MODIFIABLE RISK FACTORS FOR OROFACIAL CLEFTS AMONG HISPANIC AND NON-HISPANIC INDIVIDUALS IN THE UNITED STATES

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A dissertation submitted to the faculty at the University of North Carolina at Chapel Hill in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Epidemiology in the Gillings School of Global Public Health.

Chapel Hill
2023

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ABSTRACT

Erin Graham Sley: Modifiable risk factors for orofacial clefts among Hispanic and non-Hispanic individuals in the United States
(Under the direction of Andrew F. Olshan)

Background: Orofacial clefts (OFC) are a common birth defect in the United States (US) and prevalence varies by Hispanic ethnicity. The effect of several modifiable exposures, like maternal dietary patterns, remain largely unexplored, and recognized risk factors have only been established in primarily non-Hispanic White (NHW) cohorts. We utilized data from the National Birth Defects Prevention Study (NBDPS), a population-based case-control study, to better understand the effect of maternal diet on OFC and the impact of other OFC risk factors on Hispanics in the US.

Methods: To assess the impact of maternal diet, we used a Latent Class Analysis to identify unknown dietary patterns among the NBDPS population. We used these patterns in crude and adjusted logistic regression to estimate the odds of OFC for each class. To assess the impact of risk factors specific to Hispanics, we performed crude and adjusted logistic models for 15 established risk factors among Hispanic NBDPS participants. We estimated average adjusted population attributable fractions (aaPAFs) for the 6 most established risk factors. aaPAFs were stratified by acculturation to partially account for cultural heterogeneity within this population. aaPAFs were also created for NHW NBDPS participants to provide context for results.

Results: A dietary pattern with a relatively higher intake of fruits, vegetables, fish, and dark bread significantly reduced the odds of all OFC phenotypes, when compared to a diet with a relatively higher intake of white bread, chips, and soda, and lower intake of fruits and vegetables. This comparison was true of patterns derived for both the full (Aim 1) and Hispanic (Aim 2) NBDPS population. Among Hispanic individuals, secondhand smoke was associated with a larger increase in OFC than smoking. Diet was associated with the largest aaPAF for most phenotypes, regardless of Hispanic ethnicity. Most aaPAFs varied by acculturation although estimates were imprecise.

Conclusions: The persistent effect of maternal diet in both populations and the unique trends observed in our Hispanic risk factor profile warrants further investigation. Additional focus on modifiable risk factors for OFC, and their specific influence on Hispanics, may inform public health prevention priorities and increase efficacy of current prevention strategies.

To my mom, Dr. Carol Anne Graham, the strongest person I know.

ACKNOWLEDGEMENTS

First and foremost, this work would not have been possible without the individuals who participated in the National Birth Defects Prevention Study (NBDPS). Participation in this study not only required time and effort, but also vulnerability during a unique, and perhaps trying, time of life. To the NBDPS participants, thank you for your participation - it continues to have a far-reaching impact. I was honored to have the opportunity to use your data for this project.

Next, I could not have asked for a better committee to guide me through this process. Andy, thank you for being a phenomenal mentor and chair. You always gave me a seat at the table and made my success a priority. You knew when to push for independence and when to guide – always with kindness and humor. My confidence and knowledge grew so much under your guidance. Tania, thank you for creating a safe space to ask hard questions – of the analytical and personal kind. Your wisdom constantly reminded me of the importance of being human. I am a bolder researcher and person because of you. Suzan, thank you for sharing the breadth and depth of your knowledge with me, not only in nutrition but also research in general. Asia, thank you for always encouraging me to think critically about how my work influences communities and populations. I could not have asked for a better teacher for this. Daniela, thank you for your warmth and patience as you guided me through these analyses and reminded me that I could do hard things. Mollie, thank you for encouraging me to form my own opinions on these methods and creating space for any and every question.

I am also deeply grateful to the staff at the North Carolina Center for Birth Defects Research and Prevention and the UNC epidemiology department who provided analytic and academic support when I needed it most. Thank you for your tireless efforts to ensure that I had access to the resources required to complete this work. You taught me so much about best practices in research and, most importantly, you gave me the gift of feeling truly supported in this setting. Similarly, the support I received from the NCCBDRP trainee group provided a unique combination of methodological help, inspiration, and friendship. Thank you all for digging deep into this project with me with incredible kindness.

Additionally, my family and community were integral in this process. I am forever grateful to my mom's circle of incredibly strong and wise women who held me as their own and loved me through this process. Thank you for reminding me of my mom's strength, my strength, and your strength. It propelled me forward and kept me on solid ground. I am also thankful to my family who reminded me that I always had a place to belong, and a home to come back to, throughout this journey. And to my friends, my chosen family, thank you for sticking by my side and holding me close. The incredible support you provided and constant belief in me rejuvenated and inspired me.

I also want to acknowledge my extraordinary husband who sustained me through this journey. Phil, thank you for your tangible and unwavering love. You truly walked every step of this journey with me, sometimes knowing what I needed before I did. You took care of things big and small and made many sacrifices – many of which I am sure I don't know of. Your love is so empowering and just what I needed to achieve a dream like this one. Thank you for believing in me on days that I couldn't and for the happy dances on the days that I could. You are one of the greatest gifts in my life.

To conclude, it seems fitting to close this acknowledgement section by recognizing the two individuals who made it possible for me to begin and complete this process. First, my dad was, and is, my forever fan. Pops, thank you for making me your "sunshine" – not only in word but also in action. I can still feel the pride and love that radiated from you whenever I was near. Your pride and love were fierce and unconditional, no matter the day's successes or failures. Being fiercely loved like that made me believe that I was, and am, capable of things like this. What an incredible gift that I will take with me always.

Finally, my mom embodied strength and love. Her actions inspired me daily and led me to this doctoral program. Mama, thank you for showing me what it looks like to be an advocate for those who need it most, without the need for accolades or praise. Thank you for always doing the right, and often hard, work so that I knew that I could do it too. Thank you for giving me a love that was so pure and strong that it has continued to engulf me ever since you left this earth. Thank you for making sure that I knew my worth and own strength so that I could also store your strength within me as a reserve that constantly buoyed me throughout this process. You convinced me that I was capable of big things – including a dream like this. Thanks for letting me stand on your shoulders, Mama. You are, and will always be, my heart.

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LIST OF ABBREVIATIONS

AIC	Akaike Information Criteria
BIC	Bayesian Information Criteria
BMI	Body Mass Index
CDC	Centers for Disease Control
CI	Confidence Interval
CL	Cleft Lip
CLP	Cleft Lip with Cleft Palate
CL/P	Cleft Lip with or without Cleft Palate
CP	Cleft Palate
CVD	Cardiovascular Disease
DFE	Dietary folate equivalents
DQI	Diet Quality Index
DQI-P	Diet Quality Index for Pregnancy
EDD	Estimated Delivery Date
FFQ	Food Frequency Questionnaire
HEI	Healthy Eating Index
IPDTOC	International Perinatal Database of Typical Orofacial Clefts
IRB	Institutional Review Board
LCA	Latent Class Analysis
MDS	Mediterranean Diet Score
NAS	National Alcohol Survey
NBDPN	National Birth Defects Prevention Network
NBDPS	National Birth Defects Prevention Study
NH	Non-Hispanic
NHW	Non-Hispanic White
NTD	Neural Tube Defect
OFC	Orofacial Clefts

OMB	US Office of Management and Budget
OR	Odds Ratio
PAS	Proxy Acculturation Scale
PAF, aPAF, aaPAF, cPAF	Population Attributable Fraction (Adjusted, Average Adjusted, and Crude)
PCA	Principal Component Analysis
RBC	Red Blood Cell
RRR	Reduced Rank Regression
SES	Socioeconomic Status
TBDR	Texas Birth Defects Registry
US	United States

CHAPTER 1: INTRODUCTION AND SPECIFIC AIMS

Orofacial clefts (OFC, including cleft lip, cleft palate, and cleft lip with cleft palate) as a group represent one of the most commonly diagnosed birth defects in the United States (US).[1] The latest prevalence estimates from the National Birth Defects Prevention Network (NBDPN) report that, per every 10,000 live birth, 10.3 are diagnosed with cleft lip without or without palate (CL/P), 6.7 are diagnosed with cleft lip with palate (CLP), 3.5 are diagnosed with cleft lip alone (CL), and 5.9 diagnosed with cleft palate alone (CP).[1]

Hispanic individuals make up the largest minorized racial-ethnic subgroup in the US. While Hispanic health is often understudied, health disparities from inadequate access to healthcare have been noted.[2] The heterogeneity in this population stemming from acculturation may influence observed outcomes as 30% of Hispanic individuals in the US are foreign-born.[2,3] Hispanic, compared to non-Hispanic White (NHW) individuals, have a higher prevalence of CLP (7.7 Hispanic v. 6.5 NHW) but a lower prevalence of CL (2.9 Hispanic v. 4.1 NHW) and CLP (5.5 Hispanic v. 6.6 NHW), per 10,000 live births in the US.[1,4–6]

The etiology of OFC is largely unknown but it has been suggested that a combination of genetic and environmental factors may influence OFC occurrence.[7] OFC develop between the fourth and twelfth week of conception, often before pregnancy is recognized.[8] Therefore, it is important to consider the effect of modifiable risk factors in the larger US population and potential differences in this observed effect by ethnicity. Several OFC risk factors have been thoroughly assessed but few studies have considered the potentially unique impact of these exposures on Hispanic populations. Risk factors established in primarily NHW study samples include, but are not limited to: folic acid supplementation,[9], smoking,[10] alcohol consumption,[11] education,[12] body mass index (BMI),[13] folate deficiency, and pregestational diabetes .[14]; yet other risk factors, such as periconceptional maternal dietary patterns, have rarely been explored.[15–17] Only one study has assessed the effect of a comprehensive diet measure

on OFC in the US.[15] All other studies have focused on the effect of single nutrients on OFC. [15,18] A focus on comprehensive intake considers the effect of realistic food intake and nutrient interactions.

To assess the effect of maternal dietary patterns on OFC and the effect of previously established OFC risk factors specific to Hispanics, this project will utilize the National Birth Defects Prevention Study (NBDPS), a large case-control study from 1997-2011. The NBDPS is optimal for our larger dietary analysis as it provides a robust study sample (n= 2,480 CL/P cases, 1,169 CP cases, and 10,584 controls) with extensive dietary data collected from a modified Willett Food Frequency Questionnaire (FFQ).[19] For our analysis of established risk factors among Hispanics, the NBDPS also provides a large sample size (n= 190 CL cases, 245 CP cases, 483 CLP cases, and 2,830 controls), along with several acculturation variables that will help us better understand the diversity in heritage and cultural identity within this population and its impact on OFC risk. Overall, this project aims to provide an updated estimate on the impact of modifiable risk factors, like periconceptional maternal diet, on OFC and to better understand how these risk factors specifically affect risk among Hispanics in the US.

The overall objective of this project is to better estimate the effect of potential modifiable risk factors, such as periconceptional maternal dietary patterns, on OFC and understand how these risk factors impact Hispanics in the US. The ability to restrict to Hispanic individuals, and partially account for the cultural heterogeneity in this group by considering acculturation, will allow for a better understanding of the effects of maternal diet, a scarcely explored OFC exposure, and previously identified OFC risk factors on a large minority population with a diverse cultural background. Specific aims include:

Aim 1 will estimate the association between periconceptional maternal diet and isolated OFC (infants diagnosed with an OFC but no other birth defect) among all eligible participants (n= 2,480 CL/P cases, 1,169 CP cases, and 10,584 controls) in the National Birth Defects Prevention Study (NBDPS). Dietary patterns one-year prior to conception will be derived from a latent class analysis (LCA), which allows for an efficient description of nutrient interactions. SubAim 1.1 will determine if the association between diet and OFC differs by race/ethnicity. A multi-group LCA will be used to assess whether dietary classes are different across racial/ethnic groups. If classes are similar, primary results will be stratified by race/ethnicity. *We hypothesize that individuals with LCA patterns associated with a*

healthier diet will have a lower likelihood of having an infant with an OFC, and the magnitude of this association will differ by race/ethnicity.

Aim 2 will assess the impact of established risk factors, including periconceptional diet, on OFC among all NBDPS Hispanic individuals (n= 190 CL cases, 245 CP cases, 483 CLP cases, and 2,830 controls). We will estimate the effect of diet (as represented by LCA) and other recognized modifiable risk factors (smoking, alcohol, etc.) on OFC among Hispanic individuals only. SubAim 2.1 will calculate the crude and average adjusted population attributable fraction (aaPAF) for select risk factors. Results will estimate the observed OFC burden attributed to each risk factor. PAFs will then be stratified by acculturation status using the Proxy Acculturation Scale-3 (PAS-3), a validated five-point score that is dichotomized to identify "high" versus "low" acculturation status. Additional aaPAFs will also be created for a NHW subset of NBDPS in order to assess differences in OFC risk factor profiles between Hispanic and NHW individuals. Findings will aid in clarifying the impact of OFC risk factors specifically for Hispanic individuals. *We hypothesize that known OFC risk factors will influence OFC prevalence among Hispanics differently than that of the NHW subgroup and differences will be observed within the Hispanic population by acculturation status.*

This analysis will provide further evidence as to whether periconceptional maternal diet may reduce the risk of OFC in a large US population. It will also investigate the effect of other known OFC risk factors on OFC prevalence trends unique to Hispanics in the US, while considering acculturation status. A focus on modifiable risk factors specific to this diverse minority group could help inform targeted public health messaging and interventions that are relevant to the unique OFC trends observed in Hispanics.

CHAPTER 2: BACKGROUND AND SIGNIFICANCE

2.1. Outcome Background and Significance: Orofacial Cleft

2.1.1. Embryology of OFC

Cleft lip and palate occur in isolation or together between the fourth and twelfth week of pregnancy, as the coordinated formation of the lip, palate, nose, and mouth occur.[20,8] While cleft lip and palate can co-occur, their embryonic development differs.[8,21] The lip usually closes by the eighth week of development, if not earlier. The palate then begins to form. Timing, and physical traits specific to each anomaly, contribute to the varying embryonic makeup of CL and CP.[22]

Embryology of Cleft Lip

Between the fourth and eighth week of gestation, the lip develops.[8] Maxillary prominences, which eventually make up the upper lip, upper jaw, and secondary palate, begin to grow towards the middle of the face and fuse with the lateral nasal process. This fusion begins the formation of the upper lip.[8] The maxillary prominences continue to grow inward in order to fuse the medial nasal prominence on either side, which brings the nostrils closer to one another by the fifth week of gestation. [8] This fusion creates the intermaxillary segment. This segment combines with the maxillary prominences and begins to form the philtrum, the middle part of the upper lip; the primary palate; the nose; and nasal septum. If this process is postponed or a maxillary prominence is absent, a CL will occur. [23] A unilateral CL will occur if the maxillary prominence on the specified side fails to merge with the nasal prominence. A bilateral CL will occur if tissue on both sides fails to merge (**Figure 1**).[23] The failure of the upper lip to fuse may influence the fusion of the palate at a later time in fetal development, which can lead to a CLP.[24] Specifically, the opening(s) in the lip extends further back into the primary, and sometimes secondary, palate as palate formation begins between the fifth and twelfth week of gestation (**Figure 3**). Among all OFC cases, 86% of bilateral CL cases have a CP and 68% of unilateral CL cases have a CP.[23]

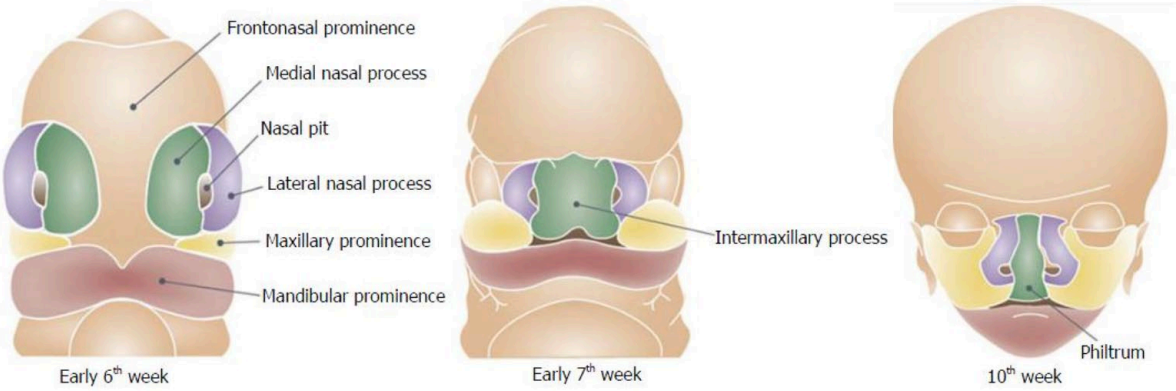


Figure 1. Fetal development of the lip
Figure and caption adapted from Smarius et al. [25]

Embryology of Cleft Palate

Between the fifth and twelfth week of gestation, palate formation begins. When the maxillary prominences join with the medial nasal prominence under the nasal openings, a mass of mesenchymal tissue is formed and the critical primary palate begins to form.[8,23] This palate sits directly behind the gum and ends at the incisive foramen (**Figure 2**).[23] The secondary palate begins as a set of mesodermal projections, stemming from the maxillary prominences. These projections are originally located on either side of the emerging tongue.[23] Over time, these shelves grow towards one another and, during the seventh week, rotate horizontally. By the ninth week, these shelves begin to fuse and, by the twelfth week, convergence is completed.[23] At this time, the hard palate extends from the maxillary and palatine bones to the palatal shelves (**Figure 2**). The part of this palate that does not harden is considered the soft palate and uvula. A CP occurs when the aforementioned fusion fails. [8,23]

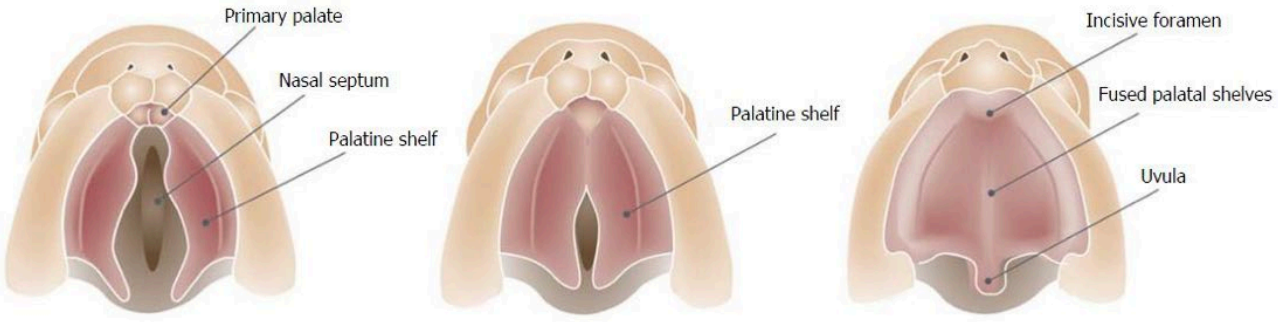


Figure 2. Fetal development of the palate
Figure and caption adapted from Smarius et al. [25]

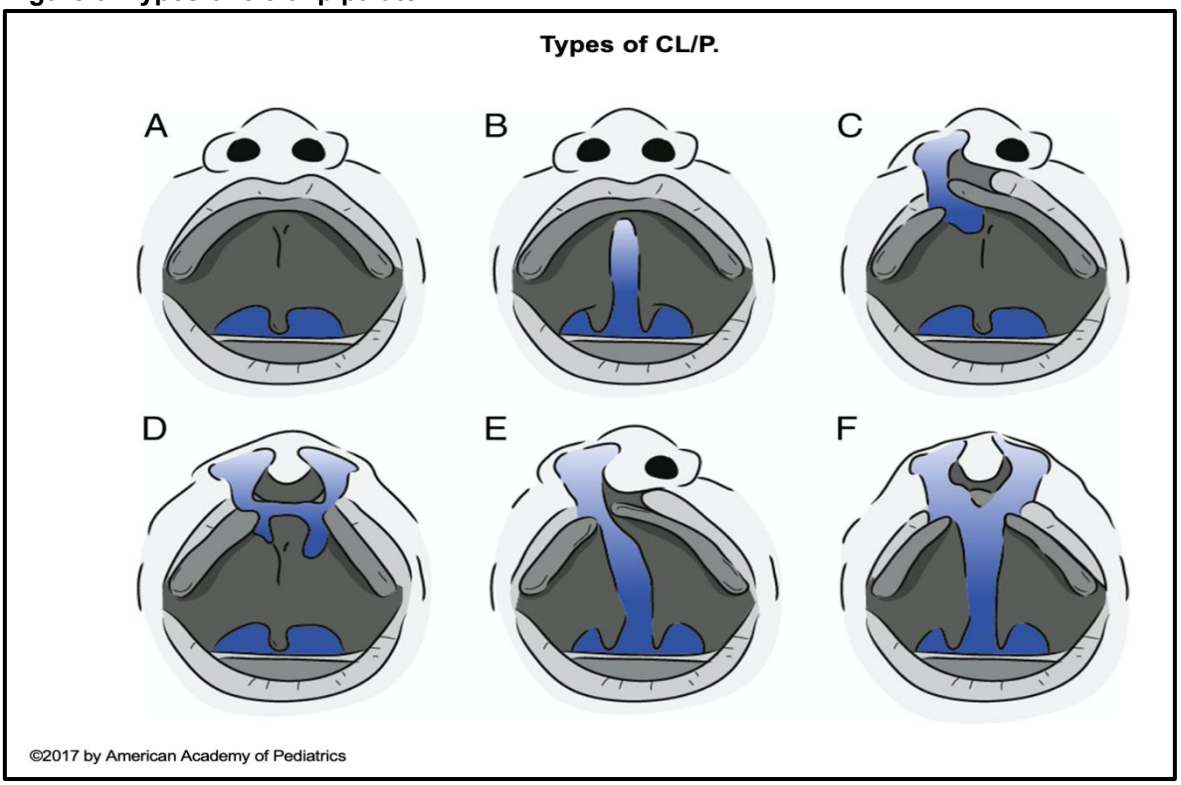
Overall, a CL occurs when the maxillary and medial nasal elevations fail to converge, on one or both sides. CPs occur when the lateral palatine processes fail to merge with one another.[23] CLP occur when the cleft lip extends back into the primary, and possibly secondary palate. CL/P distinctly differ from CP in both etiology and embryology.[23]

2.1.1.1. Clinical characteristics of OFC

Clefts can be diagnosed prenatally; however, the accuracy of these results is questionable. OFC are often found through anatomic two-dimensional ultrasonography, which often occurs between the 18th and 20th week of gestation.[26] One study reported that transabdominal ultrasound screening for CL/P has an estimated sensitivity rate of 88% yet most studies report that CP goes undetected until birth.[25,27] The palate is often obscured by other anatomical structures.[25] Of note, three-dimensional ultrasonography has been reported to have a better diagnostic accuracy overall.[27] It has been suggested that three-dimensional ultrasound screening provides a more accurate view of the fetal palate, which aids in CP detection.[28,29] When available, early detection is key as treatment is often important soon after birth.[26] Regardless of early detection, a clinical evaluation is critical within the first few days of birth.[26]

At birth, a CL presents as a small slit stemming through the lip or a large opening that stretches into the nose (**Figure 3**). A CL can develop unilaterally, on one side of the lip, or bilaterally, on both sides of the lip.[23] Severity ranges from a small notch on the lip to bilateral clefts that detach the upper lip philtrum and premaxilla from the maxillary arch.[23] A CP presents as a gap in the hard and/or soft palate.[20] The co-occurrence of CL and CP (CLP), occurs when the CL (unilateral or bilateral) spans from the incisive foramen to the palatine suture, which is located in the middle of the palate (**Figure 3**).[23] CLP severity can vary.[23]

Figure 3. Types of cleft lip/palate



(A) Normal. (B) Cleft palate alone. (C) Unilateral cleft lip and alveolus. (D) Bilateral cleft lip and alveolus. (E) Unilateral cleft lip and palate. (F) Bilateral cleft lip and palate.
Caption and figure adapted from Lewis et al.[30]

OFC cases can occur without the presence of another defect (isolated), with other birth defects (non-isolated), or as part of a known syndrome (syndromic).[31] A recent study using the Texas Birth Defects Registry (TBDR) found that 19.1% of CL/P cases and 21.6% of CP cases were non-isolated.[32] Heart anomalies were the most common co-occurring defect.[32] The International Perinatal Database of Typical Orofacial Clefts (IPDTOC) estimated that, internationally, 76.8% of OFC cases are isolated, 15.9% of OFC cases are non-isolated, and 7.3% of OFC cases are syndromic.[33] Among the 12 US areas included in this analysis, the distribution was similar. It was estimated that, in the US, 74.6% of OFC cases are isolated, 17.3% are non-isolated, and 8.1% are syndromic.[33]

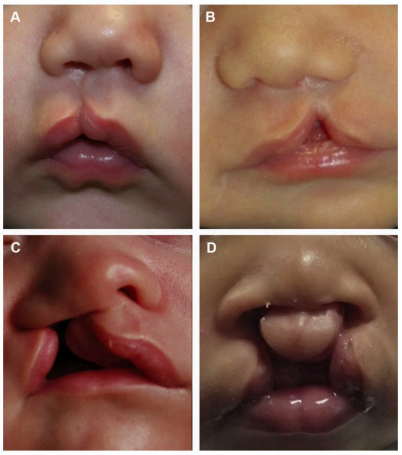
Specific to OFC phenotype, CL/P is often non-syndromic with only 10% of cases having a related syndrome.[23] Among cases with CL only, 30% of cases have an associated syndrome (such as Van der Woude or Waardenburg syndromes).[23] Among cases with CP only, 50% of cases have an associated syndrome (such as Apert, Stickeler, or Treacher-Collins syndrome).[23] OFC management and care is often dependent on whether the OFC is syndromic.[34]

Clinical classification is dependent on development in utero and the magnitude of the physical impairment.[23] A CL can be labeled as microform, incomplete, or complete.[26] A microform CL is a minor anomaly in which there is a notch at the vermillion-cutaneous junction but all lip tissue is present (**Figure 4A**).[26] An incomplete CL occurs when tissue disconnects at the orbicularis oris.[26] This classification varies in the amount of skin that is involved in the anomaly (**Figure 4B**).[26] A complete CL includes a Simonart band, soft tissue that expands through the superior part of an incomplete CL at the nasal sill.[26] Complete CLs extend through the lip and the nasal sill. The orbicularis oris is then abnormally inserted in to the ala and columella (**Figure 4C**).[26] When a complete CL is bilateral, the intermaxillary segment is displaced and the orbicularis oris is missing from the intermaxillary segment (**Figure 4D**).[26]

CP classification can also vary by development and severity.[23] Submucous CPs occur when there is a gap in the palatal musculature yet the mucosa lies over this gap; thus, detection of submucous CPs can be hard.[26] Characteristics of a submucous CP may include a notch in the hard palate and bifid uvula or a blue line in the soft palate, caused by the musculature deficit and subsequent transparency in this region of the mouth (**Figure 5A**).[26] A CP in the secondary, softer, palate stretches from the incisive foramen to the soft palate and ends at the uvula.[26] Alternatively, a CP in the primary, harder, palate spans from the palate anterior to the incisive foramen and ends at the alveolar arch.[26] The combination of a primary and secondary CP is called a complete CP (**Figure 5D**).[26]

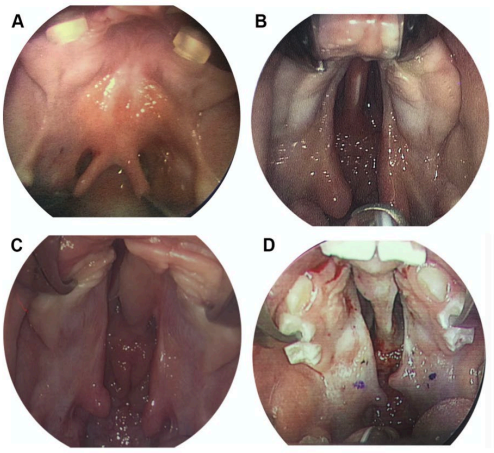
Postnatally, there are many OFC classification scales and categorization schemes. [8,34,35] Currently, there is not one universal classification system; however, most, if not all, consider: laterality, width, completeness, and atypical tissue surrounding the cleft.[35,36] These clinical classification systems often differ by their focus on an embryologic or anatomical evaluation.[37] Accurate classification is critical for effective treatment plans.

Figure 4. Types of Cleft Lip



(A) Microform right CL. (B) Incomplete left CL. (C) Complete right CL. (D) Bilateral complete CL.
Caption and figure adapted from Worley et al.[26]

Figure 5. Types of Cleft Palate



(A) Submucous CP. (B) Incomplete CP. (C) Unilateral complete CP. (D) Bilateral complete CP.
Caption and figure adapted from Worley et al.[26]

2.1.1.2. Recommended clinical care in the United States

The effectiveness of preventive OFC interventions is limited by the largely unknown etiology of OFC and the early critical window of infant lip and mouth development; thus, interventions are primarily, if not solely, performed after birth.[20,38] OFC can result in problems with feeding, speech, hearing, and psychological wellbeing, which are all associated with increased long-term morbidity and mortality risks.[30] Medical, surgical, dental, and social aspects of OFC must be considered for treatment plans. Coordinated, interdisciplinary teams are necessary to provide optimal care.[39] While surgical repairs often begin at three months of age,[26,40] it is imperative that evaluation and other treatments are initiated within the first few days after birth so that newborn feeding efficacy can be assessed and treatment plans can be created.[40] The American Cleft Palate-Craniofacial Association suggests that early surgical repairs are optimal. Specifically, CL and CP repairs should be performed by at least 12 and 18 months, respectively.[38]

Treatment often includes surgical interventions, extensive dental treatments, speech therapy, and psychological care.[20] Multiple medical interventions are often required throughout the lifespan to correct OFC.[41] OFC often impose both psychosocial and economic hardships on families and society. US hospital charges associated with OFC average up to $126,000,000 per year.[38]

Notable disparities in the quality of clinical care for OFC exist within the US.[42–47] For instance, Mullen et al. found that Black and Hispanic patients had an increased risk of hospital readmission after CL or CP repair.[42] Wu et al. noted that, compared to their NHW counterparts, these minority subgroups were also more likely to incur higher hospital charges for CP repair. They further suggested that Black patients undergoing CP repair had a higher prevalence of complications and extended stays compared to non-Black patients.[45]

Disparities in access to care have also been observed. Minority patients, including Hispanic individuals, often have a longer delay in repair compared to NHW patients.[39,43,44,47] Spanish as a primary language has previously been associated with delays in OFC repair; however, results from the same study also suggested that delays were associated with non-Hispanic ethnicity, which is contrary to other published findings.[46] OFC repair has also been associated with insurance coverage.[47] In 2019, 18.7% of Hispanic individuals were uninsured, noticeably higher than the 6.3% of uninsured NHW individuals in the US.[48] Additionally, in 2014, it was reported that Hispanic individuals had a household income that was 52% lower than that of NHW individuals in the US, which is important to note as time to OFC repair has previously been associated with income.[43,47,49] High charges incurred by patients may partially result from the common use of teaching hospitals to perform repairs, which is costly but is also associated with fewer surgical complications.[50]

Overall, minority status in the US has been associated with discrimination in the healthcare system along with decreases in both quality and access to care, all of which influence OFC care and repair.[51–54] It is also possible that quality and access to care may differ by acculturation status among Hispanics living in the US. Increased years in the US and English as a primary language, both single indicators of acculturation, may increase both access and quality of care.[55]

2.1.2. Descriptive epidemiology of OFC

2.1.2.1. Prevalence by race and ethnicity in the United States

OFC are one of the most prevalent birth defects in the US.[1] From 2010-2014, the National Birth Defects Prevention Network found that the following prevalence estimates, per 10,000 live births, were 10.25, 6.67, 3.51, and 5.91 for CL/P, CLP, CL, and CP, respectively. Upon adjustment for maternal

race/ethnicity, the prevalence estimates were 10.0 6.4, 3.6, and 5.9 for CL/P, CLP, CL, and CP, respectively.[1,4–6]

OFC patterns among race and ethnicity groups vary by OFC type (**Table 1**).[1] However, across all OFC types, non-Hispanic American Indians/Alaska natives have the highest OFC prevalence while non-Hispanic Black individuals tend to have the lowest OFC prevalence.[1,4–6] Hispanic individuals, compared to NHW, have a lower prevalence of CL and CP, but a higher prevalence of CLP. A similar trend is observed when comparing Hispanic estimates to non-Hispanic Asian/Pacific Islander estimates and to larger US prevalence estimates that combine all racial/ethnic groups (**Table 1**). In contrast, the prevalence of all Hispanic OFC phenotypes is higher than that of non-Hispanic Black individuals, but lower than that of all non-Hispanic American Indian/Alaska Native individuals (**Table 1**).[1]

Table 1. OFC prevalence (per 10,000 live births) by race/ethnicity in the NBDPN from 2010-2014[1]

	Total Prevalence (95%CI)	NH White Prevalence (95%CI)	NH Black Prevalence (95%CI)	Hispanic Prevalence (95%CI)	NH Asian/ Pacific Islander Prevalence (95%CI)	NH American Indian/ Alaska Native Prevalence (95%CI)
CL/P	10.3 (10.0, 10.5)	10.7 (10.3, 11.1)	6.6 (5.9, 7.2)	10.6 (10.1, 11.1)	9.4 (8.2, 10.7)	15.2 (12.5, 18.4)
CLP	6.7 (6.5, 6.9)	6.5 (6.2, 6.9)	4.0 (3.6, 4.5)	7.7 (7.3, 8.1)	5.8 (4.9, 6.8)	9.8 (7.7, 12.4)
CL	3.5 (3.4, 3.7)	4.1 (3.8, 4.3)	2.5 (2.1, 2.9)	2.9 (2.7, 3.2)	3.7 (3.0, 4.6)	5.2 (3.7, 7.2)
CP	5.9 (5.7, 6.1)	6.6 (6.3, 6.9)	4.1 (3.6, 4.6)	5.5 (5.2, 5.9)	6.2 (5.2, 7.2)	6.7 (5.0, 8.9)

Abbreviations: NBDPN: National Birth Defects Prevention Network; NH: Non-Hispanic
1. Values from Mai et al.[1]

2.1.3. Hispanicity, acculturation, and birth outcomes in the US

Roughly 18.5% of the US population self-identifies as “Hispanic,” “Latino,” or “Latinx.” These terms are often used interchangeably and inconsistently.[56,57] For the purposes of this project, the term “Hispanic” will refer to any individual who self-identifies with Latin American or Spanish heritage.[58,59] Within the last ten years, it is estimated that the Hispanic population has accounted for half of the population growth in the US. Specifically, the Hispanic population grew by 23% and is expected to

account for 25% of the entire US population by 2035.[59] Hispanics are the largest US minority group to date.[48]

There is great heterogeneity within the Hispanic subpopulation.[60–62] Approximately 30% of all Hispanic individuals in the US are foreign-born.[2] Health outcomes differ within the Hispanic population and, in part, are influenced by nativity.[63–68] Country of origin varies among foreign-born Hispanics, resulting in notable heterogeneity in both culture and genetics.[69] Cultural differences result in varying distributions of factors that influence health, such as education, income, access to health insurance, health behaviors and concerns, and beliefs on assimilation.[63,64,69,70]

It is essential to consider how acculturation influences health outcomes.[61,68,71] Acculturation is defined as the multifaceted social process in which an individual acclimates to, or internalizes, their new host culture.[61] Theories regarding the impact of acculturation on health suggest that health risks and behaviors change over time as an individual assimilates to their new host culture.[61,68,71] This can lead to the internalization of both healthier and unhealthier behaviors, dependent on the native and newly dominant culture.

Fox et al. explained the acculturation process and considered the sociocultural context in which it occurs.[72] The process begins when an individual has extended exposure to a new cultural group. Acculturative stress may occur if one's native cultural beliefs, habits, or customs diverge from those of their new host culture.[72] Exposure may encourage unhealthy behaviors and, to some extent, lead to internalization of the dominant culture.[61] The sociocultural context in which acculturation occurs impacts lifestyle and environmental exposures, acculturative stress, and the unhealthy behaviors accrued through the process.[61] Fox et al. defined this sociocultural context as: neighborhood composition, discrimination, attitudes towards assimilation, and the host culture's policies and resources (**Figure 6**).[61] They further suggested that several sociocultural features impact acculturative stress and health behaviors only. These features include: differences in the native and host culture environment (i.e. population size, urbanization, and socioeconomic conditions), cultural norms (i.e. predominant language, religion, and work norms),[73] and migration selection bias.[61] Overall, the impact of internalization on health is mediated by acculturative stress and health behaviors and this process is influenced by the sociocultural context in which it occurs (**Figure 6**).[61]

Figure 6. Proposed impact of acculturation on health by Fox et al.

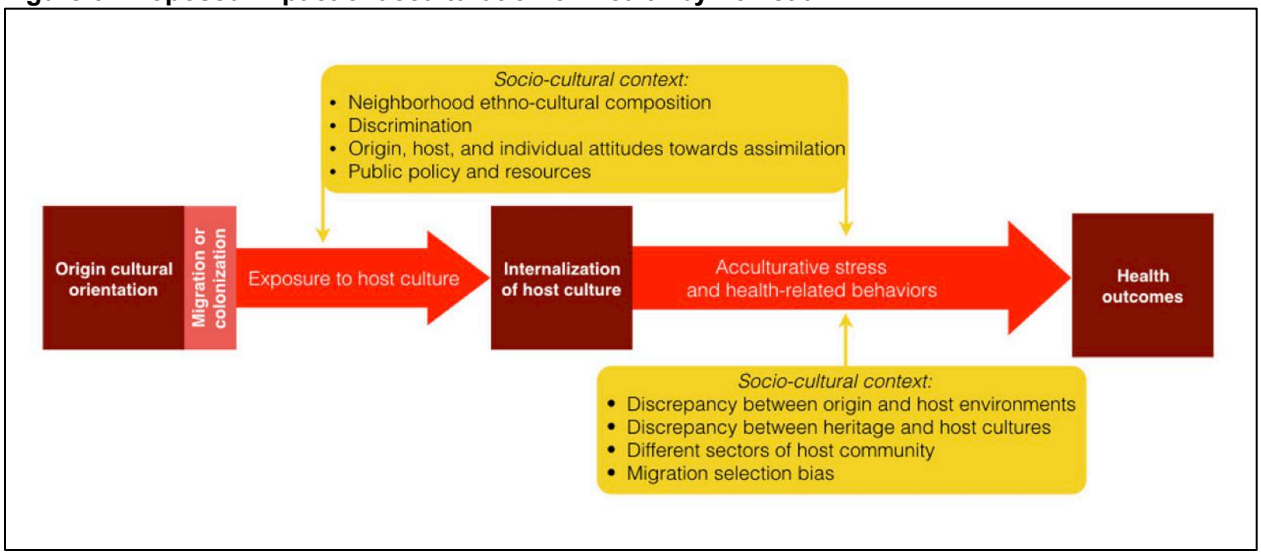


Figure adapted from Fox et al.[61]

Research suggests that this process has varied effects on health, although study results are inconsistent.[61,64] This is similar to the small body of research that has assessed the impact of acculturation on OFC specifically. For instance, Zhu et al. found that, among Hispanics in New York, foreign-born individuals had a lower prevalence of CP compared to their US-born counterparts.[74] The consideration of country of origin also impacted OFC prevalence as, compared to US-born Hispanics, individuals from Mexico had a decreased prevalence of CP but an increased CLP prevalence.[74] A decreased prevalence of CP was also observed in individuals from Cuba and Central and South America.[74] Alternatively, three studies found no difference in the risk of OFC by nativity[75–77] or country of origin among Hispanic individuals.[75] The most recent study utilized data from 2007. Of note, the aforementioned studies are vulnerable to migrant selection bias and solely evaluated acculturation through nativity or country of origin.[78] To our knowledge, current research has not explored the relationship between OFC and acculturation measures that better reflect the acculturative process proposed by Fox et al. (**Figure 6**). More recent studies are needed as the impact of acculturation is partially dependent on the current social climate of the host culture.[61]

Several other birth outcomes have also been assessed in relation to acculturation. Some, but not all, studies have observed heterogeneity in birth outcomes. For example, the Centers for Disease Control (CDC) found that the 2018 infant mortality rate, per 1,000 live births, was higher among all Hispanics (4.9)

than all NHWs (4.6).[79] However, when the Hispanic rate was stratified by nativity, the Puerto Rican infant mortality rate was higher (5.6) than both the overall Hispanic and NHW rate. Conversely, the Cuban infant mortality rate was lower (3.8) then both rates.[79] Without stratification by this measure of acculturation, the diversity in these rates would be missed. Heterogeneity in birth outcomes by acculturation, broadly defined, has also been observed in several birth defects, such as neural tube defects (NTDs).[75,80,81] For example, Carmichael et al. found that Hispanic individuals who were US-born and reported English as a primary language or those born outside of the US had increased risk of spina bifida, compared to US-born Hispanic individuals who reported Spanish as a primary language.[81] Other birth defects that may also differ by acculturation include: select heart defects,[75] anotia and microtia,[75,82] craniosynotosis,[74] gastroschisis,[83] trisomy 13, 18, and 21,[77] pyloric stenosis,[77] and hypospadias.[77] Research has also suggested that other birth outcomes such as preterm birth,[84–87] preeclampsia,[84] and birthweight[71,88,89] differ by various measures of acculturation status. Acculturation in birth outcomes research is mostly measured by a single indictor. Few studies consider the complex acculturation process; thus, the use of a more comprehensive and uniform measure across studies would aid in clarifying the effect of acculturation on prenatal health.

2.2. Exposure Background and Significance: Maternal Diet and Other Established Risk Factors

2.2.1. Maternal diet background and significance

Maternal diet quality has been found to have a critical influence on fetal growth and development, especially during the periconceptional and early pregnancy period.[90,91] Therefore, maternal nutrition must be enhanced for most individuals prior to conception so that the preimplantation environment is optimized for both fetal and placental development.[92,93] The fetus is constantly assessing its nutritional availability and regulates its development accordingly.[92] If inadequate maternal nutrition occurs at conception or during pregnancy, fetal growth is limited (**Figure 7**).[92] This then leads to an increased insulin response and decreased growth in muscle, bone, and nephrons at birth.[92] Further, each negative adaption that occurs in-utero increases the risk of poor health outcomes throughout the life course.[91,92,94,95]

Therefore, there are dietary recommendations specific to pregnancy.[92,96,97] Dietary requirements are unique during this time as pregnancy increases the body's need for nutrients and dietetic energy.[98,99]

Because maternal diet is modifiable and essential to the long-term health of the fetus, it is an important exposure to focus on in order to increase both maternal and fetal health. Further, existing periconceptional/prenatal clinic visits may be optimal for nutritional interventions.

Figure 7. Proposed model of how maternal diet impacts fetal development

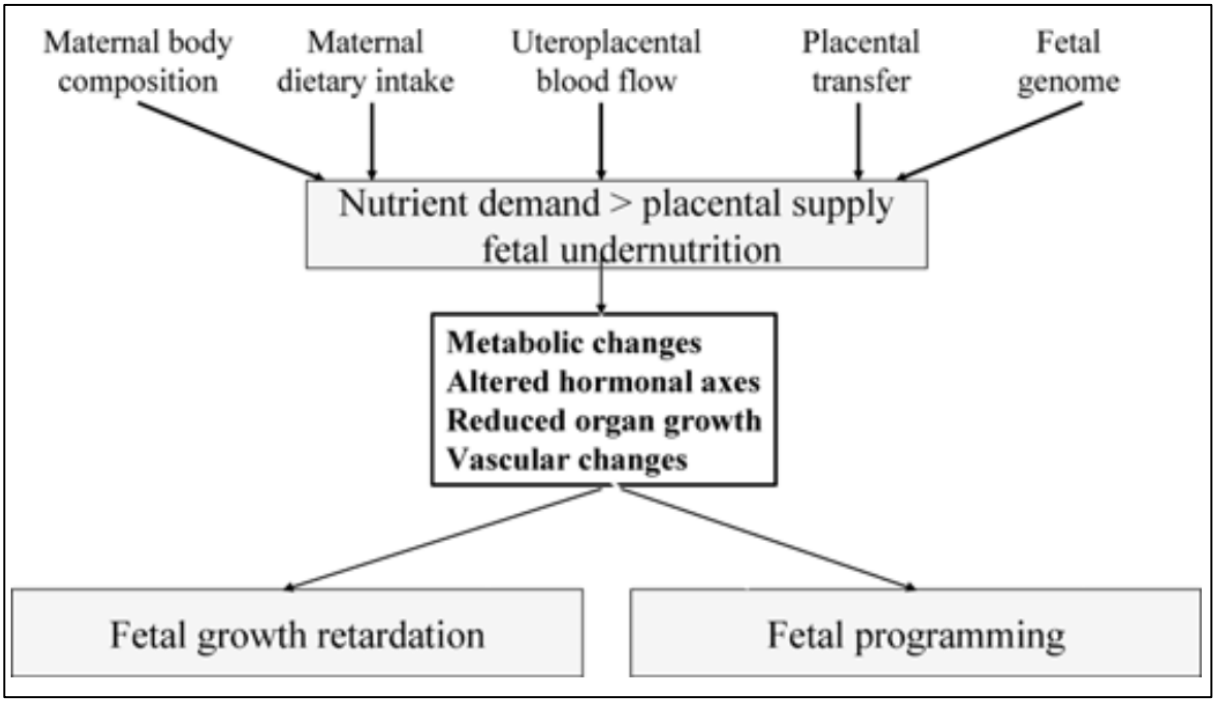


Figure adapted from Phillips et al. [95]

2.2.1.1. Measures of maternal diet

There are multiple methods to assess diet. These methods include a focus on individual nutrients, dietary indices, or dietary patterns established a posteriori. While many previous studies have evaluated diet by assessing individual nutrient intake, current literature suggests that it is important to consider the complexity in which nutrients interact with one another.[92] Evaluating nutrition through indices or patterns gives insight into the biologic mechanism in which nutrients interact.[100,101] This is especially important to consider as nutrients are not often consumed in isolation.[92] Indices and patterns offer a more holistic assessment of dietary intake.[92]

Diet quality indices utilize current nutrition hypotheses and dietary recommendations to holistically evaluate, and score, an individual's dietary intake.[102] There are several validated diet quality indices that are often used to evaluate maternal diet, either periconceptionally or prenatally. Diet quality indices score

food, nutrients, and, sometimes, lifestyle factors based on how they compare to established dietary guidelines.[103] Highly recognized indices include the Diet Quality Index (DQI),[104] Healthy Eating Index (HEI),[105,106] the Alternative Healthy Eating Index,[107] the Mediterranean Diet Score (MDS),[108] and the Healthy Food Diversity Index.[103,109] Because there are unique nutritional requirements during pregnancy, it has been argued that indices should be created specifically for prenatal needs. For example, one study found that the validated HEI did not emphasize the vitamin and mineral needs specific to a group of pregnant individuals.[110,111] Indices specific to prenatal nutrition include the Alternative Healthy Eating Index for Pregnancy [112,113] and the Diet Quality Index for Pregnancy (DQI-P).[111,114]

Nutrition intake can also be assessed through the creation of dietary patterns defined a posteriori. Identification of patterns is data-driven, rather than defined based on criteria that are defined a priori.[115] Because these patterns are data-driven, they may provide a more realistic representation of the variation in nutrient intake within a study population.[116] Patterns often rely on information from a dietary intake assessment like the validated Willett FFQ.[117] Once nutrient information is collected from study participants, nutrient intake is analytically evaluated to create dietary patterns. Analytical techniques include principal component analysis (PCA),[101] factor analysis,[101,118] cluster analysis,[101,118] and latent class analysis (LCA).[115]

2.2.1.1.1. Latent class analysis for maternal diet

This project will explore maternal dietary intake using a LCA. LCA is a data reduction technique that identifies underlying latent classes (dietary patterns) in a population. LCA will help us better understand unknown dietary patterns within the NBDPS. Individuals are assumed to belong to one exclusive, unknown class. To derive these classes, a multinomial regression model is created and may include covariates (such as energy intake) to help predict classes (dietary patterns). This model considers the relationship between the observed variables of interest (food consumption frequency) assuming participants belong to one of *K* mutually exclusive classes (dietary patterns) but for which class membership is unknown. The number of classes is specified before the model is run, so multiple models are usually run to identify the number of classes that best fit the data. Model fit, and subsequently the

optimal number of derived classes, is assessed by considering the Akaike Information Criteria (AIC), Bayesian Information Criteria (BIC), entropy value, and interpretability of results for each model.

The final model outputs regression coefficients for class membership (for each class along with any covariates included in the model) and the conditional probability of each indicator (relative consumption of each food item), given a specific class. These probabilities are then combined using Bayes' theorem to create a class membership probability for each participant. Participants are then assigned to the class with their highest probability of membership. A LCA also allows for multi-group analysis, which tests for measurement invariance of classes (dietary patterns) between population subgroups (racial/ethnic groups).

LCA differs from other data reduction methods by its ability to estimate class membership probabilities, instead of just assigning participants to one permanent class.[119] This probability is important as it allows us to better understand how well a participant fits into their assigned dietary pattern. By restricting analyses to individuals with high probabilities only, we are able to evaluate the impact of low membership probabilities on our estimates. These probabilities also help us assess the homogeneity of dietary responses within each pattern. LCA has been previously recommended for the assessment of maternal diet, especially for understanding the effect of exclusive classes (dietary patterns) on outcomes of interest.[115] Patterns created by an LCA provide flexibility to capture complex dietary patterns based on realistic nutrient and food consumption.[115]

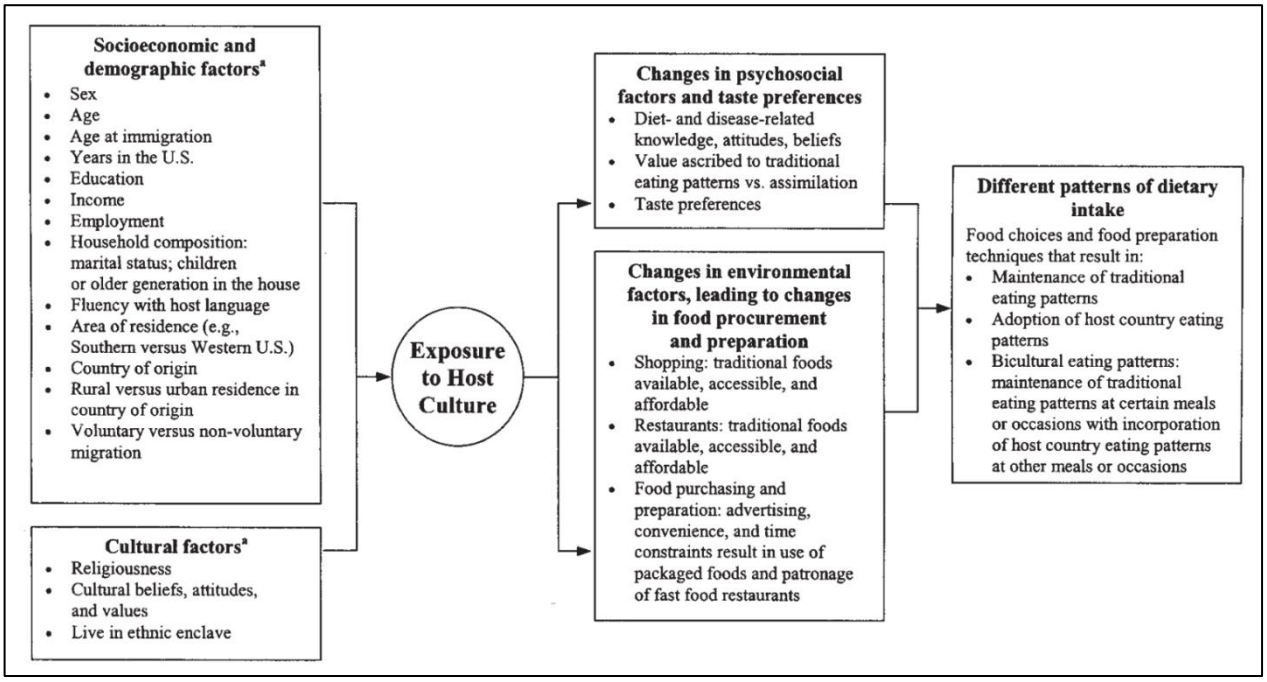
In general, the evaluation of dietary patterns allows for a better understanding of the metabolic pathways in which everyday foods interact, and ultimately influence maternal and fetal health.[115,120] Increasingly, health professionals have been focusing on dietary patterns as evidenced by a shift in focus from nutrient to pattern-based dietary guidelines in the *Dietary Guidelines for Americans, 2020-2025*.[121]

2.2.1.2. Maternal diet and acculturation

Less acculturated Hispanic individuals, compared to US-born and more acculturated Hispanic individuals, have been found to have a healthier diet.[122–124] For example, a systematic review from 2008 found that less acculturated Hispanic individuals in the US had a higher intake of fruit, rice, and beans and a lower intake of sugar, compared to more acculturated individuals.[125] Another review found that less

acculturated Hispanic individuals often retained a diet similar to that of their Latin American heritage, while more assimilated individuals consumed a more westernized diet.[126] Due to these trends, individuals who are less acculturated are more likely to abide by national dietary guidelines compared to their assimilated counterparts.[126] As Hispanic immigrants begin to assimilate to their host country, they start adopting the nutritional practices of that country. This is known as dietary acculturation.[126,127] Satia-Abouta et al. propose a model for dietary acculturation in which an individual's socioeconomic, demographic, and cultural factors impact their exposure to a new host culture. This exposure then influences changes in psychosocial factors, dietary preferences, and environmental factors. Over time, these changes influence actual dietary patterns (**Figure 8**).[127]

Figure 8. Proposed model of dietary acculturation: The process by which racial/ethnic immigrant groups adopt the eating patterns of their host country



[a] Some factors may also be influenced by host country.
Figure and caption adapted from Satia-Abouta et al.[127]

A small body of literature has evaluated the impact of acculturation on maternal diet among pregnant Hispanic individuals. Results are slightly dated, inconsistent, and specific to small geographic locations.[128–133] In 2004, Harley et al. evaluated measures of acculturation and prenatal dietary intake among low-income individuals of Mexican descent living in California. Individuals who were born in

Mexico had a significantly higher consumption of calories, fiber, vitamin A, vitamin E, vitamin C, folate, calcium, and zinc in their diet, compared to their US-born counterparts.[128] Foreign-born individuals were also more likely to meet national prenatal dietary recommendations, compared to their US-born counterparts.[128] In 2011, Hromi-Fielder et al. evaluated the influence of country of origin on dietary intake among Hispanic individuals in Connecticut. Specifically, participants were divided into groups based on Puerto Rican descent (yes/no), regardless of nativity. Compared to Puerto Rican individuals, non-Puerto Ricans were more likely to consume more fruits and vegetables and less processed foods and sweetened beverages.[130] Of note, non-Puerto Rican individuals were less acculturated (defined as foreign-born, fewer years in the US, and more likely to speak Spanish) than the Puerto Rican group.[130]

A more dated study from 1989-1991 in California assessed the effect of nativity on maternal diet three months prior to conception. Results suggested that foreign-born Hispanics, compared to US-born Hispanics and NHWs, had the highest intake of carbohydrates, fiber, cholesterol, fiber, protein-rich foods, products with grain, folate, vitamin C, iron, and zinc.[131] Foreign-born Hispanics also had the lowest fat to energy intake contribution.[131] In 2004, a larger literature review suggested that healthier pregnancy outcomes often observed in Mexican immigrants, compared to US-born individuals, may partially stem from a healthier prenatal diet.[133] Alternatively, in 1999, Gutierrez found that duration in the US did not impact adequacy of prenatal diet among a small sample of individuals who identified as Mexican American.[132] Overall, it has been suggested that cultural beliefs on conception, fetal development, and maternal health influence prenatal health behaviors. It is possible that these beliefs result in differences in prenatal diet by cultural identity.[132] Therefore, further research is needed to understand how cultural identity, including acculturation status, may influence changes in prenatal diet.

2.2.1.3. Maternal diet and OFC

During the critical OFC development window, embryonic nutritional status is completely reliant on maternal dietary consumption and metabolism.[134] Maternal nutrition largely impacts gene expression for embryonic tissue development, which greatly influences the risk of OFC.[134] The effect of several isolated nutrients on OFC has been previously studied. Many studies have assessed the impact of folic acid-

containing supplements, including multivitamins, on OFC[135–144] since folic acid has been proven highly effective in reducing the risk of NTDs, which occur during the same critical development window as OFC.[145] Although study results have been mixed, two meta-analyses, one as recent as 2018, suggest that findings in aggregate estimate a modest protective effect of folic acid-containing supplements on OFC occurrence.[9,146] The effect of dietary folate has also been found to have a modest effect on OFC, which is especially relevant since the start of mandatory folic acid fortification in the US.[138,141,143,147,148] Since fortification, a significant drop in NTD prevalence (roughly 19%) has been observed,[149] but the magnitude of its effect on OFC remains unclear. Yazdy et al. found a slight decrease in OFC post-fortification (PR: 0.94, CI: 0.92-0.96); however, upon stratification, this decline was only observed among NHW individuals, nonsmoking individuals, or those who received prenatal care early in pregnancy.[150] Canfield et al. noted a larger reduction, but only for CP (PR: 0.88, CI: 0.82-0.95).[151] When restricted to Hispanic individuals, the reduction was even more notable (PR: 0.80, CI: 0.66, 0.97).

Other nutrients have also been evaluated in the context of OFC risk. Wallenstein et al. estimated the nutrient intake of individuals in a California case-control study. They found that, among individuals who did not take vitamin supplements, the risk of CLP and CP was at least doubled with low intake of vitamin B12, riboflavin, and calcium. The risk of CP was further influenced by riboflavin, magnesium, and zinc, while the risk of CLP was also influenced by niacin.[18] Alternatively, Shaw et al. found that, among individuals who did not take vitamins in the NBDPS, increased protein, choline, and methionine were associated with a decrease in CLP and increased cysteine was associated with a decrease in CP. However, upon adjustment for all other nutrients, iron and riboflavin were the only nutrients that proved protective (for CLP only).[15] Nutrients such as vitamin A, iron, magnesium, β carotene, thiamine, pyridoxine, and zinc have also been studied. Most nutrients are found to decrease OFC in some populations but have a null effect in others.[152–156] Further, some studies suggest that the effects of certain nutrients are only found among certain subsets of individuals (e.g. those who take vitamins v. those who do), which makes comparison across studies difficult. It is important to consider the impact of isolated nutrients and holistic dietary patterns on OFC as both are modifiable.

Dietary patterns allow investigators to understand the synergistic effect of consuming multiple nutrients at once. A limited body of literature suggests that healthier periconceptional maternal dietary

patterns are associated with a decreased prevalence of OFC in infants.[16,17,114] This association remains after controlling for folic acid supplementation and food folate. Carmichael et al. utilized the MDS and DQI-P, both defined a priori.[114] Results suggested that higher (healthier) quartiles of both patterns were associated with a decreased risk of OFC in a large sample from the NBDPS.[114] Interestingly, Hispanic individuals were more likely to be in these higher dietary quartiles.[114] This is the only study conducted in the US, which is important to note since region of residence can largely influence diet.[114,157]

Vujkovic et al. conducted a factor analysis in which dietary patterns were derived a posteriori (similar to our analysis).[16] The analysis found that an unhealthy diet (defined as the highest v. lowest tertile of their "Western" diet) was associated with an increased risk of CL/P among a sample of Dutch women.[16] However, the highest (v. lowest) tertile of the "Prudent" diet had a null effect on CL/P occurrence. This study also included the ascertainment of blood samples from a large sample of their population (n=170), which measured folate deficiency through serum folate, red blood cell (RBC) folate, vitamins B6 and B12 levels, and total plasma homocysteine. Results suggested that measures of folate deficiency increased as with higher levels of the "Western" diet. Finally, Neogi et al. found that exclusive vegetarianism (defined a priori) was associated with an increased risk of OFC in India.[17] Neogi posited several explanations for their findings, one being that vegetarianism is often associated with a vitamin B12 deficiency, which may influence folic acid metabolism.[158] However, this study was unable to account for the heterogeneity of dietary patterns within subgroups of vegetarian and non-vegetarian diets.

It is difficult to disentangle the effect of isolated nutrients from comprehensive dietary measures, aside from controlling for folic acid supplementation. Both Carmichael and Vujvokic considered the association between their derived dietary patterns and individual nutrients. Carmichael found that the DQI, v. MDS, had a noticeably higher correlation with nutrient categories and that higher nutrient intake was associated with higher quartiles of both indices. Vujvokic found that higher "Western" tertiles were associated with a decrease in vitamins B12 and B6, along with RBC folate, and an increase in homocysteine. Participants in the higher tertiles of the "Prudent" diet had higher levels of polyunsaturated fats, β carotene, fiber, B12, and folate. While further research is needed to better understand how to isolate the effect of specific nutrients from overall dietary trends, it also important to acknowledge that

these holistic measures are attempting to capture nutrient interactions unique to each dietary group and their combined effect on OFC.

2.2.1.4. Maternal diet and other birth defects

Optimal periconceptional and prenatal diet reduces the risk of other birth defects as well.[98] Aside from individual nutrients, several studies have found that specific dietary patterns reduce the risk of certain birth defects. The effect of holistic diet measures on NTDs has been of specific interest since it has already been proven that NTD occurrence is strongly influenced by a single dietary exposure, folic acid.[159] This finding not only impacted prenatal dietary recommendations at a global level but also led to policy changes, such as mandatory folic acid fortification in the US.[159–161] When Carmichael et al. evaluated diet quality in relation to OFC in the NBDPS, they also evaluated the association in NTDs. After adjustment for relevant covariates including folic acid supplementation, they observed that, among selected NTDs, anencephaly was most strongly influenced by diet. Individuals in the highest quartile of the MDS had 0.64 (95% Confidence Interval [CI]: 0.45, 0.92) times the odds of having an infant with anencephaly compared to those in the lowest quartile. A similar pattern was observed when comparing individuals in the highest and lowest quartiles of the DQI (Odds Ratio [OR]: 0.49, 95%CI: 0.31,0.75).[114] Sotres-Alvarez et al. also assessed the impact of maternal diet on NTDs in the NBDPS, but measured diet through patterns derived a posteriori using a LCA, similar to our dietary assessment.[162] Four dietary pattern classes were derived from NBDPS FFQ data and labeled as: Prudent, Western, low-calorie Western, and Mexican. Among individuals who did not report folic acid supplementation, the Prudent diet, compared to all other patterns, was found to significantly decrease the risk of NTDs.[162] Vujkovic et al. also assessed the effect of diet on NTDs using a PCA, another data reduction method. They found that a pattern similar to the Mediterranean diet reduced the risk of spina bifida.[163] Most recently, Desrosiers et al. assessed the effect of a low carbohydrate diet on NTDs and found that individuals who reported restricted carbohydrate intake were more likely to have an infant with an NTD, compared to those who did not. This effect remained after controlling for folic acid supplementation.[164]

Among other NBDPS studies, researchers have observed that higher periconceptional diet quality is associated with a decreased risk of rare eye malformations,[165] biliary atresia,[166] and selected heart

defects.[162,167] Interestingly, Feldkamp et al. found that better diet quality was associated with a decreased risk of gastroschisis only among Hispanic and foreign-born individuals.[168] To date, better diet quality has not been found to reduce the risk of microtia or hypospadias among the NBDPS study population.[169,170] Diet quality and birth defect risk has also been assessed outside of the NBDPS. For example, international studies have suggested that higher diet quality, measured periconceptionally or prenatally, is associated with a decrease in both hypospadias[171] and congenital heart defects.[172]

The aforementioned studies evaluated diet differently at different time points, which has important implications for interpretation. Several studies assessed maternal diet through pre-specified indices such as the DQI, DQI-P, and MDS.[114,165–168,170] Other studies evaluated maternal diet through the creation of a posteriori patterns.[162,163,165,171,172] Statistical methods to create specific patterns included: PCA,[163] reduced rank regression (RRR),[163,172] LCA,[162,165] and cluster analysis.[171]

2.2.2. Other established risk factors

Aside from maternal diet, and previously mentioned nutrients, several other OFC risk factors have been described. There is a large, and mostly consistent, body of literature which suggests that maternal smoking increases the risk of OFC. [10,137,173–187] The magnitude and consistency of this evidence has been convincing enough that the 50th anniversary of the US Surgeon General's report on the adverse effects of smoking suggested that maternal smoking was causally linked to an increased risk in OFC.[188] Several other modifiable risk factors are thought to influence OFC prevalence; however, study results are slightly inconsistent.[181,189] These risk factors include: maternal exposure to secondhand smoke one month prior to two months after conception,[190–194] maternal alcohol consumption the month before or first month of pregnancy,[11,137,173,176,181,182,195–201] increased maternal age,[5,135,173,178,181,202–211] increased BMI,[13,140,176,211–216] household socioeconomic status (SES),[12,140,217–220] pregestational diabetes,[14,178,221,222] higher parity (two or more pregnancies),[210,223,224] and maternal fever during the month before or first month of pregnancy.[173,176,225,226] Several nonmodifiable OFC risk factors have also been identified. These risk factors include: infant sex (male for CL and CLP, female for CP),[5,135,140,177,178,208,210,223,227–230] familial history of OFC,[17,135,140,173,181,196,227,228,231,232] and maternal ethnicity (**Table 1**).[178,208,233] Of note, the pattern of association for most noted risk factors is dependent on OFC phenotype.

To date, several researchers have performed meta-analyses to better understand the magnitude of these risk factors on OFC.[9,10,146,147,176,184–187,190,198,200,203,214] Others have evaluated the interactions among these risk factors or the interaction between these risk factors and genetic markers.[182,234–244] Most interaction studies have evaluated maternal genetic interactions with either alcohol consumption, smoking, or folate.

Studies have also described the impact of OFC risk factors using population attributable fractions (PAFs).[175,191,245,246] PAFs determine the fraction of cases attributable to a specified exposure.[247,248] Specifically, PAFs are population-level estimates, and can be interpreted as the percent of the disease that would be removed, if the exposure was eliminated.[249] Raut et al. provide the most recent OFC PAF estimation in a US population; however, this study did not consider maternal diet as an exposure and did not restrict to Hispanic individuals.[245] The study estimated PAFs for 11 key OFC risk factors in the NBDPS from 1997-2011. Results suggested that these selected risk factors contributed to 50% and 43% of CL/P and CP cases, respectively. Of note, the modifiable risk factor that attributed most to both CL/P and CP risk was maternal smoking; however, the magnitude was fairly low at 4.0% and 3.4% for CL/P and CP, respectively.[245] Prior estimates in the US were similar for combined OFC at 4.0% and 6.1%.[175,246] Recently, Sato et al. explored CL/P risk factors in Japan from 2011-2014. The combined PAF for CL/P, specific to their identified risk factors, was 34.3%. The greatest contributing risk factors were folic acid supplementation at 15.1%, maternal passive smoking at 10.8%, and maternal active smoking at 9.9%.[191]

It is important to consider whether OFC risk among Hispanic individuals differs from that of NHW individuals by the aforementioned risk factors but research is limited.[151,178,250–252] Two studies using the TBDR found that parental occupation and neighborhood SES differentially influenced OFC risk by Hispanicity.[252] Specifically, Brender et al. found that NHW nursing aides had an increased odds of OFC (adjusted OR:3.7, 95%CI:1.2,11.7) but Hispanic White nursing aides had a decreased, albeit imprecise, odds of OFC (adjusted OR:0.86, 95%CI: 0.20,2.60).[252] Lupo et al. found, that among their total TBDR population, infants with CL/P were more likely to live in an area with high neighborhood deprivation, compared to infants without OFC (OR:1.20, 95%CI:1.05,1.37). This effect was strongest when the population was restricted to Hispanic infants, in which Hispanic infants with CL/P were more likely to live

in an area with high neighborhood deprivation, compared to Hispanic infants without OFC (OR:1.32, 95%CI:1.07,1.62).[250]

Tolarova analyzed one of the only US OFC studies that solely included a Hispanic cohort. The cohort included Hispanic births in California from 1983-1993. This study found that Hispanic individuals who were at highest risk for CL/P and CP were aged 20-24 and less than 20 years old, respectively.[251] Additionally, Lebby et al. utilized 2005 US Natality Data and observed that Hispanic individuals with pregnancy-related hypertension had a lower OFC risk compared to NHW individuals with pregnancy-related hypertension (OR:0.79, 95%CI: 0.63,0.98).[178]

Further, the distribution of these OFC risk factors may differ by acculturation measures among US Hispanics. For instance, in 2019 the CDC reported that 8.8% of NHW individuals and 1.5% of Hispanic individuals smoked during pregnancy in the US.[79] There were further differences within this specified Hispanic subset by maternal country of origin. Specifically, 4.9% of individuals from Puerto Rico reported prenatal smoking compared to only 0.4% of individuals from Central and South America.[79] Zhu et al. found that, among Hispanic individuals who gave birth from 1993-2001 in New York, foreign-born individuals, compared to those US-born, were 51% less likely to use an illicit drug, 63% less likely to consume alcohol, and 76% less likely to smoke.[74] Additionally, higher acculturation, defined as a composite score of nativity, language used at home, and time in the US, has been associated with an increased risk of diabetes among Hispanics.[253] Similarly, Ramadhani et al. found that, in the NBDPS, foreign-born Hispanics were twice as likely to have gestational diabetes compared to US-born Hispanics (OR:2.23, 95%CI:1.36,3.66).[254]

2.3. Limitations of current literature and innovation of proposed study

The three existing studies that have evaluated the effect of holistic maternal dietary measures on OFC provide a foundation for this proposed study to build upon. Each study is unique in its population and diet assessment. Carmichael et al. utilized a population sample from NBDPS, the same dataset that we are proposing to utilize.[114] However, this study focused on two diet quality indices and only included NBDPS data from 1997-2005 (n=1,622 CL/P cases, 853 CP cases, and 6,147 controls). Our sample is able to include a larger, updated sample from 1997-2011, which also includes a larger Hispanic

population.[114] The analysis by Vujkovic et al. included births from 1998-2000 with a noticeably smaller sample (n=171 CL/P cases, 32 CP cases, and 178 controls) from the Netherlands. This study was only able to analyze one outcome (all OFC phenotypes combined) due to their small sample of CP cases. The study also excluded individuals who were pregnant or breastfeeding at the time of interview.[16] Additionally, blood samples were collected after pregnancy under the assumption that dietary habits, and subsequent folate levels, would remain relatively unchanged from the prenatal period. Finally, Neogi et al. evaluated the effect of vegetarianism on OFC risk in India from 2015-2016 with 157 cases (all OFC phenotypes combined) and 628 controls. This study was limited by both small numbers and a dichotomous diet measurement. Unlike the use of FFQs in the Carmichael and Vujkovic studies, Neogi assessed dietary intake with a single question inquiring if a participant followed a vegetarian diet (yes/no), which led to the inability to further address heterogeneity within both vegetarian and non-vegetarian dietary patterns.[17] For example, individuals in the vegetarian group may have different intake patterns, with some consuming more starchy foods, such as white bread, potatoes, and pasta, and others consuming much less.

In regard to the aforementioned literature that focuses on maternal diet and OFC risk, our proposed study will analyze the largest population to date. We will have the ability to differentiate between OFC phenotype, as only one other study has done.[114] This is important as each phenotype has a distinct etiology.[34] Further, this will only be the second study to evaluate this association in the US, which is vital for generalizability to US populations as diet is closely linked to geographic residence.[157] Finally, this study will evaluate maternal diet a posteriori using an LCA. Our proposed LCA will provide flexibility to capture accurate and complex dietary patterns based on realistic nutrient and food consumption.[115]

In regard to additional OFC risk factors specific to Hispanics, literature is sparse. US studies typically control for ethnicity but few display stratified estimates by ethnicity like the aforementioned studies that assessed the effect of risk factors such as parental occupation, neighborhood SES, folic acid fortification, and hypertension on OFC by Hispanicity.[151,178,250–252] Without this type of stratification, it is hard to understand how a risk factor, such as neighborhood SES or folic acid fortification, impacts specific ethnic subgroups.

To our knowledge, there are no studies to date that evaluate a spectrum of risk factors solely among Hispanics in the US. The ability to evaluate OFC risk factors among Hispanics only is important for public health messaging. For example, a NBDPS study found that Hispanic participants had significantly different intake of several nutritional factors compared to NHW participants.[255] By restricting to Hispanic participants only, we will be able to better account for the unique distribution of both dietary and other OFC risk factor information specific to this population. Further, our study is strengthened by the ability to partially account for the heterogeneity in our Hispanic population through the use of the PAS-3, a validated measure of acculturation.[256] Our results will be situated to comment on effect measure modification of the diet-OFC association by acculturation among Hispanics, which, with additional research, could help inform targeted subgroup interventions.

Overall, our use of a LCA to assess maternal diet, our inclusion of a diverse study population, and our ability to restrict to a large Hispanic population, has the potential to meaningfully contribute to this current body of evidence.

CHAPTER 3: RESEARCH DESIGN AND METHODS

3.1. Overview

Our study is uniquely strengthened by the use of population-based data from NBDPS. We will employ a case-control study design in which we utilize a subset of NBDPS data. The NBDPS has a total of 10,692 controls and 4,792 OFC cases, leading to a large and diverse study population. The use of NBDPS data allows us access to extensive dietary, risk factor, acculturation and physician-validated OFC information.

3.2. NBDPS study design and population

The NBDPS is a multi-state case-control study that was created to identify unknown causes of birth defects from 1997-2011. The study solely focused on birth defects in which etiology was unknown at the time of study. Cases with selected birth defects, including OFC, were identified through surveillance systems and controls (liveborn infants without a birth defect) were randomly selected from vital or birth records within ten states in the US (**Figure 9**).[257] Cases include terminations, stillborns and liveborns. Controls are comprised of liveborn infants selected from the same region and enrollment period as cases. Eligible cases were verified through clinical reviews of terminations, stillbirths and livebirths up to 2 years of age. All eligible mothers were invited to complete a standardized, one-hour interview via telephone within 6 weeks to 24 months after their infant's estimated delivery date (EDD).

All eligible NBDPS pregnancies had an EDD between October 1997 and December 2011. Eligibility requirements included fluency in English or Spanish and legal custody of the index infant at time of interview. Individuals were ineligible if they were incarcerated, due to the personal nature of the interview. Several centers further excluded eligible individuals under the age of 18 due to legal restrictions related to minors.[257] Overall, the NBDPS dataset includes 42,892 participants (n=32,200 cases and 10,692 controls) who completed the interview from 1997-2011.

Figure 9. States participating in the National Birth Defects Prevention Study

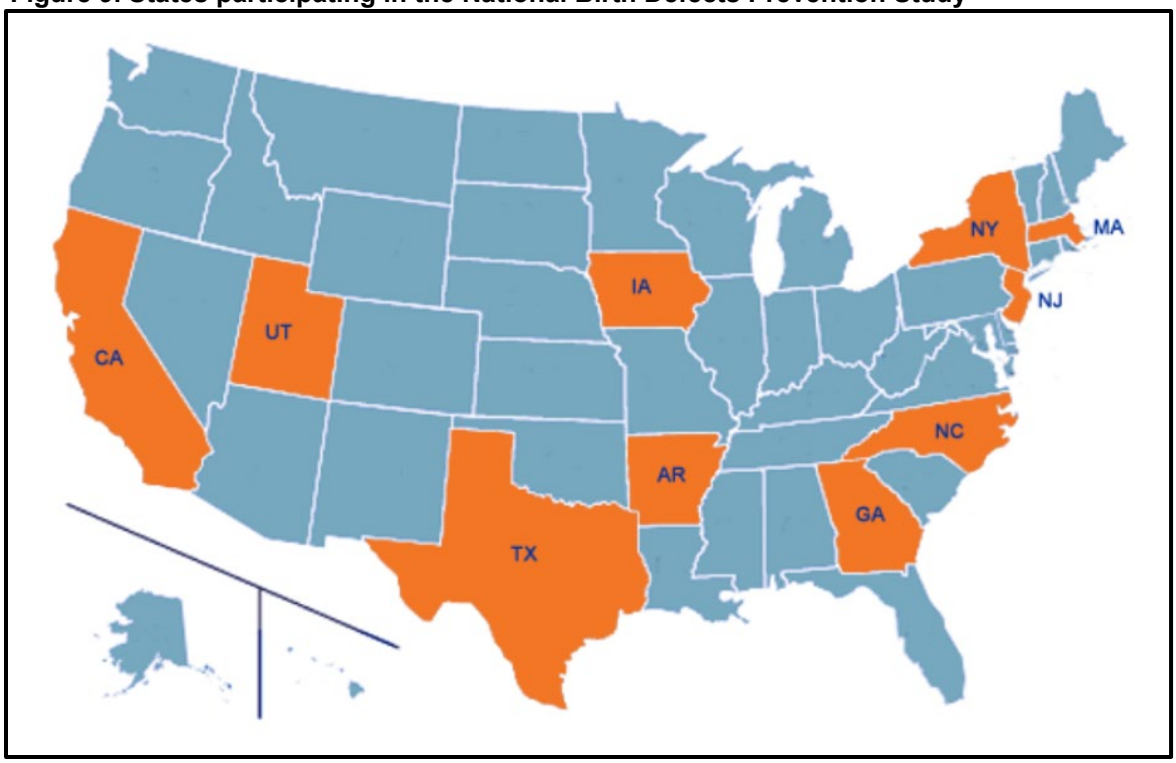
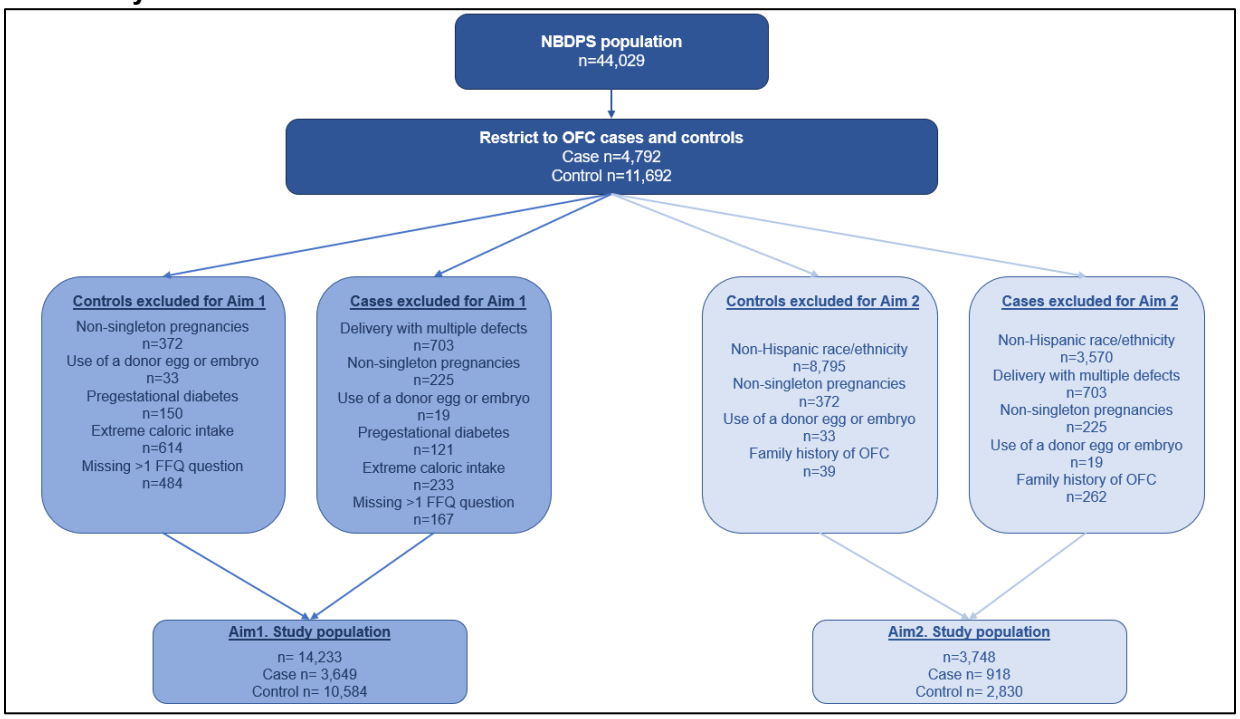


Figure adapted from the National Birth Defects Prevention Study.[258]

3.3. Proposed analytic study design and population

Our case-control study design will use a subset of NBDPS data (**Figure 10**). Both Aim 1 and 2 analyses will exclude Utah controls prior to 2004 (n=137). Utah did not include OFC infants in their case classification until 2004; therefore, all controls ascertained in Utah prior to 2004 are not representative of the time period in which Utah OFC cases were observed. The Aim 1 population will further exclude cases with multiple birth defects (n=703), non-singleton pregnancies (or missing) (n=597), and pregnancies using a donor egg or embryo (n=52) due to chromosomal or biological etiologies that likely differ from other OFC cases. Mothers with pregestational diabetes (n=271) will also be excluded since diet may differentially impact OFC occurrence among this population. Finally, participants with extreme caloric intake compared to reported diet (n=847) or those missing more than one food FFQ question (n=629) will be excluded to ensure a more valid diet measurement. Some individuals will be excluded based on multiple restrictions. The final sample will include 10,584 controls, 2,480 CL/P cases and 1,169 CP cases.

Figure 10. Flow chart of participants from the National Birth Defects Prevention Study for Aim 1 and 2 analyses



Abbreviation: OFC, orofacial clefts

Similar to Aim 1, the Aim 2 population will be restricted to non-singleton pregnancies (or missing) (n=597), pregnancies with a donor egg or embryo (n=52) and cases with multiple birth defects (n=703). Unlike Aim 1, we will further exclude infants with a family history of OFC (n=301) due to likely differences in etiology compared to other OFC cases, and non-Hispanic individuals (n=12,365). Again, participants may be excluded based on multiple factors. Our final sample will include 2,830 controls, 190 CL cases, 245 CP cases and 483 CLP cases (**Figure 10**). The NHW reference group used in our secondary analysis, will be derived from NBDPS using these same exclusion criteria and will include 610 CL cases, 772 CP cases, 898 CLP cases, and 6,425 controls. OFC phenotypes will be stratified into three groups, rather than the two in Aim 1, because differences in Hispanic OFC prevalence trends are unique to all three phenotypes, when compared to NHW phenotypes. Additionally, since this is the first study to estimate the effect of several of these risk factors among Hispanics, it is important to assess impact for each phenotype.

3.4. Outcome classification

We will utilize NBDPS validated OFC classifications for this analysis. NBDPS OFC cases were first identified via birth records within the ten participating NBDPS states. Upon ascertainment, each case was verified through a medical review by a trained clinical geneticist.[259] During these reviews, OFC phenotype was confirmed. Isolated, non-isolated, and syndromic status was also determined.[259] In order to better assess causality of risk factors related to OFC, rather than syndromes that include OFC, this analysis will be restricted to isolated OFC cases. Further, because the etiology of OFC is still largely unknown and it has been suggested that OFC phenotypes have differing etiologies, we will evaluate OFC occurrence by phenotype, rather than one combined OFC outcome.[20,34,260,261]

3.5. Exposure classification

All exposure measurements for this analysis will be ascertained through NBDPS interview data, which were collected by trained interviewers, in English or Spanish, using a standardized interview protocol at a central location.[257]

3.5.1. Maternal diet measurement

Maternal dietary patterns will be derived from self-reported responses on a modified Willett FFQ, ascertained from the hour-long NBDPS interview.[19] The Willett FFQ has been validated in several populations. One of the first validation studies enrolled a subgroup of participants from the Nurses' Health Study, in which they previously completed the Willett FFQ. Between two and four months after completion of the FFQ, participants were asked to record their food and beverage intake for one week. Upon comparison of these responses, it was concluded that Willett FFQ responses were fairly correlated with the weekly diet responses, especially after adjustment for caloric intake.[117] More specific to our population, Baddour et al. found that total iron intake in the third trimester, as calculated from food frequencies reported on the Willett FFQ, showed adequate agreement with iron biomarkers collected at delivery among French-Canadian individuals.[262] The Willett FFQ has also been assessed among Cuban Americans and was found to adequately estimate intakes of energy, cholesterol, macronutrients, and alcohol intake, when compared to a three-day food record.[263] While there is a solid amount of evidence

for the validity and efficacy of the Willet FFQ, it is important to note that the populations in these studies may not be representative of the diversity in acculturation measures that we may observe in our Hispanic subgroup. Further, the semi-quantitative nature of this tool is acceptable for the purposes of our study, which is to assess relative intake, but is limited in assessing actual nutrient intake.

During the NBDPS interview, individuals reported their dietary intake from the year prior to pregnancy using the Willett FFQ. Nutritional intake the year prior to conception is often correlated with dietary trends in early pregnancy,[264] which is also the critical development window for OFC.[20] The NBDPS added additional food items to the FFQ in the middle of their study but we will only include foods that were measured throughout the entire study to ensure that diet measurement is uniform. Specifically, 64 food items will be included. Cereals will be coded by folic acid fortification and sodas will be classified as diet or regular. 16 frequency responses were available for each food on the FFQ. Responses ranged from "six or more servings"/day to "less than one serving or no consumption"/month. Grams per day for each food will be calculated by multiplying the frequency of consumption by the number of grams/serving for that specific food. Total grams can then be calculated by summing these daily grams.

Dietary patterns, using relative intake of these foods, will be derived through LCA. To calculate relative intake, we will divide the daily intake of each food by total grams/day to estimate the percentage of daily intake that is attributed to each food. We will then categorize relative consumption for each food into four levels, which will include tertiles for consumers and a nonconsumption level. A multinomial model will derive dietary patterns from these consumption levels. The model will be adjusted for total energy intake. Adjustment for energy intake reduces confounding that stems from the variation in participants' physical characteristics and physical activity, which influence how foods are metabolized.[265,266] The multinomial model will be run multiple times so that we can specify two to six classes to determine the optimal number of classes that fit these responses. This model will output two regression coefficients that estimate class probability (for both dietary pattern and energy intake) and the conditional probability of each consumption of each food item, given a specific class. Participants will then be assigned to the class in which they had the highest probability of membership.

The consideration of these dietary patterns during pregnancy is optimal as patterns account for the interaction between nutrients that are often consumed together.[115] LCA is a useful method to identify

latent underlying classes in a population (such as dietary patterns) while adjusting for pertinent covariates and accounting for correlated errors.[115]

3.5.2. Measurement of other risk factors

Aim 2 will assess maternal diet as an OFC risk factor along with other established risk factors among Hispanics. Risk factors will be selected based off of the current state of the literature. Maternal risk factors that we will explore include: maternal age, education, BMI, maternal smoking, secondhand smoke, alcohol consumption, gravidity, folic acid supplementation, dietary folate, pregestational diabetes, fever, access to prenatal care, and infant sex. Measurement for Aim 2 risk factors can be found in **Table 2**.

Table 2. Risk factor measurements

Exposure	Definition	Measurement
Maternal age	Age at delivery (years)	<20 20-25 26-35 36+
Maternal education	Academic years completed	<12 years 12 years (high school) >12 years
Pre-pregnancy BMI	Calculated by self-reported measures of pre-pregnancy weight and height. NIH BMI classification will be used.[267]	Underweight (BMI < 18.5) Normal (18.5 ≤ BMI<25) Overweight (25 ≤ BMI < 30) Obese (BMI ≥ 30)
Maternal smoking	Smoking during the first two months of pregnancy	Yes No
Secondhand smoke	Exposure to cigarette smoke at home or work/school during the first two months of pregnancy	Yes No
Composite smoking	Exposure to a combination of smoking and secondhand smoke	Neither Smoking only Secondhand smoke only Both
Maternal alcohol consumption	Binge drinking (≥4 drinks in 1 sitting) one month prior to three months after conception	Yes No
Maternal diet	Periconceptional dietary patterns derived from a LCA using self-reported food frequencies on the NBDPS FFQ[1]	Patterns defined a posteriori to best fit data
Previous pregnancies	Number of prior pregnancies, regardless of birth outcome	0 1 >1
Folic acid-containing supplementation	Folic acid, multivitamin, or prenatal vitamin consumption during the first two months of pregnancy	Daily use (≥ 28 days) Some use (< 28 days) No use (0 days)

Table 2 continued.

Exposure	Definition	Measurement
Lowest quartile of dietary folate	Quartiles of dietary folate calculated from self-reported food frequencies on the NBDPS FFQ	Yes No
Folic acid antagonist medication use	Any use	Yes No
Pregestational diabetes	Diagnosis prior to pregnancy of interest	Yes No
Fever	Any reported fever during B1P3	Yes No
Access to prenatal care	Any prenatal care	Yes No
Infant sex	Clinical determination at birth	Female Male
Familial history of OFC	OFCs in first- or second-degree relative	Yes No

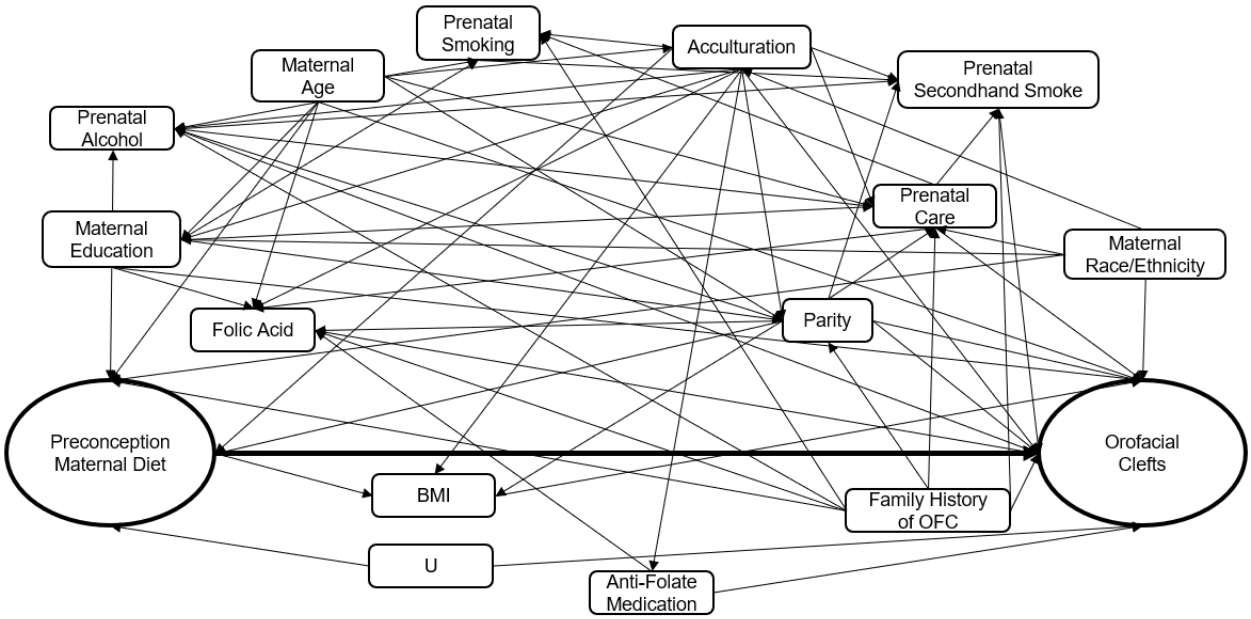
Abbreviations: BMI: body mass index; OFC: orofacial clefts
1. One-year prior to conception

3.6. Covariate selection and measurement

The NBDPS hour-long interview provides extensive maternal demographic, behavioral and nutritional information. Analytic covariates and confounders will be selected through a Directed Acyclic Graph (DAG) for all analyses (**Figure 11**). Available covariates include maternal: demographic measures (race/ethnicity and age), health behaviors (smoking status, alcohol use, and medication/supplement uptake), health outcomes (pregestational diabetes and fever), education, BMI, access to prenatal care, and family history of OFC. Covariate measurements can be found in **Table 2**.

Figure 11 provides the primary DAG that models the relationship between all covariates of interest. This DAG will be used to identify confounders for both Aim 1 and Aim 2. While the DAG is currently displaying maternal diet as the exposure, the DAG is similar for both aims since the evaluation of different risk factors on OFC should not change the observed relationship between all other covariates. For instance, regardless of whether we are assessing the effect of maternal diet on OFC in the larger NBDPS population (Aim 1) or the effect of smoking on OFC in the NBDPS Hispanic population (Aim 2), the mechanism in which prenatal care influences folic acid supplementation will not change.

Figure 11. Directed Acyclic Graph displaying the conceptual association between periconceptional maternal diet and orofacial clefts



Abbreviation: BMI, Body Mass Index; OFC, Orofacial Clefts; U, unmeasured confounders

3.6.1. Acculturation measurement

As we explore the influence of multiple risk factors on OFC prevalence among Hispanics in the US, we will stratify our aaPAF results by a validated acculturation measure to better describe the heterogeneity within this population.[2,61,62] We will incorporate the PAS-3 by Cruz and colleagues, rather than a single indicator of acculturation, in an attempt to better capture the complex and dynamic nature of acculturation.[64,66,256] Compared to single acculturation proxy measurements, the PAS-3 has been shown to have higher validity and reliability.[256] The PAS-3 combines interview language, language spoken at home, and proportion of life lived in the US (**Table 3**).

This proxy scale was validated against a more extensive acculturation scale given to a nationally representative sample of Hispanic individuals in the US (n=1,437) from the 1984 National Alcohol Survey (NAS).[256] Authors validated the PAS-3 against results from the 12-question NAS acculturation scale in the same Hispanic population.[256] The NAS population from 1984 was specifically chosen because there was an intentional oversampling of Hispanics that year and researchers utilized the full NAS acculturation scale, rather than a shortened version that would be used in subsequent years. Results suggested that

the PAS-3 performed well in both validity and reliability.[256] While longer acculturation scales are preferred, this scale is an optimal measure when extensive acculturation information is unavailable.[256]

The PAS-3 will be ascertained through NBDPS interview data and include participants' interview language, language used most at home, and the proportion of life lived in the US.[256] As tested by Cruz et al., results from the PAS-3 can be dichotomized into "medium/high acculturation" (>1 points) versus "low acculturation" (≤ 1 points) groups.[256] It is important to note that Cruz and colleagues measured "language spoken at home" by participant responses to the questions "do you speak Spanish or English with [your wife/husband/person you live with, your children, your brothers/sisters, your parents] or do you use both about the same?"[256] Response options included: "mostly Spanish" (1 point), "both about the same" (2 points), and "mostly English" (3 points). If the average of these responses was greater than or equal to two, individuals were assumed to mostly speak English at home and were assigned two points for this part of the PAS-3. Individuals with an average less than two points were assumed to mostly Spanish at home and were assigned zero points. NBDPS only has one question about language spoken at home that more generically asks the participant what language they speak at home. While this may be considered a limitation, it is still important to consider the PAS-3 in analysis because of its comprehensive nature, which may better encompass the multidimensionality of the acculturation process.

Variable	Score
Interview language	0 if Spanish 2 if English
Language spoken at home	0 if Spanish 2 if English
Proportion of life lived in the US	Value from 0 to 1

Table 3. Acculturation-related variables, coding schemes, and scoring of the PAS-3
Values and title from Cruz et al. [256]

3.7. Statistical analyses

Before the primary analysis is performed for each aim, a descriptive analysis will be conducted for each population (total NBDPS population in Aim 1 and Hispanic NBDPS population in Aim 2). Differences

in key covariate distributions between OFC cases and controls will be described. All analyses will be completed using SAS version 9.4 (SAS Institute, Cary, NC, USA).

3.7.1. Aim 1 Analysis

Aim 1: Estimate the association between periconceptional maternal diet and isolated OFC among all eligible NBDPS participants (n=2,480 CL/P cases, 1,169 CP cases, and 10,584 controls).

Dietary patterns, one-year prior to conception, will be derived from the consumption frequencies for 64 food item indicators ascertained from the NBDPS FFQ. We will assess relative consumption by comparing the percent of daily intake attributed to each food item among controls. There will be four levels of consumption (tertiles for consumers and an additional nonconsumption level). Foods with notably low intake across all controls (<20% report any consumption) will be dichotomized since consumption is substantively in the higher tertiles. To avoid sparse nonconsumption levels, the nonconsumption level and first tertile of consumption will be combined for foods consumed across almost all controls (at least 90%). A multinomial model, adjusted for total energy intake, will then identify unknown dietary patterns using these relative consumption levels. We will run this model multiple times, specifying a different number of classes (two to six) each time. We will determine the final model, and optimal number of classes for our data, by comparing the AIC, BIC, entropy value and ease of interpretation of each model.[119] We will then use combine the two regression coefficients that provide estimated class membership (for both dietary patterns and energy intake) and the conditional probability of consuming each food given a specific class using Baye's Theorem. This value gives us each participant's probability of class membership, dependent on reported food consumption and energy intake. All individuals are then assigned to the class in which they have the highest predicted probability of membership. Individuals assigned to a specified class are assumed to have a similar dietary intake. Classes will be labeled based on notable intake trends (e.g. foods with relatively high probability of consumption or relatively low probability of no consumption, etc.).

Sotres-Alvarez and colleagues provide an example of how our dietary patterns may look. Stores-Alvarez previously estimated dietary patterns in the NBDPS using a LCA.[162] Although their study focused on different outcomes (NTDs and congenital heart defects) and their population only included deliveries

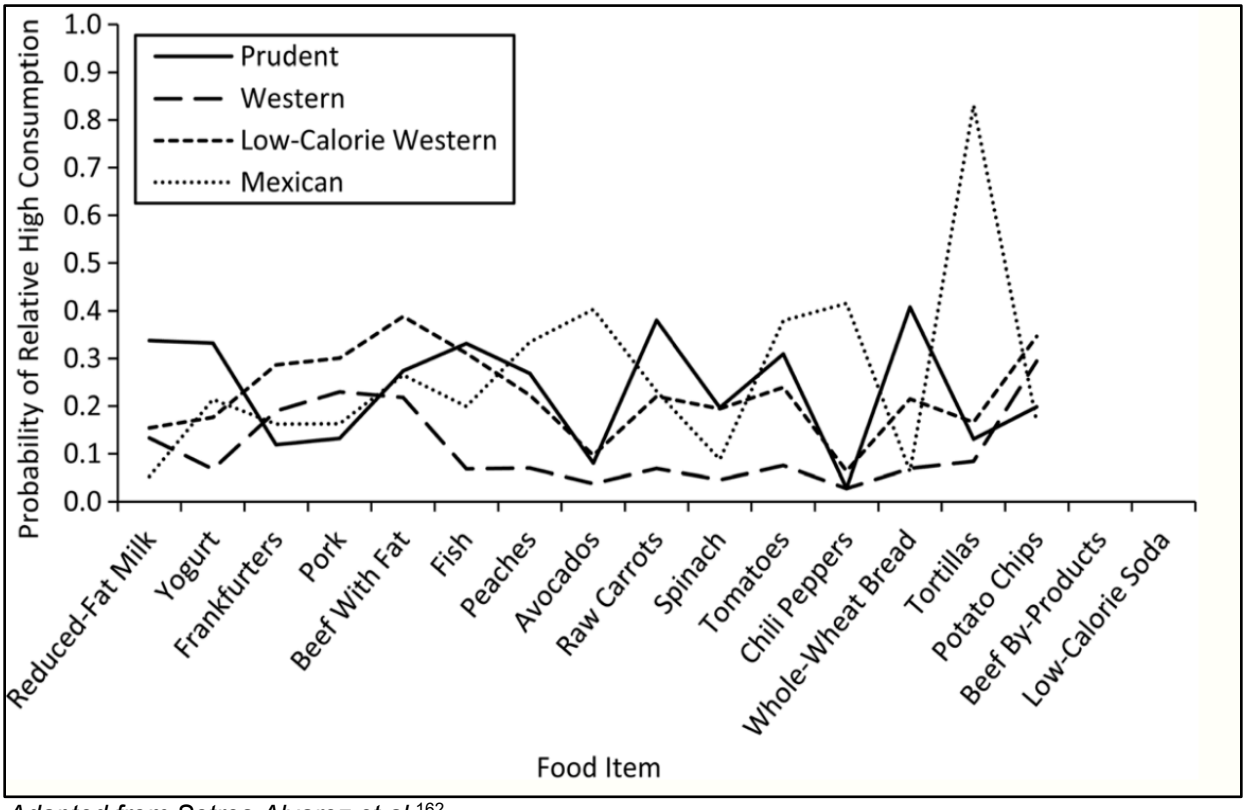
up to 2005 (rather than 2011), we expect that our dietary patterns may be similar to theirs since they will also derived patterns from control data only. **Figure 12** is one of two line plots that Stores-Alvarez provide as a visual representation of relative food consumption within the patterns they derived.[162] This plot displays the probability of relatively high consumption, compared to all controls, for specific foods by pattern. For instance, there is a peak in which participants consuming a "Mexican" diet had a high likelihood of consuming tortillas, relative to all other groups. Two other peaks are also observed in which participants on the "Prudent diet" had a relatively high likelihood of consuming raw carrots and whole-wheat bread.[162]

Once classes are created, logistic regression will be used to evaluate the odds of having an infant with an OFC based on membership to each dietary pattern. Models will be created separately for CL/P and CP cases. Results will include both crude and adjusted odds ratios. Adjusted models will control for a minimally sufficient set identified through the aforementioned DAG (**Figure 11**). Specifically, the minimally sufficient set will include: age at conception, race/ethnicity, intake of folic acid-containing supplement in the first two months of pregnancy, education level, family history of OFC, gravidity, and study center. We will use a complete-case analysis for this aim, as all variables in the minimally sufficient have <1% missing data. **SubAim 1.1** will determine if the impact of diet on OFC differs by race/ethnicity. We will first conduct a multiple group LCA to assess if dietary classes are measured differently across racial/ethnic subgroups. A multiple group LCA compares two models, one that imposes measurement invariance by assigning identical item response (food consumption) probabilities for each subgroup's dietary classes and another than allows these parameters to vary to best fit the data of each subgroup [119] We will determine if measurement invariance is observed by comparing the AIC, BIC, and entropy values for both models. If measurement invariance is observed, we will evaluate effect measure modification of the observed diet-OFC association by race/ethnicity. We will stratify primary results (odds ratios) by race/ethnicity, as collected in the NBDPS (NHW, non-Hispanic Black, Hispanic, Asian/Pacific Islander, Native American/Alaskan Native, and other). This analysis will explore if the impact of our identified dietary patterns on OFC differs by race/ethnicity.

Sensitivity Analyses

Two sensitivity analyses will also be conducted using our primary results. To evaluate the effect of membership classification quality on results, we will exclude participants with a low class membership probability (<(*K*-1)/*K* for *K* classes) from the crude and logistic regression models. We will then compare these ORs to the ORs produced in our primary analysis. Next, to assess the influence of severe morning sickness on dietary changes and the odds of OFC, we will exclude individuals who reported taking nausea medication or treatments during pregnancy, under the assumption that this captures participants with the most severe nausea in early pregnancy. Again, we will rerun the crude and adjusted logistic regression models and compare these ORs to those from our primary analysis.

Figure 12. Example of LCA results displaying the relative likelihood of specific food consumption based on dietary patterns from controls in the National Birth Defects Prevention Study, 1997-2005



Adapted from Sotres-Alvarez et al.[162]

3.7.2. Aim 2 Analysis

Aim 2: Assess the impact of established risk factors, including periconceptional diet, on OFC among NBDPS Hispanic individuals (n= 685 CL/P cases, 351 CP cases, and 2,573 controls).

Crude and adjusted logistic regression models will estimate the effect of 15risk factors, identified through a literature review, on the odds of OFC among eligible Hispanic participants from the NBDPS. Each risk factor will have three models to account for the three OFC phenotypes of interest: CL, CP, and CLP. Identified risk factors will be defined by the measurement listed in **Table 2**. Of note, two new LCAs will be run for maternal diet specific to Hispanic individuals and our NHW comparison group so that our dietary measure best fits data from these subgroups. Adjusted models for each risk factor-phenotype combination will include confounders unique to that association. All confounders will be identified using the covariate relationships posited in the DAG from **Figure 11**. **SubAim 2.1** will calculate the crude and average adjusted population attributable fractions (PAFs) for each risk factor among this Hispanic subgroup. Due to the causal nature of PAFs, only risk factors with strong priors were included in this analysis. Risk factor selection criteria included ≥5 published studies with most studies reporting an aOR≥1.5. Meta-analyses and statistical precision (95% CIs) will also be considered. For instance, it has been suggested that smoking may have the most established causal effect on OFC, as evidenced by the Surgeon General's acknowledgement of this association.[188] Most studies suggest a modest aOR that is slightly less than 1.5; however, due to the multiple meta-analyses, precise estimates, and the observed persistent effect that provide strong priors for this association, smoking will be included in our PAF analysis.[10,186] A total of six risk factors were identified using this criteria: maternal smoking, secondhand smoke, maternal diet, lack of folic acid supplementation, pregestational diabetes, and infant sex.

These risk factors will be dichotomized and crude and adjusted ORs will be estimated through multivariable logistic regressions that will include confounders previously identified for each risk factor in our primary analysis. aaPAFs will then be estimated for the six identified risk factors. Final aaPAF results will be stratified by acculturation status to assess differences in risk factor profiles by a factor that influences the cultural heterogeneity, and subsequent health outcomes, of this population. Additionally, aaPAFs will be estimated for a NHW comparison group from NBDPS to assess differences in Hispanic and NHW risk factor profiles.

Overall, PAFs are measurements used to quantify the effect of risk factors on specified outcomes, such as OFC, at the population-level. Specifically, PAFs quantify the fraction of cases attributable to the exposure of interest.[247,248] PAFs can be interpreted as the percent of the disease that could be removed, if the exposure was completely eradicated at the population-level.[249] The crude PAF (cPAF) will be calculated using formula below:

$$cPAF = \frac{p(OR-1)}{p(OR-1)+1}, \textit{where } p = \frac{Number\ Exposed}{(Number\ Exposed + Number\ Unexposed)}$$

Average adjusted PAFs (aaPAFs) will be calculated using an approach by Eide and Gefeller. This approach has been used twice in the NBPDS. Simeone et al. assessed aaPAFs for select congenital heart defects and, more specific to our study, Raut et al. estimated aaPAFs for OFC among the larger NBDPS population.[245,268,269] The aaPAF is the fraction of disease that could be prevented if the disease risk in the exposed where shifted to levels of disease risk in the unexposed, among all levels of relevant covariates.[268] Sequential PAFs, used in aaPAF calculations, are reliant on the sequence in which risk factors are removed. Ruckinger and colleagues provide an example of this for cardiovascular disease (CVD).[270] A model of CVD risk factors was created and risk factors were removed in the following order: age, sex, hypertension, cholesterol, HDL-cholesterol, smoking, and finally diabetes. Based on this sequential removal, the PAF for age was 54%; however, when risk factors were removed in the exact opposite order, the PAF for age was only 13%. Of note, there are 7! Removal permutations (n=5,040) in this model due to 7 covariates.[270]

To account for the effect of sequential removal on PAF results, the aaPAF estimates an adjusted PAF (aPAF) for each risk factor in every possible removal sequence using a specified multivariable model that will include our six risk factors with strong priors and confounders, all other risk factors. Once all sequences have been completed, PAF estimates for each risk factor are averaged together to create an aaPAF specific to each risk factor.[268] Once crude and aaPAFs are calculated, we will also calculate the total proportion of cases explained by our selected risk factors. We will use a modified SAS algorithm created by Ruckinger et al. to estimate aaPAFs and bootstrap sampling to calculate corresponding 95% CIs.[271]. We will then compare risk factor profiles between Hispanic and NHW subgroups and Hispanic PAFs will be stratified by PAS-3 status (“medium/high acculturation” versus “low acculturation”).

It is important to note that some previous studies include two additional steps to estimate aaPAFs. Because PAFs estimate a decrease in disease dependent on the removal of a risk factor, some previous analyses do not estimate PAFs for risk factors act unexpectedly in their population (e.g. increase disease occurrence as evidenced by an aOR≤1). Additionally, some studies truncate the lower limit of CIs at zero, assuming that the removal of that risk factor will not increase disease occurrence. In our analysis, we did not include either step due to the novelty of our research question and causal interpretation of PAFs. Most established risk factors for OFC have never been evaluated in Hispanic individuals and, to our knowledge, the effect of diet has not yet been included in an OFC aaPAF analysis. To provide full transparency of our results, we estimated aaPAFs for all risk factors, regardless of the specific effect on our study population, and allowed CIs to fluctuate to fully describe precision.

Missing Data

Due to notable missingness in several covariates among our NBDPS Hispanic sample, we will create an imputation model for each risk factor-OFC combination in our primary analysis and run five imputation cycles with fully conditional specification, under the assumption that data are missing at random. Models will include auxiliary variables that are associated with the missingness of the specified exposure and outcome, along with the outcome, exposure and identified confounders. We then use these five imputed datasets in the logistic regression models for our primary analysis. Models will produce crude and adjusted odds ratios (aORs), with corresponding 95% CIs and results will then be pooled using proc mianalyze in SAS.[272] We will compare imputed aORs with those estimated in a complete case analysis to determine if missing data significantly influenced our results and imputation for SubAim 2.2 (aaPAFs) is warranted.

3.7.3. Protection Of Human Subjects

NBDPS study protocol, consent forms, and data collection have been approved by the Institutional Review Boards (IRB) of each participating state center, along with the Centers for Disease Control's IRB and the US Office of Management and Budget (OMB). Before data collection, all NBDPS

participants provided informed consent. A dissertation IRB for this specific project will be created and submitted to the University of North Carolina at Chapel Hill upon proposal approval.

3.8. Power Analyses

This study is well powered due to NBDPS's large, and fairly diverse, sample size. Both Aim 1 and 2 have adequate power to detect substantively meaningful estimates. All power calculations were conducted using PS Power (https://vbiostatps.app.vumc.org/ps/dichot) and QUANTO (https://quanto.software.informer.com/1.2/) software. Minimally detectable ORs were calculated using a fixed sample size, as all NBDPS sample sizes are known. Power was set to 80%. We evaluated different prevalence values for the Western diet versus all other dietary patterns. With a total of 3,649 OFC cases and further delineations by OFC type (n=1,169 CP and 2,480 CL/P cases), our Aim 1 analysis has sufficient power to identify even small changes in estimates (**Table 4**), including among specific OFC groups. Based on the previous study that also estimated the effect of maternal diet on OFC through a posteriori dietary patterns, we expect to find an OR of roughly 1.90.[16] The highest minimally detectable OR among our Aim 1 population is 1.3. We are also well powered to detect estimates after restriction to the NBDPS Hispanic subgroup (n=918 cases and 2,830 controls (**Tables 4 and 5**). **Table 4** displays minimally detectable ORs for this subgroup related to measuring the association between maternal diet and OFC risk. **Table 5** provides additional minimally detectable ORs for Aim 2, which will have a wide range of exposure rates due to our focus on multiple exposures in the Hispanic population.

Table 4. Minimally detectable odds ratios of dietary effects on orofacial clefts[1]

	Percent in Western Diet class[2]			
	0.10[3]	**0.20**	**0.30[4]**	**0.40**
Aim 1 (Larger NBDPS population)[5]				
Cleft Palate n=1,169	1.3	1.2	1.2	1.2
Cleft lip w/wo cleft palate n=2,480	1.2	1.2	1.1	1.1
Aim 2 (Hispanic NBDPS population)[6]				
Cleft Lip n=190	1.8	1.6	1.6	1.5
Cleft Palate n=245	1.7	1.5	1.5	1.5
Cleft Lip and Palate n=483	1.5	1.4	1.3	1.3

1. β set at 0.80, α set at 0.05 (two-sided), results may seem similar due to rounding
2. Diet frequency is based on trends found in the NBDPS Sotres-Alvarez et al. study, which conducted an LCA on 64 food items to define dietary patterns.[162] One of their identified diets, labeled as "Western," was characterized by relatively high intake of bacon, French fries, white bread, potato chips, and soda (**Figure 12**). We expect similar trends in intake, since our NBDPS controls are comparable to the existent controls in Sotres-Alvarez et al.
3. Prevalence most likely based on most recent NBDPS diet study for Hispanic NBDPS population.[162]
4. Prevalence most likely based on most recent NBDPS diet study for general NBDPS population.[162]
5. 10,584 Aim 1 controls
6. 2,830 Aim 2 controls

Table 5. Minimally detectable odds ratios for OFC risk factors among NBDPS Hispanics[1]

	Exposure Rates in Controls (n=2,830)			
	0.50	**0.30**	**0.10**	**0.01**
Cleft Lip n=190	1.5	1.6	1.8	3.9
Cleft Palate n=245	1.5	1.5	1.7	3.5
Cleft Lip and Palate n=483	1.3	1.3	1.5	2.8

1. α set at 0.05, β set at 0.80

CHAPTER 4: THE EFFECT OF MATERNAL DIET ON OROFACIAL CLEFTS

4.1. Introduction

Orofacial clefts (OFC) are a commonly diagnosed birth defect in the United States (US). For every 10,000 livebirths, approximately ten infants are diagnosed with cleft lip with or without palate (CL/P) and six are diagnosed with cleft palate alone (CP).[1] Prevalence varies by race and ethnicity, which may partially be due to risk factors such as maternal diet. The critical development window for OFC occurs within the first nine weeks of pregnancy, often before conception is recognized.[34,273] These anomalies can cause complications in feeding, speech, hearing, and psychological wellbeing, which are associated with increased morbidity across the lifespan.[30,31,274] Optimal OFC care consists of extensive medical interventions and includes a coordinated interdisciplinary care consisting of medical, surgical, dental, and psychological professionals.[39] The actual etiology of most OFC is largely unknown, as an estimated eight percent of cases are chromosomal.[8,33] Current research suggests that the interaction of genetic and environmental factors. Prenatal smoking is widely recognized as the strongest risk factor for OFC, but many risk factors are still unknown.[7,10,187,188,190,245] Thus, it is important to continue assessing the effect of other modifiable exposures on OFC risk, such as maternal diet.

Maternal diet during the preconception and prenatal period has a substantial influence on fetal growth and development. Periconceptional nutrition aids in the creation of a favorable preimplantation environment for the placenta and fetus[90,92,93] and is often correlated with nutrition during early pregnancy, the critical development window for OFC.[34,264] Optimal periconceptional diet quality has been associated with a decreased risk of multiple types of birth defects.[162,165–167] The majority of the published scientific literature has assessed the effect of individual nutrients or single food items on the risk of OFC.[15,18,134,144,153,275,276] Current findings suggest that adequate consumption of nutrients such as zinc, magnesium, calcium and vitamins B, C, and E may decrease the risk of OFC. It has also been suggested that, like neural tube defects (NTDs), OFC risk may decrease with maternal folic acid and multivitamin

supplementation.[9,146] However, few studies have holistically evaluated the effect of dietary patterns and diet quality on OFC occurrence.[16,17,114]

A focus on overall dietary intake explores the realistic, complex, and synergistic effect of nutrient interactions, since nutrients are rarely consumed in isolation.[100,101] To further explore the association between periconceptional diet and OFC, we measured the effect of maternal dietary patterns, defined a posteriori with a latent class analysis (LCA), on OFC using data from a large multi-site case-control study of birth defects. We then assessed effect measure modification (EMM) of this association by race/ethnicity.

4.2. Methods

Study Population

The National Birth Defects Prevention Study (NBDPS) is a multi-site, population-based case-control study designed to identify unknown causes of major structural birth defects in the US among pregnancies from 1997-2011. Cases with OFC included liveborn infants along with terminations and stillbirths, dependent on state and year. Statewide surveillance systems identified cases in Arkansas, Iowa, New Jersey, and Utah and county-specific systems were used in California, Georgia, Massachusetts, New York, North Carolina, and Texas. Because Utah did not ascertain OFC cases until 2004, Utah controls prior to 2004 were excluded. Upon ascertainment, cases were clinically confirmed by a clinical geneticist.[259] Controls (liveborn infants without major birth defects) were selected from vital or birth records within the same region and enrollment period of cases.[257] NBDPS eligibility requirements included fluency in English or Spanish and legal custody of the index infant at time of interview. Exclusion criteria included incarceration and, for some NBDPS sites, maternal age under 18.[257] Eligible mothers were interviewed up to two years after their estimated delivery date and participated in an hour-long computer-assisted telephone interview. The interview ascertained information on maternal and paternal demographics, reproductive health, nutrition, health behaviors, and home and work environment.[257]

Characterization of Maternal Diet

Maternal diet was measured through a modified 58-question Willett FFQ administered during the NBDPS interview in which participants recalled their frequency of consumption for specific food items during the year prior to conception.[19] The interview also ascertained coffee, tea, cereal and soda intake, which allowed for the measurement of 64 food items in total. Cereals were classified by folic acid fortification status (highly fortified versus not) and soft drinks were classified as diet or regular. Soft drinks with an unknown diet status were excluded from the analysis. A total of 16 frequency responses were available for each food (ranging from “six or more servings”/day to “less than one serving or no consumption”/month). Grams/day for each food were calculated by multiplying the frequency of consumption by the number of grams/serving in each food. Total grams consumed/day was calculated by summing daily grams of all food items. Food and vitamin supplements were not included in these calculations. Nutrients were assigned to food items using the Department of Agriculture National Database, SR27.[277] Dietary folate equivalents (DFE) were calculated by upweighting synthetic folic acid by 1.7 and adding this to naturally occurring folate, to account for the greater bioavailability of synthetic folic acid.[278]

Statistical Analyses

Dietary patterns among controls were derived through a LCA, a data reduction method that identifies underlying “classes” or group patterns in a population. Individuals are assumed to belong to one unknown class of individuals with similar indicators.[279] The use of LCA for NBDPS dietary assessment has previously been used.[162] In short, we used a multinomial model, adjusted for energy intake (kcal/day), to derive dietary patterns from the relative consumption of 64 food items among controls. To assess relative consumption of each food item, we divided the daily intake of each food item (grams/day) by the total grams/day consumed to better understand the percentage of total daily intake attributed to each food. As an example, this relative percentage would highlight differences in consumption patterns between two individuals who consumed the same grams of a specific food (e.g., 112 g/day of fish) but had a significantly different energy intake (e.g.,1,000 versus 2,000 kcal/day). We categorized relative

consumption of each food into four levels, tertiles of consumption among consumers and an additional nonconsumption level. Foods with notably low intake (<20% report any consumption), including liver, organ meat, low calorie soda, and cereal fortified with folic acid, were dichotomized (any versus none) since higher tertiles still represented a substantively low number of grams consumed. To avoid sparse nonconsumption levels, foods consumed by most of the population (at least 90%), including rice/pasta, cheddar cheese, eggs, and potatoes, only had 3 intake levels with the small number of non-consumers combined with the lowest tertile.

To identify the optimal number of patterns specific to these consumption values, we conducted the multinomial model multiple times with different seeds and specified two to six classes. We then compared the Akaike Information Criteria (AIC), Bayesian Information Criteria (BIC), entropy value, and interpretability of each model. The final model, determined by these four criteria, produced regression coefficients that estimated class membership (for each dietary pattern and energy intake) and the conditional probability of food item consumption given a specific class. These two probabilities were combined, using Bayes' theorem, to predict each participant's probability of class membership dependent on reported food consumption and energy intake.[119] Participants were assigned to the class in which they had the highest probability of membership.

Of the 4,792 OFC cases and 11,692 controls available in the NBDPS, we excluded deliveries with multiple birth defects (n=703 cases), non-singleton pregnancies (n=225 cases, 372 controls), and pregnancies using a donor egg or embryo (n=19 cases, 33 controls) due to chromosomal or biological etiologies that likely differ from other OFC cases. Mothers with any type of pregestational diabetes (n= 121 cases, 150 controls) were also excluded since diet may differentially impact OFC occurrence among this population. Finally, participants with extreme caloric intake compared to reported diet (n=233 cases, 614 controls) or those missing more than one answer on the food frequency questionnaire (FFQ) (n=167 cases, 484 controls) were excluded to ensure a more valid diet measurement among all participants in our analytic sample. Some individuals were excluded based on multiple restrictions. Our final analytic sample consisted of 3,649 cases and 10,584 controls. Because OFC etiology likely differs by phenotype,[280] we differentiated CL/P cases (n=2,480) from CP cases (n=1,169). We examined the distribution of sociodemographic and pregnancy-related factors by case-control status.

After dietary patterns were derived, we estimated maternal demographic characteristics and mean nutrient intake among controls by class. We then used multivariable logistic regression models to assess the effect of dietary patterns on CL/P and CP. Crude and adjusted odds ratios (aOR) were estimated, along with corresponding 95% confidence intervals (CIs). For adjusted models, a minimally sufficient set was identified through a Directed Acyclic Graph and included maternal: age at conception (<20, 20-25, 26-35, or ≥36 years), race/ethnicity (non-Hispanic White, non-Hispanic Black, Hispanic, Other), intake of any folic acid-containing supplement (yes/no for the first two months of pregnancy), education level as a proxy for socioeconomic status (<12, 12, or >12 years), family history of OFC in a first degree relative or prior pregnancy (yes/no), gravidity (0,1, or >1 pregnancies), and study center (**Figure 11**).

Three sensitivity analyses were also conducted. To evaluate the effect of membership classification quality on results, we excluded participants with a low probability of class membership (n=355 cases and 939 controls), defined as lower than the probability of random assignment to their specific class (<(*K*-1)/*K* for *K* classes). Next, to assess the influence of severe morning sickness on dietary changes and OFC risk, we excluded individuals who reported taking nausea medication or treatments (n=507 cases and 1,580 controls), under the assumption that this captured participants with the most severe nausea whose usual diet may have been altered in early pregnancy. Finally, since smoking was not identified as a confounder in our DAG (**Figure 11**) but is the most recognized risk factor for OFC, we adjusted the final models by smoking to assess potential residual confounding.

Finally, to determine if the impact of diet on OFC differed by race/ethnicity, we conducted a multiple group LCA to assess if the measurement of dietary classes was similar across racial/ethnic subgroups. This analysis compared two models, one that imposed measurement invariance by assigning identical food consumption probabilities for each subgroup's dietary classes and another that allowed these parameters to vary to best fit the dietary intake of each subgroup.[119] We then determined if measurement invariance was observed by comparing the AIC, BIC, and entropy values for both models. If measurement invariance is observed, it is appropriate to stratify results and assess EMM because classes are similarly measured for each subgroup.

4.3. Results

Participants with a control infant were primarily non-Hispanic White (58.3%) with more than 12 years of education (59.4%) (**Table 6**). Most of the control group took a supplement containing folic acid (75.3%) and did not smoke (88.1%) or report exposure to secondhand smoke (78.5%) or binge drinking (62.8%) at the start of pregnancy. Compared to controls, CL/P and CP cases were more likely to report prenatal smoking (controls: 11.9%, CL/P: 16.7%, CP: 15.4%), exposure to secondhand smoke (controls: 21.5%, CL/P: 27.1%, CP: 25.5%), and family history of OFC (controls: 0.3%, CL/P: 6.1%, CP: 5.7%).

LCA models that specified 4, 5, and 6 classes had similar AIC, BIC, and entropy values (**Table 7**). We selected the 4 class solution to improve interpretability. Dietary patterns were labeled as Prudent (31.4%), Western (27.0%), Low-Calorie Western (26.3%), and Mexican (15.4%); labels were adopted from previous studies[162,165] and were based on similarly notable consumption probabilities (**Figure 13**). Of note, the Western diet class was more reflective of a diet found in North America, rather than other Western countries. Participants in the Prudent class had a relatively high probability of consuming dairy, nuts, and fish along with a low probability of not consuming vegetables, fruits, and dark breads. The Mexican diet class, comprised of primarily Hispanic participants, closely followed the dietary intake of the Prudent class, comprised of primarily non-Hispanic White participants, aside from a higher probability of consuming of salsa, chili peppers, and tortillas. Although similar in food intake, there were notable differences in education between participants in these two dietary classes. Among controls, 86.4% of participants in the Prudent diet class, compared to 21.7% of participants in the Mexican diet class, reported more than 12 years of education (**Table 8**).

The other two classes, Western and Low-Calorie Western, tended to have a high probability of consuming white bread, meat, and chips, and no consumption of vegetables and yogurt. Unlike the Western class, the Low-Calorie Western class was associated with a decrease in class membership probability as energy intake increased, a relatively low likelihood of caffeine intake, and extreme probabilities of relative intake for most food items (high and low). The consistently high probability of no consumption for most foods may suggest a more restrictive diet among the Low-Calorie Western class. In general, we observed differences in classes by caloric intake and macronutrients (**Table 9**).

After adjustment, membership in the Western diet class was associated with higher odds of CL/P (aOR: 1.3, CI: 1.2,1.5) and CP (aOR: 1.2, CI: 1.1,1.5), compared to the Prudent diet. Associations between OFCs and the Low-Calorie Western and Mexican diets were weaker with wider confidence intervals. Specifically, the aORs for the Low-Calorie Western diet (v. Prudent) were 1.2 (CI: 1.0,1.4) and 1.0 (CI: 0.9,1.2) for CL/P and CP, respectively. Similarly, aORs associated with the Mexican diet were 1.1 (CI: 0.9,1.3) for CL/P and 0.8 (CI: 0.6,1.1) for CP (**Table 10**). Similar results were observed when we excluded 1,294 participants with a low probability of class membership (<75%, since *K*=4 classes) or 2,087 participants with severe morning sickness (**Tables 11 and 12**). The inclusion of smoking in our adjusted models did not have a material difference on final estimates (results not shown).

Results from our multiple group LCA suggested that diet was measured similarly across racial/ethnic subgroups (results not shown). Upon stratification of our primary aORs by race/ethnicity, the Prudent diet appeared to be protective against CL/P for all groups, although most CIs included one (**Table 14**). The Western diet was associated with the strongest and most precise increase for NHW CL/P (aOR: 1.3, CI: 1.1,1.5) and CP (aOR:1.3, CI:1.1,1.6). Similarly, the Low-Calorie diet, when compared to Prudent, was associated with a notable increase of Hispanic CL/P (aOR: 2.1, CI: 1.3,3.2). Most other observed effects by subgroup were weak. However, EMM results should be interpreted with caution due to small cell sizes upon stratification by race/ethnicity (**Tables 13 and 14**).

Table 6. Maternal and infant characteristics among cases with cleft lip with or without palate (CL/P) or cleft palate (CP) and controls without a birth defect, National Birth Defects Prevention Study, 1997-2011

	Controls (n=10584) n(%)	CL/P (n=2480) n(%)	CP (n=1169) n(%)
Age at Conception, years			
<20	1368 (12.9)	344 (13.9)	124 (10.6)
20-25	3037 (28.7)	772 (31.1)	318 (27.2)
26-35	5307 (50.1)	1180 (47.6)	600 (51.3)
36+	872 (8.2)	184 (7.4)	127 (10.9)
Missing	0	0	0
Race/Ethnicity			
non-Hispanic White	6168 (58.3)	1551 (62.5)	777 (66.5)
non-Hispanic Black	1147 (10.8)	127 (5.1)	74 (6.3)
Hispanic	2577 (24.4)	625 (25.2)	235 (20.1)
Other	688 (6.5)	177 (7.1)	83 (7.1)
Missing	4 (0.04)	0	0
Maternal Education			
<12 years	1744 (16.6)	493 (20.0)	171 (14.7)
12 years	2522 (24.0)	660 (26.8)	296 (25.4)
>12 years	6241 (59.4)	1311 (53.2)	697 (59.9)
Missing	77 (0.7)	16 (0.7)	5 (0.4)
Pre-Pregnancy BMI (kg/m^2)			
Underweight (<18.5)	557 (5.5)	148 (6.3)	68 (6.0)
Normal (18.5-24.9)	5442 (53.6)	1259 (53.4)	573 (50.3)
Overweight (25.0-29.9)	2327 (22.9)	507 (21.5)	273 (24.1)
Obese (>=30)	1829 (18.0)	445 (18.9)	220 (19.4)
Missing	429 (4.1)	121 (4.9)	35 (3.0)
Maternal Smoking[1]			
Any	1206 (11.9)	388 (16.7)	170 (15.4)
None	8899 (88.1)	1940 (83.3)	936 (84.6)
Missing	479 (4.5)	152 (6.1)	63 (5.4)
Secondhand Smoke[1]			
Yes	2262 (21.5)	668 (27.1)	297 (25.5)
No	8253 (78.5)	1795 (72.9)	867 (74.5)
Missing	69 (0.7)	17 (0.7)	5 (0.4)
Maternal Alcohol[2]			
Binge (>=4 drinks)	1326 (12.7)	327 (13.3)	143 (12.4)
Some	2560 (24.5)	581 (23.7)	321 (27.7)
None	6568 (62.8)	1543 (63.0)	693 (59.9)
Missing	130 (1.2)	29 (1.2)	12 (1.0)
Prior Pregnancies			
0	3132 (29.6)	720 (29.1)	315 (27.0)
1	3019 (28.5)	731 (29.5)	352 (30.1)
>1	4430 (41.9)	1027 (41.4)	502 (42.9)
Missing	3 (0.03)	2 (0.1)	0

Table 6 continued.

	Controls	CL/P	CP
Folic Acid-Containing Supplement[1]			
Any	7967 (75.3)	1845 (74.5)	900 (77.1)
None	2612 (24.7)	633 (25.5)	268 (23.0)
Missing	5 (0.1)	2 (0.1)	1 (0.1)
Prenatal Care			
Yes	10477 (99.1)	2443 (98.6)	1156 (99.0)
No	101 (1.0)	35 (1.4)	12 (1.0)
Missing	6 (0.1)	2 (0.1)	1 (0.1)
Infant Sex			
Female	5183 (49.0)	842 (34.0)	676 (57.9)
Male	5391 (51.0)	1635 (66.0)	492 (42.1)
Missing	10 (0.1)	3 (0.1)	1 (0.1)
Family History of OFC			
Yes	32 (0.3)	152 (6.1)	66 (5.7)
No	10552 (99.7)	2328 (93.9)	1103 (94.4)
Missing	0	0	0
Folate-Antagonistic Medication			
Yes	77 (0.7)	21 (0.9)	13 (1.1)
No	10497 (99.3)	2456 (99.2)	1155 (98.9)
Missing	10 (0.1)	3 (0.1)	1 (0.1)
Maternal Residence			
Arkansas	1377 (13.0)	296 (11.9)	138 (11.8)
California	1187 (11.2)	404 (16.3)	147 (12.6)
Georgia	1053 (10.0)	249 (10.0)	128 (11.0)
Iowa	1216 (11.5)	261 (10.5)	121 (10.4)
Massachusetts	1217 (11.5)	278 (11.2)	181 (15.5)
New Jersey	542 (5.1)	93 (3.8)	57 (5.0)
New York	932 (8.8)	202 (8.2)	108 (9.2)
North Carolina	926 (8.8)	186 (7.5)	103 (8.8)
Texas	1217 (11.5)	254 (10.2)	92 (7.9)
Utah	917 (8.7)	257 (10.4)	94 (8.0)

Abbreviations: BMI, body mass index; CL/P, cleft lip with or without palate; CP, cleft palate; OFC, orofacial clefts.

1. First two months of pregnancy
2. One month prior to conception through three months after conception.

Table 7. Model fit statistics for unadjusted candidate models, differing by number of specified classes, in a latent class analysis deriving dietary patterns among controls from the National Birth Defects Prevention Study, 2005-2011

Classes in model (n)	Latent class	Controls (n)	Average probability of membership (%)	AIC[1]	BIC[1]	Entropy
2	1	4734	95.5	1414291.8	1416886.1	0.86
	2	5850	96.1			
3	1	3109	93.9	1393512.0	1397407.2	0.87
	2	3827	94.3			
	3	3648	94.8			
4	1	2754	94.1	1376297.5	1381493.4	0.90
	2	1648	95.0			
	3	2814	94.3			
	4	3388	94.4			
5	1	2326	94.0	1366645.7	1373142.5	0.90
	2	2436	93.2			
	3	2312	93.3			
	4	1485	95.2			
	5	2025	91.0			
6	1	1518	91.6	1359124.2	1366921.8	0.89
	2	1910	90.8			
	3	2099	91.6			
	4	1541	92.6			
	5	2128	93.6			
	6	1388	95.2			

Abbreviations: AIC: Akaike information criteria; BIC: Bayesian information criteria; LC: latent class.
1. Lower AIC and BIC values indicate better goodness-of-fit.

Figure 13. Probability of periconceptional high food intake (3rd tertile), low food intake (1st tertile), or non-consumption by dietary class among controls in the National Birth Defects Prevention Study, 1997-2011

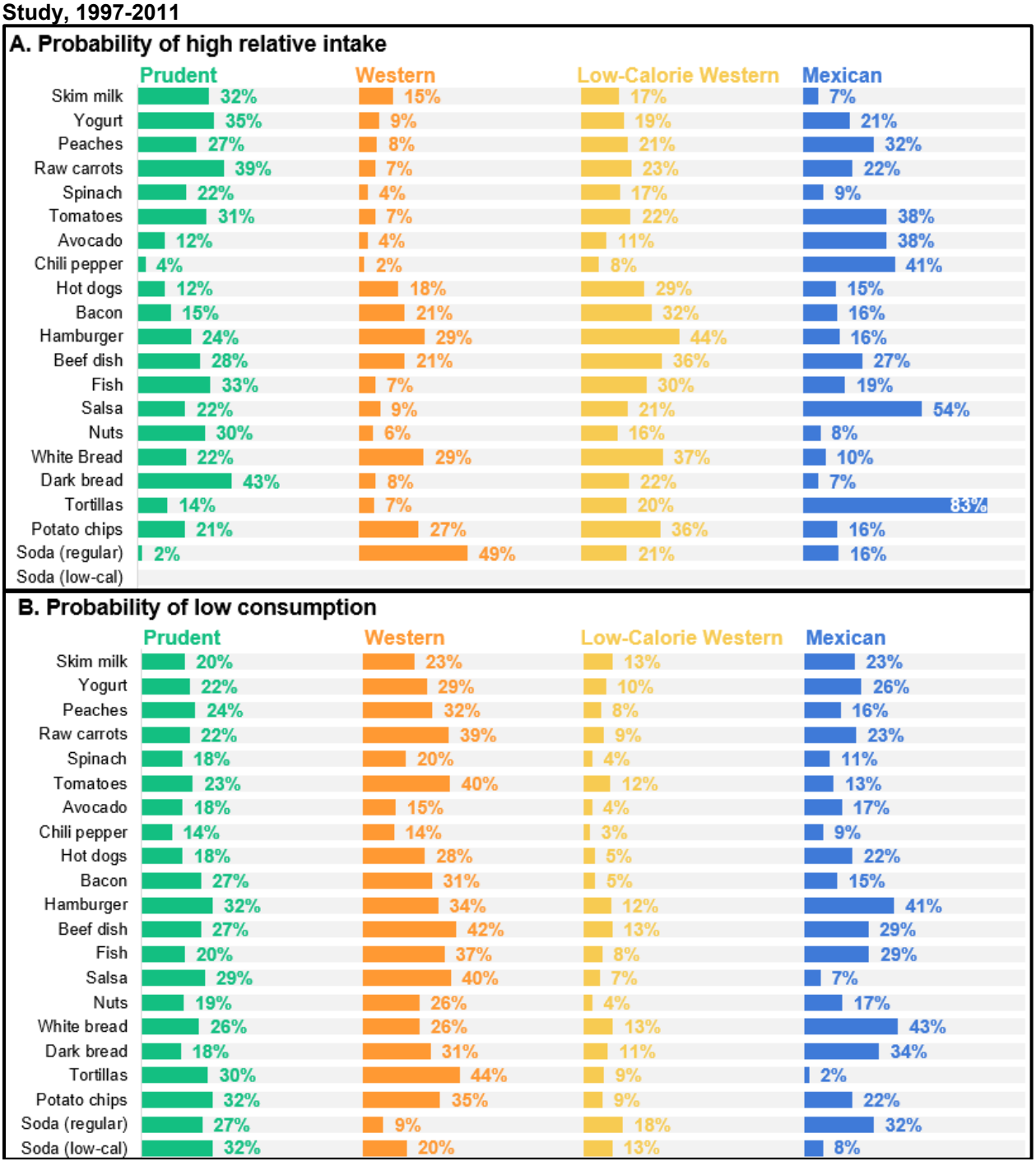


Figure 13 continued.

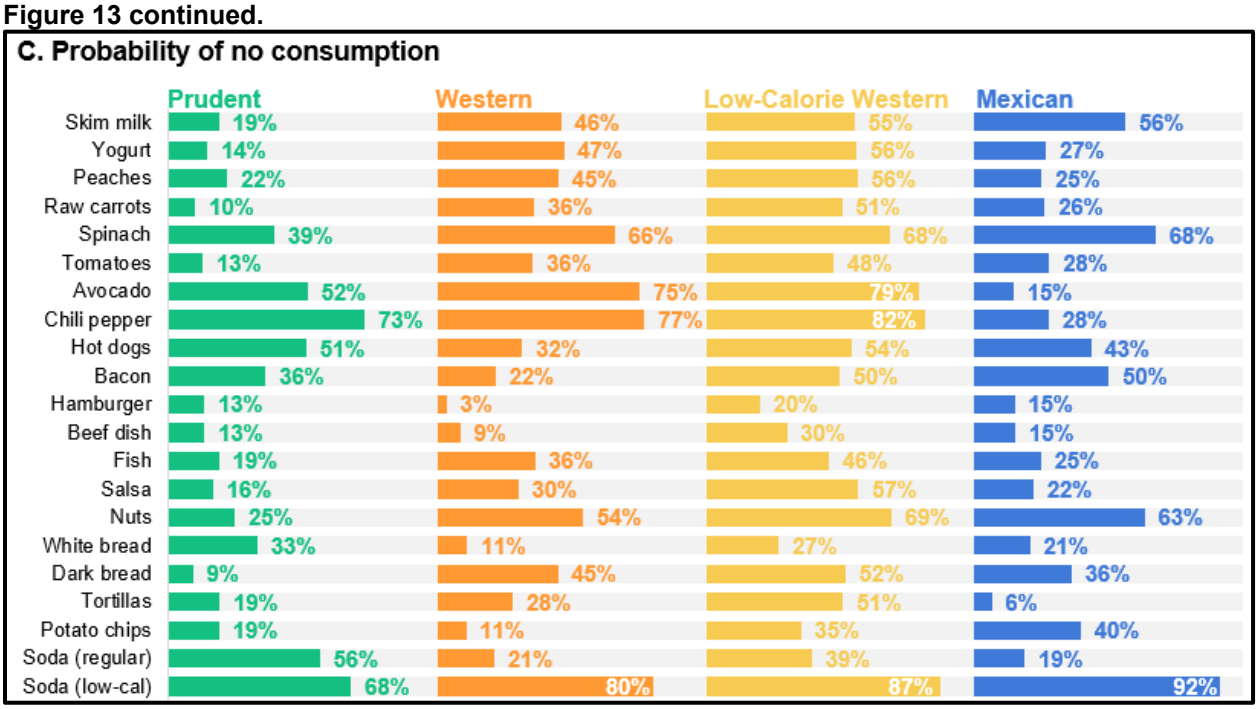


Figure 13.A: The probability of high relative intake (third tertile) dependent on dietary class and adjusted for energy intake. Low-calorie soda does not have a probability of high consumption because it was rarely consumed (<10%) so intake was dichotomized (yes/no). **Figure 13.B:** The probability of low consumption (first tertile) dependent on dietary class and adjusted for energy intake. **Figure 13.C:** The probability of no consumption dependent on dietary class and adjusted for energy intake.

Table 8. Maternal characteristics by dietary pattern among controls without a birth defect, National Birth Defects Prevention Study, 1997-2011

	Prudent (n=3396) n(%)	Western (n=2744) n(%)	Low-Calorie Western (n=2802) n(%)	Mexican (n=1642) n(%)
Age at Conception				
<20	86 (2.5)	461 (16.8)	524 (18.7)	297 (18.1)
20-25	649 (19.1)	946 (34.5)	889 (31.7)	553 (33.7)
26-35	2243 (66.1)	1180 (43.0)	1189 (42.4)	695 (42.3)
36+	418 (12.3)	157 (5.7)	200 (7.1)	97 (5.9)
Missing	0	0	0	0
Race/Ethnicity				
Non-Hispanic White	2730 (80.4)	1965 (71.6)	1403 (50.1)	70 (4.3)
Non-Hispanic Black	216 (6.4)	350 (12.8)	563 (20.1)	18 (1.1)
Hispanic	217 (6.4)	283 (10.3)	594 (21.2)	1483 (90.4)
Other	232 (6.8)	146 (5.3)	240 (8.6)	70 (4.3)
Missing	1 (0.03)	0	2 (0.7)	1 (0.6)
Education				
<12 years	77 (2.3)	384 (14.1)	503 (18.2)	780 (48.0)
12 years	385 (11.4)	851 (31.1)	794 (28.7)	492 (30.3)
>12 years	2924 (86.4)	1499 (54.8)	1466 (53.1)	352 (21.7)
Missing	10 (0.3)	10 (0.4)	39 (1.4)	18 (1.1)
Pre-Pregnancy BMI (kg/m^2)				
Underweight (<18.5)	142 (4.2)	165 (6.1)	179 (6.6)	71 (5.3)
Normal (18.5-24.9)	2075 (61.4)	1330 (48.8)	1420 (52.3)	617 (46.2)
Overweight (25.0-29.9)	704 (20.8)	615 (22.6)	631 (23.3)	377 (28.2)
Obese (>=30)	461 (13.6)	614 (22.5)	484 (17.8)	270 (20.2)
Missing	14 (0.4)	20 (0.7)	88 (3.1)	307 (18.7)
Smoking[1]				
Any	270 (8.0)	836 (30.6)	459 (16.5)	79 (4.8)
None	3119 (92.0)	1900 (69.4)	2322 (83.5)	1558 (95.2)
Missing	7 (0.2)	8 (0.3)	21 (0.8)	5 (0.3)
Alcohol[2]				
Binge (>=4 drinks)	378 (11.2)	512 (18.9)	314 (11.4)	122 (7.5)
Some	1138 (33.8)	670 (24.7)	545 (19.8)	207 (12.7)
None	1849 (55.0)	1526 (56.4)	1897 (68.8)	1296 (79.8)
Missing	31 (0.9)	36 (1.3)	46 (1.6)	17 (1.0)
Folic Acid-Containing Supplement[1]				
Any	2982 (87.8)	2027 (73.9)	1984 (70.9)	974 (59.4)
None	414 (12.2)	716 (26.1)	815 (29.1)	667 (40.7)
Missing	0	1 (0.04)	3 (0.1)	1 (0.1)

Abbreviations: BMI, body mass index

1. First two months of pregnancy
2. One month prior to conception through three months after conception.

Table 9. Average maternal daily dietary nutrient consumption[1] by dietary pattern among controls without a birth defect, National Birth Defects Prevention Study, 1997-2011

Nutrient	Prudent (n=3396) Mean (SD)	Western (n=2744) Mean (SD)	Low-Calorie Western (n=2802) Mean (SD)	Mexican (n=1642) Mean (SD)
Energy, kcal	1488.3 (519.1)	1693.2 (748.6)	1416.0 (620.5)	2035.8 (802.5)
Total grams of food/day	1712.2 (657.2)	2486.7 (1090.2)	1555.6 (792.5)	2476.1 (1020.1)
Calcium (Ca), mg	895.3 (422.5)	818.8 (499.9)	676.3 (411.0)	991.9 (518.0)
Caffeine, g	80.5 (102.2)	159.7 (152.7)	55.9 (86.5)	75.9 (85.6)
α-carotene, µg RE	797.9 (726.8)	413.5 (663.7)	524.3 (813.3)	1047.4 (1344.6)
β-carotene, µg RE	3591.3 (2616.4)	1864.2 (1962.2)	2416.7 (2881.8)	4513.6 (4281.3)
Carbohydrate, g	197.0 (76.0)	243.8 (125.7)	191.8 (97.4)	300.9 (128.0)
Cholesterol, mg	227.2 (112.0)	236.9 (145.2)	231.5 (149.8)	297.2 (166.9)
Choline, g	314.3 (124.6)	301.6 (149.4)	293.5 (155.5)	446.9 (197.9)
Fatty acids, total monounsaturated, g	17.5 (8.1)	18.7 (9.5)	16.6 (8.9)	22.6 (11.5)
Fatty acids, total polyunsaturated, g	7.6 (3.5)	7.7 (4.3)	6.9 (3.7)	8.8 (4.1)
Fatty acids, total saturated, g	19.4 (8.2)	21.0 (11.1)	17.8 (9.4)	21.7 (10.2)
Total fat, g	49.8 (20.6)	53.5 (26.6)	46.7 (23.5)	59.8 (27.4)
Fatty acids, total trans, g	1.1 (0.7)	1.2 (0.9)	0.9 (0.7)	0.8 (0.6)
Iron (Fe), mg	13.0 (7.3)	13.4 (9.0)	12.5 (8.7)	18.2 (10.1)
Fiber (total dietary), g	18.1 (8.6)	13.6 (7.7)	15.3 (9.6)	33.1 (16.0)
Folic acid, µg	172.5 (151.5)	188.3 (218.0)	173.9 (195.3)	184.9 (176.6)
Folate, µg DFE	516.6 (293.7)	491.1 (402.6)	480.4 (371.9)	649.6 (383.9)
Folate, food, µg	223.5 (103.9)	171.2 (89.6)	185.0 (109.0)	335.6 (177.6)
Retinol, µg	470.7 (350.9)	502.3 (368.8)	446.9 (489.7)	734.4 (781.6)
Vitamin E, mg α-TE	6.2 (3.3)	5.3 (3.5)	5.2 (3.4)	8.7 (4.7)
Vitamin A, IU	8357.7 (5321.9)	5241.4 (4192.7)	6082.0 (5859.4)	11097.5 (9408.9)
Vitamin A, µg RAE	808.8 473.8)	679.9 (443.4)	675.9 (587.2)	1166.2 (993.2)
Vitamin B_{12}, µg	5.3 (3.8)	5.5 (3.7)	5.1 (5.2)	8.7 (8.3)
Vitamin B_6, mg	2.1 (0.9)	2.1 (1.2)	2.0 (1.1)	3.0 (1.4)
Vitamin C, mg	112.3 (69.5)	93.2 (83.4)	104.5 (83.8)	202.9 (133.5)
Vitamin D, IU	99.3 (63.5)	106.8 (84.3)	85.1 (66.9)	113.6 (74.0)
Vitamin K, µg	116.7 (106.4)	65.3 (63.2)	85.9 (109.6)	120.4 (126.2)
Zinc (Zn), mg	11.2 (4.7)	11.5 (6.0)	10.3 (5.6)	13.6 (6.3)

Abbreviations: DFE, dietary folate equivalents; g, grams; IU, international units; kcal, kilocalories; mg, milligrams; RAE, retinol activity equivalents; RE, retinol equivalent; TE, tocopherol equivalent.
1. Arithmetic means are presented for total energy and grams per day. Geometric means, controlled for energy intake, are presented for all other nutrients.

Table 10. Association between maternal dietary patterns and orofacial clefts, National Birth Defects Prevention Study (n=14,233), 1997-2011

	N cases	%	OR[1]	(95% CI)	OR[2]	(95% CI)
CL/P						
Prudent	692	27.9	REF		REF	
Western	740	29.8	1.3	(1.2,1.5)	1.3	(1.2,1.5)
Low-Calorie Western	643	25.9	1.1	(1.0,1.3)	1.2	(1.0,1.4)
Mexican	405	16.3	1.2	(1.1,1.4)	1.1	(0.9,1.3)
CP						
Prudent	380	32.5	REF		REF	
Western	356	30.5	1.2	(1.0,1.3)	1.2	(1.1,1.5)
Low-Calorie Western	293	25.1	0.9	(0.8,1.1)	1.0	(0.9,1.2)
Mexican	140	12.0	0.8	(0.6,0.9)	0.8	(0.6,1.1)

Abbreviations: CI, confidence interval; CL/P, cleft lip with or without palate; CP, cleft palate; OR, odds ratio.

1. Odds ratio adjusted for energy intake
2. Odds ratio adjusted for energy intake, age, race/ethnicity, education, supplement with folic acid, gravidity, family history of orofacial cleft, and state.

Table 11. Association between maternal dietary patterns and orofacial clefts among eligible participants with a high probability of class membership (n=12,939),[1] National Birth Defects Prevention Study, 1997-2011

	N cases	%	OR[2]	(95% CI)	OR[3]	(95% CI)
CL/P						
Prudent	616	27.4	REF		REF	
Western	678	30.1	1.4	(1.2,1.6)	1.4	(1.2,1.6)
Low-Calorie Western	586	26.0	1.2	(1.0,1.3)	1.2	(1.1,1.4)
Mexican	370	16.44	1.2	(1.1,1.4)	1.1	(0.9,1.3)
CP						
Prudent	342	32.8	REF		REF	
Western	322	30.8	1.2	(1.0,1.4)	1.3	(1.1,1.5)
Low-Calorie Western	257	24.6	0.9	(0.8,1.1)	1.0	(0.9,1.2)
Mexican	123	11.8	0.7	(0.6,0.9)	0.8	(0.6,1.1)

Abbreviations: CI, confidence interval; CL/P, cleft lip with or without palate; CP, cleft palate; OR, odds ratio.

1. Low class membership is defined as *<(K-1)/K for K* classes (<75%). 1,298 participants with a class membership
probability less than 75% were excluded.
2. Adjusted for energy intake
3. Adjusted for energy intake plus: age, race/ethnicity, education, supplement with folic acid, gravidity, family history of OFC, and state.

Table 12. Association between maternal dietary patterns and orofacial clefts among eligible participants without extreme nausea (n=12,127),[1] National Birth Defects Prevention Study, 1997-2011

	N cases	%	OR[2]	(95% CI)	OR[3]	(95% CI)
CL/P						
Prudent	598	28.0	REF		REF	
Western	624	29.2	1.3	(1.2,1.5)	1.3	(1.2,1.5)
Low-Calorie Western	561	26.2	1.1	(1.0,1.3)	1.2	(1.0,1.4)
Mexican	356	16.6	1.2	(1.0,1.4)	1.1	(0.9,1.4)
CP						
Prudent	323	32.4	REF		REF	
Western	299	30.0	1.2	(1.0,1.4)	1.2	(1.0,1.5)
Low-Calorie Western	251	25.2	0.9	(0.8,1.1)	1.0	(0.9,1.3)
Mexican	124	12.4	0.8	(0.6,0.9)	0.9	(0.7,1.2)

Abbreviations: CI, confidence interval; CL/P, cleft lip with or without palate; CP, cleft palate; OR, odds ratio.

1. Extreme nausea is defined as taking nausea medication/treatment at any time during pregnancy
2. Adjusted for energy intake
3. Adjusted for energy intake plus: age, race/ethnicity, education, supplement with folic acid, gravidity, family history of OFC, and state.

Table 13. Number of cases with orofacial clefts in each dietary pattern by race/ethnicity, National Birth Defects Prevention Study, 1997-2011

	NH White		NH Black		Hispanic		Other	
	N cases	%	N cases	%	N cases	%	N cases	%
CP								
Prudent	314	40.4	19	25.7	27	11.5	20	24.1
Western	288	37.1	15	20.3	32	13.6	20	24.1
Low-Calorie Western	167	21.5	38	51.4	52	22.1	36	43.4
Mexican	8	1.0	2	2.7	124	52.8	7	8.4
CL/P								
Prudent	593	38.2	19	15.0	32	5.1	49	27.7
Western	581	37.5	46	36.2	63	10.1	50	28.2
Low-Calorie Western	356	23.0	59	46.5	167	26.7	59	33.3
Mexican	21	1.4	3	2.4	363	58.1	19	10.7

Abbreviations: CL/P, cleft lip with or without palate; CP, cleft palate; NH, Non-Hispanic.

Table 14. Association between maternal dietary patterns and orofacial clefts among eligible participants by race/ethnicity, National Birth Defects Prevention Study, 1997-2011

	NH White		NH Black		Hispanic		Other	
	OR_{ADJ}[1]	(95% CI)	OR_{ADJ}[1]	(95% CI)	OR_{ADJ}[1]	(95% CI)	OR_{ADJ}[1]	(95% CI)
CP								
Prudent	REF		REF		REF		REF	
Western	1.3	(1.1,1.6)	0.7	(0.3,1.4)	0.9	(0.5,1.6)	1.5	(0.7,3.0)
Low-Calorie Western	1.0	(0.8,1.2)	0.9	(0.5,1.7)	0.8	(0.5,1.4)	1.7	(0.9,3.2)
Mexican	0.7	(0.3,1.6)	1.2	(0.2,5.7)	0.7	(0.4,1.1)	1.2	(0.4,3.0)
CL/P								
Prudent	REF		REF		REF		REF	
Western	1.3	(1.1,1.5)	1.4	(0.8,2.6)	1.5	(0.9,2.5)	1.4	(0.9,2.4)
Low-Calorie Western	1.1	(0.9,1.3)	1.1	(0.6,2.0)	2.1	(1.3,3.2)	1.0	(0.6,1.5)
Mexican	1.1	(0.7,1.9)	1.9	(0.5,7.6)	1.5	(1.0,2.4)	1.0	(0.5,2.0)

Abbreviations: CL/P, cleft lip with or without palate; CP, cleft palate; NH, Non-Hispanic.
1. Adjusted for energy intake plus: age, race/ethnicity, education, supplement with folic acid, gravidity, family history of OFC, and state.

4.4. Discussion

These results further our knowledge regarding the influence of maternal nutrition, before and during pregnancy, on the etiology of birth defects. Specific periconceptional dietary patterns found in this study population had a significant impact on the risk of CL/P and CP, even after controlling for folic-acid supplementation. These patterns were derived from a LCA, which identified 4 underlying dietary classes (Prudent, Western, Low-Calorie Western, and Mexican) related to energy intake and the relative consumption of 64 food indicators. Participants were assigned to the class with their greatest likelihood of membership. The strongest OFC odds were observed when comparing the Prudent (high in dairy, fish, dark breads, vegetables, and fruits) to the Western diet (high in white bread, chips, and soda). The Western, compared to Prudent, diet was associated with a significant increase in the odds of both CP and CL/P. The more restrictive Low-Calorie Western diet (similar to Western with a lower energy intake), compared to the more diverse Prudent diet, was associated with a weaker increase in CL/P but had a null effect for CP. The Mexican diet (similar to Prudent but also high in salsa, peppers, and tortillas) had a mostly null effect on both phenotypes, when compared to Prudent.

Only two studies have evaluated the effect of comprehensive dietary measures on OFC by considering the intake and interaction of multiple food items.[16,114] These studies showed that, after adjustment for folic acid intake, a healthier overall diet quality decreased OFC risk. Our results, although smaller in magnitude, align with these findings. Vujkovic et al. evaluated this association in the Netherlands by deriving dietary patterns using a factor analysis a posteriori.[16] Similarly, the highest tertile of their "Western" pattern, which overlaps with our "Western" definition (high in red or processed meat, potatoes, and legumes and low in fruits) was associated with a significant increase in the odds of any OFC (aOR: 1.9, CI: 1.2,3.1) compared to the lowest tertile of this pattern. Carmichael et al. assessed the effect of diet on OFC in a slightly smaller subset of our NBDPS population using two diet quality indices.[114] To our knowledge, this is the only prior U.S. study, which is important since diet is closely linked to geographic residence.[157] Higher (healthier) quartiles of the indices decreased the odds of OFC when compared to the lowest quartiles. These indices positively scored dietary components like grains, fruits, vegetables, and several nutrients and negatively scored items such as meat and sweets.[111,281] Interestingly, Hispanic participants were more likely to be in these higher quartiles which is also emphasized in the dietary patterns we derived. Although we observed an increased risk of OFC associated with both Western diets, the Mexican diet had a null effect on OFC occurrence, when compared to the Prudent diet.

Our results emphasize the need for holistic measures of dietary patterns and diet quality, as a complement to investigation of individual nutrients. Like dietary indices used previously in the NBDPS data,[114] our newly derived patterns were more predictive of OFC risk than single nutrient estimates previously reported in the NBDPS.[15] To date, this is the largest individual study to assess the impact of maternal diet on OFC occurrence. The NBDPS provides a diverse, population-based study sample with clinically verified OFC phenotypes and robust dietary information. Our ability to derive dietary patterns, compared to individual nutrients, allowed for a more accurate and comprehensive assessment of food and nutrient interactions. LCA is optimal for deriving maternal dietary patterns due to its ability to identify complex patterns while adjusting for covariates, such as energy intake.[115]

Our study was limited by sample size, upon stratification by race/ethnicity, for our EMM analysis, which made the interpretation of results difficult. EMM of this association by race/ethnicity warrants further

research since diet is closely linked to cultural norms.[282] In terms of other limitations, our results are potentially susceptible to selection bias given that 65.8% of eligible subjects agreed to participate in the NBDPS. It is possible that these individuals were inherently different than those who opted out of the study; yet, it has been noted that bias from these differences may be minimal.[283] Additionally, due to the NBDPS case-control study design, exposures were collected after OFC occurrence. Self-reported exposures may have been influenced by case/control status, which could lead to differential misclassification. The extended time between delivery and interview (up to 24 months) could also lead to recall error, which could lead to differential or non-differential misclassification.[284]

Further, the FFQ ascertained dietary intake before, rather than during, pregnancy; however, nutritional intake the year prior to pregnancy has been previously correlated with early pregnancy diet.[264] The semi-quantitative nature of our FFQ warrants cautious interpretation of actual nutrient intake values but is a good tool for our purpose of assessing relative dietary patterns.[285,286] Self-reported dietary intake reported through the FFQ is vulnerable to intake-related bias, in which individuals with high consumption report lower than actual consumption and vice versa, and social desirability bias, in which individuals attempt to align their responses to broader social norms.[287–289] Additionally, the LCA assumes that dietary intake is similar within each class, which may be a strong assumption. However, results were robust to the exclusion of participants that had a lower likelihood of class membership, due to their individual intake differing from that of their assigned class. Finally, residual confounding is likely as several factors that influence both OFC risk and maternal diet were unmeasured in the NBDPS database, such as household income and other socioeconomic measures that impact access to healthy foods.

Dietary patterns that are more likely to include white bread, chips, and soda, compared to diets comprised of dairy, fish, dark breads, vegetables, and fruits, increased the odds of CL/P and CP in our study population. These results remained robust after controlling for factors such as folic acid supplementation. With confirmation of these findings, dietary counseling/interventions centered on healthy, attainable dietary patterns may be considered as a preventative OFC measure. Further research could also influence dietary recommendations and food assistance program practices for pregnant individuals, or those considering conception.

CHAPTER 5: THE EFFECT OF KNOWN RISK FACTORS ON OROFACIAL CLEFTS SPECIFIC TO HISPANICS IN THE UNIITED STATES

5.1. Introduction

Orofacial clefts (OFC) are a common birth defect in the United States (US). For every 10,000 births, 3.5 are diagnosed with cleft lip (CL), 5.9 are diagnosed with cleft palate (CP), and 6.7 are diagnosed with cleft lip with palate (CLP).[1] Compared to non-Hispanic white (NHW) individuals, Hispanic individuals have a higher prevalence of CLP, but a lower prevalence of CL and CP.[1] OFC has been associated with challenges in speaking, hearing, and feeding and is known to significantly impact the psychological wellbeing of affected individuals and their communities.[30] Treatment for OFC consists of extensive and invasive multidisciplinary care, often required throughout the lifespan.[39] OFC care in the US is associated with hospital charges upwards of $126,000,000.00 per year.[38]

OFC occur early in pregnancy, often before conception is recognized.[34] Most cases are non-chromosomal and, while etiology is largely unknown, it has been suggested that cases are likely caused by an interaction of genetic and environmental factors.[7,8,33] Thus, it is important to focus on the impact of modifiable risk factors for OFC. A relatively large body of research has identified several modifiable risk factors, such as smoking and pregestational diabetes, but the effect of these exposures has only been explored in primarily NHW cohorts.[14,186] Given that the prevalence of OFCs is different for Hispanic and NHW individuals, examining risk factors among Hispanics is important to understanding potential differences in etiology and/or targets for intervention.

As the largest minoritized racial-ethnic subgroup in the US, the Hispanic population is fast growing and diverse.[290] Disparities in healthcare and health outcomes among the US Hispanic population have been noted.[53,291] Within this US population, heterogeneity in health outcomes has been observed and may partly be explained by acculturation, as roughly 30% of this population is foreign-born.[2] To assess the impact of established OFC risk factors specific to Hispanic individuals, we evaluated the effect of 14 risk factors on OFC occurrence among a large Hispanic population, with varying levels of

acculturation, from a multi-center case-control study. Acculturation was considered as a risk factor and included as a confounder in other risk factor models, to partially account for the cultural diversity in lived experiences and health behaviors within this population. We also estimated average adjusted population attributable fractions (aaPAFs) for the Hispanic population, as well as a corresponding NHW population, to assess the population impact of the studied risk factors. Additionally, we stratified Hispanic aaPAFs by a validated acculturation score to further explore the effect of acculturation on OFC among Hispanic individuals living in the US.

5.2. Methods

Study Population

The National Birth Defect Prevention Study (NBDPS) is a multi-state population-based case control study, in which 25% of participants identified as Hispanic. Most Hispanic controls in this analysis lived in Texas (38.0%) or California (26.4%) at the time of delivery and spoke primarily Spanish at home (61.6%), although 63.6% completed the NBDPS interview in English. 42.6% of controls were born outside of the US, with 80.1% reporting Mexico as their country of origin.

The NBDPS was designed to identify causes of major structural birth defects among deliveries in ten US states from 1997-2011. Cases, including terminations, stillbirths and liveborn infants with select birth defects, were identified through state surveillance systems and confirmed by clinical geneticists.[259] Controls, liveborn infants without a birth defect, were selected from birth or vital records at a similar time and region to that of cases. Eligible participants spoke English or Spanish, had custody of the index infant, and were 18 or older (dependent on state).[257] Participants were ineligible if incarcerated, due to the sensitive nature of the study interview. Eligible participants, identified up to two years after their estimated delivery date, were recruited to participate in a standardized hour-long telephone interview conducted in either English or Spanish. The interview collected extensive information on race and ethnicity, other demographics, acculturation, and periconceptional and prenatal exposures.[257]

There are 4,792 OFC cases and 10,692 controls in NBDPS. Of these participants, we excluded non-Hispanic individuals (n=12,365), non-singleton pregnancies (or missing) (n=597), pregnancies with a donor egg or embryo (n=52), cases with multiple birth defects (n=703), and infants with a family history of

OFC (n=301) due to likely differences in etiology compared to other OFC cases. Several participants were excluded based on multiple factors. Our final sample included 918 OFC cases and 2,830 controls. Cases were further differentiated by phenotype (CL=190, CP=245, CLP=483). Our secondary analysis used a NHW reference group that was derived from NBDPS using these same exclusion criteria and included 2,280 cases (CL=610, CP=772, CLP=898) and 6,425 controls.

Risk Factors

Risk factors considered as established were identified through a literature review and ascertained from the NBDPS interview. Risk factors included: maternal age at conception (<20, 20-25, 26-35, or >35 years),[203] education (0-11, 12, or >12 years),[220] folic acid-containing supplement intake (any/none during the first two months of pregnancy),[9] smoking and secondhand smoke (smoking only/secondhand smoke only/both/neither during the first two months of pregnancy),[186,188,192,246192] binge drinking (≥4 or <4 drinks per sitting from one month prior to three months after conception),[177,197] preconception body mass index (≥25.0 or <25.0 kg/m^2),[176,214] pregestational diabetes (yes/no),[14] maternal fever (yes/no from one month prior to three months after conception),[225,292] gravidity (≤1 or >1 pregnancy),[223] prenatal care (any visits/none),[218,220] and dietary folate intake (lowest quartile versus higher, preconception).[143]

Using information about language and nativity reported during the interview, we assessed acculturation using the validated Proxy Acculturation Score-3 (PAS-3), which incorporates interview language (2=English, 0=Spanish), language spoken at home (2=English, 0=Spanish), and proportion of life lived in the US ranging from 0 to 1 (years in the US/age).[256] A validation study showed that the PAS-3, when dichotomized (“medium/high” [>1] v. “low” [≤1] acculturation), outperformed several single indictors of acculturation.[256]. If a participant in our sample was missing a part of this score but their observed score was already greater than 1, they were assigned to the “medium/high” group. Otherwise, if the observed score was ≤1 and missing an indicator, the score was set to missing.

Periconceptional maternal diet was measured during the NBDPS interview for the year before conception using a modified Willett food frequency questionnaire (FFQ) with 58 food items.[19] Beverage and cereal items were additionally added for a total of 64 food items. Using a Latent Class Analysis (LCA), we derived dietary patterns based on the relative consumption of these food indicators and daily

energy intake. The use of LCA for dietary assessment has been previously described.[162] In brief, we first calculated relative food consumption among controls by dividing daily grams consumed for each food by total daily grams and then calculated tertiles from these food-specific percentages (with an additional nonconsumption level). To avoid sparse cells, foods with extremely low intake (<20%) were dichotomized (none v. any), while the nonconsumption level and first tertile were combined for foods with extremely high intake (≥90%). We ran a multinomial model, adjusted for daily energy intake, several times to identify the optimal number of dietary classes for these food-specific tertiles. The final model was selected based on the Akaike Information Criteria (AIC), Bayesian Information Criteria (BIC), entropy value, and model interpretability. This model produced regression coefficients for class membership and the conditional probability of each food belonging to each class. We then combined these probabilities, using Baye's theorem, to predict each participant's class membership probability, dependent on relative intake of foods consumed and energy intake. Participants were then assigned to the class with their highest probability of membership.[119] LCA models were run separately for NHW and Hispanic subgroups.

Primary Analysis

Confounders specific to each risk factor model were identified through a Directed Acyclic Graph (DAG) (**Table 15**)**.** Missing values in any of the 14 variables in our risk factor analysis were imputed under the assumption that data was missing at random. We created an imputation model for each risk factor-OFC combination and ran five imputation cycles with fully conditional specification. We then used these imputed datasets in crude and adjusted multivariable logistic regression models created for each risk factor-phenotype combination. Models produced crude and adjusted odds ratios (aORs), with corresponding 95% confidence intervals (CI). Results were then pooled in SAS using proc mianalyze.[272]

Secondary Analysis

As a secondary analysis, crude and average adjusted population attributable fractions (aaPAFs) were calculated for select risk factors among our Hispanic and NHW populations. The aaPAF estimates the fraction of the outcome (OFC) that could be prevented if the risk in the exposed shifted to the risk in the unexposed, among all levels of relevant covariates which necessitated dichotomization of our

variables.[268] Due to the causal nature of PAFs, we only estimated aaPAFs for risk factors with strong priors. Risk factor selection was based on the number of published studies (≥5) with most studies reporting an aOR of 1.5 or higher. Meta-analyses and statistical precision (95% CIs) were also considered. Six risk factors were identified: maternal smoking, secondhand smoke, maternal diet (derived patterns), lack of folic acid supplementation, pregestational diabetes, and infant sex. Of note, aaPAF populations were estimated using a complete-case analysis. Upon exploring the impact of imputation on results from our primary analysis, we found that aORs in the imputed versus complete-case analysis were similar and that imputation did not meaningfully impact results (data not shown).

Crude and adjusted ORs for selected dichotomized risk factors were estimated through multivariable logistic regression models that included previously identified confounders (**Table 15**). aORs were further stratified by PAS-3 scores. cPAFs were then derived using the formula below.[249]

$$cPAF = \frac{p(OR-1)}{p(OR-1)+1}, \textit{where } p = \frac{Number\ Exposed}{(Number\ Exposed + Number\ Unexposed)}$$

To estimate aaPAFs, we used an approach from Eide and Gefeller that has been modified for case-control studies and used in prior NBDPS studies.[245,268,269] aaPAFs were calculated using an algorithm from Ruckinger et al .[270] First, adjusted PAFs (aPAFs) for each risk factor were estimated using a logistic regression model for each OFC phenotype using all risk factors. aPAF estimations are dependent on the order in which risk factors are removed so we calculated aPAFs for every possible sequence of risk factor removal and then averaged these aPAF estimates to get the aaPAF. We then used bootstrap sampling to produce 95% CIs for cPAFs and aaPAFs.[271] aaPAFs for Hispanic and NHW populations were then compared. Hispanic aaPAFs were further stratified by PAS-3 scores to assess differences in risk factor profiles by acculturation status.

In some previous studies two additional steps are considered when estimating aaPAFs. Because PAFs estimate a decrease in disease dependent on the removal of a risk factor, some previous analyses do not estimate PAFs for risk factors that increase disease occurrence in their study population (aOR≤1). Also, some studies truncate the lower limit of CIs at zero, under the assumption that the removal of that specific risk factor will not increase disease occurrence. We did not include either step due to the novelty of our research question and causal interpretation of PAFs. Most established risk factors for OFC have never been evaluated in Hispanic individuals and, to our knowledge, the effect of diet has not yet been

included in an OFC aaPAF analysis. To provide full transparency of our results, we estimated aaPAFs for all risk factors, regardless of the specific effect on our study population, and allowed CIs to fluctuate to fully describe precision.

5.3. Results

Within our NBDPS sample of Hispanic participants, the majority of controls were 26-35 years old (40.2%) with a high acculturation (PAS-3) score (65.8%) and less than 12 years of schooling (42.5%). Most controls did not smoke (93.6%) or drink (76.3%), and 84.2% reported no exposure to secondhand smoke during early pregnancy (**Table 16**).

Risk Factor Associations Among Hispanics

Specific dietary patterns had a notably strong effect on all OFC phenotypes (**Table 19**). Based on the AIC, BIC, entropy value, and interpretability of results, three classes best fit our Hispanic population. The Prudent class was characterized by a relatively high consumption of fruits, vegetables, beans/lentils, and fish and a low probability of consuming high amounts of soda and chips. The Low-Calorie class closely followed Prudent trends but was differentiated by a slightly higher likelihood of consuming bacon, hot dogs, and French fries, extreme probabilities of intake (e.g. relatively higher probabilities of high consumption and no consumption), and a class membership probability that decreased as total energy increased. The last class, labeled Western, had a notably low likelihood of high consumption for most vegetables and fruits, but a relatively high probability of consuming hamburgers, white bread, French fries, chips, and soda (**Tables 17 and 18**). When compared to Prudent, the Western diet increased the odds of all phenotypes (CL aOR: 1.7, CI: 1.1,2.6; CP aOR: 1.7, CI: 1.2,2.5; CLP aOR: 1.3, CI: 1.0,1.7), and the Low-Calorie dietary pattern increased the odds of CL (aOR: 1.6, CI: 1.1,2.4) and CLP (aOR: 1.3; CI: 1.0,1.7).

Prenatal exposure to secondhand smoke had a strong impact on all OFC phenotypes as well (CL aOR: 1.8, CI: 1.2,2.7; CP aOR: 1.9, CI: 1.3,2.8; CLP aOR: 1.7, CI: 1.3,2.2). The relative strength of other risk factors differed by phenotype. Smoking (aOR: 2.4, CI: 1.2,4.5) and maternal fever (aOR: 1.6, CI: 1.1,2.5) were associated with an increase in CL, but CP was more influenced by acculturation (aOR: 1.4,

CI: 1.0,1.9), gravidity (aOR: 1.4, CI: 1.1,1.9), and pregestational diabetes (aOR: 3.6, CI: 1.6,7.9). CLP was the only phenotype to be influenced by education. Higher education (>12 v. 12 years) was associated with a notable reduction in CLP (aOR: 0.7, CI: 0.5,0.9) (**Table 19**).

Risk Factor Associations Among Non-Hispanic Whites

Similar to the LCA conducted in our Hispanic subgroup, three classes best fit the dietary data for our NHW subgroup. The NHW Prudent class was characterized by a higher probability of consuming fish, fruits, vegetables, dark bread and nuts, along with an overall lower probability of nonconsumption for most foods, perhaps suggesting a more diverse diet. The Western class was comprised of higher consumption probabilities for hot dogs, bacon, chips, sodas, and desert foods **(Tables 17 and 18)**. Unlike the Hispanic Low-Calorie class, the NHW Low-Calorie class followed Western consumption trends (aside from soda) but, similar to the Hispanic Low-Calorie class, the probability of class membership decreased as total energy increased and extreme probabilities of intake were observed. To dichotomize this dietary exposure for aaPAF analyses, a non-Prudent (Western and Low-Calorie classes combined) and Prudent group were created. The effect of the non-Prudent pattern (v. Prudent) was associated with a notable increase in CLP (aOR: 1.4, CI: 1.2,1.6), a relatively smaller increase in CP (aOR: 1.1, CI: 1.0,1.3), and a null effect for CL (aOR: 1.0, CI: 0.8,1.2).

Most other dichotomized risk factors had a fairly weak effect on OFC occurrence among NHW individuals (**Table 21**). Aside from the effect of diet on CLP, the strongest observed association was for smoking (aOR: 1.4, CI: 1.2,1.7) on CLP. Smoking was also associated with a slight increase in CP (aOR: 1.2, CI: 1.0,1.5). All associations for CL were relatively small in magnitude with confidence intervals that included one.

Average Adjusted Population Attributable Fractions

The total proportion of Hispanic CL, CP, and CLP cases explained from our aaPAF models were 47.1% (CI: 24.0%,65.7%), 46.2% (CI: 27.3%,63.4%), and 43.1% (CI: 26.7%,56.9%), respectively (**Table 22**). The highest modifiable aaPAF for all Hispanic phenotypes was the non-Prudent (v. Prudent) diet (CL: 23.5%, CI: 5.2%,41.3%; CP: 10.2%, CI: -6.27%,25.2%; CLP: 14.8%, CI: 0.6%,27.7%) followed by

prenatal exposure to secondhand smoke (CL: 6.3%, CI: -1.1%,14.2%; CP: 8.0%, CI: 2.1%,14.2%; CLP: 2.0%, CI: -2.4%,6.5%) (**Table 22**).

Upon stratification of Hispanic aaPAFs by acculturation, the impact of diet (other v. Prudent) on CL (low PAS-3 aaPAF: 18.4%, CI: -11.8%,46.9%; high PAS-3 aaPAF: 24.6%, CI: -0.2%,48.5%) and secondhand smoke on CP (low PAS-3 aaPAF: 20.4%, CI: 9.7%,33.2%; high PAS-3 aaPAF: 3.0%, CI: -4.2%,10.2%) were associated with the largest differences in aaPAFs between individuals with low and high PAS-3 scores (**Table 25**). Otherwise, risk factor profiles were fairly similar for Hispanic participants across PAS-3 scores. Of note, these estimates should be interpreted with caution due to small sample size and, subsequently, wide CIs (**Table 25**).

Among NHW individuals, the largest total proportion of cases explained was found for CLP (53.9%, CI: 46.2%,61.3%), followed by CL (35.6%, CI: 25.1%,46.6%) and CP (21.7%, CI: 9.8%,32.0%). Diet (Other v. Prudent) was the highest modifiable aaPAF for NHW CP (4.7%, CI: -4.6%,14.3%) and CLP (13.8%, CI: 6.3%,21.2%), while smoking was the highest for CL (2.4%, CI: -2.0%,7.0%) (**Table 23**). Diet aaPAFs had the widest confidence intervals for both populations.

Table 15. Confounders specific to each OFC risk factor model among Hispanic participants in the National Birth Defects Prevention Study, 2007-2011[1]

Risk factor model	Minimally sufficient set
Maternal Age	Center
Maternal Education	PAS-3, age, center
BMI	PAS-3, education, gravidity, diet, diabetes, center
Composite Smoking Measure (smoking/secondhand smoke)	PAS-3, age, education, gravidity, alcohol, prenatal care, center
Maternal Drinking	PAS-3, age, education, gravidity, prenatal care, center
Maternal Diet	Energy, age, education, gravidity, diabetes, center
Pregestational Diabetes	Center
Prior Pregnancies	PAS-3, age, education, diabetes, center
FA Supplement	PAS-3, anti-folate medications, age, education, gravidity, diabetes, prenatal care, center
Low Dietary Folate	Diet, center
Any Fever	Center
Prenatal Care	PAS-3, age, education, gravidity, diabetes, center
Infant Sex	Center
High Acculturation (PAS-3)	Age, center

Abbreviations: OFC, orofacial cleft; PAS-3, proxy acculturation score-3.
1. Confounders identified through Directed Acyclic Graphs reflective of covariate relationships from **Figure 11**

Table 16. Maternal and infant characteristics among Hispanic cases with OFC and controls without a birth defect in the National Birth Defects Prevention Study, 1997-2011

	Controls (n=2830) n(%)	CL (n=190) n(%)	CP (n=245) n(%)	CLP (n=483) n(%)
Age at Conception				
<20	586(20.7)	31(16.3)	40(16.3)	90(18.6)
20-25	950(33.6)	70(36.8)	85(34.7)	177(36.7)
26-35	1138(40.2)	74(39.0)	105(42.9)	185(38.3)
36+	156(5.5)	15(7.9)	15(6.1)	31(6.4)
Missing	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Education				
<12 years	1147(42.5)	69(37.3)	95(40.6)	217(46.9)
12 years	775(28.8)	58(31.4)	73(31.2)	143(30.9)
>12 years	774(28.7)	58(31.4)	66(28.2)	103(22.3)
Missing	134(4.7)	5(2.6)	11(4.5)	20(4.1)
Pre-Pregnancy BMI (kg/m^2)				
Underweight (<18.5)	119(5.0)	8(4.9)	11(5.1)	23(5.8)
Normal (18.5-24.9)	1145(47.7)	79(48.8)	93(43.3)	176(44.3)
Overweight (25.0-29.9)	627(26.1)	43(26.5)	69(32.1)	94(23.7)
Obese (>=30)	508(21.2)	32(19.8)	42(19.5)	104(26.2)
Missing	431(15.2)	28(14.7)	30(12.2)	86(17.8)
Smoking[1]				
Any	175(6.4)	20(10.8)	23(9.8)	30(6.5)
None	2543(93.6)	165(89.2)	211(90.2)	435(93.6)
Missing	112(4.0)	5(2.6)	11(4.5)	18(3.7)
Secondhand Smoke[1]				
Yes	429(15.6)	40(21.7)	56(23.9)	100(21.6)
No	2277(84.2)	144(78.3)	178(76.1)	363(78.4)
Missing	124(4.4)	6(3.2)	11(4.5)	20(4.1)
Alcohol Intake[2]				
Binge (>=4 drinks/sitting)	230(8.5)	18(10.0)	22(9.5)	49(10.7)
Some but not binge	411(14.2)	27(15.0)	46(19.8)	62(13.5)
None	2059(76.3)	135(75.0)	164(70.7)	248(75.8)
Missing	130(4.6)	10(5.3)	13(5.3)	24(5.0)
Pregestational Diabetes				
Yes	17(0.6)	3(1.6)	4(1.7)	10(2.1)
No	2791(99.4)	187(98.4)	238(98.4)	469(97.9)
Missing	22(0.8)	0(0.0)	3(1.2)	4(0.8)
Prior Pregnancies				
0	808(28.7)	50(26.5)	47(19.3)	125(26.1)
1	766(27.2)	56(29.6)	66(27.1)	125(26.1)
>1	1245(44.2)	83(43.9)	131(53.7)	229(47.8)
Missing	11(0.4)	1(0.5)	1(0.4)	4(0.8)
FA-Containing Supplement[1]				
Any	1676(60.4)	111(59.0)	135(57.0)	276(58.4)
None	1099(39.6)	77(41.0)	102(43.0)	197(41.7)
Missing	55(1.9)	2(1.1)	8(3.3)	10(2.1)
Maternal Dietary Patterns[3]				
Western	753(29.0)	57(32.8)	79(35.0)	131(29.9)
Prudent	956(36.8)	48(27.6)	70(31.0)	142(32.4)
Low-Calorie	887(34.2)	69(39.7)	77(34.1)	165(37.7)
Missing	234(8.3)	16(8.4)	19(7.8)	45(9.3)

Table 16 continued.

	Controls	CL	CP	CLP
Dietary Folate, DFE[4]				
Lowest Folate Quartile	691(25.0)	50(26.6)	70(29.5)	134(28.5)
>Lowest Folate Quartile	2072(75.0)	138(73.4)	167(70.5)	336(71.5)
Missing	67(2.4)	2(1.1)	8(3.3)	10(2.7)
Anti-Folate Medications				
Yes	10(0.4)	1(99.5)	0(0.0)	2(0.4)
No	2817(99.7)	189(0.5)	244(100.0)	480(99.6)
Missing	3(0.1)	0(0.0)	1(0.004)	1(0.002)
Any Fever[2]				
Yes	277(9.9)	27(14.3)	28(11.6)	48(10.0)
No	2534(90.2)	162(85.7)	213(88.4)	431(90.0)
Missing	19(0.7)	1(0.5)	4(1.6)	4(0.8)
Access to Prenatal Care				
Yes	2762(98.2)	187(98.9)	237(97.5)	467(97.5)
No	51(1.8)	2(1.1)	6(2.5)	12(2.5)
Missing	17(0.6)	1(0.5)	2(0.8)	4(0.8)
Language at Home				
English	982(36.3)	74 (40.0)	101(43.2)	153(33.1)
Spanish	1664(61.6)	110(59.5)	133(56.8)	302(65.2)
Other	57(2.1)	1(0.5)	0(0.0)	8(1.7)
Missing	127(4.5)	5(2.6)	11(4.5)	20(4.1)
Language of Interview				
English	1800(63.6)	130(68.4)	172(70.2)	302(62.5)
Spanish	1016(35.9)	59(31.1)	73(29.8)	178(36.9)
Translated	14(0.5)	1(0.5)	0(0.0)	3(0.6)
Missing	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Nativity				
US-Born	1150(42.6)	81(43.8)	112(47.9)	183(39.5)
Non-US-Born	1549(57.4)	104(56.2)	122(52.1)	280(60.5)
Missing	131(4.6)	5(2.6)	11(4.5)	20(4.1)
Proxy Acculturation Score-3				
High	1819(65.8)	131(69.7)	172(71.4)	306(65.1)
Low	947(34.2)	57(30.3)	69(28.6)	164(34.9)
Missing	64(2.3)	2(1.1)	4(1.6)	13(2.7)
Infant Sex				
Female	1352(47.9)	71(37.4)	154(62.9)	166(34.4)
Male	1471(52.1)	119(62.6)	91(37.1)	316(65.6)
Missing	7(0.6)	0(0.0)	0(0.0)	1(0.2)

Table 16 continued.

	Controls	CL	CP	CLP
Maternal Residence				
Arkansas	119(4.2)	6(3.2)	8(3.3)	17(3.5)
California	746(26.4)	74(39.0)	86(35.1)	158(32.7)
Georgia	222(7.8)	18(9.5)	20(8.2)	48(9.9)
Iowa	60(2.1)	1(0.5)	5(2.0)	9(1.9)
Massachusetts	117(4.0)	12(6.3)	16(6.5)	14(2.9)
New Jersey	112(4.0)	6(3.2)	5(2.0)	9(1.9)
New York	103(3.6)	8(4.2)	14(5.7)	28(5.8)
North Carolina	163(5.8)	5(2.6)	18(7.4)	23(4.8)
Texas	1076(38.0)	56(29.5)	64(26.1)	155(32.1)
Utah	112(4.0)	4(2.1)	9(3.7)	22(4.6)
Missing	0(0.0)	0(0.0)	0(0.0)	0(0.0)

Abbreviations: BMI, body mass index; CL, cleft lip; CP, cleft palate; CLP, cleft lip with palate; DFE, dietary folate equivalent; OFC, orofacial clefts.
1. First two months of pregnancy, 2. One month prior to conception through three months after conception, 3. One year prior to conception (measured with Latent Class Analysis), 4. Quartiles based on DFE levels in controls.

Table 17. Probability of high relative intake of specific food items by dietary classes derived from LCAs created for Hispanic and non-Hispanic controls in the National Birth Defects Prevention Study, 1997-2011[1]

	Probability of high relative intake LCA for Hispanics			Probability of high relative intake LCA for NHWs		
	Low Calorie	Prudent	Western	Low Calorie	Prudent	Western
Skim milk	0.17	0.19	0.09	0.15	0.30	0.23
Whole milk	0.23	0.16	0.20	0.10	0.04	0.11
Yogurt	0.24	0.28	0.12	0.09	0.36	0.20
Ice cream	0.31	0.27	0.22	0.17	0.31	0.36
Cottage cheese	0.12	0.17	0.05	0.09	0.23	0.15
Cheese[2]	0.31	0.27	0.33			
Margarine	0.14	0.13	0.23	0.16	0.20	0.25
Butter	0.18	0.14	0.27	0.12	0.24	0.23
Apples[2]				0.11	0.43	0.30
Oranges	0.34	0.34	0.15	0.11	0.33	0.26
Orange juice	0.35	0.27	0.23	0.17	0.31	0.30
Peaches	0.26	0.30	0.09	0.08	0.31	0.20
Bananas	0.36	0.33	0.19	0.13	0.41	0.30
Other fruit	0.26	0.27	0.17	0.12	0.42	0.24
Tomatoes	0.23	0.29	0.15	0.10	0.38	0.24
String beans	0.18	0.19	0.11	0.15	0.28	0.32
Broccoli	0.29	0.31	0.09	0.09	0.37	0.32
Cabbage/Cauliflower	0.27	0.26	0.04	0.06	0.21	0.18
Carrots, raw	0.27	0.29	0.10	0.08	0.41	0.25
Carrots, cooked	0.22	0.26	0.04	0.08	0.23	0.23
Corn[2]	0.33	0.28	0.25			
Peas/Lima beans	0.12	0.15	0.06	0.11	0.23	0.23
Yams/Sweet potatoes	0.11	0.13	0.03	0.03	0.19	0.10
Spinach	0.10	0.15	0.03	0.04	0.22	0.11

Table 17 continued.

	Low Calorie	Prudent	Western	Low Calorie	Prudent	Western
Beans/Lentils	0.29	0.26	0.13	0.07	0.27	0.18
Yellow squash	0.13	0.17	0.02	0.05	0.20	0.12
Eggs[2]						
Chicken/Turkey	0.32	0.22	0.27	0.14	0.28	0.38
Bacon	0.17	0.12	0.25	0.20	0.18	0.31
Hot dogs	0.22	0.15	0.23	0.16	0.12	0.28
Processed Meats	0.27	0.18	0.29	0.17	0.15	0.29
Liver[3]	0.10	0.09	0.02			
Hamburger[2]	0.32	0.16	0.42			
Beef, mixed dish	0.28	0.23	0.23	0.19	0.29	0.37
Beef, main dish	0.31	0.26	0.24	0.20	0.29	0.41
Fish	0.30	0.28	0.11	0.07	0.34	0.23
Chocolate	0.23	0.16	0.33	0.21	0.33	0.38
Candy without chocolate	0.14	0.13	0.25	0.20	0.21	0.30
Pie	0.16	0.18	0.13	0.10	0.17	0.20
Cake	0.27	0.21	0.19	0.14	0.23	0.34
Cookies	0.27	0.26	0.24	0.20	0.31	0.39
White bread	0.26	0.19	0.37	0.24	0.18	0.38
Dark bread	0.21	0.24	0.15	0.06	0.39	0.23
French fries	0.28	0.16	0.37	0.28	0.19	0.47
Potatoes[2]	0.30	0.23	0.29			
Rice/Pasta[2]						
Chips	0.19	0.11	0.39	0.24	0.22	0.37
Nuts	0.09	0.16	0.11	0.06	0.31	0.16
Peanut butter	0.13	0.16	0.13	0.11	0.36	0.28
Oil	0.10	0.15	0.08	0.07	0.33	0.20
Cantaloupe	0.23	0.27	0.06	0.08	0.26	0.17
Avocado	0.28	0.29	0.17	0.04	0.18	0.09
Chile	0.20	0.22	0.14			
Salsa	0.24	0.27	0.19	0.14	0.31	0.30
Chicken Liver[3]						
Organ meats[3]	0.20	0.13	0.18			
Tortillas	0.36	0.34	0.16	0.11	0.33	0.29
Refried beans	0.32	0.30	0.19	0.09	0.20	0.18
Coffee	0.17	0.20	0.18	0.20	0.17	0.11
Tea	0.09	0.13	0.23	0.26	0.10	0.13
Soda	0.19	0.11	0.52	0.41	0.02	0.18
Soda (low-cal)[3]				0.17	0.05	0.07
Cereal, FA[3]						
Cereal, non-FA	0.27	0.21	0.25	0.16	0.28	0.32

1. NHW and Hispanic probabilities cannot be directly compared because separate LCAs were run to best fit the dietary intake of each population, 2. Foods that were consumed by > 90% of the population (Hispanic or non-Hispanic) have 3, rather than 4, consumption levels, 3. Foods that were consumed by <10% of the population (Hispanic or non-Hispanic) were dichotomized.

Table 18. Probability of no consumption for specific food items by dietary classes derived from LCAs created specifically for Hispanic or non-Hispanic controls in the National Birth Defects Prevention Study, 1997-2011[1]

	Probability of no consumption LCA for Hispanics			Probability of no consumption LCA for NHWs		
	Low Calorie	Prudent	Western	Low Calorie	Prudent	Western
Skim milk	0.55	0.43	0.66	0.40	0.16	0.42
Whole milk	0.45	0.48	0.34	0.66	0.87	0.75
Yogurt	0.43	0.14	0.51	0.43	0.13	0.50
Ice cream	0.34	0.15	0.12	0.16	0.11	0.26
Cottage cheese	0.75	0.48	0.75	0.56	0.35	0.69
Cheese	0.22	0.06	0.07	0.49	0.26	0.33
Margarine	0.68	0.45	0.42	0.28	0.46	0.46
Butter	0.60	0.38	0.31	0.44	0.34	0.49
Apples	0.32	0.29	0.62	0.19	0.03	0.24
Oranges	0.25	0.05	0.19	0.35	0.16	0.45
Orange juice	0.26	0.08	0.14	0.21	0.16	0.32
Peaches	0.48	0.13	0.43	0.48	0.20	0.59
Bananas	0.20	0.03	0.13	0.16	0.06	0.25
Other fruit	0.45	0.16	0.30	0.22	0.08	0.35
Tomatoes	0.45	0.17	0.37	0.32	0.11	0.42
String beans	0.66	0.33	0.63	0.25	0.15	0.41
Broccoli	0.42	0.09	0.42	0.26	0.05	0.37
Cabbage/Cauliflower	0.52	0.15	0.64	0.59	0.40	0.71
Carrots, raw	0.47	0.14	0.42	0.30	0.08	0.40
Carrots, cooked	0.58	0.24	0.59	0.49	0.35	0.61
Corn	0.29	0.04	0.12	0.48	0.39	0.30
Peas/Lima beans	0.80	0.46	0.80	0.47	0.28	0.61
Yams/Sweet potatoes	0.80	0.53	0.87	0.76	0.48	0.82
Spinach	0.82	0.50	0.86	0.71	0.39	0.79
Beans/Lentils	0.38	0.13	0.46	0.52	0.27	0.69
Yellow squash	0.75	0.44	0.84	0.70	0.43	0.79
Eggs	0.35	0.39	0.39	0.53	0.30	0.34
Chicken/Turkey	0.25	0.21	0.13	0.13	0.31	0.23
Bacon	0.70	0.44	0.28	0.23	0.36	0.46
Hot dogs	0.63	0.37	0.25	0.37	0.53	0.53
Processed Meats	0.44	0.22	0.16	0.33	0.44	0.51
Liver	0.83	0.67	0.91	0.98	0.97	0.98
Hamburger	0.27	0.11	0.04	0.40	0.49	0.27
Beef, mixed dish	0.40	0.16	0.25	0.12	0.15	0.27
Beef, main dish	0.32	0.09	0.18	0.07	0.13	0.19
Fish	0.42	0.13	0.41	0.39	0.18	0.53
Chocolate	0.51	0.27	0.16	0.10	0.05	0.19
Candy without chocolate	0.70	0.45	0.33	0.27	0.29	0.44
Pie	0.74	0.41	0.46	0.50	0.46	0.69
Cake	0.50	0.26	0.22	0.29	0.25	0.41
Cookies	0.41	0.18	0.13	0.12	0.06	0.20
White bread	0.34	0.18	0.12	0.13	0.35	0.26
Dark bread	0.54	0.19	0.51	0.42	0.07	0.44
French fries	0.39	0.19	0.06	0.06	0.14	0.20
Potatoes	0.38	0.12	0.11	0.44	0.37	0.25
Rice/Pasta	0.28	0.36	0.42	0.57	0.22	0.28

Table 18 continued.

	Low Calorie	Prudent	Western	Low Calorie	Prudent	Western
Chips	0.58	0.34	0.13	0.12	0.19	0.30
Nuts	0.84	0.47	0.62	0.55	0.22	0.64
Peanut butter	0.75	0.50	0.50	0.27	0.13	0.36
Oil	0.80	0.54	0.65	0.46	0.20	0.53
Cantaloupe	0.53	0.20	0.56	0.54	0.29	0.67
Avocado	0.42	0.10	0.25	0.78	0.49	0.84
Chile	0.54	0.28	0.52	0.79	0.74	0.88
Salsa	0.46	0.17	0.28	0.25	0.14	0.43
Chicken Liver	0.95	0.85	0.97	0.97	0.97	0.97
Organ meats	0.63	0.42	0.44	0.98	0.99	0.98
Tortillas	0.21	0.07	0.11	0.25	0.16	0.43
Refried beans	0.28	0.11	0.19	0.51	0.44	0.70
Coffee	0.56	0.34	0.51	0.48	0.47	0.67
Tea	0.72	0.54	0.44	0.42	0.56	0.65
Soda	0.33	0.27	0.10	0.29	0.58	0.45
Soda (low-cal)	0.92	0.86	0.93	0.73	0.66	0.80
Cereal, FA	0.93	0.87	0.91	0.84	0.77	0.84
Cereal, non-FA	0.35	0.27	0.24	0.25	0.23	0.33

1. NHW and Hispanic probabilities cannot be directly compared because separate LCAs were run to best fit the dietary intake of each population.

Table 19. Association between established risk factors and OFC occurrence among Hispanic participants in the National Birth Defects Prevention Study, 1997-2011

	CL (n=190)		CP (n=245)		CLP (n=483)	
Risk Factor	**cOR (95% CI)**	**aOR[1] (95% CI)**	**cOR (95% CI)**	**aOR[1] (95% CI)**	**cOR (95% CI)**	**aOR[1] (95% CI)**
Maternal Age						
<20	0.7(0.5,1.1)	0.7(0.5,1.1)	0.8(0.5,1.1)	0.8(0.5,1.2)	0.8(0.6,1.1)	0.9(0.6,1.1)
20-25	REF	REF	REF	REF	REF	REF
26-35	0.9(0.6,1.2)	0.9(0.6,1.2)	1.0(0.8,1.4)	1.0(0.8,1.4)	0.9(0.7,1.1)	0.9(0.7,1.1)
36+	1.3(0.7,2.3)	1.3(0.7,1.4)	1.1(0.6,1.9)	1.1(0.6,1.9)	1.1(0.7,1.6)	1.1(0.7,1.6)
Maternal Education						
0-11 years	0.8(0.6,1.1)	0.8(0.6,1.2)	0.9(0.7,1.2)	0.9(0.7,1.3)	1.0(0.8,1.3)	1.0(0.8,1.3)
12 years	REF	REF	REF	REF	REF	REF
>12 years	1.0(0.7,1.5)	1.0(0.7,1.4)	0.9(0.6,1.3)	0.8(0.6,1.2)	0.7(0.5,0.9)	0.7(0.5,0.9)
Pre-Pregnancy BMI						
Normal/Underweight (BMI<25.0)	REF	REF	REF	REF	REF	REF
Obese/Overweight (BMI≥25.0)	0.9(0.7,1.2)	0.9(0.7,1.2)	1.2(0.9,1.6)	1.1(0.8,1.5)	1.1(0.9,1.4)	1.1(0.8,1.4)
Composite Smoke Exposure[2]						
Neither	REF	REF	REF	REF	REF	REF
Smoking only	2.4(1.3,4.4)	2.4(1.2,4.5)	1.5(0.8,3.0)	1.5(0.8,3.0)	1.2(0.7,2.0)	1.1(0.6,1.9)
Secondhand smoke only	1.7(1.1,2.5)	1.8(1.2,2.7)	1.7(1.2,2.4)	1.9(1.3,2.8)	1.6(1.2,2.1)	1.7(1.3,2.2)
Both	1.3(0.5,3.0)	1.5(0.6,3.6)	1.9(1.0,3.6)	2.0(1.0,3.8)	1.0(0.6,1.9)	1.0(0.5,1.9)
Maternal Drinking[3]						
<4 drinks/setting (or none)	REF	REF	REF	REF	REF	REF
≥4 drinks/setting	1.2(0.7,2.0)	1.2(0.7,2.0)	1.1(0.7,1.8)	1.1(0.7,1.8)	1.2(0.9,1.7)	1.3(0.9,1.8)
Maternal Diet[4]						
Western	1.5(1.0,2.2)	1.7(1.1,2.6)	1.5(1.0,2.0)	1.7(1.2,2.5)	1.1(0.9,1.5)	1.3(1.0,1.7)
Prudent	REF	REF	REF	REF	REF	REF
Low-Calorie	1.5(1.0,2.2)	1.6(1.1,2.4)	1.2(0.8,1.7)	1.3(0.9,1.9)	1.3(1.0,1.6)	1.3(1.0,1.7)
Pregestational Diabetes						
No	REF	REF	REF	REF	REF	REF
Yes	2.5(0.7,8.7)	2.8(0.8,9.9)	2.8(0.9,8.4)	2.9(0.9,8.9)	3.5(1.6,7.6)	3.6(1.6,7.9)
Prior Pregnancies						
≤1 pregnancy	REF	REF	REF	REF	REF	REF
>1 pregnancy	1.0(0.7,1.3)	0.9(0.7,1.3)	1.5(1.1,1.9)	1.4(1.1,1.9)	1.2(1.0,1.4)	1.1(0.9,1.4)
FA-Containing Supplement[2]						
None	REF	REF	REF	REF	REF	REF
Any	1.0(0.7,1.3)	0.9(0.6,1.2)	0.8(0.6,1.1)	0.8(0.6,1.1)	0.9(0.7,1.1)	1.0(0.8,1.2)
Low Dietary Folate, DFE[5]						
>Lowest Quartile	REF	REF	REF	REF	REF	REF
Lowest Quartile	1.1(0.8,1.5)	1.0(0.7,1.4)	1.2(0.9,1.7)	1.2(0.9,1.6)	1.2(1.0,1.5)	1.2(0.9,1.4)

Table 19 continued.

	CL		CP		CLP	
Risk Factor	**cOR (95% CI)**	**aOR[1] (95% CI)**	**cOR (95% CI)**	**aOR[1] (95% CI)**	**cOR (95% CI)**	**aOR[1] (95% CI)**
Any Fever[3]						
None	REF	REF	REF	REF	REF	REF
Any	1.5(1.0,2.4)	1.6(1.1,2.5)	1.2(0.8,1.8)	1.2(0.8,1.9)	1.0(0.7,1.4)	1.0(0.8,1.4)
Prenatal Care						
No visits	REF	REF	REF	REF	REF	REF
Any visits	1.7(0.4,7.2)	1.5(0.3,6.1)	0.7(0.3,1.8)	0.7(0.3,1.6)	0.7(0.4,1.4)	0.7(0.4,1.4)
Infant Sex						
Female exposure	0.6(0.5,0.9)	0.7(0.5,0.9)	1.8(1.4,2.4)	1.9(1.4,2.4)	0.6(0.5,0.7)	0.6(0.5,0.7)
Male exposure	1.5(1.1,2.1)	1.5(1.1,2.1)	0.5(0.4,0.7)	0.5(0.4,0.7)	1.7(1.4,2.1)	1.7(1.4,2.1)
High Acculturation						
Low PAS-3	REF	REF	REF	REF	REF	REF
High PAS-3	1.2(0.9,1.7)	1.2 (0.9,1.7)	1.3(1.0,1.8)	1.4(1.0,1.9)	1.0 (0.8,1.2)	1.0(0.8,1.2)

Abbreviations: BMI, body mass index; CI, confidence interval; CL, cleft lip; CLP, cleft lip with palate; CP, cleft palate; DFE, dietary folate equivalent; FA, folic acid; PAS-3, proxy acculturation score-3.
1. aORs adjusted for confounders identified in DAGs created for each risk factor (**Table 15**)
2. First two months of pregnancy
3. One month prior to conception through three months after conception
4. One year prior to conception (measured with Latent Class Analysis)
5. Quartiles based on DFE levels in controls.

Table 20. Adjusted association between dichotomized risk factors and OFC among Hispanic participants in the National Birth Defects Prevention Study, 1997-2011

Risk Factor	**CL aOR[1] (95% CI)**	**CP aOR[1] (95% CI)**	**CLP aOR[1] (95% CI)**
Maternal Smoking[2]			
None	REF	REF	REF
Any	1.7(1.1,2.8)	1.4(0.9,2.3)	1.0(0.6,1.5)
Secondhand Smoke[2]			
None	REF	REF	REF
Any	1.4(0.9,2.0)	1.6(1.2,2.3)	1.4(1.1,1.9)
Diet[3]			
Prudent	REF	REF	REF
Other	1.6(1.1,2.2)	1.4(1.0,1.9)	1.2(1.0,1.6)
Pregestational Diabetes			
No	REF	REF	REF
Yes	2.6(0.8,9.1)	2.8(0.9,8.3)	3.5(1.6,7.7)
Lack of FA[2]			
No	REF	REF	REF
Yes	1.1(0.8,1.5)	1.1(0.9,1.5)	1.0(0.8,1.2)
Infant Sex			
Female	0.6(0.5,0.9)	1.8(1.4,2.4)	0.6(0.5,0.7)
Male	1.5(1.1,2.1)	0.5(0.4,0.7)	1.8(1.4,2.1)

Abbreviations: aOR, adjusted odds ratio; CI, confidence interval; CL, cleft lip; CLP, cleft lip with palate; CP, cleft palate; FA, folic acid; OFC, orofacial clefts.
1. aORs adjusted for dichotomized confounders identified in DAGs specific to each risk factor (**Table 15**)
2. First 2 months of pregnancy
3. Dietary patterns are measured one year prior to conception using a Latent Class Analysis, specific to NBDPS Hispanic participants

Table 21. Adjusted association between dichotomized risk factors and OFC among non-Hispanic White participants in the National Birth Defects Prevention Study, 1997-2011

Risk Factor	CL aOR[1] (95% CI)	CP aOR[1] (95% CI)	CLP aOR[1] (95% CI)
Maternal Smoking[2]			
None	REF	REF	REF
Any	1.1(0.9,1.3)	1.2(1.0,1.5)	1.4(1.2,1.7)
Secondhand Smoke[2]			
None	REF	REF	REF
Any	1.1(0.9,1.4)	1.1(0.9,1.3)	1.2(1.0,1.4)
Diet[3]			
Prudent	REF	REF	REF
Other	1.0(0.8,1.2)	1.1(1.0,1.3)	1.4(1.2,1.6)
Pregestational Diabetes			
No	REF	REF	REF
Yes	1.3(0.5,3.8)	1.9(0.8,4.3)	2.1(1.0,4.4)
Lack of FA			
No	REF	REF	REF
Yes	1.1(0.8,1.3)	1.0(0.8,1.3)	1.0(0.8,1.2)
Infant Sex			
Female	0.5(0.4,0.6)	1.3(1.1,1.5)	0.5(0.4,0.5)
Male	2.0(1.7,2.4)	0.8(0.7,0.9)	2.2(1.9,2.5)

Abbreviations: aOR, adjusted odds ratio; CI, confidence interval; CL, cleft lip; CLP, cleft lip with palate; CP, cleft palate; FA, folic acid; OFC, orofacial clefts.

1. aORs adjusted for dichotomized confounders identified in DAGs specific to each risk factor (**Table 15**)
2. First 2 months of pregnancy
3. Dietary patterns are measured one year prior to conception using a Latent Class Analysis, specific to NBDPS NHW participants.

Table 22. Crude and average adjusted population attributable fractions for select OFC risk factors among Hispanic participants in the National Birth Defects Prevention Study from 2007-2011

	CL		CP		CLP	
Risk Factor	**cPAF (95% CI)**	**aaPAF (95% CI)**	**cPAF (95% CI)**	**aaPAF (95% CI)**	**cPAF (95% CI)**	**aaPAF (95% CI)**
Lack of FA[1]	2.2 (-9.5,14.0)	1.6 (-8.6,12.2)	5.7 (-5.1,16.5)	3.5 (-5.6,12.3)	3.4 (-4.6,11.4)	-0.9 (-8.2,6.0)
Diet (not Prudent)[2]	25.1 (6.9,43.3)	23.5 (5.2,41.3)	15.9 (-0.6,32.3)	10.2 (-6.2,25.2)	12.0 (-0.9,24.8)	14.8 (0.6,27.7)
Maternal Smoking[1]	4.7 (-0.1,9.4)	2.3 (-2.5,7.8)	3.6 (-0.7,7.9)	2.1 (-2.0,6.9)	0.01 (-2.6,2.6)	-0.9 (-3.7,1.8)
Secondhand Smoke[1]	7.0 (-0.03,14.0)	6.3 (-1.1,14.2)	9.6 (3.0,16.2)	8.0 (2.1,14.2)	6.8 (2.3,11.3)	2.0 (-2.4,6.5)
Pregestational Diabetes	1.0 (-0.7,2.7)	0.5 (-0.3,2.3)	1.1 (-0.6,2.7)	0.9 (-0.5,2.5)	1.5 (0.2,2.8)	1.4 (0.2,2.9)
Infant Sex						
Female	-	-	28.7 (17.1,40.3)	21.5 (9.9,34.4)	-	-
Male	22.0 (6.8,37.1)	12.9 (-1.0,26.8)	-	-	28.1 (18.9,37.3)	26.7 (16.3,36.8)
Total % of cases explained		47.1 (24.0,65.7)		46.2 (27.3.63.4)		43.1 (26.7,56.9)

Abbreviations: aaPAF, average adjusted population attributable fraction; CI, confidence interval; cPAF, crude population attributable fraction; CL, cleft lip; CLP, cleft lip with palate; CP, cleft palate; FA, folic acid; OFC, orofacial cleft.

1. First two months of pregnancy
2. One year prior to conception (measured with Latent Class Analysis).

Table 23. Crude and average adjusted population attributable fractions for select OFC risk factors among non-Hispanic White participants in the National Birth Defects Prevention Study from 2007-2011

	CL		CP		CLP	
Risk Factor	**cPAF (95% CI)**	**aaPAF (95% CI)**	**cPAF (95% CI)**	**aaPAF (95% CI)**	**cPAF (95% CI)**	**aaPAF (95% CI)**
Lack of FA[1]	1.7 (-2.0,5.4)	0.5 (-2.4,3.6)	0.3 (-3.0,3.6)	0.4 (-2.6,3.6)	3.4 (-4.5,11.3)	-1.2 (-3.7,1.2)
Diet (not Prudent)[2]	1.8 (-8.9,12.4)	-1.8 (-11.6,7.4)	7.7 (-2.1,17.5)	4.7 (-4.6,14.3)	25.4 (17.3,33.5)	13.8 (6.3,21.2)
Maternal Smoking[1]	3.0 (-1.2,7.3)	2.4 (-2.0,7.0)	4.0 (0.03,8.0)	4.1 (-0.2,8.2)	11.7 (7.8,15.6)	6.1 (2.9,9.8)
Secondhand Smoke[1]	3.3 (-1.4,8.1)	1.9 (-2.2,6.5)	2.5 (-1.5,6.5)	0.4 (-3.4,4.8)	6.8 (2.3,11.4)	2.9 (-0.6,6.5)
Pregestational Diabetes	0.2 (-0.5,0.8)	0.1 (-0.3,0.7)	0.5 (-0.3,1.2)	3.9 (-0.2,1.1)	0.5 (-0.1,1.2)	0.4 (-0.1,1.0)
Infant Sex						
Female	-	-	11.9 (4.7,19.1)	11.7 (4.9,19.0)	-	-
Male	33.0 (24.8,41.2)	32.5 (24.7,40.3)	-	-	28.1 (18.3,37.8)	32.0 (26.9,38.1)
Total % of cases explained		35.6 (25.1,46.6)		21.7 (9.8,32.0)		53.9 (46.3,61.3)

Abbreviations: aaPAF, average adjusted population attributable fraction; CI, confidence interval; cPAF, crude population attributable fraction; CL, cleft lip; CLP, cleft lip with palate; CP, cleft palate; FA, folic acid; OFC, orofacial cleft.

1. First two months of pregnancy
2. One year prior to conception (measured with Latent Class Analysis)

Table 24. Association between dichotomized risk factors and OFC stratified by the Proxy Acculturation Scale-3 (PAS-3) among Hispanic participants in the National Birth Defects Prevention Study, 1997-2011

	CL		CP		CLP	
Risk Factor	**Low PAS-3 aOR[1] (95% CI)**	**High PAS-3 aOR[1] (95% CI)**	**Low PAS-3 aOR[1] (95% CI)**	**High PAS-3 aOR[1] (95% CI)**	**Low PAS-3 aOR[1] (95% CI)**	**High PAS-3 aOR[1] (95% CI)**
Maternal Smoking[2]						
None	REF	REF	REF	REF	REF	REF
Any	2.5(0.7,8.7)	1.6(0.9,2.8)	1.5(0.3,6.5)	1.5(0.9,2.4)	0.6(0.1,2.4)	1.0(0.7,1.6)
Secondhand Smoke[2]						
None	REF	REF	REF	REF	REF	REF
Any	1.4(0.7,3.0)	1.3(0.8,2.1)	2.7(1.5,5.0)	1.3(0.9,2.0)	1.6(1.0,2.5)	1.4(1.0,1.9)
Diet[3]						
Prudent	REF	REF	REF	REF	REF	REF
Other	1.5(0.8,2.7)	1.5(1.0,2.4)	1.1(0.6,1.8)	1.4(1.0,2.1)	1.4(1.0,2.0)	1.1(0.9,1.5)
Pregestational Diabetes						
No	REF	REF	REF	REF	REF	REF
Yes	5.7(1.1,28.9)	1.3(0.2,9.8)	NA[4]	3.9(1.2,12.4)	1.0(0.1,8.0)	5.0(2.1,12.2)
Lack FA Supplement[2]						
No	REF	REF	REF	REF	REF	REF
Yes	1.1(0.6,1.9)	1.2(0.8,1.7)	1.0(0.6,1.6)	1.2(0.8,1.6)	1.1(0.8,1.5)	0.9(0.7,1.2)
Infant Sex						
Female	0.6(0.4,1.1)	0.6(0.4,0.9)	2.5(1.5,4.3)	1.6(1.2,2.2)	0.6(0.4,0.8)	0.6(0.5,0.8)
Male	1.6(0.9,2.7)	1.5(1.1,2.2)	0.4(0.2,0.7)	0.6(0.5,0.9)	1.8(1.3,2.5)	1.7(1.3,2.2)

Abbreviations: aOR, adjusted odds ratio; CI, confidence interval; CL, cleft lip; CLP, cleft lip with palate; CP, cleft palate; FA, folic acid; OFC, orofacial clefts; NA, not applicable; PAF, population attributable fraction.

1. aORs adjusted for dichotomized confounders identified in DAGs specific to each risk factor (**Table 15**)
2. First 2 months of pregnancy
3. One year prior to conception (measured with Latent Class Analysis)
4. All cases are unexposed

Table 25. Average adjusted population attributable fractions for select OFC risk factors stratified by the Proxy Acculturation Scale-3 (PAS-3) among Hispanic participants in the National Birth Defects Prevention Study from 2007-2011

	CL		CP		CLP	
Risk Factor	**Low PAS-3** aaPAF (95% CI)[1]	**High PAS-3** aaPAF (95% CI)[1]	**Low PAS-3** aaPAF (95% CI)[1]	**High PAS-3** aaPAF (95% CI)[1]	**Low PAS-3** aaPAF (95% CI)[1]	**High PAS-3** aaPAF (95% CI)[1]
Lack of FA[2]	-6.6 (-31.8,14.0)	4.4 (-8.9,16.0)	-1.2 (-23.5,16.0)	6.0 (-4.9,16.1)	2.4 (-11.7,16.2)	-2.3 (-10.9,5.7)
Diet (not Prudent)[3]	18.4 (-11.8,46.9)	24.6 (-0.2,48.5)	-0.6 (-23.9,20.4)	17.4 (-4.6,36.2)	17.8 (-2.0,35.9)	10.7 (-10.4,29.1)
Maternal Smoking[2]	1.9 (-3.4,9.0)	2.7 (-4.0,10.0)	1.3 (-1.6,7.4)	3.0 (-2.2,8.7)	-1.1 (-4.0,1.8)	-1.1 (-5.3,3.0)
Secondhand Smoke[2]	7.3 (-7.5,22.3)	5.8 (-2.7,16.4)	20.4 (9.7,33.2)	3.0 (-4.2,10.2)	-0.3 (-7.6,5.7)	3.7 (-2.3,10.5)
Pregestational Diabetes	1.5 (0.0,6.6)	0.2 (-0.2,2.0)	NA[4]	1.4 (-0.3,3.6)	0.1 (-1.0,2.4)	1.9 (0.3,3.9)
Infant Sex						
Female	-	-	30.5 (10.4,53.2)	17.4 (2.9,32.6)	-	-
Male	14.3 (-22.0,50.3)	12.5 (-5.7,30.0)	-	-	27.5 (9.6,46.0)	27.4 (15.0,39.7)
Total % of cases explained	36.8 (-11.8,71.3)	50.2 (20.7,72.4)	50.2 (12.7,78.4)	48.1 (26.3,66.4)	46.3 (20.0,67.1)	40.3 (16.5,58.2)

Abbreviations: aaPAF, average adjusted population attributable fraction; CI, confidence interval; CL, cleft lip; CLP, cleft lip with palate; CP, cleft palate; FA, folic acid; NA, not applicable; NHW, non-Hispanic White; OFC, orofacial cleft.
1. Lower limit of CIs is truncated at 0, assuming that the removal of the specified risk factor would not increase OFC cases
2. First two months of pregnancy
3. One year prior to conception (measured with Latent Class Analysis).
4. All cases are unexposed

5.4. Discussion

Periconceptional diet and secondhand smoke had the strongest effect on OFC among Hispanic individuals. These risk factors were also associated with the largest modifiable aaPAFs for all phenotypes in the Hispanic population. Other risk factors associated with an increase in OFC included: maternal fever (CL), smoking (CL), education (post-high school v. high school, CLP), and high acculturation (PAS-3, CP).

Our aaPAF analysis explored potential reductions in the prevalence of OFC among Hispanics for strong risk factors that have been established in primarily NHW cohorts. Among modifiable risk factors, the percentage of cases attributable to diet was the highest. However, it should be noted that these estimates, compared to all other aaPAFs, were the most imprecise. Relatively large aaPAFs were also

observed for secondhand smoke. The magnitude of all other aaPAFs varied by phenotype, but the total proportion of cases explained by our selected risk factors was fairly similar across phenotype (~40%) among Hispanics.

Comparison of aaPAFs estimated in our Hispanic v. NHW populations should be interpreted with caution. As previously noted, a separate LCA was run for each population so that derived dietary patterns were unique to each population's intake, thus patterns differed by group. While this is an appropriate method to measure the effect of diet within a group, the effect of diet should not be directly compared across groups. These novel aaPAF results are intended to be used as preliminary evidence that, with further research, may inform prevention priorities among Hispanics. Eventually, prevention strategies may focus on risk factors that are consistently associated with the relatively largest aaPAFs among Hispanics. Our inclusion of aaPAFs for NHW starts to explore whether these priorities may differ between ethnic groups.

For instance, results suggest that the effect of diet, although imprecise, was associated with one of the largest aaPAFs for our Hispanic and NHW (CP and CLP) populations. However, the relative strength of other risk factors, and subsequent risk profile, differed within each group. Among Hispanic participants, secondhand smoke had a relatively large aaPAF, but the aaPAF for smoking was relatively weak when compared to other risk factors within the population. Alternatively, for our NHW population, smoking, compared to most other risk factors, was associated with a relatively large effect on OFC.

Unlike Hispanic results, the total proportion of NHW cases explained had great variation by phenotype (21.7% [CP] to 53.9% [CLP]). This may be a commentary on how risk factors uniquely impact racial/ethnic subgroups, phenotypes, or a combination of both. It is important to note that these aaPAFs are relatively small, suggesting that most risk factors, whether environmental or genetic, are still unknown.

The observed persistent effect of diet on OFC among our Hispanic population aligns with prior studies that have assessed this association in primarily NHW cohorts; however, this is the first study to include diet in an aaPAF analysis. The magnitude of these aORs is similar to that of past studies that also measured holistic diet through the use of multiple food-item intake responses.[16,114] Multiple studies have assessed the effect of individual nutrients on OFC, [18,134,144,152] but few have considered a holistic dietary measure. Dietary patterns and indices provide insight into nutrient interactions and overall food intake.

Food consumption patterns may be especially important to consider when evaluating diet among ethnic subgroups. Diet is often linked to cultural norms and specific food-item intake may generally differ by ethnicity.[282] Consumption patterns unique to Hispanic individuals in the US and their impact on OFC warrant further investigation.

Unlike the expected effect of diet, the strong effect of secondhand smoking among all Hispanic phenotypes, and the weaker effect for personal smoking, was inconsistent with prior effect estimates from primarily NHW cohorts. Maternal smoking is often acknowledged as one of the most influential risk factors for OFC.[7] Results, although moderate, have been so persistent that the US Surgeon General's report on smoking noted a causal link between maternal smoking and OFC, but an unknown effect of secondhand smoke.[188] Among aaPAFs recently estimated for OFC among the larger NBDPS population, smoking was associated with the largest aaPAF among modifiable risk factors (Cleft lip with/without palate: 4.0%, CP: 3.4%).[245] Our NHW aORs and aaPAFs align with current literature; however, maternal smoking was only statistically significantly associated with an increase in Hispanic CL. Otherwise, smoking had a weaker (CP) or null (CLP) effect. Exposure to secondhand smoke at home or work, regardless of maternal smoking status, was generally more influential than maternal smoking in the Hispanic population. NHW estimates for secondhand smoke were notably weaker. The notable effect of secondhand smoke, and lesser effect of smoking, on Hispanic OFC warrants further research, as most public health messaging and prevention strategies for OFC do not address the effect of secondhand smoke.

This is the first study to assess the effect of multiple risk factors on OFC specific to Hispanic individuals in the US. The NBDPS is ideal for this study as it provides a well-powered Hispanic population with thorough exposure information and clinically verified OFC cases. Our study was also strengthened by the inclusion of the PAS-3, a validated acculturation scale, and a LCA that is optimal for identifying complex dietary patterns related to pregnancy. Further, the use of a NHW comparison group and aaPAF analyses provided context for our primary findings. The inclusion of average adjusted PAFs, rather than adjusted PAFs, allowed us to account for bias stemming from the sequential removal of risk factors.[115,256]

However, stratification of our Hispanic aaPAFs by acculturation status (PAS-3) was limited due to sample size. Other limitations are also possible. Selection bias is possible as only 65.8% of recruited

individuals participated in NBDPS. However, bias stemming from inherent differences in enrolled versus unenrolled individuals is likely minimal.[283] The NBDPS retrospectively collected self-reported exposures after OFC occurrence. Thus, exposure information may be influenced by case/control status, potentially leading to differential misclassification. Self-reported exposures may also be vulnerable to social desirability bias, which is largely influenced by cultural norms. Finally, there is potential for residual confounding. For example, the Hispanic NBDPS population had high missingness for household income and other socioeconomic indicators, which limited our measure of socioeconomic status to a single indicator (education). Thus, this study could have been strengthened by a more comprehensive proxy for socioeconomic status.

We found that, among Hispanic participants in the NBDPS, secondhand smoke and periconceptional maternal diet had the strongest effect on OFC. The adverse effect of secondhand smoke, rather than smoking, observed for Hispanic, but not NHW, OFC cases warrants further investigation. The exploration of other comprehensive dietary measures on OFC occurrence among Hispanics is also needed. Results highlight several differences in risk factor profiles for OFC among Hispanic and NHW populations. With confirmation, public health messaging and prevention priorities can be tailored to address risk factors specific to OFC occurrence among Hispanic individuals in the US.

CHAPTER 6: DISCUSSION AND CONCLUSIONS

6.1. Summary of specific aims and findings

Although there is a large body of research that has assessed risk factors for OFC, the effect of maternal diet and the effect of most other risk factors specific to Hispanics is largely understudied. It is important to consider the effect of maternal diet, and other risk factors like smoking, on OFC since these exposures are modifiable and can be easily intervened upon at the individual-level and all the way up to the policy-level. Our Aim 1 analysis assessed the effect of periconceptional maternal dietary patterns on OFC. We evaluated dietary patterns using a LCA to reflect the holistic intake of food items and nutrient interactions. In Aim 2, we examined the effect of maternal diet along with previously established risk factors on OFC occurrence among Hispanics living in the US. We then explored how the impact of these risk factors, and potential prevention priorities, may differ for Hispanic and NHW individuals.

6.1.1. Aim 1

Four dietary classes best fit the dietary intake of our study population. The Western class (relatively high likelihood of consuming white bread, chips, and meat, and not consuming vegetables) statistically significantly increased the odds of CL/P and CP, when compared to the Prudent class (relatively high likelihood of consuming dairy, fish, dark breads, vegetables, and fruits). This association remained after controlling for folic acid supplementation. Compared to the Prudent class, the Low-Calorie Western class (similar to Western intake trends but was associated with a lower energy intake) and the Mexican class (similar to Prudent trends with the addition of tortillas, beans/lentils, and salsa) had a fairly weak effect on both CL/P and CP.

6.1.2. Aim 2

Among Hispanic NBDPS participants, maternal dietary patterns, derived solely from Hispanic dietary intake, had a notably strong effect on all OFC phenotypes. Specifically, the Western diet (higher likelihood of hamburgers, white bread, French fries, chips, and soda) significantly increased the odds of CL, CP, and CL/P when compared to the Prudent diet (higher likelihood of fruits, vegetables, beans/lentils, and fish). We also observed a strong effect of secondhand smoke on all phenotypes. However, the effect of maternal smoking, the most established modifiable OFC risk factor to date, only statistically significantly impacted the odds of CL. We also estimated Hispanic and NHW aaPAFs to provide more context for these results. Aside from NHW CL, diet was associated with the largest aaPAF for modifiable risk factors, regardless of racial/ethnic subgroup. Relatively large aaPAFs were observed for maternal smoking for NHW phenotypes, but not Hispanic phenotypes. Conversely, Secondhand smoke was associated with relatively large aaPAFs for Hispanic OFC, but not NHW OFC.

Our dietary findings from both Aim 1 and Aim 2 align with the two previous studies that have evaluated the effect of a comprehensive dietary measure on OFC. However, the relatively strong observed effect of secondhand smoke, compared to smoking, among the Hispanic population was slightly unexpected. Exposure to secondhand smoke has been identified as a risk factor, but evidence of its effect is weaker than that of smoking. The effect of most established OFC risk factors, and subsequent risk factor profiles, has not been evaluated among Hispanics in the US. Our observed Hispanic risk factor profile may suggest that the impact of OFC risk factors differs for Hispanic individuals, compared to NHW individuals in the US. However, due to the novelty of our research question, further investigation is needed.

6.2. Strengths and Limitations

Our study is strengthened by the use of the large, population-based NBDPS data which is optimal for our analysis. Case-control studies are ideal for rare outcomes, such as OFC (n=7,000 diagnosed per year in the US).[293,294] The NBDPS provides a large, diverse, population-based study sample with thorough information on OFC outcomes, multiple exposures, and covariates of interest. All OFC cases were clinically verified through the NBDPS case classification process. Phenotypes were also clinically

verified, which is important due to their differing etiology and our individual focus on each OFC type.[34] We also had the ability to restrict to isolated OFC cases, which is important to better define etiologic pathways.[34] To date, this study included one of the largest sample sizes of infants with OFC, which allowed for subgroup analysis by race/ethnicity (Aim 1) and the further ability to restrict to a sizeable Hispanic subgroup (Aim 2). Access to the large Hispanic NBDPS population allowed us to evaluate a spectrum of risk factors specific to this minoritized group, which has not been done before.[16,17,114]

NBDPS exposure information is rich and diverse. We had access to extensive maternal dietary information through the hour-long NBDPS interview. Our diet analysis was further strengthened by the use of a LCA, a unique dietary assessment tool that identifies latent dietary patterns, that allows for a comprehensive evaluation of food and nutrient interactions .[115] The NBDPS interview also provided information on an extensive list of recognized OFC risk factors and confounders. Finally, our study was strengthened by our ability to focus on an understudied racial/ethnic group. Overall, we were able to study a large and diverse Hispanic subgroup and account for the contribution of acculturation to health risks, while providing context for these results through the use of a NHW comparison group.[256]

In terms of possible limitations, it is estimated that two-thirds of invited individuals participated in NBDPS. If enrolled controls are inherently different than controls who opted out of the study, NBDPS controls would not be representative of the base population in which they were selected, leading to selection bias. However, it has been suggested that this bias may be minimal.[283] Participation may also differ by Hispanicity, as it there is a lack of representation of Hispanic individuals in clinical trials; however, Hispanic participation rates in observational studies are largely unknown.[295] Further, some of our results are susceptible to information bias due to the long window spanning from the periconceptional period to the interview date (roughly 6 weeks – 24 months after delivery). For example, it may be hard for a participant to accurately report periconceptional dietary intake three years prior. Errors in recall solely stemming from this extended timeframe are likely non-differential, which often biases results towards the null for binary exposures but an unknown direction for all other exposures.[248,296] It has been suggested that this type of error in dietary recall may be fairly minimal.[19,117,283,297,298]

Additionally, dietary recall may be influenced by intake-related bias in which individuals with higher intakes of certain foods report lower intake and individuals with lower intakes of certain food report

higher intake.[287] It could be argued that all participants may be prone to this bias, regardless of OFC outcome; thus, our entire population may report a healthier diet. This may lead to non-differential bias, in which the heterogenous dietary patterns on OFC prevalence would be diluted, thus also biasing our estimate towards the null. However, it could also be argued that dietary, along with exposure and covariate, information may differ by case-control status. Our self-reported measures are vulnerable to recall bias (differential misclassification) due to our case-control study design. Because case-control studies collect information after the health outcome has occurred, participants' exposure and covariate recall may be dependent on the outcome, thus leading to potential differential misclassification. For example, mothers who have an infant with OFC may be more acutely aware of their pregnancy exposures, compared to mothers who have an infant without a birth defect. Also, mothers with an infant with OFC may want to disclose all health behaviors in an attempt to better understand OFC etiology or they may not want to share any health tendencies out of fear that they will be unjustly blamed. This may further be influenced by social desirability bias in which individuals report behaviors based on practices that are considered acceptable based on larger societal and cultural norms.[288,289,299] This is particularly relevant to dietary and risk factor data as participants may want to provide answers that are deemed healthier by society, especially during the fragile time of pregnancy. Overall, this recall may lead to differential misclassification based on OFC outcome and the direction of the resulting bias is unknown.[248]

It is also important to note the semi-quantitative nature of the NBDPS FFQ. This FFQ is limited in assessing actual nutrient intake, yet it is an appropriate tool to measure relative intake, which is the focus of our dietary measure. A focus on relative intake, using a data driven approach such as LCA, can be hard to generalize to a larger population so replication of this analysis in other populations is needed. Moreover, our LCA assumes that dietary intake is similar for all individuals within a class, so there is likely some variation in consumption within each class that we are missing. However, each participant's probability of class membership allowed us to quantify how well their intake aligned with their assigned class. Statistical assumptions related to our aaPAF methods should also be acknowledged. PAFs are inherently dependent on the exposure prevalence and its observed effect specific to the study population of interest; therefore, issues with generalizability to populations outside of NBDPS are possible. Further, these methods assume that the removal of one risk factor will not affect the impact of other risk factors on

OFC and that total removal of an exposure is possible, thus aaPAF results should be interpreted with caution.

Finally, it is important to acknowledge that unmeasured confounding is possible and likely as there are covariates that are not measured in NBDPS that influence both OFC risk and our exposures of interest. For example, our acculturation measure, while validated, is susceptible to bias and confounding. If foreign-born participants have experienced societal discrimination, they may be less likely to disclose acculturation measures. Additionally, our NBDPS data does not include the exact questioning for "language spoken at home" specific to the PAS-3; thus, our measure may not fully encompass this scale. While we were able to define acculturation using multiple indicators, it is likely that our measure did not fully capture the multidimensional process of acculturation. This study could be enhanced by the availability of a larger, more extensive acculturation scale. Similarly, the addition of more thorough socioeconomic measures could better capture bias stemming from access to healthy foods and healthcare.

6.3. Public health impact and future directions

6.3.1 Public health impact

The prevalence of OFC is higher than most other birth defects in the US and its etiology is largely unknown.[1,7] Thus, it is important to further explore modifiable OFC risk factors, such as maternal diet. Maternal dietary patterns have scarcely been explored in relation to OFC and has never been estimated for Hispanic individuals specifically. As more research is conducted, evidence may suggest that specific patterns are associated with a reduction in OFC occurrence. Individuals can be advised of these patterns that capture realistic intake at routine periconceptional and prenatal clinic visits. Additional evidence may also influence dietary recommendations and food assistance program protocols for pregnant individuals, or those considering conception.

Overall, modifiable risk factors for OFC are often studied internationally or in US cohorts that are mostly NHW. While US studies often have several races and ethnicities represented in their sample, they rarely provide results focused on specific race and/or ethnicity-based groups. This lack of attention makes it challenging for researchers to understand how risk factors directly influence minoritized individuals in

the US. To our knowledge, there is little research that has evaluated OFC risk factors among US Hispanics only. A focus on Hispanics individuals is important as they are one of the largest minoritized subgroups in the US and OFC trends differ by Hispanicity in the US.[1,2] Further, the influence of these risk factors on OFC among Hispanics has not been evaluated in the context of acculturation, which partially accounts for the cultural heterogeneity in this large subgroup. Acculturation is important to consider as it may influence the effect of OFC risk factors previously identified among US cohorts.[61,300] These analyses, along with further research, have the potential to help inform specific public health prenatal messaging for Hispanic individuals in the US.

6.3.2. Future directions

To better understand the impact of maternal diet on OFC, further investigation of comprehensive dietary measures, such as dietary patterns, in conjunction with individual nutrients is required. It is important to understand if similar patterns can be replicated in other populations or if there are specific characteristics within these patterns that may be persistent across populations in the US. A focus on patterns aligns with national dietary recommendations that have recently shifted from an emphasis on individual foods and nutrients to more comprehensive dietary patterns.[121] The use of patterns in national dietary recommendations not only emphasizes the need to consider dietary patterns, but also the need for a clear interpretation of these patterns so that intake of recommended patterns is more attainable. Along with a focus on dietary patterns, future studies could be strengthened by the prospective collection of self-reported dietary intake, and other relevant exposures, to reduce differential misclassification by OFC status. The influence of race and ethnicity on these dietary patterns should also be considered. The specific impact of diet on OFC likely differs by racial and ethnic subgroups as dietary choices and access to food is likely influenced by culture and the lived experiences of minority groups in the US.[282]

To further our knowledge on OFC occurrence among Hispanics in the US and, subsequently, inform public health prevention strategies specific to this population, confirmation of the relatively strong observed effect of diet and secondhand smoke, rather than smoking, on OFC is needed. The effects of residual confounding on these associations should be addressed since these, and most other risk factors, are influenced by factors such as SES. Future exploration of established risk factors among Hispanic

individuals should also include a more extensive acculturation measure to partially account for the impact of cultural heterogeneity and observed health disparities within this population. Similarly, the intersection of race and ethnicity should be investigated further as there is heterogeneity in self-reported race among Hispanics in the US.[57] As our knowledge of modifiable risk factors, such as maternal diet, and their specific effect on Hispanics deepens, public health prevention strategies can prioritize risk factors most influential on OFC occurrence specific to Hispanic and NHW individuals.

6.4. Conclusions

Although OFC is a common birth defect in the US, etiology and risk factors that significantly reduce occurrence are relatively unknown. Our study found that diets relatively high in fruits, vegetables, fish, and dark bread, compared to diets less likely to consume fruits and vegetables and more likely to consume white bread, chips, and soda, were protective against all OFC phenotypes in a large population-based study sample and its Hispanic subset. Results also suggested that secondhand smoke, rather than smoking, may have a relatively stronger effect on OFC, a trend that may be unique to Hispanics. The effect of modifiable risk factors on OFC occurrence warrants further attention. A focus on modifiable risk factors and their specific influence on Hispanics, the largest minoritized group in the US, prioritizes exposures that can be intervened upon at the individual-, community-, and policy-level.

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