
Invited Editorial

Is There a Relationship Between Risk Factors for Oral Clefts?

Oral clefts include cleft lip (CL), cleft palate (CP), and cleft lip and palate (CLP) and collectively these 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 (Mitchell et al., '02). The incidence of oral clefting varies from 0.6 to 1.7 per 1,000 Caucasian births. African Americans show a lower incidence (0.4/1,000) and Japanese have a higher frequency (1.7/1,000). Cleft lip with cleft palate comprise about 45% of all cleft cases, cleft palate only about 30%, and cleft lip only about 25% (Gorlin et al., '90).

Identification of modifiable risk factors for oral clefts is the first step toward primary prevention. A few such risk factors have been described. Maternal smoking (Kallen, '97; Chung et al., '00) and folic acid deficiency are two factors that have been associated with increased risk for oral clefts (Khoury et al., '87; Shaw et al., '95). Factoring in genetic information complicates matters further, as gene-environment interaction seems to play a role in the etiology of clefts (Hwang et al., '94; Shaw et al., '98; Romitti et al., '99).

In this issue, Vieira and Orioli present a meta-analysis of eight studies where another possible risk factor for oral clefts is described, increasing birth order. When considering isolated cleft cases only, the highest risk was seen in the "four or more" birth order category; however, risk was also increased in the second and third birth order category. Interestingly, when multiple anomaly cases were included in the analysis, these risks were even greater. As the authors point out, adjustment for maternal age was not possible. Maternal smoking status and folate levels were not included as well; birth order could be a surrogate for these factors.

Meta-analysis can be a useful tool to untangle the often disparate results from several small studies, especially when studying rare diseases such as birth defects. It is difficult for any one study to recruit enough subjects to obtain sufficient power to detect a modest association. It is also important to realize that detecting a statistical association between a risk factor and a disease does not automatically represent a biological mechanism. The association could arise from the risk factor playing a direct role in disease etiology;

an indirect association between the risk factor and another risk factor; and confounding due to population stratification (Hwang et al., '94).

In 1987, Khoury et al. ('87) found a positive relationship between smoking and cleft lip and palate (OR = 3.33; 95% CI = 1.3–8.4). Wyszynski et al. ('97) performed a meta-analysis utilizing the results from 11 studies and found an overall odds ratio of 1.29 (95% CI = 1.18–1.42) for oral clefts in children of women who smoked and calculated an attributable risk of 11%, suggesting that smoking is a general risk factor for all oral clefts. Lieff et al. ('99) found a positive dose-response relationship between CLP and smoking (light smokers: OR = 1.09, 95% CI = 0.6–1.9; moderate smokers: OR = 1.84, 95% CI = 1.2–2.9; heavy smokers: OR = 1.85, 95% CI = 1.0–3.5). In a large study, Chung et al. ('00) found an adjusted odds ratio for smokers versus nonsmokers of 1.34 (95% CI = 1.16–1.54). Honein et al. ('01) found that cigarette smoking increased the risk for several birth defects, including oral clefts.

Folic acid has been recognized as an important component of early fetal development. In 1952, Theirsch suggested an association between neural tube defects (NTDs) and low maternal folate levels (Steegers-Theunissen, '95). Case control studies (Werler et al., '93), randomized trials (MRC Vitamin Research Group, '91; Czeizel and Duda, '92), and observational studies (Milunsky et al., '89) have shown that periconceptual use of folic acid (doses ranged from 0.35 to 5 mg/d) reduces the occurrence and recurrence of NTDs from 60% to 100%. A recent meta-analysis of four clinical trials in which women were supplemented with folic acid periconceptually found a significant reduction in the incidence of NTDs (Lumley et al., '02).

Folic acid is thought to play an important role in the prevention of clefts. Tolorova and Harris ('95) found a

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65% decrease in risk of clefting among children of women who were given a multivitamin supplement (with high levels of folic acid). Itikala et al. ('01) saw a 48% reduction in risk for CLP when women were folic acid-supplemented, and Loffredo et al. ('01) found a protective effect of multivitamins against oral clefts.

While the mechanisms by which decreased levels of folic acid cause birth defects are still unknown, it has been hypothesized that homocysteine, not folic acid, is the teratogenic agent; homocysteine levels increase when folate levels decrease (Rosenquist et al., '96). Studies have shown that women with metabolic disorders of folate metabolism are at increased risk for having a child with a neural tube defect (Stegers-Theunissen et al., '95; Mills et al., '96), and Wong et al. ('99) showed that maternal hyperhomocysteinemia may also be a risk factor for oral clefts.

Several factors affect folate and homocysteine levels. Cigarette smoking is one such factor. An inverse relationship between cigarette smoking and red cell and serum folate levels has been observed (Walmsley et al., '99), and smokers have higher levels of total homocysteine and lower levels of whole blood folate than non-smokers (Christensen et al., '99; Kato et al., '99). Regular use of multivitamins increased serum folate almost four times and cut plasma homocysteine in half (Kato et al., '99). Serum total homocysteine levels increase with age (Rasmussen et al., '00; Jacques et al., '01).

Pregnancy also affects homocysteine and folate levels, such that homocysteine levels increase with each trimester of pregnancy and folate levels decrease (Walker et al., '99; Smits and Essed, '01). Additionally, folate levels continue to decrease after pregnancy (Mackey and Picciano, '99; Smits and Essed, '01). Women who supplemented with folic acid postpartum had higher folate and lower homocysteine concentration than nonsupplemented women (Mackey and Picciano, '99; Walker et al., '99).

Short interpregnancy interval (< 6 months) has been associated with negative birth outcomes such as NTD (Todoroff and Shaw, '00), congenital heart defects (Fedrick and Adelstein, '73), small-for-gestational age, low birth weight, prematurity (Zhu et al., '01), anemia, and third-trimester bleeding (Conde-Agueldo and Belizan, '00). Smits and Essed ('01) postulated that these negative outcomes may arise from depleted folate stores that have not had enough time to replenish. An estimated 6%–12% of nonfirst pregnancies are conceived within 6 months after a previous pregnancy (Smits and Essed, '01).

Gene-environment interaction may also account for some cases of oral clefts. Hwang et al. ('95) examined whether there was an association between the rarer C2 allele at the *Taq1* site in the *TGF α* gene and oral clefts. They showed a significantly increased risk of CP among infants carrying the C2 allele if the mother smoked (OR = 5.5, 95% CI = 2.1–14.6). Shaw et al. ('98) found an increased risk for having a child with a cleft lip and palate if the mother did not use multivitamins

during the periconceptual period and the infant carried the rare C2 allele at *TGF α* . Evidence of an association between allelic variants at *TGF β 3* and *MSX1* and cleft palate has been shown; this association was increased if the mother smoked or consumed alcohol during the pregnancy, suggesting the presence of gene-environment interaction (Romitti et al., '99). Beaty et al. ('02) also found some evidence for interaction between *MSX1* and smoking.

The association between birth order and oral clefts described by Vieira and Orioli is intriguing, especially when taking into consideration that other studies have found an association between birth order and other congenital anomalies. Sheiner et al. ('99) found that higher birth order was associated with major congenital malformations such as NTDs and cardiac defects. Grover ('00) found that the incidence of congenital anomalies was highest among women over 35 years of age and gravida four or more.

The question arises, are these associations caused by genetic factors, environmental factors, or both? If this association were purely genetic in nature, we would expect a random pattern of risk among siblings (given that there was not an already affected sibling), and we would not expect a trend where risk increases steadily for each subsequent pregnancy. This raises the possibility that environmental factors or gene-environment interaction could contribute to this association with birth order.

Several possibilities exist. Maternal age probably plays a role in some way. Maternal age has been identified as a risk factor on its own; Hollier et al. ('00) found a significant increase in risk for non-chromosomal congenital malformations in women over 25, and this age-related risk continues to increase every 5 years. Women who have had four pregnancies are generally older than women having their first pregnancy. Age alone could affect homocysteine levels. As women age, their levels of homocysteine rise, which increases risk for negative pregnancy outcomes. Second, among women who smoke, the cumulative effect of smoking could either increase risk to oral clefts by itself or act through decreased folate levels. Finally, it could be due to low folate levels from short interpregnancy intervals. Women with four or more pregnancies may have perpetually low folate levels, especially if the pregnancies are closely-spaced. Each of these risk factors could differentially influence risk depending on the infant's genotype. As all of these factors increase risk to birth defects other than oral clefts, it could explain the increase in risk seen among the cleft cases with multiple anomalies.

Most of the studies included in Vieira and Orioli's meta-analysis were from the 1960s and 1970s. Other subsequent studies have looked at birth order and oral clefts have been conducted. It would have been useful for the authors to include the data from these more recent studies, such as the study conducted in Shanghai (Cooper et al., '00). The authors also acknowledge the need to examine this issue further. The 2000 U.S.

Census showed that over the last 3 decades, family size has decreased; in 1970, 17% of families had four or more children and by 1990 and 2000 this dropped to 6%. This would make replicating such a study difficult in the United States.

If indeed there is a real association between birth order and risk to oral clefts, is there a way to estimate its effect for use in genetic counseling? A study conducted in 1998 by the March of Dimes revealed that only 7% of women of childbearing age knew that folic acid should be taken prior to pregnancy, 13% knew that folic acid prevents birth defects, and 32% of women were taking a folic acid supplement (CDC, '99). Knowledge about folic acid is associated with higher social class and education (Sayers et al., '97; Sen et al., '01). An estimated 25% of women aged 18 to 44 smoke and at least 14% of these women smoke during pregnancy (Siener et al. '00). Since it seems that factors such as folic acid and cigarette smoking play a role in risk to oral clefts, counseling should focus on these modifiable factors.

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