Review on Genetic Variants and Maternal Smoking in the Etiology of Oral Clefts and Other Birth Defects

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Abstract

A spectrum of adverse pregnancy outcomes, including preterm birth, low birth weight, and birth defects has been linked with maternal smoking during pregnancy. This article includes a review of studies investigating interactions between genetic variants and maternal smoking in contributing to birth defects using oral clefting as a model birth defect. The primary gene-smoking studies for other major birth defects are also summarized. Gene-environment interaction studies for birth defects are still at an early stage with several mixed results, but evolving research findings have begun to document clinically and developmentally important interactions. As samples and data become increasingly available, more effort is needed in designing innovative analytical methods to study gene-environment interactions.

Keywords

cleft lip; cleft palate; birth defects; smoking; gene-environment interaction

INTRODUCTION

Maternal smoking during pregnancy has been linked with a spectrum of adverse pregnancy outcomes including preterm birth, low birth weight, and birth defects. Among the birth defects, cleft lip and/or palate have been particularly associated with smoking. These facial defects require surgical, nutritional, dental, speech, medical, and behavioral interventions and impose a substantial economic and societal burden. Clefts are common birth defects (birth prevalence ranges from 1/500 to 1/2500, depending on geographic origins) whose etiology includes multiple genetic and environmental factors (Reviewed by Jugessur and Murray, 2005; Lidral and Moreno, 2005). The complex etiology of clefts affords opportunities to identify gene–gene or gene-environment interactions that can shed light on human embryology and its disturbances. This manuscript focuses on oral clefting as a model disease and includes a review of studies investigating interactive effects between genetic variants and maternal smoking. A brief summary of gene-smoking studies for other primary birth defects is also provided.

Oral clefts were historically classified as either those that involve the lip with or without the palate (CL/P) or those that involve the palate only (CPO) (Fraser, 1970). Clefts can also be

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divided into nonsyndromic (isolated) and syndromic forms. In isolated clefts, affected individuals have no other physical or developmental anomalies. Most studies suggest that about 70% of cases of CL/P and 50% of CPO are nonsyndromic. An environmental component to clefts was recognized when Warkany et al. (1943) associated nutritional deficiencies with cleft palate. Recognized teratogens that contribute to clefts include rare exposures, such as phenytoin and Thalidomide, and also common environmental exposures, such as maternal alcohol or cigarette use (Hayes, 2002). Increased risks from exposures can suggest metabolic pathways whose disruption may play a role in the development of CL/P. Common variables such as alcohol, smoking, or nutrition may have their effects amplified by pharmacogenetic variation. Studies of gene-environment interactions for oral clefts are still at an early stage, but evolving research findings have begun to document clinically and developmentally important interactions.

GENE-ENVIRONMENT INTERACTION

Common complex traits are presumed to arise under the joint action of genetic and environmental factors. Interaction can be defined either biologically or statistically, and the definition of interaction between two factors is dependent on the underlying model (Ottman, 1990, 1996). Interaction can be measured on an additive or a multiplicative scale with much debate on the choice of scale. Recently, Weinberg (2007) has advocated a log-complement scale for measuring interaction effects, which under some situations may have causal interpretation. Interaction on a multiplicative scale, that is, the differential effect of an exposure on disease risk among individuals with different genotypes, or the differential effect of a genotype on disease risk among individuals with different degrees of exposure, is a commonly used definition, partially due to its simplicity of statistical modeling. This manuscript refers to interaction on a multiplicative scale when mentioning testing for interaction without explicitly defining the scale. A number of study designs and analytical methods have been proposed for testing gene-environment interactive effects, including case–control (Rothman and Greenland, 1998), case-only (Piegorsch et al., 1994), and case-parent (Umbach and Weinberg, 2000) approaches, each with its own strengths and weaknesses, discussed elsewhere (Umbach and Weinberg, 1997, 2000).

It is worth noting that an interaction is different from a joint effect, where the former is a measurement of differential effects of one factor with the changing levels of another factor and the latter measures the effect when the factors are jointly at certain levels. A significant joint effect does not imply a significant interaction and vice versa. Caution needs to be practiced when using the term "interaction".

GENE-ENVIRONMENT INTERACTION ON HUMAN EMBRYONIC DEVELOPMENT

Human embryonic development is determined by both the fetal genetic properties and the intrauterine environment, which in turn depends on the maternal genetic attributes and behaviors. Susceptible variants of genes can function through multiple biological scenarios: through fetal genes to increase the susceptibility to diseases, the mother's genes to cause a less favorable environment for the developing fetus, the interaction between the environmental exposure and fetal or maternal genes, the interaction between the fetal and maternal genes, or through a combination of several scenarios. The essential message is that studying the exposure, fetal and maternal genomes is needed to better understand the pathological processes, which in turn can lead to improved prevention and treatment methods. For such environmental factors as maternal cigarette smoking, variations in the maternal and/or fetal ability to detoxify the exposed compounds may play a role in the causation of diseases. Allelic variants in genes

in the detoxification pathway are thus natural candidates for studying gene-smoking interaction effects.

DETOXIFICATION PATHWAY

Humans have complex enzymatic mechanisms to detoxify a wide array of xenobiotics absorbed by ingestion, inhalation, or surface contact. The biotransformation of xenobiotics into nonreactive compounds involves two phases. In phase I, a reactive group is added to the parental chemical compound by oxidation, reduction, hydrolysis, or dehalogenation reactions. Generally speaking, phase I reactions result in reactive molecules that may be even more toxic than their parental molecules. The cytochrome P450 gene family is the major phase I enzyme. Phase II reactions are largely considered detoxifying. During this phase, a water-soluble group is added to the reactive site of intermediate compounds by conjugation reactions including glucuronidation, sulfation, and acetyl, glutathione, or amino acid conjugation. As a result, these intermediate compounds are transformed into water-soluble forms which can be excreted. The phase II detoxification enzymes include several gene families, such as epoxide hydrolases, glutathione transferases, arylamine *N*-acetyltransferases, quinone oxidoreductases, sulfotransferases, and UDP-glycosyltransferases.

GENES OF SPECIAL INTERESTS TO GENE-SMOKING EFFECTS ON CLEFT DEVELOPMENT

AHR-ARNT Pathway

The aryl hydrocarbon receptor (AhR) is a ligand-activated transcription factor that regulates endo-, xenobiotic metabolism and cell proliferation and differentiation. One AhR ligand is 2, 3, 7, 8-tetra-chlorodibenzo-*p*-dioxin (TCDD), a teratogen that has been shown to induce cleft palate in mouse, and TCDD is found in tobacco smoke. The binding of TCDD to the Ah receptor leads to translocation of the ligand attached-AhR from cytosol to nucleus, where it interacts with the aryl hydrocarbon receptor nuclear translocator (ARNT) to form a heteromeric DNA-binding complex. The formed AhR/ARNT complexes in turn bind to the xenobiotic response element, therefore activating gene transcription. The expression of *CYP1A1*, a major phase I detoxification enzyme, is regulated by the AhR-ARNT pathway (Whitlock, 1999). AhR is also a player in the signaling pathway that regulates the expression of *TGFB* genes, major candidate genes for oral clefts, and it was hypothesized that AhR signals might interfere with palatal shelf fusion by down-regulating TGF*β*3 signals (Puga et al., 2005). Interestingly, and consistent with the hypothesis, Thomae et al. (2005) reported that the addition of TGF*β*3 to dioxin-exposed in vitro palatal culture restored palatal fusion. Mouse embryos from *Ahr* null mothers were reported to be five times more sensitive to dioxin-induced cleft palate, suggesting potential maternal effects of the *AhR* gene (Thomae et al., 2004).

Genes in Palate Development Pathways

Because of the complex interplays between genetic and environmental factors, candidate genes for oral clefts are also strong candidates for studying gene-environment interactions (Murray, 2002; Jugessur and Murray, 2005). In fact, one of the candidate genes, *TGFA*, is the most studied gene for gene-smoking interaction effects on clefting.

CIGARETTE SMOKING AND ORAL CLEFTS

The adverse effects of cigarette smoke on cleft development have been demonstrated in several animal studies, both in vitro (Kang and Svoboda, 2003; Saito et al., 2005) and in vivo (Saad et al., 1990; Seller and Bnait, 1995). Cigarette smoke exposure, along with alcohol (Romitti et al., 2007) and nutrition (Yazdy et al., 2007) are the most studied environmental factors in

the causes of oral clefts. Positive association between maternal cigarette smoking and CL/P or CPO in their offspring have been reported in several studies (Khoury et al., 1987, 1989; Beaty et al., 1997; Kallen, 1997a; Wyszynski et al., 1997; Chung et al., 2000; Wyszynski and Wu, 2002); nevertheless, this association failed to be confirmed in some other studies (Werler et al., 1990; Christensen et al., 1999; Lieff, 1999; Mitchell et al., 2001). Little et al. (2004) performed a meta-analysis of the association between maternal smoking during pregnancy and oral clefts using data from 24 case–control and cohort studies. A relative risk of 1.34 (95% CI: 1.25–1.34) was found between maternal smoking and CL/P and a relative risk of 1.22 (95% CI: 1.10–1.35) between maternal smoking and CPO. Evidence of a moderate dose-response effect for CL/P was also reported. Using data from the Swedish Medical Birth Registry, Meyer et al. (2004) found an association between smoking and CPO with a dose response. Studying data collected from National Birth Defect Prevention Study, Honein et al. (2007) reported an odds ratio (OR) of 1.3 (95% CI: 1.0–1.6) for CL/P, an even stronger association with bilateral CL/P (OR = 1.7; 95% CI: 1.2–2.6), and a weaker association for CPO. An OR of 1.50 (95% CI: 1.05–2.14) for CL/P was reported in a Danish study (Bille et al., 2007). Despite the positive association identified between maternal smoking and clefting in Denmark, the decrease in smoking prevalence among pregnant women in the last 20 years in Denmark did not seem to reduce the rate of cleft prevalence as suggested by the similar occurrence of clefts in 1988–2001 to the occurrence in 1962–1987 (Bille et al., 2005). As hypothesized by the authors, this might be due to the weak causal association between smoking and oral clefting.

EFFECTS OF GENE-SMOKING ON ORAL CLEFTS

Listed below is a review of studies investigating interaction effects of genes and maternal smoking on oral clefts categorized by gene. Table 1 lists a summary of the population, study design, sample, genes, markers and results of these studies in chronological order. All of these studies investigated the joint effects of maternal smoking and genetic variants, although some did not test formally for gene-smoking interactions.

METABOLIC GENES

Cytochrome P450 Proteins

The cytochrome P450 related enzymes are important in metabolizing a variety of endogenous and xenobiotic compounds. In humans, 18 CYP families and 43 subfamilies have been identified and 86 human CYP genes, including 27 pseudogenes, have been sequenced (Nelson, 2003). The CYP genes are highly polymorphic with most having multiple functional alleles. CYP1A1 is the most important enzyme in catalyzing smoking metabolites, such as the PAHs and B[α]P, and is induced by TCDD via the AHR-ARNT pathway. CYP1A1 protein expression and enzymatic activities were detected in the first trimester human placenta and the activities were significantly elevated in the placentas of smoking mothers (Collier et al., 2002). Compelling evidence for the presence of constitutive expression of CYP1A1 enzyme in the fetus as early as the first trimester has been shown (Hines and McCarver, 2002). Van Rooij et al. (2001) performed a study using case–control samples collected in the Netherlands and did not find any association between offspring and maternal genotypes and smoking. Shi et al. (2007) also reported a negative finding for *CYP1A1*-smoking interaction in two large populations. They also studied genetic variants in several other members of the CYP superfamily, *CYP1A2*, *CYP1B1*, and *CYP2E1*, but with no significant interactions identified for any of the CYP genes. Besides their role in detoxification, the CYP class genes may also play a role in the metabolism of endogenous morphogens in the developing fetus, creating another avenue by which variation in them might affect structural development (Stoilov et al., 2001).

Epoxide Hydrolase

Epoxide hydrolases (EPHX) are a class of proteins that catalyze the hydration of chemically reactive epoxides into their corresponding dihydrodiol products. Microsomal epoxide hydrolase (EPHX1) plays an important role in both the bio-activation and detoxification of exogenous chemicals such as PAHs, which are present in cigarette smoke. Multiple studies have reported *EPHX1* expression and activity in several developing tissues as early as 7.5 weeks gestation (reviewed by McCarver and Hines, 2002). Hartsfield et al. (2001) found no association between the fetal Y113H polymorphism in *EPHX1* with maternal smoking and oral clefting. Negative results were also reported by Ramirez et al. (2007), who performed a study based on a larger set of samples. Shi et al. (2007) however did find a significant interaction effect between the *EPHX1* Y113H variant in the fetus and maternal smoking on oral clefts in an Iowan population.

Glutathione Transferase Gene Family

Glutathione transferases (GST) are families of dimeric phase II enzymes that catalyze the conjugation of reduced glutathione with electrophilic groups of a wide variety of environmental agents. The reactions carried out by GSTs are generally considered to be detoxifying, which serve to protect cellular molecules from damage caused by cytotoxic and carcinogenic agents (Watson et al., 1998). The currently known soluble human GSTs are categorized into four main classes: alpha, mu, theta, and pi. Null genotypes (caused by deletion of all or part of the gene) have been identified in both *GSTM1* and *GSTT*. *GSTM1* is the major gene detoxifying PAHs and has been widely studied in a variety of disorders including many cancers. The frequency of the homozygous *GSTM1* deletion varies across populations and is approximately 50% in Caucasians. Joint effects of the *GSTM1* deletion polymorphism and maternal smoking on oral clefts have been investigated in four studies, two of which reported negative results (Hartsfield et al., 2001; Shi et al., 2007). Hozyasz (2005) identified an association between maternal GSTM1 deletion and oral clefts in the fetus, but no gene-smoking interaction effects were identified. Lammer et al. (2005) reported an OR of 6.8 (95% CI: 2–24) for CL/P in infants carrying the *GSTM1* null genotype and whose mothers smoked ≥20 cigarettes/day.

Two human θ-class isoenzymes, *GSTT1* and *GSTT2*, have been identified, both of which are located on chromosome 22q11.2 separated by approximately 50 kb. The theta class is highly expressed in human adult liver but not in the fetal liver. It is also expressed in a variety of tissues/organs, such as erythrocytes, lung, kidney, brain, skeletal muscles, heart, and small intestine (Landi, 2000). The wide expression of the *GSTT1* suggests that it may play a more global role than *GSTM1*. Although expression of *GSTT1* was not detected in embryonic and early fetal tissues in a study by Raijmakers et al. (2001), expression data from the COGENE project showed an elevated expression profile for *GSTT1* at the craniofacial regions during embryonic development (Shi et al., 2007). Similar to the *GSTM1* deletion, the prevalence of the *GSTT1* deletion varies across populations 15%–20% homozygous for the deletion in Caucasians. Significant joint effects of the *GSTT1* deletion and maternal cigarette smoking on oral clefts were reported in several studies. Van Rooij et al. (2001) reported increased risk of having a cleft child in *GSTT1*-null mothers who smoked during pregnancy, OR = 3.2 (95% CI: 0.9–11.6). They also reported an OR of 1.9 (95% CI: 0.5–6.6) for oral clefts in mothers who smoked and had an infant with the *GSTT1*-null genotype and an OR of 4.9 (95% CI: 0.7–36.9) in mothers who smoked where both the mother and the infant were homozygous for the *GSTT1*-deletion allele compared with nonsmoking mothers where both the mother and the infant were noncarriers of the *GSTT1* deletion. Using samples from a case–control study in California, Lammer et al. (2005) found an increased risk for CL/P in smoker mothers whose infants were carrying the GSTT1 null genotype OR = 2.9 (95% CI: 1.2–7.2). The joint effects of the GSTT1 null genotype and maternal smoking on clefting, however, were not found in a study from Poland (Hozyasz et al., 2005). Using samples collected from two populations, Shi

et al. (2007) reported significant interaction between maternal smoking and offspring GSTT1-null genotype in a Danish population (*p* value for interaction is 0.03) and an Iowan population (*p* value for interaction is 0.002). In the combined samples, the joint effects of GSTT1 and maternal smoking were reported to be OR = 2.23 (95% CI: 1.20–3.71) for all cleft cases, and the effects were most elevated for CPO with an OR of 4.25 (95% CI: 1.51–7.33). Interestingly, Shaw et al. (2003) detected an elevated relative risk, also for CPO, in infants with the GSTT1 null genotype and whose mothers were exposed to certain occupational chemicals.

GSTP1 is another major enzyme involved in the inactivation of cigarette smoke metabolites. It is widely expressed in normal human epithelial tissue and is particularly abundant in the lung, esophagus, and placenta, and it is reported to be the most important *GST* isoform at the embryonic and early fetal developmental stages (Raijmakers et al., 2001). Elevated expression of *GSTP1* was detected in both embryos at 8-weeks gestation (Robertson et al., 1986; Raijmakers et al., 2001) and fetuses at 12-week of gestation (Raijmakers et al., 2001). Overexpression of *GSTP1* in a variety of tumors has been reported. Mice with deleted *Gst-π* gene cluster (*Gst-π1*, and *Gst-π2*), when treated with PAH 7,12-dimethylbenz anthracene and 12-*o*-tetracannoylphorbol-13-acetate, showed a highly significant increased rate of papillomas (Henderson et al., 1998). This suggests that variable *GSTP1* expression may be an important determinant of susceptibility to environmental chemicals. Significant interaction between offspring *GSTP1* and maternal smoking for CL/P was reported by Shi et al. (2007), but this interaction effect was not found by Ramirez et al. (2007). Shi et al. (2007) also studied two other members of the GST family, *GSTA4* and *GSTM3*, and reported significant interaction between offspring *GSTA4* and maternal smoking for CPO in a Danish population.

Hypoxia-Induced Factor-1

One mechanism by which maternal cigarette smoking may affect embryonic development is through hypoxia, possibly caused by the production of carbon monoxide which interferes with oxygen transfer to the placenta, or nicotine which constricts the uterine wall resulting in hypoxia (Philipp et al., 1984). Experiments in mice also supported the involvement of hypoxia in cleft lip (CL) pathogenesis, in that hypoxia increases the rate of CL in mouse strains with a spontaneous rate of CL while hyperoxia decreases the rate (Millicovsky and Johnston, 1981a, b). HIF1A, a member of the bHLH/PAS family, is a master enzyme involved in the cellular responses to hypoxia. HIF1A regulates the expression of genes involved in energy metabolism, angiogenesis, and apoptosis. *Hif1a* knockout mouse embryos die in mid-gestation, showing cardiovascular malformations and open neural tube defects (Iyer et al., 1998). Cross-talk between the HIF1A/ARNT and AHR/ARTN pathways has also been hypothesized (Puga et al., 2005). Shi et al. (2007) reported significant interaction between fetal HIF1A and maternal smoking in an Iowan population but no association with AHR.

Arylamine *N*-Acetyltransferase Gene Family

Aromatic amines such as 4-aminobiphenyl and heterocyclic amines such as PhIP are present in cigarette smoke. N-conjugation of arylamine by the action of *N*-acetyltransferases (NATs), UDP-glucuronosyltransferases (UGTs), or sulfotransferases (SULTs) produces nontoxic compounds. In humans, two arylamine *N*-acetyltransferases, *NAT1* and *NAT2*, and a pseudogene, *NATP1*, have been identified which are located at chromosome 8p23.1-8p21.3. NAT2 has a limited distribution found in the liver and epithelial cells of the intestine, while *NAT1* is expressed in many tissues, including erythrocytes, bladder, lymphocytes, neural tissues, liver, and intestines. Expression of *NAT1* was found as early as blastocyst stage and in human placenta at 5.5 gestational weeks (Sim et al., 2000; Smelt et al., 2000). Acetylation activity in humans varies dramatically across different populations, which may be attributable to the fact that both *NAT1* and *NAT2* are highly polymorphic with more than 25 alleles identified in each. Several investigations of the joint effects of *NAT* genes and oral clefts have been

conducted. Van Rooij et al. (2002) studied the potential association between maternal NAT2 acetylator status and births with oral clefts as well as the possible interaction between maternal NAT2 and maternal smoking in clefting. No significant effects for either maternal acetylator status alone or its interactions with smoking were detected. Lammer et al. (2004) in a California population-based case–control study did not find independent associations for either *NAT1* or *NAT2* variants with maternal smoking in clefting. They did find an OR of 3.9 (95% CI: 1.1–17.2) for CL/P among mothers who smoked and whose infants had the *NAT1* 1088 genotype AA versus nonsmoking mothers whose infants had the TT genotype, and OR = 4.2 (95% CI: 1.2–18.0) for smoking mothers with infants of NAT1 1095 genotype AA versus nonsmoking mothers with infants of CC genotypes. No significant joint effects of NAT2 and smoking were reported by Lammer et al. (2004). Significant interaction effects were nonetheless reported by Shi et al. (2007) between a maternal *NAT2* variant and maternal smoking in a Danish population.

Methylenetetrahydrofolate Reductase

Methylenetetrahydrofolate reductase (*MTHFR*) plays a key role in the metabolism of folate by reducing methylenetrahydrofolate to 5-methyltetrahydrofolate, the primary methyl donor for methionine synthesis. The C677T nucleotide variant in the *MTHFR* gene results in an amino acid variation encoding a thermally unstable protein with reduced activity resulting in elevated plasma homocysteine levels. Considerable heterogeneity in the prevalence of the C677T polymorphism throughout the world has been reported (Pepe et al., 1998; Botto and Yang, 2000). Joint effects of *MTHFR* C677T variant and maternal smoking have been studied in cleft cases from a US population (Beaty et al., 2002) and a Norwegian population (Jugessur et al., 2003), with negative results in both studies.

Other Metabolic Genes

Shi et al. (2007) also studied several other detoxification genes, including: NAD(P)H quinone oxidoreductase (NQO1), a flavoenzyme that catalyzes two-electron reduction of quinone compounds to hydroquinone and is inducible by oxidative stress, dioxin, and PAHs found in cigarette smoke; SULT1A1 which catalyzes the transfer of the sulfonate group from the active sulfate to a substrate to form the respective sulfate or sulfamate ester; and UDP-glycosyltransferases (UGTs) UGT1A7, an enzyme that catalyzes the conjugation reactions where hydrophobic chemicals are transformed into water-soluble compounds. They did not detect effects for either *NQO1* or *SULT1A1*. Both maternal genetic effect and maternal gene-smoking interaction effect were seen for variants in *UGT1A7* but in a different direction. Interestingly, Collier et al. (2002) reported a significant elevation of UGT1A activity in the first trimester placenta of mothers who smoked (Collier et al., 2002), lending support for potential maternal effects of *UGT1A7* on embryonic development. Potential interaction between a fetal variant in UGT1A7 and maternal smoking was also reported (Shi and Christensen et al., 2007). Shaw et al. (2005a, b) studied endothelial nitric oxide synthase (NOS3), which regulates nitric oxide production and may be involved in folate catabolism. They reported an OR of 1.6 (95% CI: 1.0–2.6) for CL/P in smoking mothers with *NOS3* A (−922)G homozygote infants. Increased risk for CL/P was observed in smoking mothers who did not use vitamins and whose infants had at least one variant allele (for SNP A(−922)G, OR = 4.6, 95% CI: 2.1–10.2; for SNP 894T, OR = 4.4, 95% CI: 1.8–10.7). Test for *NOS3*-smoking interaction was not significant.

DEVELOPMENTAL GENES FOR ORAL CLEFTS

Transforming Growth Factor *α* (TGFA)

TGFA is a trans-membrane protein that is expressed at the medial edge epithelium (MEE) of fusing palatal shelves. Its receptor, epidermal growth factor receptor (*EGFR*), is strongly

expressed in the degenerating MEE. Even though *Tgfa* null mice do not exhibit craniofacial anomalies, mice with *Egfr* deleted are born with open-eyes and facial medio-lateral defects including high incidence of cleft palate (Miettinen et al., 1999). Ardinger et al. (1989) first associated the TaqI polymorphism in *TGFA* with CL/P in a case–control study. Many more studies on *TGFA* have been performed since then, but with conflicting results (reviewed by Vieira, 2006). *TGFA* is the most extensively studied gene for gene-environment interaction effects on oral clefts. Significant interaction between *TGFA* TaqI variant and maternal smoking was first reported by Hwang et al. (1995) by analyzing data from a case–control study in Maryland, US. An increased risk for CPO (OR = 7.02, 95% CI: 1.78–27.6) was observed in smoking mothers with infants carrying the *TGFA* TaqI C2/C2 genotype. Analyzing data from a case-parent study, Maestri et al. (1997) also reported a significant *TGFA*-smoking interaction. Even though the test for interaction was not significant in a California case–control study, elevated risk for *TGFA* TaqI C2-allele carrier in smoking mothers was observed, with OR = 6.1 (95% CI: 1.1–36.1) for CL/P and OR = 9 (95% CI: 1.4–61.9) for CPO (Shaw et al., 1996). Negative results were also reported in several studies (Beaty et al., 1997; Christensen et al., 1999; Romitti et al., 1999; Beaty et al., 2002; Jugessur et al., 2003). Zeiger et al. (2005) performed a meta-analysis and reported an increased risk (OR = 1.95, 95% CI: 1.22–3.10) for CPO in smoker mothers if the infant carried the TaqI C2 allele.

Transforming Growth Factor *β* 3

The TGF *β* family consists of over 30 ligands, regulating a wide variety of biological processes including proliferation, differentiation, epithelium-mesenchymal transformation, and apoptosis. Expression of one of the family members, transforming growth factor *β* 3 (*TGFB3*), is detected in the epithelial component of the vertical palatal shelf and peaks at day 14 to 14.5, just before immediate contact of the palatal shelves. Potential roles of TGF*β*3 in palate formation have been demonstrated both in vitro (Sun and Vanderburg et al., 1998; Gato et al., 2002; Cui et al., 2003) and in vivo (Cui et al., 2003). Using a case-parent study design, Maestri el al. (1997) identified significant interaction between maternal smoking and a *TGFB3* variant in the offspring (*p* value for interaction = 0.04). An elevated risk for CPO in mothers who smoked >10 cigarettes/day and whose infants were *TGFB3* variant allele carriers was reported by Romitti (1999). Studies by Mitchell (2001) and Beaty (2002) identified only genetic main effects for TGF*β*3 but no interaction effects.

Muscle Segment Homeobox 1

Muscle segment homeobox 1 (MSX1) is a transcriptional repressor which is important in craniofacial, limb, and nervous system development. In mice, *Msx1* is expressed in the palatal mesenchyme confined to the anterior portion of the developing palatal shelves (Alappat et al., 2003) and all *Msx1* homozygous −/− mice manifest cleft secondary palate, where the paired palatal shelves elevate normally but fail to make contact and fuse, a phenotype mimicking cleft palate in human. Potential involvement of *MSX1* in the etiology of clefting has been demonstrated in several sequencing (van den Boogaard et al., 2000; Alappat et al., 2003; Jezewski et al., 2003) and association studies (Lidral et al., 1997; Jumlongras et al., 2001; Vieira et al., 2003; Schultz et al., 2004). Mitchell et al. (2001) studied variants in MSX1 and maternal smoking using a Danish case–control study and did not find genetic main or interaction effects. However, an elevated risk for CPO was observed in mothers who smoked >10 cigarettes/day and whose infants were *MSX1* variant carriers in an Iowan population based case–control study (Romitti et al., 1999) and significant interaction (*p* value for interaction = 0.028) was reported in a Maryland case-parent study (Beaty et al., 2002).

Other Developmental Genes

Gene-smoking interaction effects have been investigated for some other candidate genes, such as retinoic acid receptor (Maestri et al., 1997), and the proto-oncogene (BCL3) (Maestri et al., 1997; Beaty et al., 2002), all with negative results.

EFFECTS OF GENE-SMOKING ON BIRTH DEFECTS OTHER THAN ORAL CLEFTING

Several studies have evaluated the role of maternal smoking on other birth defects besides oral clefting. While some consensus has emerged for some birth defects, the results are rather mixed for others, likely due to different designs, sample sizes, and different extents of confounding bias. Listed below is a summary of the results for the other most common birth defects.

Congenital Heart Disease

A few studies have evaluated the effects of smoking on Congenital heart disease (CHD) but without strong evidence that smoking has a large risk effect on CHD. A study using Swedish registry data found a small effect of smoking (OR = 1.09; 95% CI: 1.01–1.19) on CHD, but larger effects were observed for specific types including atrial septal defects and persistent ductus arteriosus (Kallen, 1999a). Using a population based case–control sample from California, a larger effect was observed for conotruncal heart defects with both parents being smokers compared to none (OR = 1.9; 95% CI: 1.2–3.1), but maternal smoking had no significant effect by itself (Wasserman et al., 1996).

There have been few studies of interactions between smoking and selected genetic variants but some suggestive interactions have been reported. Larger joint risk effects of maternal smoking and the null type of *NOS3* A(−922)G (OR = 1.9; 95% CI: 1.1–3.4) and glu298asp (OR = 2.2; 95% CI: 1.2–4.0) on conotruncal heart defects were observed using the California sample (Shaw et al., 2005a, b). Using a case–control sample from Arkansas, smoking had a moderate effect on CHD (1.7 OR; 95% CI: 0.95–3.14), significant joint effects between smoking, and high homocysteine levels (OR = 11.8; 95% CI: 2.59–53.3) were observed relative to nonsmoking and low homocysteine for the subgroup with the CC genotype of *MTHFR* 677C>T (Hobbs et al., 2006).

Neural Tube Defects

The few studies that have evaluated the effects of smoking on Neural tube defects (NTDs) suggest no or minimal adverse risk effect on NTDs. Using a Swedish registry sample, Kallen (1998) reported a protective effect of smoking on NTDs (OR = 0.75; 95% CI: 0.61–0.91), but no effects of smoking were observed with the California sample (Wasserman and Shaw et al., 1996). Interaction between smoking and fetal *NAT1* C1095A variant has been reported using a family-based study with larger risk effects of smoking on spina bifida in infants carrying the 1095A allele (Jensen et al., 2005). One recent study (Suarez et al., 2007) did find effects of maternal smoking on NTD risk in a Mexican-American population.

Limb Defects

An increased risk of terminal transverse limb deficiency (OR = 1.48; 95% CI: 0.98–2.23) was reported with maternal smoking using a case–control sample from a Hungarian birth registry (Czeizel et al., 1994). Maternal smoking had no significant effects on limb deficiency using the California sample but intensive paternal smoking (≥20 cigarettes/day) doubled the risk (OR = 2.1; 95% CI: 1.3–3.6) (Wasserman et al., 1996). Using the Swedish registry sample, maternal smoking was reported to increase the risk (OR = 1.26; 95% CI: 1.06–1.50) (Kallen, 1997b). In a recent study grouping polydactyly, syndactyly, and adactyly, maternal smoking was

reported to increase the birth defect risk (OR = 1.31; 95% CI: 1.18–1.45) though this grouping of limb defects has been criticized for its heterogeneity (Kirby, 2006).

Significant joint effects of active maternal smoking with homozygote variant allele of *NAT1* 1095 (OR = 7.5; 95% CI: 1.2–45.3), *NAT1* 1088 (OR = 16.9; 95% CI: 1.7–166.2), and null-type *GSTT1* (OR = 7.0; 95% CI: 1.2–40.1) in fetus on limb deficiency defects relative to nonsmoking and wild-type alleles have been reported using the California registry case–control sample (Carmichael et al., 2006a, b). Significant joint effects of secondhand smoking and *NAT1* 1088 (OR = 3.4; 95% CI: 1.0–11.9), heterozygote *NOS3* A(−922)G (OR = 2.7; 95% CI: 1.0–7.2), and heterozygote *NOS3* G894T (OR = 2.7; 95% CI: 1.0–7.2) were also reported. Using the same sample, Carmichael et al. (2006a, b) reported significant joint effects between maternal smoking and a *β*-2 adrenergic receptor (*ADRB2*) variant (OR = 1.9; 95% CI: 1.0–3.8). In the same study, they also reported significant joint effects between smoking, lack of vitamin use and variants in several genes, including serpin peptidase inhibitor (*SERPINE1* 11053G>T) (OR = 3.0; 95% CI: 1.0–8.8), integrin, *α* 2 (*ITGA2* 873G>A) (OR = 3.5; 95% CI: 1.0–12.0), G protein *β* 3 subunit (*GNB3* 825C>T) (OR = 3.3; 95% CI: 1.0–11.3), and *ADRB2* Arg16Gly (OR = 3.9; 95% CI: 1.2–12.6). These variants are implicated in blood pressure. No interaction between smoking and *MSX1* was found using the same sample (Carmichael et al., 2004).

Gastroschisis

Gastroschisis is of particular interest for environmental exposures as it is associated with both low maternal age and has been increasing in birth prevalence over the last two decades (Alvarez and Burd, 2007). There is no consensus of a large risk effect of maternal smoking on gastroschisis. A few case–control studies have showed an increased risk for smoking. Using a sample from Washington State, Goldbaum et al. (1990) reported a significant doubled risk with smoking (OR = 2.0; 95% CI: 1.03–3.8). A similar but statistically insignificant risk estimate was reported using a case–control sample from Maine (Haddow et al., 1993) (OR = 2.1; 95% CI: 0.9–4.8).[1] However, no significant effect of maternal smoking was observed using a sample from the California birth defects registry (Torfs et al., 1994). Werler et al. (1992) also reported no effects of smoking in a case–control study that used as controls infants with other malformations.[2]

Significant joint effects between smoking and variants in *NOS3* glu298asp (OR = 5.2; 95% CI: 2.4–11.4), intracellular adhesion molecule 1 (*ICAM1* gly24larg) (OR = 5.2; 95% CI: 2.1–12.7), and atrial natriuretic peptide (*NPPA* 2238 T > C) (OR = 6.4; 95% CI: 2.8–14.6) were reported using the California sample (Torfs et al., 2006). The *ICAM1* variant was related to peripheral arterial disease and the NPPA variant was related to cardiac hypertrophy. Significant joint effects between BMI and intensive smoking (defined by smoking more than one pack per day or smoking marijuana more than once within 3 months before pregnancy) have also been reported (OR = 26.5; 95% CI: 7.9–89.4) relative to mothers without either risk factor (Lam and Torfs, 2006).

Other Birth Defects

Smoking has been reported to increase the risk of craniosynostosis in three case–control studies using samples from Colorado (Alderman et al., 1994), Sweden (Kallen, 1999b) (OR = 1.67; 95% CI: 1.27–2.18 for isolated forms), and Atlanta (Honein and Rasmussen, 2000) (OR = 1.92; 95% CI: 1.01–3.66 for isolated forms). A dose response effect was suggested but not confirmed

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[1]This study involved 22 cases and about 58, 559 controls.
[2]Using a birth defect sample as a control is problematic given that smoking may contribute to these birth defects as well, and so finding no effects of smoking in this design is not sufficient to suggest that smoking has no effect on gastroschisis.

in these studies which had also mixed findings for the types of craniosynostosis that are likely to be mostly affected by smoking (sagittal versus coronal). No effects of smoking was reported in a case–control study involving samples from the Baltimore-Washington area, including sagittal craniocynostosis cases (Zeiger and et al., 2002). There have been no studies of gene-smoking interactions for craniocynostosis.

Maternal smoking has been reported to slightly increase the overall risk (OR = 1.15; 95% CI: 1.02–1.29) of having multiple malformations using the Swedish registry data (Kallen, 2000). This risk increased with intensive smoking (OR = 1.34; 95% CI: 1.13–1.58 for smoking ≥10 cigarettes per day). Smoking has not been shown to increase the risk of Down Syndrome (Chen et al., 1999), but an increased risk has been reported for heart defects (OR = 2.0; 95% CI: 1.2–3.2) among births with Down Syndrome, particularly for atrioventricular canal, tetralogy of Fallot, and atrial septal defects (Torfs and Christianson, 1999).

DISCUSSION

Investigations into gene-smoking interactions in birth defects have produced some insightful and interesting results; however, failure of replication and conflicting results are still major unresolved issues. Many of these studies, both positive and negative, are limited by a small sample size; consequently, comprehensive investigations of environment and gene-environment interactive effects with a larger sample size are needed. Multi-institutional collaborations such as the International Clearing-house for Birth Defects Surveillance and Research (Botto et al., 2006), ECLAMC (Castilla and Orioli, 2004), and CDC sponsored Birth Defects Prevention Study (Yoon and Rasmussen, 2001) have already been successful in providing large, epidemiologic datasets for analysis, and in some settings have added DNA samples as well. Comprehensive collaborations between institutions and investigators can provide the large sample sizes required by studies of gene-environment interactions. The recently initiated Gene Association Information Network (GAIN) and Genes, Environment, and Health Initiative (GEI) are steps in the direction of promoting such collaborations that can also include genome wide association studies that can move past candidate gene analysis to search for common variants contributing to disease with no constricting, underlying models (Christensen and Murray, 2007). GAIN is a public–private partnership established to investigate the genetics of complex disease through a series of whole genome association studies, using samples from existing case–control studies (Manolio et al., 2007). GEI is a unique collaboration between geneticists and environmental scientists, which will take advantage of the innovative genetic technology as well as new instrument for measuring environmental factors to understand the genetic contributions and gene-environment interactions in common diseases. They provide new models of collaboration, data sharing, and intellectual protection. Such studies will also require accurate phenotyping and subphenotyping to maximize power, as has been demonstrated by several cleft studies (Rahimov et al., 2007; Suzuki et al., 2007).

In addition to adequate sample size, assessment of gene-environment interaction effects also depends upon the accurate and detailed measurement of exposures and the proper statistical evaluation. New methods of environmental variable measures that can be noninvasive and longitudinal will enhance detection efforts (Schwartz and Collins, 2007). One important limitation that is common to previous studies is self-selection on smoking based in part on risk preferences and anticipated pregnancy outcomes which might result in biased estimates of the effects of smoking. Genetic instrumental variable models are being applied to address self-selection when estimating the average and interactive effects on smoking (Wehby et al., 2007). These present a promising approach that can also be adopted for studying interactions between smoking and genetic factors.

Case–control is the traditional design for investigating gene-environment interactions. Interactions on either an additive or a multiplicative scale can be estimated using such a design; this design is however susceptible to population stratification. Case-only design is more powerful in detecting interaction on a multiplicative scale, but it cannot test for exposure main effects and its validity heavily relies on the assumption that gene and environment are independent in the control population. Case-parent design has the robustness to population stratification, but similar to case-only design it forfeits the ability to detect exposure main effects and it can only study interaction on a multiplicative scale. A hybrid design that can combine the advantages of the case–control and case-parent designs is desired. As samples and data become more available, the challenges will shift more towards identifying more efficient and robust statistical and analytical methods and approaches to interpret the results.

Interactions between genes, environments, and behaviors will continue to be a growing field to identify health risk factors that can be modified through policy or behavioral interventions. Studying the role of interactions with maternal smoking in adverse birth outcomes such as birth defects is one area with substantial successes and that can benefit significantly from further work.

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TABLE 1

Literature Review of Studies on Gene-Smoking Interaction (GxS) in Oral Clefts

Year	First author	Population	Study design	Phenotype, sample size	Gene	Marker	Results
1995	Hwang	US (MD)	case-control	114 CL/P; 69 CPO; 284 controls	TGFA	Taq1	Fetal GxS
1997	Maestri	US (MD, DC)	case-parents	31 CL; 79 CLP; 50 CPO	TGFA	Taq1	Fetal GxS
					TGFA	D2S443	Fetal GxS
					TGFB3	D14S61	Fetal GxS
					RARA	4 markers	No GxS
					BCL3	THR1	No GxS
1996	Shaw	US (CA, white population)	case-control	191 CL/P; 87 CPO; 379 controls	TGFA	Taq1	No GxS; Some significant joint effects with fetal genes
1997	Beaty	US (MD)	case-control	114 CL/P; 69 CPO; 284 controls	TGFA	Taq1	No GxS
1999	Christensen	Denmark	case-control	192 CL/P; 65 CPO; 457 controls	TGFA	Taq1	No GxS
1999	Romitti	US (IA)	case-control	90-118 CL/P; 41-51 CPO; 166-338 controls	TGFA	Taq1	Some significant joint effects with fetal TGFB3 or MSX1
					TGFB3	CA, X5.1, 5;UTR.1	
					MSX1	CA, X1.1, X1.3, X2.1, X2.4	
2001	Hartsfield	US (CA)	case-control	85 CL/P; 110 controls; 79 CL/P, 51 controls	EPHX1	Y113H	No GxS
2001	van Rooij	Netherlands	case-control (child + mother)	89 CL/P; 24 CPO; 105 controls	GSTM1	null	No GxS
					GSTT1	deletion	No GxS
2001	Mitchell	Denmark	case-control	174-198 CL/P; 56-68 CPO; 420-473 controls	CYP1A1	1 SNP	No GxS
					MSX1	CA, N8	No GxS
2002	Beaty	US (MD)	triad	52 CL; 134 CLP; 83 CPO	TGFB3	X5.1, CA	No GxS
					TGFA	D2S443	No GxS
					TGFB3	D14S61	No GxS
					MSX1	CA	Fetal GxS
					BCL3	BCL3	No GxS
					MTHFR	C677T	No GxS
2002	van Rooij	Netherlands	case mother + control mother	45 mothers with cleft offspring; 73 controls mothers	NAT2	urinary AAMU/1X ratio (acetylator status)	No fetal GxS (offspring genotype)
2003	Jugessur	Norway	case triad	173 CL/P; 88 CPO	TGFA	Taq1	No fetal GxS
2004	Lammer	US (CA)	case-control	309 CL/P; 128 CPO; 299 controls	MTHFR	C677T	No GxS; Some significant joint effects with fetal gene
					NAT1	1088, 1095	No GxS
2005	Hozyasz	Poland	case mother -control mother	121 case; 80controls	NAT2	857, 481, 590	No GxS
					GSTM1	null	No GxS
2005	Lammer	US (CA)	case-control	297 CL/P; 125 CPO; 299 controls	GSTT1	null	No GxS; significant joint effects
					GSTT1	null	No GxS; significant joint effects
					GSTM1	null	No GxS; significant joint effects
2005	Shaw	US (CA)	case-control	244 CL/P; 99 CPO; 588 controls	NOS3	A(−922)G, G894T	No GxS; significant joint effects
2005	Zeiger		case-control	5 studies	TGFA		Significant joint effects and GxS for CPO
2007	Shi	US (IA); Denmark; Denmark	case triad-control triad; case triad; case-control	260 CL/P; 110 CPO; 419 controls; 357 CL/P; 101 CPO; 202 CL/P; 68 CPO; 485 controls	AhR	2 SNPs	No GxS
					CYP1A1	T3801C	No GxS
					CYP1A2	−164 C->A intron 1	No GxS
					CYP1B1	V432L, N453S	No GxS
					CYP2E1	1SNP	No GxS
					EPHX1	Y113H, H139R	Significant fetal GxS
					GSTA4	2 SNPs	Significant fetal GxS for Danish CPO
					GSTM1	null, 1 SNP	No GxS
					GSTM3	1 SNP	No GxS

Year	First author	Population	Study design	Phenotype, sample size	Gene	Marker	Results
					GSTP1	A313G, C341T, G->T	Significant fetal GxS for Danish CL/P
					GSTT1	null	Significant fetal GxS
					HIF1A	A->C	Significant fetal GxS in Iowan sample
					NAT2	C481T, G590A	Significant fetal GxS in Danish sample
					SULT1A1	R213H	No GxS
					UGT1A7	T387G, C391A, G392A, T662C	Significant maternal and fetal GxS
2007	Ramirez	US (CA)	case-control	305 CL/P; 126 CPO; 299 controls	EPHX1	Y113H, H139R	No GxS
					GSTP1	I105V, A114V	No GxS

CL/P: cleft lip with/without cleft palate, CPO: cleft palate only.