HHS Public Access

Author manuscript

Birth Defects Res. Author manuscript; available in PMC 2024 November 15.

Published in final edited form as:

Birth Defects Res. 2023 November 15; 115(19): 1835–1850. doi:10.1002/bdr2.2226.

Epigenetic implications in maternal diabetes and metabolic syndrome-associated risk of orofacial clefts

Bo Sun[1,2,#], **Kurt S. Reynolds**[1,2,#], **Michael A. Garland**[1,2], **Moira McMahon**[1,2], **Subbroto K. Saha**[1,2], **Chengji J. Zhou**[1,2,*]

[1]Department of Biochemistry and Molecular Medicine, University of California at Davis, School of Medicine, Sacramento, CA 95817.

[2]Institute for Pediatric Regenerative Medicine of Shriners Hospitals for Children, University of California at Davis, School of Medicine, Sacramento, CA 95817.

Abstract

Orofacial clefts (OFCs) are one of the most common birth defects. The etiologies are complicated, with genetic, epigenetic, and environmental factors involved. Studies have found that maternal diabetes and metabolic syndrome are associated with a higher risk of OFCs in offspring. Metabolic syndrome is a clustering of several disease risk factors, including hyperglycemia, dyslipidemia, obesity, and hypertension. Metabolic disease during pregnancy can increase risk of adverse outcomes and significantly influence fetal development, including orofacial formation and fusion. An altered metabolic state may contribute to developmental disorders or congenital defects such as OFCs, potentially through epigenetic modulations, such as histone modulation, DNA methylation, and non-coding RNAs to alter activities of critical morphogenetic signaling or related developmental genes. This review summarizes the currently available evidence and underlying mechanisms of how the maternal metabolic syndrome is associated with OFCs in mostly human and some animal studies. It may provide a better understanding of the interactions between intrauterine metabolic status and fetal orofacial development which might be applied towards prevention and treatments of OFCs.

Keywords

orofacial clefts; maternal metabolic syndrome; diabetes; obesity; dyslipidemia; hypertension; epigenetics

Introduction

Orofacial clefts (OFCs) impact about 1 in 700 newborns globally (S. E. Watkins, Meyer, Strauss, & Aylsworth, 2014). The common types of OFCs are cleft lip with or without

*Corresponding author: cjzhou@ucdavis.edu.
#Equal contributions.

CONFLICT OF INTEREST STATEMENT
The authors declare no conflict of interest.

DATA SHARING AND DATA ACCESSIBILITY
Data sharing is not applicable to this article as no new data were created or analyzed in this study.

cleft palate (CL/P) and cleft palate only (CPO), and affected children experience significant difficulties in feeding, speech, and social integration. Although plastic and maxillofacial surgery, speech therapy, and psychosocial intervention are available, OFCs are associated with significant long-term health, life-style wellbeing, and socio-economic burdens for patients and their families (Dixon, Marazita, Beaty, & Murray, 2011). Facial primordia become evident by the fourth week of gestation in humans and embryonic day 9.5 in mice (Tarr, Lambi, Bradley, Barbe, & Popoff, 2018). It is a complicated and highly integrated process involving a precise series of morphological changes driven by extensive cell proliferation, differentiation, migration, and apoptosis (Y. Ji et al., 2020). Disruption of any of these processes may result in OFCs which may occur alone (nonsyndromic) or associated with other structural birth defects (syndromic OFCs) (Arias Urueña, Briceño Balcazar, Martinez Lozano, Collins, & Uricoechea Patiño, 2015; Mossey & Modell, 2012; Tolarová & Cervenka, 1998).

OFCs have complex and multifactorial etiology including genetic and epigenetic factors and can be influenced by gene-environment interactions (Garland, Reynolds, & Zhou, 2020; Garland, Sun, et al., 2020; Iwaya, Suzuki, & Iwata, 2023; Reynolds et al., 2020). Genome-wide association studies have identified loci (e.g. 8q24) or candidate genes (e.g., *CREBBP*, *DICER1*, *FGFR1*, *GRHL3*, *IRF6*, *MSX1*, *MYC*, *NOG*, *PTCH1*, *SPRY2*, *TFAP2A*, *VAX1*, *WNT9B*, and others) whose polymorphisms are associated with OFCs (Butali et al., 2018; Leslie et al., 2016; Mangold et al., 2010; Yu et al., 2017). Both human and animal studies demonstrate that environmental factors (e.g., folate deficiency, maternal diabetes, and smoking) increase the incidence of OFCs (reviews in (Garland, Reynolds, et al., 2020; Mossey & Modell, 2012; Waller et al., 2007)); for example, carriers of certain polymorphisms in transforming growth factor alpha (*TGFA*), whose mothers smoked during gestation, had significantly higher risks of developing cleft palate (Beaty et al., 1997; Hwang et al., 1995). Another example is that maternal supplementation with folic acid or vitamin A may reduce the occurrence rate of OFCs (Gildestad et al., 2015; Johansen, Lie, Wilcox, Andersen, & Drevon, 2008; Y. Zhou et al., 2020). Maternal health conditions, such as diabetes and metabolic syndrome, also contribute to OFCs. Metabolic syndrome is a clustering of several disease risk factors, including hyperglycemia, obesity, dyslipidemia, and hypertension (Huang, 2009). The purpose of this review is to explore the currently available evidence on how maternal metabolic syndrome is associated with OFCs and potential underlying epigenetic mechanisms. This may provide a better understanding of the interactions between intrauterine environment and orofacial development, which could help contribute to their future prevention.

Epigenetic mechanisms underlie orofacial clefts and related birth defects associated with maternal metabolic disorders

Birth defects may occur after fertilization due to teratogenic factors in the environment instead of an inherited genetic cause (Carstens, 2004; Cederberg, Picard, & Eriksson, 2003). A major link between the environment and the genome is the epigenome, such as DNA methylation and histone modification, which is influenced by the environment and regulates chromatin structure and genome functions without changing the nucleotide sequences (Messerschmidt, Knowles, & Solter, 2014; Xu, Li, Liu, & Gao, 2021). Two

critical periods for epigenetic reprogramming in mammalian embryos occur shortly after fertilization and during the formation of the primordial germ cells (Kobayashi et al., 2013; Smith et al., 2012). Epigenetic reprogramming profoundly affects cell lineage and differentiation during embryonic development. Both human and animal studies have confirmed that epigenetic alterations contribute to OFCs. Rogers and colleagues found that administration of the DNA demethylating agent 5-aza-2'-deoxycytidine during pregnancy increased occurrence of cleft palate in mice (Rogers et al., 1994). The critical window for this effect is between embryonic days 11 and 14, a crucial period for palatal development in mice (Bulut, Ozdemir, Başimoglu-Koca, Korkmaz, & Atalay, 1999). Further, Kuriyama and colleagues confirmed that maternal exposure to all-trans retinoic acid, a teratogen known to induce cleft palate in mice, is associated with profound DNA methylation changes within CpG islands and globally reduced RNA expression on embryonic day 14.5 (Kuriyama et al., 2008). In addition to DNA methylation, microRNAs are also important effectors in regulating many key signaling mediators (e.g., transforming growth factor beta [TGFβ], bone morphogenic protein [BMP] and Wnt ligands) during orofacial development (Iwaya et al., 2023; Nakamura, Inloes, Katagiri, & Kobayashi, 2011; Seelan, Pisano, & Greene, 2022; Tomé et al., 2011; Yao et al., 2011). For example, microRNA-335 (miR-335) is known to regulate proliferation in human mesenchymal stem cells through the canonical Wnt signaling pathway, while *Dnpep*, as the target of miR-140, regulates BMP signaling to influence bone morphology and craniofacial development (Nakamura et al., 2011; Tomé et al., 2011). Another study identified 17 microRNAs as potential modifiers of human cleft palate-associated genes; for example, miR-133b overexpression downregulated target cleft palate genes, including *FGFR1*, *GCH1*, *PAX7*, *SMC2*, and *SUMO1*, and resulted in reduction of cell proliferation in human palatal mesenchymal cell cultures (Suzuki et al., 2019).

The prevalence of maternal metabolic syndrome is increased due to high-calorie/high-sugar diets and sedentary lifestyle. It has become one of the greatest health concerns for pregnant women since it increases the risk of congenital anomalies (e.g., cardiovascular, neural tube, and craniofacial defects) and gestational complications (e.g., preeclampsia, gestational diabetes, and preterm delivery) (Grieger et al., 2018; Krakowiak et al., 2012; Mossey & Modell, 2012). Studies have found that altered metabolic status affects epigenetic regulation and consequently influences fetal development as well as the offspring's health in their adult life (review in (Lesseur & Chen, 2018)). For example, maternal obesity was associated with altered histone methylation during embryonic development and histone H3 Lysine 4 dimethylation (H3K4me2) was reduced in the embryos of obese mice (Pan et al., 2020). Epigenetic studies using human blood samples have demonstrated that both maternal diabetes and obesity can reprogram the DNA methylome of newborns or offspring beyond birth (Alba-Linares et al., 2023; Rizzo et al., 2020). Thus, while maternal metabolic disorders can negatively influence the fetal and offspring development acting through epigenetic alterations, it may be preventable or even reversible. Therefore, it is critical to understand the interactions between maternal risk factors and susceptible genes for specific types of congenital disorders. The associations and related mechanisms between specific metabolic disorders and orofacial clefts are discussed further in the following sections.

Maternal diabetes mellitus and orofacial clefts

Pregestational or preexisting diabetes mellitus (PGDM) contributes to an increased incidence and worsening of gestational glucose intolerance, which leads to the developing fetus being exposed to maternal hyperglycemic and/or hyperinsulin conditions *in utero* (Chung & Myrianthopoulos, 1975). Excessive glucose metabolism consumes more oxygen, exacerbating a hypoxic state which contributes to increased mitochondrial super-oxide production (R. Li, Chase, Jung, Smith, & Loeken, 2005). This hyperglycemia-induced hypoxia contributes to congenital abnormalities. For example, hypoxia in the developing fetus changes the DNA methylation status at *Pax3*, which plays a critical role in neural crest development, and may have detrimental effects neural tube closure and craniofacial development (Chang et al., 2003; R. Li et al., 2005; Wei & Loeken, 2014). A 2001 study found that infants of mothers with either PGDM or GDM had an increased prevalence of many different congenital malformations including OFCs. However, PGDM had an increased association with infants showing multiple defects, which was not observed in infants of mothers with gestational diabetes mellitus (GDM) (Aberg, Westbom, & Källén, 2001).

GDM is defined as a glucose tolerance disorder during pregnancy and that is initially diagnosed in the second or third trimester of pregnancy by a 75g oral glucose tolerance test (ADA, 2020; Schäfer-Graf et al., 2018). A population-based, nationwide analysis showed the overall prevalence of GDM in the USA is 13.2% (Melchior, Kurch-Bek, & Mund, 2017). GDM is associated with an increased rate of gestational complications (e.g., pre-eclampsia and C-section) and women with GDM showed significantly higher risks of developing type 2 diabetes in the years after the first diagnosis (Bellamy, Casas, Hingorani, & Williams, 2009; Rayanagoudar et al., 2016). GDM increases the risk of adverse effects on the heath of newborns, including macrosomia, shoulder dystocia, and hypoglycemia (Crowther et al., 2005; Landon et al., 2009; Metzger et al., 2008). Infants born to mothers with either GDM or PGDM have higher risks of having congenital anomalies, most frequently involving cardiovascular defects (Aberg et al., 2001; Moore, Singer, Bradlee, Rothman, & Milunsky, 2000; Spilson, Kim, & Chung, 2001). A recent meta-analysis of population-based studies confirmed that the relative risks (RRs) of overall congenital anomalies in offspring of women with PGDM were higher than those in offspring of women with GDM (T. N. Zhang et al., 2022).

While some early investigations had a difficult time directly connecting maternal diabetes with OFCs specifically (Correa et al., 2008b; Martinez-Frias et al., 2005; Stott-Miller, Heike, Kratz, & Starr, 2010), several more recent larger population-based association studies have shown an increased prevalence of OFCs in both babies born to mothers with PGDM and to mothers with GDM (Table 1). A report of live births in Canada found an increased overall prevalence of OFCs in infants born to mothers with PGDM with an odds ratio for type 1 DM of 2.48, and for type 2 DM of 2.77 (Liu et al., 2015). Analysis of USA births documented in the National Vital Statistics System found increased relative risk for both CL/P ($p<0.001$) and CPO ($p<0.001$) in babies born to mothers with PGDM. Mothers with GDM had an increased relative risk for babies with CL/P ($p<0.001$), and both PGDM and GDM showed increased association with all cleft subtypes combined ($p<0.0001$; $p<0.0001$) (Wu

et al., 2020). Multiple reports from the National Birth Defect Prevention Study (NBDPS) also reported an increased prevalence of all types of clefts in infants born in the USA between 1997 and 2011 to mothers with PGDM and mothers with GDM (Correa et al., 2008a; Marchincin et al., 2023; Tinker et al., 2020). Another study specifically examining singletons born in upstate New York between 2004 and 2016 found an increased OFC risk to mothers with PGDM ($p<0.001$), but not GDM (Yang, Reece, Wang, & Gabbay-Benziv, 2015).

Studies in animal models suggest that maternal diabetes contributes to a teratogenic effect that results in craniofacial malformations. A hyperglycemic environment in diabetic rat pregnancies contributes to altered metabolism of inositol and prostaglandins, and increased presence of reactive oxygen species, all of which are likely contributing mechanisms (Eriksson et al., 2000). These changes particularly affect the development of neural crest-derived organs, especially the mandible and Meckel's cartilage, causing a DiGeorge-like phenotype (Cederberg et al., 2003; Simán et al., 2000). DiGeorge Syndrome is a 22q11 deletion disorder which commonly includes micrognathia or retrognathia. This affects growth of maxillary structures, often resulting in OFCs (McDonald-McGinn et al., 2015). While clefts are most commonly observed in isolation without other defects, they often present as part of a broader syndrome. Given that altered metabolic states can affect many different craniofacial structures, it is possible that some OFC cases in diabetic pregnancy may be attributable to other primary defects resulting from altered maternal environment which affect development of the lip or palate resulting in a cleft as a secondary effect.

Maternal environment and metabolic status during pregnancy contribute to the offspring's epigenetic profile, and embryonic exposure to maternal hyperglycemia may modify the chromatin, particularly since glucose metabolism regulates histone acetylation through the citrate lyase pathway. AATP citrate lyase (ACLY) converts glucose-derived citrate into acetyl-CoA which is necessary for histone acetylation (Wellen et al., 2009). ACLY dysregulation contributes to the etiology of type 2 diabetes, and *Acly* knockdown improves glucose tolerance in a diabetes mouse model (Q. Wang et al., 2009). Another study examined the deteriorated intrauterine environment in an intrauterine growth retardation rat model and found that the expression of *Pdx1* was reduced due to the lower levels of histone acetylation markers. These altered epigenetic marks and reduced *Pdx1* expression had adverse effects on fetal development, including pancreatic islets, lasting into adulthood with diabetes (Park, Stoffers, Nicholls, & Simmons, 2008). Overall, these studies indicate that the diabetic intrauterine environment could alter gene expression through influencing epigenetic modulators.

Wnt signaling is required to regulate patterning processes during craniofacial development by modulating cell proliferation, migration, and apoptosis, and mutations in more than 20 Wnt signaling genes are associated with OFCs in humans (see review summary in (Reynolds et al., 2019)). Wnt signaling is traditionally classified into the canonical and non-canonical pathways, the latter of which is further divided into the planar cell polarity and the Wnt/Ca^{2+} pathways (Gao & Chen, 2010; Komiya & Habas, 2008). Diabetes can cause dysregulation of Wnt signaling pathways which may contribute to the etiology of defects that present in infants born of mothers with PGDM or GDM. Interestingly, one study

found that maternal diabetes-induced oxidative stress could lead to the impairment of the non-canonical Wnt5a-Ca^{2+} pathway (F. Wang, Fisher, Zhong, Wu, & Yang, 2015). Further, maternal diabetes induced the overexpression of Wnt antagonists *Sfrp1* and *Dkk1*, which consequently suppressed canonical Wnt signaling by decreasing Dvl2 phosphorylation and increasing Gsk3β activity (F. Wang et al., 2015). Another study showed that maternal diabetes increases the risk of cleft palate and caudal regression induced by the vitamin A metabolite retinoic acid in mouse embryos, with diminished *Wnt3a* expression as a contributing mechanism (Chan et al., 2002). Further evidence for the role of epigenetic modifications in the presentation of congenital defects is the fact that genetically identical individuals often show variable penetrance of phenotypes. Variability of gene expression results from the combined changes in transcription factors and chromatin structure, as well as histone modification regulators (e.g., *Ehmt2* and *Kat2a*) in diabetic pregnancy (Aberg et al., 2001; Lin et al., 2008; Wagschal et al., 2008). Differential DNA methylation may also contribute to the penetrance of OFCs, which is understudied in GDM and PGDM settings.

Maternal obesity and orofacial clefts

The consumption of highly processed food coupled with overall low physical activity both contribute to the increasing prevalence of obesity in women of reproductive age. Between 2011 and 2012, 31.8% of women aged 20–39 years were obese in the USA while the proportion was less than 10% in the 1970s (Ogden, Carroll, Kit, & Flegal, 2014; K. Rasmussen, 2012). Maternal obesity is strongly associated with pregnancy complications such as maternal diabetes, pre-eclampsia, and early pregnancy loss, as well as with stillbirth and congenital anomalies (review in (Poston et al., 2016)). Maternal obesity significantly increases the occurrence of neural tube defects, cardiovascular, orofacial, and limb anomalies (Mikhail, Walker, & Mittendorf, 2002; Moore et al., 2000; Ray, Wyatt, Vermeulen, Meier, & Cole, 2005).

Differences in sample size, different criteria for obesity, ethnicity, socioeconomic and geographical differences may contribute to variability in the reported association between maternal obesity and OFCs. While several early studies into the association between obesity and OFCs reported no association (Shaw, Todoroff, Schaffer, & Selvin, 2000; M. L. Watkins, Rasmussen, Honein, Botto, & Moore, 2003), a number of more recent larger meta-analyses have shown an increased prevalence of cleft presentation in infants born to obese or overweight mothers (Table 2). One meta-analysis of 2 studies from the USA and one from Sweden showed a significant association between obesity in mothers with a BMI above 30 and cleft lip and palate (CLP) (p=0.02) as well as CPO (p=0.02) in infants (Stothard, Tennant, Bell, & Rankin, 2009). Another meta-analysis of data from USA patients including the two aforementioned studies with one other found an odds ratio for CL/P of 1.16 (CI = 1.0–1.34) and for CPO of 1.14 (CI = 0.95–1.37) in obese mothers. A study including data from 8 groups in the USA, Australia, and Sweden found an increased odds ratio in obese mothers (BMI > 30) for either CL/P (OR = 1.13/CI = 1.04–1.23) or CPO (OR = 1.22/CI = 1.09–1.35) (Blanco, Colombo, & Suazo, 2015). Another more recent analysis of 6 cohorts from Northern Europe and the USA found the risk of any form of cleft palate increased in babies with overweight mothers, and increase correlated with the degree of obesity. Mothers with a BMI between 30 and 35 showed an odds ratio of 1.09 (CI = 0.95–

1.25) for a baby born with cleft palate, but mothers with a BMI over 35 had an odds ratio 1.36 (CI = 1.16–1.58) (Kutbi et al., 2017).

Additional differences in the linkage between obesity and cleft risk might also depend on whether the studies analyzed specific cleft types. In addition to CPO vs cleft lip forms, the difference in etiologies between CLO *versus* CLP as distinct forms of CL/P should also be considered. Studies have found different distributions and patterns of the risks of recurrence between CLO and CL/P or CPO (Harville, Wilcox, Lie, Vindenes, & Abyholm, 2005; Kutbi et al., 2017; Stoll, Alembik, Dott, & Roth, 2000; Tolarová & Cervenka, 1998). Specifically, there are a few distribution characteristics that separates CL/P from CLO in which 1) CL/P more frequently co-exists with other congenital defects compared with CLO (Harville et al., 2005; Stoll et al., 2000; Tolarová & Cervenka, 1998); 2) males have higher risks of developing CL/P than CLO, while females have higher frequencies of developing CPO, and twins have CLO more often than CL/P (Harville et al., 2005; Mossey & Modell, 2012; Shapira, Lubit, Kuftinec, & Borell, 1999). Different cleft forms similarly show differential association with maternal weight as well. For example, Villamor et al. (2008) conducted a large population-based cohort study in Sweden and found that higher maternal weight gain and longer interpregnancy intervals positively were associated with the occurrence of CPO, but not CL/P (Villamor, Sparén, & Cnattingius, 2008). Besides maternal weight gain, another study found that maternal obesity is associated with a mild increase in risk of CPO, while underweight maternity may be associated with CL/P (Waller et al., 2007). A meta-analysis concluded that maternal obesity is significantly associated with increased risk of CPO and CL/P, but not associated with cleft lip only (CLO) (Stothard et al., 2009).

The precise mechanisms underlying obesity-associated OFCs remain unclear but undiagnosed type 2 diabetes, hyperlipidemia, or maternal dietary changes in conjunction with maternal obesity could contribute. Maternal obesity is also associated with epigenetic changes. Histone H3 Lysine 4 dimethylation (H3K4me2) was reduced in the embryos of obese mice (Pan et al., 2020), which may alter signaling gene activities critical for orofacial development. A more recent animal study demonstrates that maternal high-fat diet changes DNA methylation in early embryos by disrupting the TCA cycle intermediary α-ketoglutarate, which is necessary for DNA demethylation by TET enzymes (Penn, McPherson, Fullston, Arman, & Zander-Fox, 2023). These studies suggest that maternal obesity may alter DNA and histone methylation at OFC-associated genes, and provide new directions for related mechanistic studies.

Maternal dyslipidemia and orofacial clefts

During pregnancy, maternal physical adaptations such as fat accumulation and hormonal changes are necessary for fetal development. The accumulation of fat during the first two-thirds of gestation are mainly attributed to hyperphagia and increased lipid synthesis (Murphy & Abrams, 1993; Palacín, Lasunción, Asunción, & Herrera, 1991). The levels of blood triglycerides are markedly increased (100–200%) during pregnancy, while a moderate increase (40–50%) in cholesterol levels is also observed (Basaran, 2009; Palacín et al., 1991). The presence of lipoprotein receptors in the placenta allows uptake of maternal polyunsaturated fatty acids and release into the fetal plasma (Herrera, Amusquivar,

López-Soldado, & Ortega, 2006). Maternal non-esterified fatty acids and cholesterol are also able to be transferred through the placenta and be used by the fetus. In the early stage of gestation, maternal cholesterol and cholesterol synthesis is the major source for fetal cholesterol which will further be utilized for cell proliferation, synthesis of steroid hormones, and fetal growth (Herrera et al., 2006).

Dyslipidemia is characterized by elevated levels of triglycerides and total blood cholesterol, including increased low-density lipoprotein (LDL) and reduced high-density lipoprotein (HDL) (NCEP, 2002). Studies have found that maternal obesity and the western dietary pattern, which is rich in fat and poor in fruits, are significantly associated with prevalence of CL/P (Blanco et al., 2015; Vujkovic et al., 2007). Zhou et. al. reported that a high fat maternal diet significantly increased the prevalence and severity of triamcinolone-induced cleft palate in mice (M. Zhou & Walker, 1993). One mechanism by which a maternal diet affects development is through alterations in the epigenome, and high-fat diet contributes to alterations in DNA methylation, histone methylation, and non-coding RNA expression (C. C. Li et al., 2013; Vucetic, Kimmel, Totoki, Hollenbeck, & Reyes, 2010). Another study using human palatal mesenchyme cells found that microRNAs are significantly involved in regulating cleft palate-associated genes, and KEGG pathway analysis showed several of these target genes (e.g., *DHCR7*, *DHCR24* and *PAFAH1B1*) are enriched in cholesterol and steroid metabolic processes (Suzuki et al., 2019). Maternal variants in genes associated with lipid metabolism, including *DHCR7*, which functions in cholesterol biosynthesis, contribute to Smith-Lemli-Opitz syndrome (SLOS) in which the affected infants sometimes present with CL/P. Further, variants in two other genes involved in lipid transport or uptake, *ABCA1* and *APOE*, contribute to the severity of defects observed in SLOS patients (Lanthaler, Steichen-Gersdorf, Kollerits, Zschocke, & Witsch-Baumgartner, 2013). Lipid metabolism defects are associated with reduced cell proliferation during mouse palatogenesis. *Tgfbr2* mutant mouse embryos showed cleft palate in which the ablation of *Tgfbr2* reduced *Adcy2* and *Pde4b* expression and consequently led to the accumulation of lipid droplets and a reduction in proliferation of palatal mesenchymal cells (Iwata et al., 2014). Lipid droplet accumulation in palatal tissue also occurs in RA-induced cleft palate models, and mass spectrometry showed that the lipid droplet content is altered as well, with increased triacylglycerides but reduced fatty acids and ceramides (W. Zhang et al., 2020).

Maternal hypertension and orofacial clefts

Hypertension (blood pressure > 140 mmHg systolic or > 90 mmHg diastolic) in pregnancy includes chronic hypertension, gestational hypertension, pre-eclampsia and eclampsia, which are observed in 5 to 10% of all pregnancies (Hutcheon, Lisonkova, & Joseph, 2011; Lo, Mission, & Caughey, 2013). Chronic hypertension is characterized as hypertension diagnosed before pregnancy or before 20 weeks of gestation; up to 1.5% of pregnancies are complicated with chronic hypertension (Croke, 2019; NHBPEP, 2000). Gestational hypertension is initially diagnosed during pregnancy after 20 weeks of gestation and returns to normal within 12 weeks postpartum in the absence of proteinuria (urinary excretion of ≥ 300mg of protein in 24 h. Pre-eclampsia is defined as a systemic syndrome originating in the placenta with hypertension and proteinuria during pregnancy (NHBPEP, 2000). Different from the chronic hypertension, the insufficient placental cytotrophoblast invasion is thought

to be mainly attributed to pre-eclampsia, which usually accompanies maternal endothelial dysfunction, placental ischemia, and hypoxia; consequently, this causes a reduced blood supply for the developing fetus (L. Ji et al., 2013; Sircar, Thadhani, & Karumanchi, 2015). A subset of pre-eclamptic pregnancies result in eclampsia, characterized by convulsions and possibly coma during pregnancy or in the immediate postpartum period, frequently accompanied by blurred vision, nausea, and headaches (Wallace, Harris, & Bean, 2019).

Maternal hypertension is associated with increased risk of adverse outcomes for the mother, fetus, or infant. For example, pre-eclampsia and placental insufficiency are associated with mental disorders such as autism spectrum disorder, attention-deficit-hyperactivity-disorder, and schizophrenia (Dachew, Mamun, Maravilla, & Alati, 2018; Dachew, Scott, Betts, Mamun, & Alati, 2020; Dachew, Scott, Mamun, & Alati, 2019; Maher et al., 2018). Maternal pre-eclampsia specifically has been shown to positively correlate with fetal growth restriction (S. Rasmussen & Irgens, 2003).

Several studies have demonstrated an association between maternal-related hypertension and OFCs. Uruena *et. al.* found a positive association between maternal hypertension and syndromic forms of OFCs, and suggested potential mechanisms may include mutations of essential genes with roles in both involved in angiogenesis and palatogenesis (Arias Urueña et al., 2015). One study of births in California showed relative risk of CL/P and CPO in births to mothers with pre-existing hypertension (1.74 and 1.15, respectively), gestational hypertension (1.07 and 1.14), mild pre-eclampsia (1.52 and 0.96), and severe pre-eclampsia (1.18 and 1.44), and found a more drastically increased risk for mothers with pre-existing hypertension with pre-eclampsia (1.68 and 1.95). This study also examined the joint effects of diabetic and hypertensive disorders, and showed a relative risk of 1.99 for CL/P and of 1.94 for CPO in mothers with any hypertensive disorder and any diabetic disorder (Weber et al., 2018). A study of Chinese live births did not find any increased risk of clefts with gestational hypertension, but showed that pre-eclampsia generated an increased relative risk for all types of nonsyndromic OFC of 2.02 (CI = 1.27 – 3.20) (An et al., 2022). Another analysis of birth defect data from 29 countries showed an adjusted odds ratio of OFC defects with maternal chronic hypertension of 4.2 (CI = 1.5–11.6), while in mothers with chronic hypertension in addition to pre-eclampsia, the adjusted odds ratio was increased to 8.2 (CI = 2.0–34.3) (Bellizzi et al., 2016). While pregnancy-related hypertensive disorders are typically diagnosed around gestational week 20, there is evidence to suggest that associated pathological changes may occur earlier. The pathogenic process of pre-eclampsia begins in the first trimester, well prior to clinical presentation (Gathiram and Moodley, 2016). Although the etiology of pre-eclampsia and eclampsia are incompletely understood, improper trophoblast differentiation and placental development could affect the formation of facial structures before significantly altering blood pressure or protein content of urine.

Although lacking direct study, maternal hypertension disorder may influence fetal palatal development through epigenomic modulations. Studies have found that the maternal preeclampsia is associated with altered methylation at the *IGF2* and *HSD11B2* loci in fetal cord blood, two genes which are essential for regulating embryonic development and metabolism, respectively (Ching et al., 2015; He et al., 2013; Hu et al., 2014).

A meta-analysis study including 10 cohort studies further validated that preeclampsia alone is associated with epigenome profile alterations in offspring cord blood (Kazmi et al., 2019). Another study of a US cohort of patients with maternal hypertension found significant association with alterations in DNA methylation at 24 genomic sites in placenta samples (Workalemahu et al., 2020). A follow up study specifically looking at methylation changes in specific cell types was able to replicate significance with 5 of the original 24 sites (Broséus et al., 2022), further strengthening the evidence that maternal hypertensive disorders can affect gene expression through epigenomic alterations, as a possible mechanism for congenital defects.

Conclusions

While genomic variants play a key role in the development of craniofacial abnormalities, increasing evidence in recent years has strengthened the association between OFCs and environmental factors, including maternal risk factors. One important contribution is maternal metabolic disorders, including diabetes (both pre-existing diabetes and GDM), obesity, dyslipidemia, and hypertension, which are frequently co-morbid and exacerbated by each other. With increased accumulation of clinical data along with advancing research technologies, we are becoming better able to determine not only the contributions of maternal metabolic disorders to OFCs and related birth defects, but also specific underlying mechanisms, mainly epigenetic alterations that may disrupt critical target genes (Figure 1). As our understanding of the epigenetic and metabolic contribution of uterine environment on embryonic development advances, our ability to use that knowledge for the betterment of outcomes will continue to improve. A better understanding of the role of maternal metabolism in influencing orofacial development will be helpful for the prevention of not just OFCs, but diverse congenital disorders that are influenced by external factors.

Acknowledgments

This work is supported by grants from the NIH (R01DE026737 & R01NS102261 to C.J.Z.) and the Shriners Hospitals for Children (71039 to B.S., and 85105 to C.J.Z.). We are grateful to the rest of Zhou lab members for their general supports during the manuscript preparation. We apologize to colleagues whose important work we were unable to cite due to space constraints or inadvertently overlooking.

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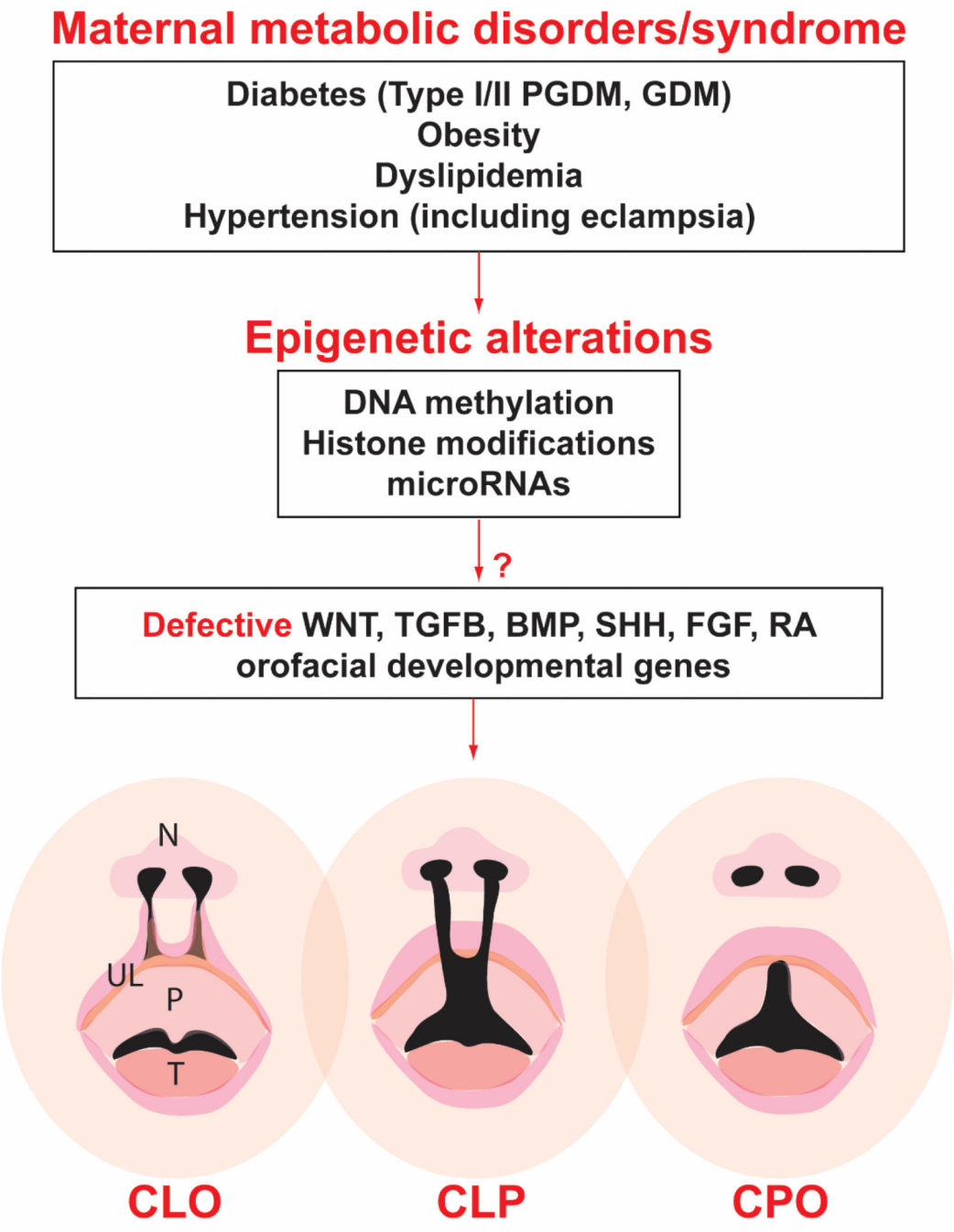

Figure 1. Epigenetic mechanisms implicated in orofacial clefts associated with maternal metabolic disorders.

Maternal metabolic disorders or syndromes can alter fetal epigenome, including DNA methylation, histone modifications, and microRNA productions which may further affect expression activities of critical signaling genes, such as Wnt and other morphogenetic signaling pathways that are required for orofacial development, leading to orofacial clefts. CLO, cleft lip only; CLP, cleft lip with cleft palate; CPO, cleft palate only; GDM, gestational diabetes; N, nose; P, palate; PGDM, pregestational diabetes; T, tongue; UL,

upper lip. Cleft lip (CLO or CLP) can be unilateral or bilateral, only the latter is shown in the diagram.

Table 1.

Representative association studies between maternal diabetes and orofacial clefts

Study Description	Subject Population and Years	Maternal Condition	OFC Number total included in study (prevalence); RR/OR (95% CI)	OFC Subtype total included in study (prevalence); RR/OR (95% CI)	Reference
Population-based study of association between **PGDM** and **GDM** and birth defects in mother-infant pairs from birth registry data	29,211,974 live births with maternal age ranging from 18–49 years old documented in the National Vital Statistics System of **USA, 2011–2018**	**PGDM** 242,600 mothers (0.83%)	412 (0.170%); **RR** [1]**2.31** (2.10–2.55), P<0.0001	**CL/P** 273 (0.113%); **RR 2.06** (1.82–2.33) P<0.001 **CPO** 139 (0.057%); **RR 2.35** (1.97–2.97), P<0.001	Wu et al 2020
		GDM 1,685,479 mothers (6.18%)	1,688 (0.100%); **RR 1.36** (1.30–1.43), P<0.0001	**CL/P** 1,145 (0.068%); **RR 1.28** (1.20–1.36), P<0.001 **CPO** 543 (0.032%); **RR 1.40** (1.28–1.53)	
Analysis of association between **PGDM** and **GDM** and birth defects using case-control data from National Birth Defects Prevention Study (**NBDPS**)	13,030 infants with and 4,895 without birth defects in **USA, 1997–2003**	**PGDM** All birth defects group: 1.7% of mothers	33 OFC cases	**CL/P** **Isolated** 14; **OR 2.92** (1.45–5.87) **Syndromic** 8; **OR 8.07** (3.05–21.39) **CPO** **Isolated** 5; **OR 1.8** (0.67–4.87) **Syndromic** 6; **OR 10.73** (3.99–28.86)	Correa et al 2008
		GDM All birth defects group: 4.7% of mothers	96 OFC cases	**CL/P** **Isolated** 54; **OR 1.45** (1.03–2.04) **Syndromic** 8; **OR 1.22** (0.52–2.86) **CPO** **Isolated** 29; **OR 1.54** (1.01–2.37) **Syndromic** 5; **OR 1.26 (0.50–3.20)**	
Expanded analysis of **NBDPS** data	31,007 infants with and 11,477 without birth defects in **USA, 1997–2011**	**PGDM** All birth defects group: 775 mothers (2.5%), Control group: 71 mothers (0.6%)	58 **CL/P** cases; **OR 3.0** (2.1–4.3) 42 **CPO** cases; **OR 4.3** (2.9–6.5)	**CL/P** **Isolated** 37; **OR 2.2** (1.4–3.3) **Syndromic** 21; **OR 8.7** (5.0–15.0) **CPO** **Isolated** 19; **OR 2.5** (1.4–4.2) **Syndromic** 23; **OR 12.3** (7.3–20.7)	Tinker et al 2020
		GDM All birth defects group: 1,653 mothers (5.3%), Control group: 536 mothers (4.7%)	161 **CL/P** cases; **OR 1.1** (0.9–1.3) 96 **CPO** cases; **OR 1.4** (1.1–1.8)	**CL/P** **Isolated** 143; **OR 1.1** (0.9–1.4) **Syndromic** 18; **OR 1.0** (0.6–1.7) **CPO** **Isolated** 79; **OR 1.4** (1.1–1.8) **Syndromic** 17; **OR 1.4** (0.9–2.4)	
Analysis of **NBDPS** data for association between **PGDM** and birth defects	29,024 infants with and 10,898 infants	**PGDM Type 1** All birth defects group: 252	27 OFC cases	**CLP** 10; **OR 2.3** (1.1–4.7) **CLO** 2[3]	Marchincin et al 2022

Study Description	Subject Population and Years	Maternal Condition	OFC Number total included in study (prevalence); RR/OR (95% CI)	OFC Subtype total included in study (prevalence); RR/OR (95% CI)	Reference
stratified by **type 1 and type 2 DM**	without birth defects in **USA, 1997–2011**[2]	mothers (0.9%); Control group: 24 mothers (0.2%)		**CPO** 15; **OR 4.1** (2.1–7.8)	
		PGDM Type 2 All birth defects group: 357 mothers (1.2%); Control group: 34 mothers (0.3%)	55 OFC cases	**CLP** 23; **OR 4.1** (2.3–7.1) **CLO** 13; **OR 4.8** (2.4–9.3) **CPO** 19; **OR 4.4** (2.4–7.8)	
Population-based study of association between **PGDM** and **GDM** and birth defects in mother-infant pairs from birth registry data	650,914 singletons born in **Upstate New York, 2004–2016**	**PGDM**: 4,134 mothers (0.6%)	12 OFC cases (0.290%); **OR 2.9** (1.6–5.2), P<0.001	**CL/P** 7 (0.169%); **OR 2.5** (1.2–5.3), P<0.05 **CPO** 5 (0.121%); **OR 3.8** (1.6–9.3), P<0.01	Yang et al 2019
		GDM: 32,605 mothers (5.0%)	32 OFC cases (0.098%); **OR 1.0** (0.7–1.4), P>0.05	**CL/P** 22 (0.067%); **OR 1.0** (0.6–1.5), P>0.05 **CPO** 10 (0.031%); **OR 1.0** (0.5–1.8), P>0.05	
Population-based study of birth defects assessing **PGDM** attributable risk percentage	2,839,680 live births in **Canada** (except Quebec), **2002–2013**	**PGDM Type 1** 0.27% (2002) – 0.28% (2012) of mothers	0.36% OFC prevalence; **OR 2.48** (1.70–3.63)	(No OFC subtype available)	Liu et al. 2015
		PGDM Type 2 0.19% (2002) – 0.47% (2012) of mothers	0.39% OFC prevalence; **OR 2.77** (2.02–3.80)		
Population-based study of association between **PGDM** and **GDM** and birth defects in mother-infant pairs from birth registry data	1,216,198 births in **Sweden, 1987–1997**	**PGDM** 3,864 mothers (0.32%)	19 OFC cases (0.492%); **OR 2.33** (1.49–3.67)	(No OFC subtype available) Isolated 11 (0.285%) Syndromic 8 (0.207%)	Aberg et al 2001
		GDM 8,688 mothers (0.72%)	18 OFC cases (0.207%); **OR 0.98** (0.62–1.56)	Isolated 14 (0.161%) Syndromic 4 (0.046%)	

[1] RR adjusted for maternal age, race/ethnicity, education levels, marital status, parity, smoking before and during pregnancy, timing of initiation of prenatal care, pregnancy BMI, infant sex, and pregestational hypertension.

[2] Mothers were excluded if they had GDM, if their first DM diagnosis was during any pregnancy, if they had an unknown DM type or diagnosis date, or if they had a type 1 PGDM diagnosis but did not use insulin during pregnancy.

[3] OR not calculated if n<3

Table 2.

Meta-analyses of association studies between maternal obesity and orofacial clefts

Study Description	Pregestational maternal condition BMI Characterization	OFC subtype OR (95% CI)	References
Analysis of 6 pooled case-control studies (1987–2008) from Northern Europe and USA for association between maternal weight and orofacial clefts	**Obese I** BMI: ≥ 30, < 35	**All cleft palate; OR 1.09** (0.95–1.25)	Kutbi et al 2017
	Obese II & III BMI: > 35	**All cleft palate; OR 1.36** (1.16–1.58);	
	Overweight BMI: 25–29.9	**All cleft palate; OR 1.02** (0.92–1.13)	
	Normal BMI: 18.5–24.9	N/A	
	Underweight BMI: <18.5	**CL/P; OR 1.16** (0.98–1.36)	
Meta-analysis of 8 studies (5 from USA, 1 from Australia, and 2 from Sweden) to assess increased orofacial cleft risk with maternal obesity	**Obese** BMI: ≥ 30 (except one cohort study > 29)	**CL/P; OR 1.13** (1.04–1.23) **CPO; OR 1.22** (1.09–1.35)	Meta-analysis: Blanco et al 2015 Selected studies: Watkins 2003 Cedergren 2005 Honein 2007 Waller 2007 Oddy 2009 Blomberg 2010 Stott-Millier 2010 Carmichael 2012
	Normal BMI: 18.5–24.9 (except one cohort study 19.8–26)	N/A	
Review and meta-analysis of 3 studies from USA for association between maternal weight and orofacial clefts	**Obese** BMI: ≥ 30	**CL/P; OR 1.16** (1.0–1.34) **CPO; OR 1.14** (0.95–1.37)	Meta-analysis: Izedonmwen et al 2015 Selected studies: Watkins 2003 Waller 2007 Stott-Millier 2010
	Overweight BMI: 25–29.9	**CL/P; OR 1.06** (0.93–1.21)	
	Normal BMI: 18.5–24.9	N/A	
Meta-analysis of several studies for association between maternal obesity and congenital anomalies	**Obese** BMI: ≥ 30 or > 29	**CLP; OR 1.20** (1.03–1.40), P=0.02 **CPO; OR 1.23** (1.03–1.47), P=0.02	Meta-analysis: Stothard et al 2009 Selected studies: Watkins 2003 Cedergren & Kallen 2005 Waller 2007
	Normal BMI: 18.5–24.9 or 19.8–26	N/A	