcohol use in the 4-mo exposure time period. Control for child sex also did not alter the maternal cigarette smoking risk estimates for the case groups, except for multiple CP. For this group, the increased risk associated with maternal smoking was observed exclusively among female offspring. In addition, risk estimates associated with maternal smoking for all five case groups were minimally influenced when those with a family history of clefting (defined as orofacial cleft in mother, father, or previous sib of the proband) were excluded from analyses.

Risks from paternal smoking in the period 3 mo before through 3 mo after conception were modestly elevated for all case groups when fathers smoked ≥20 cigarettes/d but were not consistently elevated when fathers smoked less, nor were risks as large as those observed for maternal smoking. For fathers smoking ≥20 cigarettes/d, the odds ratios were 1.6 (1.1–2.4), 1.3 (0.74–2.3), 1.3 (0.64–2.5), 1.2 (0.57–2.5), and 1.7 (0.91–3.4) for isolated CL±P, isolated CP, multiple CL±P, multiple CP, and known-etiology clefts, respectively. Because increased risks were observed for both maternal and paternal smoking, we explored whether these risks were independent or were mediated through only one parent. Table 3 shows risk estimates associated with maternal smoking in the absence or presence of paternal smoking, and vice versa. The comparison group for these analyses were those parents who were concordant for nonsmoking. Among nonsmoking women, we found no evidence of an increased risk for clefting from paternal smoking. In contrast, maternal smoking, in the absence of paternal smoking, was associated with an increased risk for isolated CL±P. If both parents smoked, however, risks were generally greater than if only the mother smoked. Risks of twofold to threefold were observed for isolated CP and CL±P groups when both parents smoked ≥20 cigarettes/d.

Among women who did not smoke cigarettes in the 4-mo period, we investigated whether reported passive smoke exposures at home, in the workplace, or in other locations such as restaurants or laundromats increased risk for having offspring with an orofacial cleft, as compared with women who did not report any passive

Table 2

Risk Estimates for Maternal Cigarette Smoking From 1 Mo Before through 3 Mo After Conception, by Case Groupings

	Maternal Cigarettes Smoked/D	No. of Cases	Odds Ratio	95% Confidence Interval
Isolated CL±P (n = 348)[a]	0	227	Reference	...
	1–19	87	1.6	1.2–2.3
	≥20	32	2.1	1.3–3.6
Isolated CP (n = 141)	0	95	Reference	...
	1–19	32	1.4	.90–2.3
	≥20	14	2.2	1.1–4.5
Multiple CL±P (n = 99)	0	70	Reference	...
	1–19	21	1.3	.73–2.2
	≥20	8	1.7	.71–4.1
Multiple CP (n = 74)	0	56	Reference	...
	1–19	13	.99	.50–1.9
	≥20	5	1.4	.45–3.8
"Known-etiology" clefts (n = 69)[a]	0	49	Reference	...
	1–19	14	1.2	.62–2.4
	≥20	5	1.6	.51–4.4

NOTE.—Use among control mothers was as follows: nonsmokers, n = 562; 1–19/d, n = 132; ≥20/d, n = 37; unknown, n = 3.
[a] Smoking status was unknown for two mothers of isolated CL±P cases and one mother of "known etiology."

smoke exposure. Nonsmoking women who reported that someone smoked tobacco in their home were not at substantially increased risk for having offspring with an orofacial cleft (odds ratios of ≤1.4). However, nonsmoking women who reported that tobacco was frequently (weekly occurrence) smoked in their home at a close distance (within 6 feet of the smoker), were at increased risk for isolated CL±P (odds ratio = 2.0 [1.2–3.4]), isolated CP (1.6 [0.71–3.4]), and for multiple CP (1.6 [0.56–4.6]). Nonsmoking women were at slightly increased risk from ambient tobacco smoke in their workplace, but risks were consistent statistically with no increase and were not higher among those women who reported frequent close exposure to coworkers who smoked. Nonsmoking women who reported "any" (home, work, or other place) smoke exposure were also not at substantially increased risk.

Among the 731 cases and 734 controls for whom maternal interview information was available, the *Taq*I TGFa polymorphism was genotyped for 306 (87.9%) isolated CL±P cases, 125 (88.7%) isolated CP cases, 55 (55.6%) multiple CL±P cases, 43 (58.1%) multiple CP cases, 42 (60.9%) known-etiology cases, and 640 (87.2%) controls. Compared with controls, the odds ratio associated with the less common A2 TGFa allele was not elevated for isolated and multiple CL±P, but it was for the three other phenotypes (table 4). Analyses of the risk for clefting in infants who had the A2 allele stratified by race/ethnic group (white, Hispanic, black, and other), revealed some variation in the magnitude of the odds ratio across race/ethnic strata (table 4) but did not reveal sufficient statistical evidence (χ^2 statistic with an associated P value ≤.20; see Selvin 1991) for heterogeneity. Stratification by family history of clefting also did not reveal heterogeneity in clefting risk associated with the uncommon allele. Among cases with a family history of clefting, 18% (9/50; three isolated CL±P, five isolated CP, and one known-etiology cleft) had the uncommon allele, and 14% did among cases without a family history of clefting.

Analyses for the risk of clefting from maternal smoking, stratified by presence or absence of the uncommon TGFa allele, revealed that the risk of clefting among white infants was much greater for infants with the less common A2 TGFa allele than for infants with the common allele when their mothers smoked ≥20 cigarettes/d (table 5). Statistical evidence for interaction was observed between TGFa genotype and maternal smoking ≥20 cigarettes/d for isolated CL±P, isolated CP, and known-etiology clefts (using $P \leq .20$ as a statistical criterion; see Selvin 1991). Elevated risks associated with heavier maternal smoking among infants with the less common A2 allele ranged from 2.9 to 10.8. These risks were only minimally influenced when those with a family history of clefting were excluded from analyses. Although risks shown in table 5 were exclusive to white infants, all race/ethnic groups combined showed similar results. Maternal smoking and the frequency of the A2 allele were too sparse to assess risk for the other individual race/ethnic groups. Among nonsmoking women who reported any passive smoking exposures, risks associated with these exposures were higher in infants with

Table 3

Risk Estimates for Maternal and Paternal Cigarette Smoking, by Case Groupings

	Paternal No. of Cigarettes Smoked/Day	Maternal No. of Cigarettes Smoked/D: 0		Maternal No. of Cigarettes Smoked/D: 1–19		Maternal No. of Cigarettes Smoked/D: ≥20	
		N[a]	Odds Ratio (95% Confidence Interval)	N[a]	Odds Ratio (95% Confidence Interval)	N[a]	Odds Ratio (95% Confidence Interval)
Isolated CL±P	0	167 (412)	Reference	31 (62)	1.2 (.75–2.0)	11 (13)	2.1 (.85–5.1)
	1–19	37 (95)	.96 (.62–1.5)	28 (41)	1.7 (.98–2.9)	6 (7)	2.1 (.62–7.1)
	≥20	19 (47)	1.0 (.55–1.8)	28 (25)	2.8 (1.5–5.1)	15 (16)	2.3 (1.1–5.1)
Isolated CP	0	74	Reference	15	1.4 (.69–2.6)	1	.43 (.02–3.2)
	1–19	14	.82 (.42–1.6)	10	1.4 (.61–3.0)	5	4.0 (1.1–14.4)
	≥20	6	.71 (.26–1.8)	7	1.6 (.59–4.0)	8	2.8 (1.1–7.2)
Multiple CL±P	0	47	Reference	6	.85 (.31–2.2)	2	1.4 (.20–6.6)
	1–19	17	1.6 (.82–3.0)	9	1.9 (.81–4.4)	1	1.3 (.06–10.5)
	≥20	3	.56 (.13–2.0)	5	1.8 (.56–5.1)	5	2.7 (.83–8.5)
Multiple CP	0	43	Reference	5	.77 (.26–2.1)	2	1.5 (.22–7.2)
	1–19	7	.71 (.28–1.7)	4	.93 (.27–2.9)	1	1.4 (.06–11.5)
	≥20	5	1.0 (.34–2.9)	4	1.5 (.43–4.9)	2	1.2 (.18–5.7)
"Known-etiology" clefts	0	36	Reference	5	.92 (.31–2.6)	2	1.8 (.26–8.7)
	1–19	5	.60 (.20–1.7)	5	1.4 (.45–4.0)	0	0
	≥20	7	1.7 (.65–4.3)	3	1.4 (.31–5.1)	3	2.2 (.47–8.4)

[a] No. of case fathers/mothers in a given category. Number in parentheses denotes no. of control fathers/mothers. Numbers do not total the overall number of subjects, because of missing information.

Table 4

Risk Estimates for Orofacial Clefts among Infants with the Uncommon (A1,A2 or A2,A2) TGF-alpha Allele, by Race/Ethnic Group

	Uncommon Allele (A1,A2 or A2,A2)[a]	Common Allele (A1,A1)	Odds Ratio[b]	95% Confidence Interval	χ^2 Homogeneity *P* Value[c]
Controls	78	562	Reference	...	
White	58	321	Reference	...	
Hispanic	11	164	Reference	...	
Black	2	18	Reference	...	
Other	7	58	Reference	...	
Isolated CL±P	37	269	.99	.64–1.5	
White	27	163	.92	.54–1.5	
Hispanic	8	77	1.6	.54–1.5	.65
Black	1	7	1.3	.04–23.9	
Other	1	22	.38	.02–3.4	
Isolated CP	23	102	1.6	.94–2.8	
White	19	68	1.6	.83–2.9	
Hispanic	1	23	.65	.03–5.3	.82
Black	1	3	3.0	.08–79.3	
Other	2	8	2.1	.25–14.4	
Multiple CL±P	6	49	.88	.33–2.2	
White	4	23	.96	.27–3.1	
Hispanic	1	17	.88	.04–.73	.93
Black	0	4	0	...	
Other	1	5	1.7	.06–19.4	
Multiple CP	9	34	1.9	.82–4.3	
White	8	19	2.3	.89–6.0	
Hispanic	0	11	0	...	.57
Black	0	3	0	...	
Other	1	1	8.3	.20–352.4	
"Known-etiology" clefts	10	32	2.3	.99–5.0	
White	9	17	2.9	1.1–7.4	
Hispanic	0	9	0	...	.52
Black	0	5	0	...	
Other	1	1	8.3	.20–352.4	

NOTE.—This table reflects genotype information from eligible liveborn cases and control infants whose mothers were interviewed.

[a] Five controls (three white, two Latino), five isolated CL±P cases (five white), and one isolated CP case (white) were A2,A2 homozygotes.

[b] Risk for orofacial clefts for those with the uncommon allele.

[c] Tests for interaction across race/ethnic strata.

the A2 allele for isolated CL±P and isolated CP than in those infants without the A2 allele (odds ratios: 9.8 [1.1–218.0] vs. 0.98 [0.57–1.7] for isolated CL±P and 5.3 [0.55–124.0] vs. 1.5 [0.68–3.2] for isolated CP.

Discussion

This study is among the first to explore gene-environment effects on the risk for a congenital anomaly, particularly one that has long been recognized as having a multifactorial etiology (Fogh-Anderson 1967). Our results suggest that maternal, and not paternal, smoking during early pregnancy is associated with an ~1.5-fold to ~2-fold increased risk for delivering offspring with an orofacial cleft defect, especially isolated defects with risks increasing with the number of cigarettes smoked. Of particular note, we observed a strong association between maternal smoking and a genetic variant of TGFa in the risk for clefting. The fraction of cases possibly attributed to this interaction was small, however; for example, only 7 case infants of 306 isolated CL±P had the uncommon A2 allele and had mothers who were heavier smokers. Our results did not reveal an association between isolated CL±P and TGFa observed elsewhere (Ardinger et al. 1989; Chenevix-Trench et al. 1991, 1992; Holder et al. 1992; Sassani et al. 1993; Shiang et al. 1993). The discrepancy in findings may reflect the potential nonrandom selection of cases and controls used in those studies, as compared with the population-based ascertainment of cases and controls employed in this study. Increased risks observed for "known-etiology" clefts from maternal smoking were perplexing, given that most of the clefts in this group had associated monogenic conditions. The increased risk observed among this group overall (table 2) appeared to be exclusive to infants with the uncommon TGFa allele (table 5). A similar pattern was not observed for isolated cleft cases; that is, even though risks from smok-

Table 5

Risk Estimates for Maternal Cigarette Smoking from 1 Mo Before through 3 Mo After Conception, by Case Groupings and TGF-alpha Genotypes for White Infants Only

	Maternal Cigarettes Smoked/D	TGF-alpha: Uncommon Allele (A1,A2 or A2,A2) No. of Cases[a]	Odds Ratio	95% Confidence Interval	TGF-alpha: Common Allele (A1,A1) No. of Cases[b]	Odds Ratio	95% Confidence Interval	χ^2 Homogeneity *P* Value[c]
Isolated CL±P (n = 191)[d]	0	14	Reference	...	94	Reference	...	
	1–19	7	1.8	.51–6.2	49	1.8	1.1–2.8	.97
	≥20	6	6.1	1.1–36.6	20	1.8	.91–3.5	.15
Isolated CP (n = 87)[d]	0	8	Reference	...	42	Reference	...	
	1–19	6	2.7	.66–11.0	17	1.4	.70–2.7	.34
	≥20	5	9.0	1.4–61.9	9	1.8	.72–4.4	.09
Multiple CL±P (n = 27)[d]	0	3	Reference	...	15	Reference	...	
	1–19	1	1.2	.04–15.3	5	1.1	.34–3.5	.84
	≥20	0	0	...	3	1.7	.36–6.7	.98
Multiple CP (n = 27)[d]	0	5	Reference	...	15	Reference	...	
	1–19	2	1.4	.17–10.2	3	.67	.15–2.6	.47
	≥20	1	2.9	.10–45.4	1	.56	.03–4.3	.30
"Known-etiology" clefts (n = 25)[d]	0	4	Reference	...	13	Reference	...	
	1–19	1	.90	.03–10.2	2	.52	.08–2.5	.61
	≥20	3	10.8	1.2–111.7	2	1.3	.19–6.5	.11

[a] Use among control mothers whose infant had the uncommon allele was 0/d, n = 43; 1–19/d, n = 12; ≥20/d, n = 3.
[b] Use among control mothers whose infant had the common allele was 0/d, n = 226; 1–19/d, n = 67; ≥20/d, n = 27.
[c] Tests for interaction between allele strata.
[d] No. of cases with genotype and maternal interview information. Maternal smoking unknown for one case.

ing were substantially higher for infants with the uncommon allele, risks remained elevated among infants with the common allele.

The increased risk observed for maternal smoking is consistent with some previous investigations (Andrews and McGarry 1972; Saxen 1974; Kelsey et al. 1978; Ericson et al. 1979; Khoury et al. 1987, 1989; Van Den Eeden et al. 1990) and contrasts with others (Saxen 1975; Evans et al. 1979; Hemminki et al. 1983; Shiono et al. 1986; Malloy et al. 1989; Werler et al. 1990). Only one (Savitz et al. 1991) of two studies (Savitz et al. 1991; Zhang et al. 1992) that described a relation with paternal smoking had information on maternal smoking habits, and no previous study has examined the clefting risk to offspring from both parents smoking. Further, only one previous study investigated potential effects associated with passive smoke exposures (Kelsey et al. 1978). Thus, the current data containing information on smoking exposures from multiple sources are not directly comparable to much of the previous research.

One other report has explored the clefting risk associated with maternal smoking and genetic variation of TGFa; that study found a possible interaction between TGFa genotype and maternal smoking for isolated CP only (Hwang et al. 1995). It found modest increased risks for maternal smoking that were not observed among those infants with the common TGFa allele. Our study and the one by Hwang et al. have two notable differences that might contribute to differences between study results. First, our study used normal controls, whereas the study by Hwang et al. used malformed controls. If the uncommon TGFa allele frequency or the frequency of maternal smoking was associated with any of the malformations contained in Hwang et al. study's control group, then the two studies might reveal different results. Second, our study asked women about their smoking behavior in early pregnancy, whereas the Hwang et al. study relied on smoking behaviors after delivery as a proxy for smoking during early pregnancy. If case and control mothers differentially quit smoking in the latter two trimesters of pregnancy, then the two studies could reveal different results.

Epidemiologic evidence points to maternal smoking as a risk factor for clefts. The myriad chemical constituents in tobacco smoke, however, make it difficult to postulate what pathogenetic mechanism(s) might underlie this association. One biologically plausible connection is that cigarette smoking may influence embryonic development via embryonic hypoxia. Both carbon monoxide and nicotine exposures, common agents from cigarette smoking, can produce tissue hypoxia (Longo 1982). Hypoxia has been shown experimentally to induce orofacial cleft defects (Millicovsky and Johnston 1981; Bronsky et al. 1986) as well as other malformations (Astrup et al. 1972). In addition to a teratogenic effect observed for nicotine (Upshall 1972), cadmium, another cigarette constituent, has been observed to induce orofacial clefts and other malformations in laboratory animals (Carmichael et al. 1982). Further, cigarettes contain N-nitroso compounds and polycyclic aromatic hydrocarbons such as benzo(a)pyrene, some of which are known or suspected teratogens in laboratory animals (Druckery et al. 1966; Givelbar and DiPaolo 1969; Lambert and Nebert 1977). Of note, placental oxidation activities (induction of cytochrome P-450) for polycyclic aromatic hydrocarbons have been observed to be lower in infants with malformations than in nonmalformed infants whose mothers were smokers (Manchester and Jacoby 1984). Another plausible connection is that cigarette smoking may decrease a pregnant woman's serum folate (Witter et al. 1982), and we have previously reported that maternal use of multivitamins containing folic acid is associated with a reduction in risk for clefting (Shaw et al. 1995). The relation of these lines of evidence to causal mechanisms is unknown and needs to be explored. Moreover, how the evidence relates to the observations made with the gene variant of TGFa is unknown but may prove informative to those investigating mechanisms underlying the relation between TGFa and lip and palate formation/closure.

Despite the plausibility of a relation between embryonic cigarette smoke exposures and risk for clefting, potential biases resulting from differential reporting and differential participation by case and control mothers need to be addressed. This study did not have an objective standard to verify reported cigarette use or passive smoke exposures. Even though self-reported cigarette use has been shown to be adequate, as compared to biochemical validation (Patrick et al. 1994), suspicion may be raised about our results being explicable to reporting bias. Many women regard smoking during pregnancy as risky behavior (Fox et al. 1987), and women were asked to recall their periconceptional smoking habits several years after delivery. Reporting bias could arise if control mothers differentially underreported their smoking. This bias is unlikely because the proportion of control mothers who smoked in our study was comparable to that found in other studies (Windham et al. 1992; Fox et al. 1994). Reporting bias could also arise if case mothers who did not smoke reported that they did. Without a plausible motive such overreporting seems unlikely. Furthermore, as part of this study we also interviewed mothers who delivered children with other congenital anomalies (conotruncal heart, neural tube, or limb defects; data not shown). These other case mothers were either not or only slightly more likely than control mothers to be smokers. Thus, reporting bias seems unlikely since there is little reason to suspect that only nonsmoking mothers of cleft cases would incorrectly report that they smoked. Moreover, recall errors are unlikely to explain the increased risks that we found among infants with the uncommon TGFa genotype

whose mothers smoked. Women could not have known the genotypes of their children. Maternal reporting of paternal smoking and passive smoke exposures could also be subject to reporting bias. Women's reporting of their partners smoking in the first trimester, however, has been shown to be reasonably reliable (Hatch et al. 1991). The accuracy of self-reported passive smoke exposures is less clear because of the difficulty associated with measuring such exposures in the population.

Our results need to be interpreted in view of the response among those eligible for study, 85% of case and 78% of control mothers. If the proportion of women who smoked cigarettes (or were exposed to another's cigarette smoke) differed among the noninterviewed mothers, then the observed risk patterns might be slightly higher or lower. It is fair to assume that the proportion of smokers among interviewed control mothers was representative of the proportion of smokers among all those eligible, because the percentage of smokers in this group, 24%, was similar to what others have observed in a similar time period among other populations of pregnant women (Windham et al. 1992; Fox et al. 1994). If one further assumes for the 15% of case mothers not interviewed, that only 24% smoked, the risk estimates would still be elevated.

This study offers additional evidence that risk of orofacial clefting is influenced by the interaction between genotype (TGFa) and an exogenous factor (smoking exposures). From the standpoint of attributable risk, in light of the substantial number of women (one in four) who smoke cigarettes during pregnancy, even the modestly increased risks observed in this study are relevant to the population burden of orofacial clefts among infants.

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