Published in final edited form as:

Birth Defects Res. 2019 July 01; 111(11): 649–658. doi:10.1002/bdr2.1502.

Evaluation of Prenatal Diabetes Mellitus and Other Risk Factors for Craniofacial Microsomia.

Babette Siebold^{1,2}, Carrie L. Heike^{1,2,3}, Brian G. Leroux⁴, Matthew L. Speltz^{1,2,3}, Amelia F. Drake⁵, Alexis L. Johns⁶, Kathleen A. Kapp-Simon^{7,8}, Leanne Magee⁹, and Daniela V. Luquetti^{1,2,3}

¹Seattle Children's Research Institute, Seattle, WA

²Seattle Children's Hospital, Seattle, WA

³University of Washington School of Medicine, Seattle, WA

⁴University of Washington School of Dentistry, Seattle, WA

⁵University of North Carolina, Chapel Hill, NC

⁶Children's Hospital Los Angeles, Los Angeles, CA

⁷Shriners Hospital for Children, Chicago, IL

⁸University of Illinois at Chicago, Chicago, IL

⁹Children's Hospital of Philadelphia, Philadelphia, PA

Abstract

Background: Craniofacial microsomia (CFM) is a congenital condition that typically involves hypoplasia of the ear and jaw. It is often associated with adverse effects such as hearing loss and sleep-disordered breathing. There is little research on its etiology.

Methods: We conducted a case-control study from maternal interview data collected from mothers of infants with and without CFM. The study included 108 children with and 84 children without CFM. Logistic regression with adjustment for demographic factors was used to evaluate associations between maternal exposures of interest and risk for CFM overall, as well as for different phenotypic sub-groups of children on the CFM spectrum.

Corresponding Author: Daniela V. Luquetti, MD, PhD, Assistant Professor, 1900 9th avenue, Seattle, WA, 98115, luquetti@uw.edu, Phone: 206-884-5120.

The Craniofacial microsomia: Longitudinal Outcomes in Children pre-Kindergarten (CLOCK) study team includes the following members: **Seattle Children's Hospital** (coordination center): Carrie Heike, MD, MS; Matthew Speltz, PhD; Craig Birgfeld, MD; Brent Collett, PhD; Daniela Luquetti, MD, PhD; Sara Kinter, MA, CCC-SLP; Susan Norton, PhD; Jessica Mendoza; Amber Sand, BA; Kathleen Sie, MD; Babette Siebold, PhD; Laura Stueckle, MPH; Erin Wallace, PhD; Alison Paolozzi, BA, CSP; Erik Stuhaug, AAS; Sharman Conner, MA; Linda Peters, BA; Kristen Daniels, MLIS; Jessica Berg, MS; Molly Wright; Suzie Park; Jessica Kahler; Cindy Ola, BA; Tristen Nash; **University of Washington:** Brian Leroux, PhD; **Children's Hospital of Los Angeles:** Alexis Johns, PhD; Mark Urata, MD, DDS; Artur Fahradyan, MD; Amanda Tyree, MA. Raquel Engilman; Madeleine Williams; **Children's Hospital of Philadelphia:** Leanne Magee, PhD; Scott Bartlett, MD; **Shriners Hospitals for Children, Chicago and University of Illinois at Chicago:** Kathleen Kapp-Simon, PhD; Janine Rosenberg, PhD; Claudia Crilly Bellucci, MS; Suzel Bautista, BA; Jody Coppersmith, MS; Amanda Lossia, MS; Stephanie McConville, PhD, Kristina Butler, MS; **University of North Carolina:** Amelia Drake, MD; Marina Pastore Rampazzo, DDS; Daniela Vivaldi, DDS. **University of Pittsburgh:** Jeffrey F. Cohen, PhD; Zakia Hammal, PhD. **New York University:** Harriett Oster, PhD. Partial results of this work were previously presented as abstracts at the American Cleft Palate Association's (March, 2015) and Pediatric Academic Society's (May, 2017) annual meetings. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Results: We found a statistically significant association between diabetes mellitus (DM) and CFM (OR 4.01, 95% CI 1.6–10.5). The association was slightly attenuated after adjustment for BMI. Higher parity was also associated with increased risk for CFM (OR 2.0, 95% CI 1.0–4.0). Vitamin A consumption and/or liver consumption was associated with a 70% lower risk compared with non-users (OR 0.3, 95% CI 0.1–0.8). Maternal age at the time of pregnancy was not associated with CFM.

Conclusions: These analyses contribute evidence linking maternal DM with an elevated risk of having an infant with CFM, which is consistent with previous research and adds to the body of knowledge about the strength of this association. Further study is warranted to understand the potential mechanisms underlying the effect of DM in the developing embryo.

Keywords

Craniofacial microsomia; hemifacial microsomia; OAVS; diabetes; microtia; anotia

Introduction

Craniofacial microsomia (CFM), also known as oculo-auriculo-vertebral spectrum (OAVS), is a congenital condition with variable presentation that often includes underdevelopment of one or both sides of the face. Features typically include microtia and/or mandibular hypoplasia in addition to preauricular tags, epibulbar dermoids, lateral oral clefts, and extracranial anomalies. Isolated microtia is the mildest form on the CFM spectrum (Beleza-Meireles et al. 2014; Bennun et al. 1985; Keogh et al. 2007).

Although CFM can have a significant clinical impact on individuals, little is known about its risk factors. As isolated microtia is considered a mild presentation of CFM, studies that evaluate the risk of isolated microtia and anotia are considered here alongside those that evaluate the risk for more heterogeneous and variable presentations of CFM described in the first paragraph. There is likely etiologic heterogeneity, with genetic and non-genetic risk factors (Barisic et al. 2014; Beleza-Meireles et al. 2014; Kaye et al. 1992). Prior investigations have detected an association between prenatal exposure to maternal diabetes mellitus, retinoids, thalidomide, and mycophenolate mofetil and risk of microtia (Anderka et al. 2009; Klieger-Grossmann et al. 2010). Other risk factors identified in the few published case-control studies on microtia and/or CFM include higher maternal parity and maternal acute illness (Castilla and Luquetti 2009; Correa et al. 2008; Luquetti et al. 2013; Ma et al. 2010; Mastroiacovo et al. 1995a; Werler et al. 2004a; Zhang et al. 2009). Low birthweight, advanced maternal age, low maternal education, and Hispanic ethnicity have been reported as risk factors for CFM in cross-sectional, population-based studies (Werler et al. 2004b). Early case-control studies suggested that maternal use of vasoactive medications in the 1st trimester in combination with cigarette smoking was associated with increased risk of CFM (Werler et al. 2004a; Werler et al. 2009). A later study in a larger cohort showed that those exposures or other potential vascular events (such as twinning) did not increase risk (Van Bennekom et al. 2013). Periconceptional intake of folic acid has been associated with reduced risk of microtia among non-obese women (Ma et al. 2010; Van Bennekom et al. 2013). The mechanisms by which these exposures cause microtia have not been fully elucidated, as most publications have focused on the risk for microtia as opposed to the

broader CFM spectrum. Furthermore, those that did evaluate the risk for CFM did not have clearly defined case definitions.

We were interested in the influence of maternal diabetes mellitus (DM) on an infant's risk for CFM since it is one of the more strongly associated risk factors for microtia. Furthermore, obesity is a risk factor for DM and an association between non-isolated microtia and obesity has been reported (Ma et al. 2010; Van Bennekom et al. 2013). We sought to evaluate whether maternal DM, obesity and other pre-pregnancy and prenatal factors are associated with risk of CFM in a well-defined sample of infants with CFM.

Methods

The current study uses data collected through a multi-center, longitudinal cohort study titled Craniofacial Microsomia: Longitudinal Outcomes in Children Pre-Kindergarten (CLOCK). The primary goal of the CLOCK study is to evaluate the neurobehavioral outcomes in children with and without CFM (Speltz et al. 2018). Data used in the current analyses were collected between November 2013 and April 2017 when children were between 12 and 24 months of age.

The study included children who were recruited through six US craniofacial centers (Seattle Children's Hospital (SCH), Children's Hospital of Los Angeles (CHLA), Children's Hospital of Philadelphia (CHOP), Shriners Hospitals for Children, Chicago (SHC) University of Illinois at Chicago (UIC), and University of North Carolina at Chapel Hill (UNC)). The recruitment and study protocol have been described in detail elsewhere (Luquetti et al. 2019).. All study procedures were approved by the IRB at each of the six participating sites.

Case participants.

Infants between 12 and 24 months of age were eligible for the study if they had been diagnosed with CFM by a regional craniofacial team and met at least one of the following inclusion criteria: (1) microtia; (2) anotia; (3) facial asymmetry and preauricular tag; (4) facial asymmetry and facial tag; (5) facial asymmetry and epibulbar dermoid; (6) facial asymmetry and lateral oral cleft (e.g. macrostomia); (7) preauricular tag and epibulbar dermoid; (8) preauricular tag and lateral oral cleft; (9) facial tag and epibulbar dermoid; and (10) lateral oral cleft and epibulbar dermoid. Infants were excluded if they had an abnormal karyotype, a syndromic diagnosis, a major medical or neurological condition (e.g., cancer, cerebral palsy) at time of recruitment, increased risk for inability to comply with or complete the study, were born before 34 weeks estimated gestational age, had a sibling already participating in the study or the consenting parent spoke neither English nor Spanish.

Potential participants were identified in the study by querying electronic hospital billing data using ICD-9 codes that matched the inclusion criteria (743.8, 744.0, 744.01, 744.02, 744.09, 744.21, 744.23, 744.1, 744.83, 754.0, 756.0). The clinical database was queried at the SCH site. Study team members also identified potential participants through review of clinic schedules for craniofacial, otolaryngology, audiology, and genetics clinics at the study sites.

Control participants

Infants without a congenital facial anomaly or injury who were between 12 and 24 months of age were eligible for the study. Individuals were excluded if they met any of the exclusionary criteria for cases. Controls were primarily enrolled through local pediatric practices that were near the study sites from which cases were recruited. Recruitment flyers were posted in practice-based network offices and, for the SCH site, we also contacted additional participants through the University of Washington Participant Pool, which is a database of individuals of all ages from the Seattle metropolitan area that are interested in participating in research studies.

We monitored the balance of case and control on the following characteristics: infant age and sex, family socioeconomic status (SES), and language spoken in the home (English or Spanish), in order to minimize group differences.

Study data collection

We recorded infant age and sex, family socioeconomic status (SES) and language spoken in the home (English or Spanish). SES categorization was determined based on marital/cohabitation status, occupation, and education of the head(s) of the household. Parents of study participants completed an in-person structured interview about their infants' medical and family history by a trained research personnel. Biologic mothers were asked about their pregnancy history using an instrument developed from questions from the National Birth Defects Prevention Study mother questionnaire (www.nbdps.org). Questions related to DM included type of diabetes ("gestational, that is during pregnancy only; insulin-dependent diabetes, also called type I or juvenile type II; or adult onset") and time of diagnosis.

Information regarding clinical CFM features was abstracted from patients' medical charts. We obtained digital photographs of the child's face (frontal, left and right lateral, and smiling views) following a simplified version of our previously published protocol (Birgfeld et al. 2011b).

Phenotype assessment

We used a modified version of the Phenotypic Assessment Tool for Craniofacial Microsomia (PAT-CFM), which includes a photographic protocol and a modified version of the Pictorial OMENS form to categorize the facial features associated with CFM (Birgfeld et al. 2011a; Birgfeld et al. 2016). We integrated data from the modified pictorial OMENS ratings, health history interview, and medical chart abstraction data to categorize the phenotype of cases and controls. These integrated dataset was compiled in an Access Database and reviewed by a craniofacial pediatrician (CH) to establish the phenotype by feature for each participant.

We developed subcategories based on CFM-related facial phenotypes, with the goal of creating subgroups of adequate size in order to prevent over-stratification.

Statistical Methods

The distributions of demographic and phenotypic characteristics were calculated, and logistic regression was used to evaluate the associations between exposures of interest and risk for CFM overall as well as within sub-groups of children with CFM.

We included the following covariates in the initial statistical models in order to adjust for their potentially confounding effect: sex, SES, study site and ethnicity. In the statistical models evaluating the association between DM and CFM we also evaluated the potentially confounding effect of parity, mothers' age at birth, and mothers' pre-pregnancy BMI, as these are all associated with both DM as well as CFM. As the results from the fully adjusted models did not meaningfully change the risk estimates (i.e., neither changed the threshold into or out of statistical significance, nor changed the magnitude sufficiently to interpret the nature of the relationship differently), the results presented here are from the unadjusted statistical models. All analyses were carried out using Stata 14 (StataCorp. 2015. *Stata Statistical Software: Release 14*. College Station, TX: StataCorp LP).

Results

Participation rates for cases was 49% and for controls 56%. Cases were more likely than controls to be male (62 versus 44%), Hispanic (55 vs 38%) and white (76 vs 58%). Age at the time of enrollment was comparable (14.5 vs 14.4 months). Mean SES score as measured by the Hollingshead method was slightly lower among case participants (mean score: 35. vs 37.5), and the proportion of cases on Medicaid was slightly higher than among controls (54 vs 48 %). Ninety-five cases had microtia (95%) and, of those, 29% were bilateral. Among those with microtia, the severity based on the more severely affected ear varied as follows: first degree microtia (8%), second degree microtia (24%), third degree microtia (63%), and anotia (5%). Mandibular hypoplasia was present in 59% of cases; most were characterized as mild (71%), 21% were moderate and 8% severe. The distribution of phenotypic characteristics was as follows: 30 cases with microtia only (28%); 59 cases with microtia in the presence of facial asymmetry with or without other characteristics such as tags or pits (55%); or 19 cases characterized as 'other' (18%) who may have included children who did not fit into the prior two categories, but who met at least one inclusion criterion 7–10 listed above. Full descriptive data for the cohort have been previously published (Luquetti et al. 2019).

Mothers with any form of DM, either prior to or during the cases' pregnancy, had a four-fold higher risk of having children with CFM (OR 4.0, 95% CI 1.5–10.5; Table 1). This association was slightly higher among women diagnosed with gestational DM than those with Type II DM (OR 4.6, 95% CI 1.5–14.2 vs. OR 2.9, 95% CI 0.6–14.9, respectively), but the difference in odd-ratios between these two subgroups was based on only 2 controls and 6 cases in the group with a history of gestational DM (data not shown). Although slightly attenuated, the increased risk of CFM associated with DM persisted even when we adjusted for mother's pre-pregnancy BMI (OR 3.65, 95% CI 1.4–9.7; Table 2). Relative to mothers who were primiparous, mothers with one, two, or more prior pregnancies were more likely to have a child with CFM, but the association within each stratum included one (OR 1.2,

95% CI 0.6–2.7 and 2.0, 95% CI 1.0–4.0; Table 1). Mothers with a previous miscarriage did not appear to have a higher risk of having a child with CFM (data not shown). Mothers had similar risk for having a child with CFM irrespective of their age (OR 1, 1, and 0.9 among 25–30, 30–35 and 35–46 year olds relative to women under 25, respectively). While higher BMI was associated with higher risk for CFM, the confidence interval included one (OR 1.7, 95% CI 0.8–3.5 and OR 1.5, 95% CI 0.8–3.0 among over weight and obese women relative to normal weight women, respectively; Table 1).

We evaluated whether use of fertility medications, vitamin A consumption during pregnancy, use of a prenatal vitamin during pregnancy were associated with risk for CFM. Although all were associated with a lower risk for CFM, confidence intervals included one and the estimates were based on small sample sizes (<10). When we looked jointly at vitamin A consumption and/or liver consumption, we found that users had a 70% lower risk of CFM than non-users (OR 0.3, 95% 0.1–0.8; Table 1).

We evaluated whether DM was associated with a specific sub-phenotype of CFM and found that, relative to controls, while the association was strongest among children with isolated microtia (OR 7.1, 95% CI 2.3–22.2), this association was also observed among those with microtia accompanied by facial asymmetry (OR 3.4, 95% 1.2–9.7) and those who met the CFM inclusion criteria but did not fit into either of the first two categories (OR 2.4, 95% CI 0.5–10.6; Table 3).

Discussion

Our analyses contribute to prior evidence linking maternal diabetes mellitus with an elevated risk of having an infant with CFM (Correa et al. 2008; Garne et al. 2012; Mastroiacovo et al. 1995a; Van Bennekom et al. 2013; Wang, Martinez-Frias, Graham 2002; Werler et al. 2004a). We found that women with DM had a four-fold risk of having a child with CFM in comparison with women without DM. Higher parity was associated with higher risk for CFM and higher BMI was associated with higher CFM risk, but these associations did not reach statistical significance. Fertility treatment, vitamin A and prenatal vitamin consumption were all associated with lower risk for CFM, but the 95% confidence intervals all included one.

A relationship between DM and microtia has been established in the literature (Correa et al. 2008; Ewart-Toland et al. 2000; Garne et al. 2012; Mastroiacovo et al. 1995b; Van Bennekom et al. 2013) with the strength of the association ranging from 1.3 to 18.5. A summary of the results from previous work is presented in Table 4. While some studies evaluated the risk associated with any DM (Mastroiacovo et al. 1995b), others tried to evaluate the risk associated with gestational versus established DM. Only three prior studies have evaluated the risk of the broader CFM spectrum in relation to DM (Barisic et al. 2014; Wang, Martinez-Frias, Graham 2002; Werler et al. 2009). In a case control study with 157 cases, Wang et al. found a 50% higher risk for CFM among mothers with established DM, and a greater than two-fold higher risk --among mothers who were diagnosed with DM during the course of their pregnancy. Similarly, Werler et al, in a case control study involving 230 children with CFM, found that children with CFM were six times more likely to have a

mother with DM than were controls. Barisic et al compared the rates of DM among mothers of children with CFM using data from the EUROCAT registry (a European network of population-based registries for the epidemiologic surveillance of congenital anomalies) and found a rate that was 10-fold higher than expected based on published population-based rates; this study did not have a comparison group (Barisic et al. 2014).

In summary, no clear pattern has been established in prior research indicating whether pre-gestational or gestational DM is more strongly associated with microtia or CFM, with some studies finding that established DM is more strongly associated (Van Bennekom et al. 2013) and others finding that gestational DM is more strongly associated (Wang, Martinez-Frias, Graham 2002). In the current analysis, we were unable to evaluate with statistical precision whether there was any difference in risk associated with CFM and type of DM.

Although maternal obesity has been linked to risk of birth defects in general, no study has looked at the impact of obesity on the risk of CFM, and to date, only two other studies have evaluated the specific association with risk of microtia (Ma et al. 2010; Waller et al. 2007). The first (Waller et al. 2007) analysis was carried out on a data set that was nested within the second (Ma et al. 2010), and neither reported an association. Although we did find that overweight women were more likely to have children with CFM, there was not a linearly increasing risk for CFM as women's BMI increased. Our findings together with those of Waller's and Ma' may indicate that obesity itself is not associated with an increased risk of microtia, and the increase in risk of CFM among overweight women may be the result of a proportion of women in the overweight group that have undiagnosed DM. According to the National Health and Nutrition Examination Survey (NHANES), the proportion of adults with undiagnosed DM was 4.3% and 3.2% among overweight and obese women, respectively (Gregg et al. 2004). In support of this explanation, in the current analysis, the 2.5-fold higher risk of having a child with CFM among overweight women was attenuated to 2.0 (0.8–5.3) when we adjusted for presence of DM.

Although the mechanisms that underlie the relation between DM and birth defects are not clear, the cellular damage that occurs in adult tissues leading to vascular disease may also occur in the differentiating embryo tissue. This is consistent with the vascular disruption hypothesis that has been posited as a potential explanation for the development of CFM. It also seems likely that the DM CFM relationship may be mediated through hyperglycemia (Eriksson, Cederberg, Wentzel 2003). Among human studies evaluating the effects of maternal glycemic control early in pregnancy, the association between DM and congenital anomalies persisted only among women with hyperglycemia (Suhonen, Hiilesmaa, Teramo 2000), which further implicates glycemic control as a mechanism underlying the relationship between maternal DM and microtia/CFM. In an evaluation of dietary intake of nutrients by mothers participating in the National Birth Defects Prevention Study, no increase in risk for microtia was observed with increases in either glycemic index or glycemic load (Ma et al. 2010). However, these findings were based on women's responses to a food frequency questionnaire querying their diet at the time of their child's conception, which was administered when their infants were between 6 weeks and 24 months of age (i.e., as long as 33 months after the time point in question), thus susceptible to recall biases. Furthermore, the interview collected data about a woman's consumption, but was not able to

measure circulating glucose levels directly (which may be impacted by her DM status, or insulin resistance). If obesity is associated with CFM as we suggest here, it is likely related to the fact that DM is more common among individuals with higher BMI. Gregg et al report that the prevalence of DM rises from 4.1 percent to 18.3 percent as BMI rises from <25 to ≥ 35 percent (Gregg et al. 2004).

Consistent with published reports evaluating the association between parity and microtia (Castilla and Orioli 1986; Harris, Kallen, Robert 1996; Mastroiacovo et al. 1995a), we found that risk for CFM increases with parity. In our cohort, women with two or more prior pregnancies had double the risk of giving birth to an infant with CFM than primiparous women.

Advanced maternal age has been reported as a risk factor for microtia (Forrester and Merz 2005; Harris, Kallen, Robert 1996), but we did not find elevated risk for CFM with increasing age, nor did we find that any maternal age group was at a particularly higher risk for CFM than others.

Periconceptional intake of supplements containing folic acid has been associated with lower risk of microtia among non-obese women, but not among obese women (Ma et al. 2012). The findings from the current analysis suggest that women who took prenatal vitamins had a substantially lower risk for CFM, but these estimates were limited in several respects: only nine women in our study reported having taken no prenatal vitamins and among these, only one was a control; our confidence intervals were wide and included 1.0 (0.02–1.19); we were not able to further stratify into subgroups defined by BMI.

We found use of vitamin A and/or liver consumption (a food rich in vitamin A) to be associated with a 70 percent lower risk for CFM. Retinoic acid is a metabolite of vitamin A and has been reported to be a risk factor for microtia (Carey, Park, Muntz 2006; Lammer et al. 1985; Stern, Rosa, Baum 1984). It may be that these apparently discrepant findings are the result of a dose effect; below a specific threshold of consumption, vitamin A is essential for normal reproduction and development, but beyond some threshold, vitamin A has a teratogenic effect on the developing fetus (Khoury, Moore, Mulinare 1996; Rothman et al. 1995).

It is important to acknowledge the limitations of this study. We relied on women's recall of their prenatal DM diagnosis as we did not have access to maternal and/or gestational history from medical records. However, high positive predictive value of self-reported DM is well reported in longitudinal follow-up studies. For example, in a study using the Pregnancy Risk Assessment Monitoring System (PRAMS), a self-reported questionnaire, and data abstracted from hospital records as a gold standard; any DM had 91 % sensitivity and 94% specificity (Dietz et al. 2014). In addition, recall bias cannot be ruled out but, previous studies on the field have showed that there is minimal to no recall bias in case-control studies comparing mothers with and without adverse pregnancy outcomes including those with children with birth defects (Feldman et al. 1989; Khoury, James, Erickson 1994; Mackenzie and Lippman 1989; Rockenbauer et al. 2001). Gestational DM is a complex disease, and often is undetected until an individual undergoes a screening evaluation during pregnancy. As a

result, women who are diagnosed with gestational DM through screening during their pregnancy may have had DM before conception. It is often difficult to differentiate between the two types of DM and to assess the temporal relationship of the period characterized by poor glucose control. In addition, we did not have data on the severity of women's associated hyperglycemia.

Conclusions

Our analyses provide additional evidence linking maternal diabetes mellitus as well as higher parity with an elevated risk of having an infant with CFM. Further studies to understand the risk factors for this heterogeneous and complex disorder will require larger sample sizes and the integration of parental exposures with genetic risk factors to clarify the biological mechanism through which risk for the development of CFM is mediated.

Acknowledgments:

We thank the children and their caregivers for their participation in the study. We are grateful for the support from advocacy organizations for their assistance with identifying study participants.

Grant number: Research reported in this presentation was supported by the National Institute of Dental and Craniofacial Research under award number R01 DE 022438 and the Center for Clinical and Translational Research at Seattle Children's Research Institute grant UL1 TR000423. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

References

- Anderka MT, Lin AE, Abuelo DN, Mitchell AA, Rasmussen SA. 2009 Reviewing the evidence for mycophenolate mofetil as a new teratogen: Case report and review of the literature. *Am J Med Genet A* 149A(6):1241–8. [PubMed: 19441125]
- Barisic I, Odak L, Loane M, Garne E, Wellesley D, Calzolari E, Dolk H, Addor MC, Arriola L, Bergman J, et al. 2014 Prevalence, prenatal diagnosis and clinical features of oculo-auriculo-vertebral spectrum: A registry-based study in europe. *Eur J Hum Genet* 22(8):1026–33. [PubMed: 24398798]
- Beleza-Meireles A, Clayton-Smith J, Saraiva JM, Tassabehji M. 2014 Oculo-auriculo-vertebral spectrum: A review of the literature and genetic update. *J Med Genet* 51(10):635–45. [PubMed: 25118188]
- Bennun RD, Mulliken JB, Kaban LB, Murray JE. 1985 Microtia: A microform of hemifacial microsomia. *Plast Reconstr Surg* 76(6):859–65. [PubMed: 4070453]
- Birgfeld CB, Heike CL, Saltzman BS, Leroux BG, Evans KN, Luquetti DV. 2016 Reliable classification of facial phenotypic variation in craniofacial microsomia: A comparison of physical exam and photographs. *Head Face Med* 12:14,016–0109-x.
- Birgfeld CB, Luquetti DV, Gougoutas AJ, Bartlett SP, Low DW, Sie KC, Evans KN, Heike CL. 2011a A phenotypic assessment tool for craniofacial microsomia. *Plast Reconstr Surg* 127(1):313–20. [PubMed: 21200224]
- Birgfeld CB, Luquetti DV, Gougoutas AJ, Bartlett SP, Low DW, Sie KC, Evans KN, Heike CL. 2011b A phenotypic assessment tool for craniofacial microsomia. *Plast Reconstr Surg* 127(1):313–20. [PubMed: 21200224]
- Carey J, Park A, Muntz H. 2006 External ear. In: *Human malformations and related anomalies* New York: Oxford University Press 329 p.
- Castilla EE and Luquetti DV. 2009 Brazil: Public health genomics. *Public Health Genomics* 12(1):53–8. [PubMed: 19023184]
- Castilla EE and Orioli IM. 1986 Prevalence rates of microtia in south america. *Int J Epidemiol* 15(3): 364–8. [PubMed: 3771073]

- Correa A, Gilboa SM, Besser LM, Botto LD, Moore CA, Hobbs CA, Cleves MA, Riehle-Colarusso TJ, Waller DK, Reece EA. 2008 Diabetes mellitus and birth defects. *Am J Obstet Gynecol* 199(3): 237.e1,237.e9. [PubMed: 18674752]
- Dietz P, Bombard J, Mulready-Ward C, Gauthier J, Sackoff J, Brozicevic P, Gambatese M, Nyland-Funke M, England L, Harrison L, et al. 2014 Validation of self-reported maternal and infant health indicators in the pregnancy risk assessment monitoring system. *Matern Child Health J* 18(10): 2489–98. [PubMed: 24770954]
- Eriksson UJ, Cederberg J, Wentzel P. 2003 Congenital malformations in offspring of diabetic mothers--animal and human studies. *Rev Endocr Metab Disord* 4(1):79–93. [PubMed: 12618562]
- Ewart-Toland A, Yankowitz J, Winder A, Imagire R, Cox VA, Aylsworth AS, Golabi M. 2000 Oculoauriculovertebral abnormalities in children of diabetic mothers. *Am J Med Genet* 90(4):303–9. [PubMed: 10710228]
- Feldman Y, Koren G, Mattice K, Shear H, Pellegrini E, MacLeod SM. 1989 Determinants of recall and recall bias in studying drug and chemical exposure in pregnancy. *Teratology* 40(1):37–45. [PubMed: 2763209]
- Forrester MB and Merz RD. 2005 Descriptive epidemiology of anotia and microtia, hawaii, 1986–2002. *Congenit Anom (Kyoto)* 45(4):119–24. [PubMed: 16359491]
- Garne E, Loane M, Dolk H, Barisic I, Addor MC, Arriola L, Bakker M, Calzolari E, Matias Dias C, Doray B, et al. 2012 Spectrum of congenital anomalies in pregnancies with pregestational diabetes. *Birth Defects Res A Clin Mol Teratol* 94(3):134–40. [PubMed: 22371321]
- Gregg EW, Cadwell BL, Cheng YJ, Cowie CC, Williams DE, Geiss L, Engelgau MM, Vinicor F. 2004 Trends in the prevalence and ratio of diagnosed to undiagnosed diabetes according to obesity levels in the U.S. *Diabetes Care* 27(12):2806–12. [PubMed: 15562189]
- Harris J, Kallen B, Robert E. 1996 The epidemiology of anotia and microtia. *J Med Genet* 33(10):809–13. [PubMed: 8933331]
- Kaye CI, Martin AO, Rollnick BR, Nagatosh K, Israel J, Hermanoff M, Tropea B, Richtsmeier JT, Morton NE. 1992 Oculoauriculovertebral anomaly: Segregation analysis. *Am J Med Genet* 43(6): 913–7. [PubMed: 1415339]
- Keogh IJ, Troulis MJ, Monroy AA, Eavey RD, Kaban LB. 2007 Isolated microtia as a marker for unsuspected hemifacial microsomia. *Arch Otolaryngol Head Neck Surg* 133(10):997–1001. [PubMed: 17938322]
- Khoury MJ, Moore CA, Mulinare J. 1996 Vitamin A and birth defects. *Lancet* 347(8997):322. [PubMed: 8569374]
- Khoury MJ, James LM, Erickson JD. 1994 On the use of affected controls to address recall bias in case-control studies of birth defects. *Teratology* 49(4):273–81. [PubMed: 8073366]
- Klieger-Grossmann C, Chitayat D, Lavign S, Kao K, Garcia-Bournissen F, Quinn D, Luo V, Sermer M, Riordan S, Laskin C, et al. 2010 Prenatal exposure to mycophenolate mofetil: An updated estimate. *J Obstet Gynaecol can* 32(8):794–7. [PubMed: 21050513]
- Lammer EJ, Chen DT, Hoar RM, Agnish ND, Benke PJ, Braun JT, Curry CJ, Fernhoff PM, Grix AW Jr, Lott IT. 1985 Retinoic acid embryopathy. *N Engl J Med* 313(14):837–41. [PubMed: 3162101]
- Luquetti DV, Saltzman BS, Lopez-Camelo J, Dutra Mda G, Castilla EE. 2013 Risk factors and demographics for microtia in south america: A case-control analysis. *Birth Defects Res A Clin Mol Teratol* 97(11):736–43. [PubMed: 24265127]
- Luquetti DV, Speltz ML, Wallace ER, Siebold B, Collett BR, Drake AF, Johns AL, Kapp-Simon KA, Kinter SL, Leroux BG, et al. 2019 Methods and challenges in a cohort study of infants and toddlers with craniofacial microsomia: The clock study. *Cleft Palate Craniofac J* : 1055665618821014.
- Ma C, Shaw GM, Scheuerle AE, Canfield MA, Carmichael SL, National Birth Defects Prevention Study. 2012 Association of microtia with maternal nutrition. *Birth Defects Res A Clin Mol Teratol* 94(12):1026–32. [PubMed: 22821770]
- Ma C, Carmichael SL, Scheuerle AE, Canfield MA, Shaw GM, National Birth Defects Prevention Study. 2010 Association of microtia with maternal obesity and periconceptional folic acid use. *Am J Med Genet A* 152A(11):2756–61. [PubMed: 20949601]

- Mackenzie SG and Lippman A. 1989 An investigation of report bias in a case-control study of pregnancy outcome. *Am J Epidemiol* 129(1):65–75. [PubMed: 2910073]
- Mastroiacovo P, Corchia C, Botto LD, Lanni R, Zampino G, Fusco D. 1995a Epidemiology and genetics of microtia-antia: A registry based study on over one million births. *J Med Genet* 32(6): 453–7. [PubMed: 7666397]
- Mastroiacovo P, Corchia C, Botto LD, Lanni R, Zampino G, Fusco D. 1995b Epidemiology and genetics of microtia-antia: A registry based study on over one million births. *J Med Genet* 32(6): 453–7. [PubMed: 7666397]
- Rockenbauer M, Olsen J, Czeizel AE, Pedersen L, Sorensen HT, EuroMAP Group. 2001 Recall bias in a case-control surveillance system on the use of medicine during pregnancy. *Epidemiology* 12(4): 461–6. [PubMed: 11416783]
- Rothman KJ, Moore LL, Singer MR, Nguyen US, Mannino S, Milunsky A. 1995 Teratogenicity of high vitamin A intake. *N Engl J Med* 333(21):1369–73. [PubMed: 7477116]
- Speltz ML, Kapp-Simon KA, Johns AL, Wallace ER, Collett BR, Magee L, Leroux BG, Luquetti DV, Heike CL. 2018 Neurodevelopment of infants with and without craniofacial microsomia. *J Pediatr* 198:226,233.e3. [PubMed: 29685618]
- Stern RS, Rosa F, Baum C. 1984 Isotretinoin and pregnancy. *J Am Acad Dermatol* 10(5 Pt 1):851–4. [PubMed: 6233342]
- Suhonen L, Hiilesmaa V, Teramo K. 2000 Glycaemic control during early pregnancy and fetal malformations in women with type I diabetes mellitus. *Diabetologia* 43(1):79–82. [PubMed: 10663219]
- Van Bennekom CM, Mitchell AA, Moore CA, Werler MM, National Birth Defects Prevention Study. 2013 Vasoactive exposures during pregnancy and risk of microtia. *Birth Defects Res A Clin Mol Teratol* 97(1):53–9. [PubMed: 23180593]
- Waller DK, Shaw GM, Rasmussen SA, Hobbs CA, Canfield MA, Siega-Riz AM, Gallaway MS, Correa A, National Birth Defects Prevention Study. 2007 Prepregnancy obesity as a risk factor for structural birth defects. *Arch Pediatr Adolesc Med* 161(8):745–50. [PubMed: 17679655]
- Wang R, Martinez-Frias ML, Graham JM Jr. 2002 Infants of diabetic mothers are at increased risk for the oculo-auriculo-vertebral sequence: A case-based and case-control approach. *J Pediatr* 141(5): 611–7. [PubMed: 12410187]
- Werler MM, Starr JR, Cloonan YK, Speltz ML. 2009 Hemifacial microsomia: From gestation to childhood. *J Craniofac Surg* 20 Suppl 1:664–9. [PubMed: 19218862]
- Werler MM, Sheehan JE, Hayes C, Mitchell AA, Mulliken JB. 2004a Vasoactive exposures, vascular events, and hemifacial microsomia. *Birth Defects Res A Clin Mol Teratol* 70(6):389–95. [PubMed: 15211707]
- Werler MM, Sheehan JE, Hayes C, Padwa BL, Mitchell AA, Mulliken JB. 2004b Demographic and reproductive factors associated with hemifacial microsomia. *Cleft Palate Craniofac J* 41(5):494–50. [PubMed: 15352870]
- Zhang QG, Zhang J, Yu P, Shen H. 2009 Environmental and genetic factors associated with congenital microtia: A case-control study in Jiangsu, China, 2004 to 2007. *Plast Reconstr Surg* 124(4):1157–64. [PubMed: 19935299]

Table 1.

Association between various pregnancy related factors and risk for craniofacial microsomia

	Controls	Cases	crude OR		
	n (%)	n (%)	OR	95% CI	p-value
Any diabetes	6 (7.2)	25 (24.0)	4.0	(1.6–10.5)	0.01
Number of prior pregnancies					
Primiparous	29 (34.5)	26 (24.1)	1	(ref)	
1 prior	25 (29.8)	28 (25.9)	1.2	(0.6, 2.7)	0.56
2+ prior	28 (33.3)	50 (46.3)	2.0	(1.0, 4.0)	0.05
Age group (years)					
< =25	19 (22.6)	25 (23.1)	1	(ref)	
>25–30	17 (20.2)	22 (20.4)	1.0	(0.4, 2.3)	0.97
>30–35	26 (31.0)	33 (30.6)	1.0	(0.4, 2.1)	0.93
>35–46	21 (25.0)	24 (22.2)	0.9	(0.4, 2.0)	0.74
Body Mass Index (BMI)					
Normoweight (18.5–25)	40 (47.6)	37 (34.3)	1	(ref)	
Overweight (>25–30)	17 (20.2)	26 (24.1)	1.7	(0.8, 3.5)	0.19
Obese (>30+)	24 (28.6)	34 (31.5)	1.5	(0.8, 3.0)	0.22
Prenatal exposures *					
Fertility drugs	9 (10.7)	8 (7.4)	0.7	(0.3, 1.9)	0.46
Vitamin A consumption	5 (6.0)	2 (1.9)	0.3	(0.1, 1.7)	0.17
Prenatal vitamin	82 (97.6)	96 (88.9)	0.1	(0.0, 1.2)	0.07
Group B streptococcus	6 (7.1)	2 (1.9)	0.3	(0.0, 1.3)	0.10
Any vitamin A or liver consumption	14 (16.7)	6 (5.6)	0.3	(0.1, 0.8)	0.02

OR: odds ratio

CI: confidence interval

* Neither cases or controls had any exposure to the following: thalidomide, mycophenolate mofetil, rubella, syphilis, cytomegalovirus or measles.

Table 2.

Adjusted risk for craniofacial microsomia associated with diabetes mellitus and body mass index.

	Controls (n=84)	Cases (n=108)	
	n (%)	n (%)	aOR 95% CI
Diabetes Mellitus^a			
No	77 (92.8)	79 (76.0)	1.00 (ref)
Yes	6 (7.2)	25 (24.0)	3.65 (1.4–9.7)
Body Mass Index (BMI)^b			
Normoweight (>18.5–25)	40 (49.4)	37 (37.4)	1.00 (ref)
Overweight (>25–30)	17 (21.0)	26 (26.3)	1.46 (0.7–3.2)
Obese (>30+)	24 (29.6)	34 (34.3)	1.21 (0.6–2.5)

^a Adjusted by BMI^b Adjusted by diabetes mellitus

aOR: adjusted odds ratio

CI: confidence interval

Table 3:

Association between diabetes mellitus and craniofacial microsomia subtypes

	Controls (n=84)	Microtia only (n=30)		Microtia + facial asymmetry (n=59)		Other (n=19)	
	n (%)	n (%)	aOR (95% CI)	n (%)	aOR (95% CI)	n (%)	aOR (95% CI)
Any diabetes mellitus							
No	77 (92.8)	18 (64.3)	1.00 (ref)	45 (78.9)	1.00 (ref)	16 (84.2)	1.00 (ref)
Yes	6 (7.2)	10 (35.7)	7.1 (2.3–22.2)	12 (21.1)	3.42 (1.2–9.8)	3 (15.8)	2.41 (0.5–10.6)

aOR: adjusted odds ratio

CI: confidence interval

Table 4.

Literature review of publications reporting the risk of microtia and/or craniofacial microsomia in relation to body mass index, diabetes and glycemic control^a

1 st author (Year)	Study Design	N. Cases ^b	Population (years of ascertainment)	Exposure	Outcome(s)	Risk of outcome among exposed relative to unexposed
Body mass index						
Ma (2010)	Case Control	420	NBDPS (1997–2005)	BMI <18.5 BMI 18.5–<25 BMI 25–<30 BMI ≥35 BMI <18.5 BMI 18.5–<25 BMI 25–<30 BMI ≥35	Non isolated microtia Non isolated microtia Non isolated microtia Non isolated microtia Isolated Microtia Isolated Microtia Isolated Microtia Isolated Microtia	1.24 (0.56–2.74) 1.00 (ref) 0.74 (0.43–1.28) 1.45 (0.90–2.36) 1.00 (0.62–1.60) 1.00 (ref) 1.00 (0.78–1.31) 1.27 (0.96–1.67)
Waller (2007)	Case Control	216	NBDPS (1997–2002)	BMI <18.5 BMI 18.5–<25 BMI 25–<30 BMI ≥35	Microtia Microtia Microtia Microtia	0.82 (0.43–1.56) 1.00 (ref) 0.86 (0.60–1.23) 1.10 (0.74–1.65)
Diabetes						
Wang (2002)	Case Control	157	ECEMC (1976–2000)	GDM PGDM	OAVS OAVS	2.28 (1.03–4.82) 1.50 (0.08–9.99)
Mastroiacovo (1995)	Case Control	172	IPIMC (1983–1992)	PGDM	Microtia	8.7 (1.2–96.4)
Game (2012)	Case Control	88	EUROCAT (1990–2005)	PGDM	Anotia	4.37 (1.36–14.03)
Correa (2008)	Case Control	286	NBDPS (1997–2002)	GDM GDM PGDM PGDM	Isolated Microtia Microtia with other defects Isolated Microtia Microtia with other defects	1.30 (0.65–2.61) 0.4 (0.06–3.20) 3.75 (1.04–13.51) 18.50 (6.95–49.24)
Barisic (2014)	Compared cases' rates with published population rates	355	EUROCAT (1990–2009)	DM ns	Goldenhar/HFM	~10-fold higher risk ^c
Van Bennekom (2013)	Case Control	421	NBDPS	PGDM	Any microtia (potentially included cases with OAVS)	7.2 (3.9–13.1)

1 st author (Year)	Study Design	N. Cases ^b	Population (years of ascertainment)	Exposure	Outcome(s)	Risk of outcome among exposed relative to unexposed
Werler (2004)	Case Control	421	US-multisite (1996–2002)	GDM	Any microtia (potentially included cases with OAVS)	1.4 (0.9–2.0)
		304		PGDM	Isolated (potentially included cases with OAVS)	3.4 (1.3–8.5)
		304		GDM	Isolated (potentially included cases with OAVS)	1.4 (0.9–2.2)
				DM ns	OAVS	no association ^d
Glycemic control	Case Control	230	US-multisite (1996–2002)	PGDM + GDM	HFM/OAVS/Goldenhar	6.0 (2.5–14.3)
				PGDM + GDM	Isolated CFM	3.1 (1.0–9.6)
				PGDM + GDM	CFM	12.6 (4.6–34.4)
Ma (2012)	Case Control	382	NBDPS (1997–2005)	Glycemic Index	Microtia	0.98 (0.71–1.35)

NBDPS: National Birth Defects Prevention Study; ECEMC: Estudio Colaborativo Español de Malformaciones Congénitas; IPIMC: Indagine Policentrica Italiana Malformazioni Congenite; EUROCAT: European Surveillance of Congenital Anomalies; UCSF: University of California, San Francisco; BMI: body mass index; DM: diabetes mellitus; GDM: gestational diabetes mellitus; PGDM: pregestational diabetes mellitus; ns: not specified; OAVS: oculoauricular vertebral spectrum; CFM: craniofacial microsomia, HFM: hemifacial microsomia

^aCases were identified as craniofacial microsomia, hemifacial microsomia, Goldenhar syndrome, or oculoauricular vertebral spectrum

^bSum of exposed and unexposed cases

^cMaternal diabetes was present in 3.9% of case pregnancies compared with a population rate of 0.3% of pregestational diabetes mellitus

^dPer published manuscript: “We did not see a relationship between maternal diabetes and OAVS in this study.”