Fall 2010

Epidemiology of choanal atresia - the National Birth Defects Prevention Study

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EPIDEMIOLOGY OF CHOANAL ATRESIA – THE NATIONAL BIRTH DEFECTS PREVENTION STUDY

by

Vijaya Kancherla

An Abstract

Of a thesis submitted in partial fulfillment
of the requirements for the Doctor of
Philosophy degree in Epidemiology
in the Graduate College of
The University of Iowa

December 2010

Thesis Supervisor: Associate Professor Paul A. Romitti

ABSTRACT

Choanal atresia is a well-defined congenital malformation; however, little is known about its prevalence and risk factors. Data from the Iowa Registry for Congenital and Inherited Disorders were used to examine prevalence, infant, and maternal characteristics of choanal artesia. Data from the National Birth Defects Prevention Study (NBDPS) were used to examine selected risk factors for choanal atresia. Overall prevalence was estimated as number of choanal atresia cases per 10,000 live births with 95% confidence intervals (CI)s. Crude and adjusted odds ratios (OR)s and 95% CIs were estimated to investigate selected risk factors. The overall prevalence of choanal atresia among live born deliveries in Iowa from January, 1998 through December, 2005 was 0.46 (95% CI=0.27, 0.78) per 10,000 live births. Using data from the NBDPS, choanal atresia cases were compared to unaffected control infants for births from October 1997 through December 2005. Overall, case infants compared to control infants were more likely to be female, preterm, and a multiple birth. For all choanal atresia cases combined, odds of high maternal zinc (OR=2.1; 95% CI=1.2, 3.9) and vitamin B-12 (OR=2.4; 95% CI=1.4, 4.3) intake in the year prior to pregnancy, and maternal periconceptional (one month before through three months after conception) exposure to anti-infective urinary tract medications (OR=3.3; 95% CI=1.3, 8.4) were significantly elevated among case compared to control mothers. For isolated choanal atresia cases (those with no additional major malformations), odds of maternal periconceptional exposure to passive cigarette smoke (OR=2.3; 95% CI=1.0, 5.3) as well as maternal intake of 3 or more cups of coffee per day one-year prior to pregnancy were increased (OR=2.9; 95% CI=1.3, 6.4) for case compared to control mothers. The reverse was found for low maternal intake of

pantothenic acid (OR=0.4; 95% CI=0.2,0.9) and vitamin A (OR=0.3; 95% CI=0.1, 0.8) one-year prior to pregnancy. The current study provided support for potential associations between maternal health behaviors before and during pregnancy and choanal atresia; however, the findings were based on a modest number of cases. The study needs to be replicated in a larger case sample, also examining the role of genetics in choanal atresia.

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Graduate College
The University of Iowa
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CERTIFICATE OF APPROVAL

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PHD THESIS

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This is to certify that the PhD thesis of

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To my mother

The important thing is not to stop questioning. Curiosity has its own reason for existing.

Albert Einstein

ACKNOWLEDGMENTS

I would like to sincerely thank my dissertation advisor, Dr. Paul Romitti for his support, guidance, and exceptional mentoring. I am very grateful to him for giving me an opportunity to be a part of the National Birth Defects Prevention Study. Special thanks to Dr. Trudy Burns, Dr. John Carey, Dr. Elizabeth Chrischilles, Dr. Charles Lynch, and Dr. Anne Helene Skinstad for sharing their expertise and providing invaluable critique. Special thanks to Lixian Sun for providing statistical advice. Thanks to the staff and researchers at the Iowa Registry for Congenital and Inherited Disorders. Sincere thanks to staff and researchers engaged in the National Birth Defects Prevention Study at various centers, and dedicating their efforts towards birth defects surveillance and research. Heart-felt thanks to families that participated and shared their experiences in this landmark study. Deepest thanks to my parents and family for being an integral part of me, and teaching me the importance of purpose, empathy, and perseverance in life. Thanks to my dearest friends for their unwavering support during this journey.

This work was funded by grants sponsored by the Centers for Disease Control and Prevention (U50/CCU 713238; U01/DD000492). The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

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Choanal atresia is a well-defined congenital malformation; however, little is known about its prevalence and risk factors. Data from the Iowa Registry for Congenital and Inherited Disorders were used to examine prevalence, infant, and maternal characteristics of choanal artesia. Data from the National Birth Defects Prevention Study (NBDPS) were used to examine selected risk factors for choanal atresia. Overall prevalence was estimated as number of choanal atresia cases per 10,000 live births with 95% confidence intervals (CI)s. Crude and adjusted odds ratios (OR)s and 95% CIs were estimated to investigate selected risk factors. The overall prevalence of choanal atresia among live born deliveries in Iowa from January, 1998 through December, 2005 was 0.46 (95% CI=0.27, 0.78) per 10,000 live births. Using data from the NBDPS, choanal atresia cases were compared to unaffected control infants for births from October 1997 through December 2005. Overall, case infants compared to control infants were more likely to be female, preterm, and a multiple birth. For all choanal atresia cases combined, odds of high maternal zinc (OR=2.1; 95% CI=1.2, 3.9) and vitamin B-12 (OR=2.4; 95% CI=1.4, 4.3) intake in the year prior to pregnancy, and maternal periconceptional (one month before through three months after conception) exposure to anti-infective urinary tract medications (OR=3.3; 95% CI=1.3, 8.4) were significantly elevated among case compared to control mothers. For isolated choanal atresia cases (those with no additional major malformations), odds of maternal periconceptional exposure to passive cigarette smoke (OR=2.3; 95% CI=1.0, 5.3) as well as maternal intake of 3 or more cups of coffee per day one-year prior to pregnancy were increased (OR=2.9; 95% CI=1.3, 6.4) for case compared to control mothers. The reverse was found for low maternal intake of

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LIST OF ABBREVIATIONS

AR	Arkansas
B1	The one month prior to conception
B2	The second month prior to conception
B3	The third month prior to conception
BDPGB	Birth Defects and Pediatric Genetics Branch
BMI	Body Mass Index
BPA	British Pediatric Association
CA	California
CATI	Computer Assisted Telephone Interview
CDC	Centers for Disease Control and Prevention
CHARGE	Coloboma, Heart Defect, Atresia of Choanae, Retarded Growth and Development, Genital Anamoly, Ear Defect
CHD	Chromodomain Helicase DNA
CT	Computerized Tomogram
DNA	Deoxyribose Nucleic Acid
EDD	Expected Date of Delivery
FDA	Food and Drug Administration
FFQ	Food Frequency Questionnaire
FGF	Fibroblast Growth Factor
GA	Georgia
IA	Iowa
IDPH	Iowa Department of Public Health
IRCID	Iowa Registry for Congenital and Inherited Disorders
MA	Massachusetts
M1	The first month after conception
M2	The second month after conception
M3	The third month after conception
NBDPS	National Birth Defects Prevention Study
NSAID	Non-Steroidal Anti-Inflammators
NJ	New Jersey
NY	New York
OR	Odds Ration
PRAMS	Pregnancy Risk Assessment Survey
RAR	Retinoic Acid Receptors
RXR	Retinoid X Receptors
SSRI	Selective Seratonin Reuptake Inhibitors
T2	Second Trimester
T3	Third Trimester
TX	Texas
UBDN	Utah Birth Defects Network
UI	University of Iowa
USDA	United States Department of Agriculture
UT	Utah

UTI	Urinary Tract Infections

PREFACE

Epidemiology of rare birth defects is hard to study due to several challenges involved in identification of cases. Designing and coordinating a population-based study for a modest number of cases is not only difficult, but also hard to justify in terms of allocation of funds, resources, and research personnel. But a collaborative effort by ten major birth defects surveillance registries in the U.S. has made possible the National Birth Defects Prevention Study, one of the largest studies on birth defects epidemiology. The National Birth Defects Prevention Study has provided a unique opportunity to examine choanal atresia, a rare birth defect, using population-based surveillance. Through this research, I have come to appreciate many aspects of design and implementation of an epidemiological study, along with the value of a collaborative and collective effort made by several individuals towards promoting healthy birth outcomes.

CHAPTER I

INTRODUCTION

Choanal atresia is a well-recognized congenital defect characterized by obstruction in the posterior nasal apertures[1]. As chronicled by Hengerer (2008), this defect was first described by Johann George Roederer in 1755, and was later characterized as an anatomical deformity of palatine bone by Adolf Otto in 1854[1]. Also in 1854, Carl Emmert first successfully corrected choanal atresia using transnasal surgery of the palate[1].

Presently, choanal atresia is one of the most common defects involving the nose[1]. It is estimated to affect 1 in 5,000 to 7,000 live births[1] and is twice as common in females as males[2]. Overall, approximately one-half of all choanal atresia diagnoses are bilateral; unilateral presentation predominantly affects the right nasal aperture[3].

Choanal atresia is the most common indication for early surgical intervention involving the nose[4]. As newborns are obligate nasal breathers, bilateral choanal atresia presents as a medical emergency at birth[1]. Infants with bilateral choanal atresia are at an increased risk of presenting with other malformations, particularly cerebral abnormalities, developmental delay, and other respiratory conditions, such as laryngeal tracheomalacia and subglottic stenosis[5]. Bilateral choanal atresia has also been associated with long-term nasal complications, repeat correction surgeries, and life-long respiratory conditions, such as nasal stuffiness, sleep apnea, and rhinorrhoea[3,6]. Additionally, affected infants with either unilateral or bilateral choanal atresia tend to screen positive for genetic syndromes and other complex conditions[7].

Although choanal atresia is the most common cause of nasal abnormality at birth, other, less common defects also cause nasal obstruction in newborns. In particular, choanal stenosis, the second most common nasal defect in newborns, presents with similar symptoms as choanal atresia. Choanal stenosis is characterized by excessive growth of the medial nasal process of the maxilla, which causes narrowing or closure of the anterior bony part of the nasal passage and leads to respiratory distress in the newborn[8]. Choanal stenosis is differentiated from choanal atresia effectively by using computerized tomographic (CT) evidence[6]. Clinical management of both choanal atresia and choanal stenosis is conservative (intubation, ventilation, and stenting) and surgical correction is advised only when the obstruction is severe[9].

Over the past century, many case reports and hospital-based studies have been published on the clinical characteristics and surgical management of choanal atresia[1,7]. As such, case characteristics have varied widely, because of these small samples. Limited, published population-based data are available for the prevalence or descriptive epidemiology of choanal atresia[10]; no published population-based data are available for choanal stenosis. Many of the previous case reports and hospital-based studies on choanal atresia have not stated their inclusion or exclusion criteria, making it difficult to determine their diagnostic specificity. Due to similarity in signs and symptoms between the two defects, there is a potential for diagnostic misclassification.

Because of its rare occurrence, little is known about the role of maternal health behaviors during pregnancy in the etiology of choanal atresia. For example, women may engage in high risk behaviors, such as cigarette smoking or alcohol use, during the initial months of their pregnancies[11-13]. Both animal and human studies have shown that such

exposures during reproductive years can trigger potential teratogenic effects in the fetus[14-18]. Also, high intakes of caffeine during periconceptional period have been shown to increase the risk of selected birth defects[19-22], whereas energy-adjusted intakes of selected macro- and micronutrients may either protect against or increase the risk of birth defects[23-26]. In addition, about one percent of all birth defects are estimated to be caused by therapeutic drugs taken close to conception or during pregnancy[27]. To elucidate the role of these well-known risk factors in the etiology of choanal atresia, a population-based study is needed to examine the association between several pregnancy-related risk factors and choanal atresia in the offspring to better understand the etiology of choanal atresia, as well as co-occurring birth defects.

Specific Aims

The current study had two specific aims. These aims were investigated using data from population-based Iowa Registry for Congenital and Inherited Disorders (IRCID) and other population-based birth defects surveillance systems that are engaged in the National Birth Defects Prevention Study (NBDPS), a multicenter, case-control study funded by the Centers for Disease Control and Prevention. One aim was to estimate prevalence of choanal atresia and choanal stenosis in Iowa and describe the characteristics of the affected infants and their mothers. For this aim, data were examined exclusively from the IRCID, as not all centers participating in the NBDPS included choanal stenosis in their surveillance activities. The second aim was to evaluate the associations between exposure to cigarette smoke, alcohol, and medications during the month before through the third month following conception, and exposure to caffeine and

nutrients one-year before pregnancy in case and control mothers, and choanal atresia in their offspring. For the second aim, data were examined from ten centers participating in the NBDPS, including the IRCID.

Aim 1

To determine the prevalence of choanal atresia and choanal stenosis and describe the characteristics of affected infants and their mothers

The first aim addressed the prevalence and descriptive epidemiology of choanal atresia and choanal stenosis in a population-based sample obtained from the IRCID. Expert clinical geneticist reviewed case diagnoses. Eligible case infants were those with an expected date of delivery (EDD) on or after January 1, 1998 and on or before December 31, 2005. Selected characteristics of infants and mothers were examined for each defect. Prevalence was estimated as cases per 10,000 live births along with 95% confidence intervals (CI)s based on the Poisson distribution. Characteristics of affected infants and their mothers were summarized using frequency distributions.

Aim 2

To examine the associations between exposure to cigarette smoke, alcohol, and medications during the month before through the third month following conception, and exposure to caffeine and nutrients one-year before pregnancy in case and control mothers, and choanal atresia in the offspring

Associations between maternal exposure to cigarette smoking, alcohol, caffeine, nutrients, medications and choanal atresia in the offspring were examined. Because of the paucity of previous epidemiologic studies on choanal atresia, a comprehensive analysis of the NBDPS data was considered for various exposures; particularly those previously identified environmental risk factors for orofacial clefts, which share embryological

origins with choanal atresia[28]. The case-control study design allowed application of logistic regression analysis, and calculation of odds ratio (OR) and 95% confidence interval (CI) estimates for these selected risk factors. Further, ORs and CIs were adjusted for covariables using multivariable logistic regression models.

Summary and Significance

Choanal atresia is a rare defect and a case-control study was the design of choice to examine potential risk factors. This study used data from the population-based birth defects surveillance systems participating in the NBDPS, the largest study of birth defects in the United States covering almost 500,000 births annually. These data provided an ideal opportunity to investigate the prevalence and descriptive epidemiology of choanal atresia and choanal stenosis. Given the rather modest number of cases estimated and the paucity of previous etiologic studies, this study was unique in investigating demographic characteristics (e.g., maternal, race and ethnicity, education, economic status, etc.) and several classes of maternal exposures, such as high risk behaviors (e.g., cigarette smoking, alcohol, and caffeine use), nutrition, and medication use before and during pregnancy. No previously published research has attempted such a comprehensive assessment of risk factors for choanal atresia. Findings from this study will provide important insights into the etiology of choanal atresia and may generate new hypotheses and guide the design of future studies.

CHAPTER II

BACKGROUND AND LITERATURE REVIEW

The research study described herein examined the prevalence and descriptive epidemiology of choanal atresia and choanal stenosis using population-based data ascertained by the Iowa Registry for Congenital and Inherited Disorders (IRCID). It also used data from the National Birth Defects Prevention Study (NBDPS) to provide the first comprehensive, population-based study to investigate epidemiologic risk factors for choanal atresia.

Several major birth defects, including heart, neural tube, and orofacial defects, have been well-studied and are frequently associated with choanal atresia[5,2,29]. These groups of defects also share similar embryologic origins[28,30,31]. In particular, orofacial clefts which include cleft lip (with or without cleft palate) and cleft palate, are closely associated with choanal atresia as each is categorized as an 'anterior skull base deformity'[31,32].

A review of both animal and human studies have shown that maternal exposures to cigarette smoking, alcohol, caffeine, and selected medications, as well as maternal sub-optimal intake of selected nutrients are associated with the risk of orofacial clefts in offspring[32]. Published data are lacking on the role of these risk factors in the development of choanal atresia. Following is a description of the supporting published literature to date.

Embryology of Choanal Atresia

In humans, development of cranial structures and the face occurs during the first 12 weeks of gestation with the choanae developing between the 4th and 11th weeks[33] (Figure 2.1). Formation of cranial structures is largely initiated by neural crest cell migration. At four weeks gestation, nasal development begins with the formation of nasal pits. At approximately five weeks gestation, the nasal pits fold inwards into the surrounding mesenchyme to form the nasal sacs, which are separated from the primitive oral cavity by oronasal membranes. At about eight weeks gestation, these membranes rupture to create a nasal cavity and primitive choanae located at the junction of the nasal cavities and the nasopharynx. Development of the nasal cavity and primitive choanae is followed by a gradual proliferation of neural crest cells, which contributes to the formation of the skull base and nasal vaults. By the end of the 10th week gestation, the nasal septum and the developing palate fuse and the primitive choanae undergo alteration and are pushed posterior. At this stage, the choanae are termed secondary choanae. In a normal fetus, secondary choanae are patent and enable a functional airway between the anterior part of the nose and the inner nasopharynx[33].

Anatomy of Choanal Atresia

Anatomically, choanae are located in the posterior section of the nose. Each choana is surrounded by a posterior border of vomer and the nasal crest of the palatine bone on the *medial side*, ala (or wing like structures) of the vomer and the sphenoid bone on the *top*, medial pterygoid process of sphenoid and the perpendicular plate of palatine bone on the *lateral side*, and the horizontal plate of palatine bone at the *base.* A fully-

developed choana measures about 6 to 8 mm vertically and 12 to 17 mm horizontally at birth. Atresia of the choanae occurs when a bony or membranous growth measuring between 1 and 10 mm is found at the posterior margin of the hard palate. Also, the surrounding tissue is affected, forming a high arched hard palate, a thick nasal septum, and narrow nasal fossae, all significantly narrowing the diameter of the nasopharynx[33].

Embryological Theories of Causation

The molecular basis of choanal atresia was extensively examined during the previous two decades[7], and four theories gained prominence: 1) persistence of the buccopharyngeal membrane from the foregut; 2) abnormal persistence or location of mesoderm forming adhesions in the nasochoanal region; 3) abnormal persistence of the nasobuccal membrane of Hochstetter; and 4) misdirection of neural crest cell migration and subsequent mesodermal flow[1]. Other, less accepted theories have included resorption of the floor of the secondary nasal fossa, incomplete dorsal extension of the nasal cavity, and migration of the dorsal part of the frontonasal process to fuse with the palatal shelves[1].

Presently, the theory of misdirection of neural crest migration and subsequent mesodermal flow is thought to offer the strongest evidence. During embryogenesis, the ectoderm at the neural plate margin produces neural crest cells, which then migrate to form the skeletal and connective tissues in the face and portions of the cranium. Deficiency in production of neural crest cells has been associated with defects in the frontonasal parts of the face (e.g., arrhinencephaly). In contrast, disturbances in migration patterns of neural crest cells are associated with caudal maxillary process and visceral

arch derivatives as seen in choanal atresia[34]. Further, craniofacial anomalies with mesenchymal damage and cell disruption were found in offspring of mothers who took high doses of vitamin A, and these changes were associated with embryonic pathways involving the migration of neural crest cells in early fetal life[34].

Clinical Presentation of Choanal Atresia

Unilateral choanal atresia is mild and presents with breathing difficulty and persistent nasal discharge; whereas, bilateral choanal atresia is severe and often becomes a neonatal emergency with asphyxia neonatorum. Bilateral choanal atresia is also associated with nursing difficulties, respiratory distress, recurrent nasal allergies, and respiratory infections. Physical examination of bilateral choanal atresia often shows a bony or membranous obstruction in the nasal cavity, stridor, marked retraction of the chest, and paradoxical cyanosis (blueness of skin relieved by crying). Frequent complications of bilateral choanal atresia include sinusitis, respiratory arrest, and aspiration of food into the lungs[3].

Choanal atresia can occur either as a solitary defect (isolated) or with two or more major defects of different organ systems (multiple). A recent study showed bilateral choanal atresia occurs more frequently with other major defects[5]. For example, orofacial, heart, and limb defects generally co-occur with bilateral choanal atresia without a recognized underlying etiology (e.g., single gene or chromosomal). Alternatively, some choanal atresia cases, mostly bilateral, may present in the form of Amniotic Band, Antley-Bixter, Apert, Bamforth, Crouzon, DiGeorge, Downs, and Treacher Collins syndromes[35-36]. About 7 to 29% of cases with choanal atresia are identified as part of the

CHARGE (Coloboma, Heart defect, Choanal Atresia, Retarded growth and development, Genital anomaly and Ear defect) syndrome[10,37,38]. Other well-described teratogenic syndromes that present with choanal atresia include methimazole embryopathy[39] and carbimazole embryopathy[40].

Diagnosis of Choanal Atresia

Definitive diagnosis of choanal atresia is made using computerized tomography (CT) of the paranasal sinuses and the skull base[7]. CT helps to accurately delineate the location of obstruction within the nasal passages and the position (anterior, posterior), laterality (unilateral, bilateral), and histological characteristics (bony, membranous or mixed) of each affected choana(e). Before the availability of CT, the histological distribution of choanal atresia cases was estimated as 90% bony and 10% membranous[41]. More recent findings using CT and tissue specimens suggest that about 30% of choanal atresia is pure bone and 70% is mixed (membranous and bone), with no pure membranous occurrence[42,43].

Treatment of Choanal Atresia

Treatment of choanal atresia aims at establishing the patency of the airway, preventing further damage to the surrounding structures and a short intervention with limited hospitalization[44]. There are two well-known treatment options for choanal atresia, transpalatal and transnasal surgeries[7]. Transpalatal surgery involves mucosal incisions to open the hard palate and correct the atresia. Initially, transpalatal surgery was favored due to excellent visualization and a high success rate; however, the method was also

associated with complications, such as blood loss during transfusion, palatal flap breakdown or fistula, increased risk of cross bite, high arched palate deformity and a need for orthodontic treatment[1,45,46]. As result, the transnasal approach was introduced to minimize some of these complications. Transnasal surgery is preferred for neonates and young children diagnosed with bilateral choanal atresia. The procedure uses primarily an endoscopic instrument to visualize the nasal cavity and establish the patency of the nasal cavity by drilling the atretic plates, followed by insertion of temporary nasal stents to maintain patency of the nasal apertures until they heal. Compared to transpalatal surgery, transnasal surgery provides a shorter hospital stay and reduced blood loss; however, it has been shown to require increased revision surgeries due to re-stenosis[47]. A recent study that used a tertiary care hospital sample examining the management and outcomes of choanal atresia repair in children with CHARGE syndrome from 1990 to 2005 showed that 82% of children with unilateral choanal atresia and 78% of children with bilateral choanal atresia were treated with transnasal surgery[48]. Overall, the median follow-up post-surgery was 24.9 months (range 1 to 144 months), and revision surgeries were needed in 45% of cases, some requiring multiple revision surgeries. Another hospital-based study[4] reported a mean of 4.6 transnasal surgeries (range 1 to 6 surgeries) to correct bilateral choanal atresia and 2.8 surgeries (range 1 to 6 surgeries) for unilateral choanal atresia. In particular, low birth weight infants with either bilateral or unilateral choanal atresia were more likely to experience unfavorable outcomes following surgery. Additional, commonly noted complications for transnasal surgery were broken ligatures, nasal septal deviation, injury to turbinates, excess pressure on nasal ala, and trauma and iatrogenic cosmetic deformities[7].

More recent approaches in choanal atresia repair use transnasal endoscopic techniques, which have shown improved outcomes with lower rates of complications[47,49]. These techniques also have been shown to reduce traumatic injury during surgery and post-operative scarring[1]. Also, Mitomycin C, computer-aided surgery and laser techniques are currently being explored as treatment options for choanal atresia[7]. Further, stents, which were traditionally used for post-operative care to maintain patency, are not currently being recommended as they can produce excessive pressure on the nasal ala, localized infection and ulceration, circumferential scarring and injury to the surrounding tissue[44].

Choanal Stenosis and its Relevance to Choanal Atresia

Although choanal atresia is the most common cause of nasal abnormality at birth, other, less common defects also cause nasal obstruction in newborns (Table 2.1). In particular, congenital nasal pyriform aperture stenosis, also referred to as choanal stenosis, is the second most common nasal defect[50]. As described, choanal stenosis is defined as an excessive growth of the medial nasal process of the maxilla during the 4th to 8th week of gestation[8,51]. It leads to narrowing or closure of the anterior bony part of the nasal passage causing respiratory distress in the newborn. Choanal stenosis is often mistaken for choanal atresia because of its similar anatomical and clinical characteristics[50].

Choanal stenosis is considered a microform of holoprosencephaly due to the common occurrence of a single central maxillary incisor in both conditions[52,53]. It occurs predominantly as a bilateral defect with sleep apnea and cyanosis being two of the

common symptoms. Definitive diagnosis of choanal stenosis is made by CT. Posterior choanae appear occluded in the CT images of choanal atresia cases, whereas in choanal stenosis, the choanae appear unblocked. Further, the maximum diameter of each transverse pyriform aperture is often less than or equal to 3 mm and the aperture width is less than 8 mm[8].

Descriptive Epidemiology of Choanal Atresia

Many case reports and hospital-based studies have been published in the last century on clinical characteristics and surgical management of choanal atresia, but few studies have examined its risk factors (Table 2.2). Choanal atresia cases included in hospital-based studies ranged from approximately 10 to 80 children and were most often recruited from tertiary care hospitals. Overall, these studies produced mixed results in terms of the sex ratio of affected children. In particular, two[2,45] of the three[1,2,45] larger studies (sample size of 70 or more) reported male:female ratios close to 1:2; whereas a third reported a 1:1 ratio[1] (Table 2.2). Conversely, the male:female ratio was reversed (2.4:1) in another study on 65 children with choanal atresia[4]. The sex ratios varied even more for studies with smaller study samples. The clinical presentation of choanal atresia also varied among hospital-based studies. Among the larger studies that reported laterality of choanal atresia, more than one-half of all choanal atresia cases were bilateral[4,2]. The laterality ratio varied among studies with smaller samples. Multiple birth defects were noted in 25 to 60% of choanal atresia cases[2,4].

The only published population-based study of prevalence and case characteristics of choanal atresia identified was conducted by Harris et al. (1997) using data for 444 cases from birth defect registries in California, Sweden, and France for the years 1976 through 1992[10]. These investigators reported an overall prevalence of choanal atresia of 0.82 per 10,000 live births with the California population having the highest prevalence (1.13 per 10,000 live births) compared to Sweden (0.54 per 10,000 live births) and France (0.78 per 10,000 live births). In contrast to hospital-based studies, no significant differences in prevalence were found by infant sex. Overall, 47% of the cases were associated with other major structural birth defects and 5.4% presented with the CHARGE syndrome. Harris et al. (1997) also examined selected maternal characteristics and found that non-Hispanic white infants had a higher prevalence compared to other racial/ethnic groups[10]. In addition, a moderate increase in choanal atresia was observed among twins compared to singleton births; maternal age and parity showed no association with choanal atresia. It is important to note that choanal atresia with known underlying chromosomal defects and cases with severe stenosis were included in the analyses.

Risk Factors for Choanal Atresia

Genetic Risk Factors

Choanal atresia is thought to be a multifactorial trait, although some studies have suggested single gene models that include both autosomal dominant and autosomal recessive transmission[54]. More commonly, choanal atresia is found to occur sporadically and to recur infrequently in siblings and in successive generations[54-56]. In several studies, about 7 to 29% of children with choanal atresia were noted to have the CHARGE

syndrome[10,57-59] which is an autosomal dominant condition with mutations in the chromodomain helicase deoxyribonucleic acid (DNA) binding protein 7 (*CHD7*) gene at chromosome 8q12.1[60,61]. Additional genetic factors that may contribute to choanal atresia are not well understood. As examples, animal studies that used knockout mouse models for suppression of retinoic acid synthesis exhibited choanal atresia and other malformations of the nasal cavity; when these mice were fed retinoic acid, it proved protective for choanal atresia[62]. Also, persistent local activation of fibroblast growth factor (FGF) pathways among knockout mice (aldh1a3 null mutants) has been suggested among animal models of choanal atresia which occur in syndromes of craniosynostosis[63]. Each finding warrants further investigation.

Environmental Risk Factors

Environmental (i.e., non-inherited) risk factors for choanal atresia have not been well studied. Maternal behaviors, including lifestyle and nutritional intake during pregnancy play an important role in optimal fetal development and outcomes. Reproductive-age women may be exposed to cigarette smoke, alcohol, caffeine, sub-optimal nutrition, and medications, each of which has been associated with birth defects.

Cigarette smoking

Animal and human studies have shown that chronic maternal exposure to cigarette smoking during reproductive years can trigger potential anti-estrogenic response[16,64] and affect glucose regulation[65], insulin resistance[16] and obesity[17]. Both active and passive cigarette smoking has been shown to affect the growth and

development of the fetus[18,66,67]. With regard to craniofacial malformations, maternal cigarette smoking has been associated with orofacial clefts in newborns[68,69].

Alcohol

A recent study using a population-based sample of pregnant women showed that an estimated 30% reported any alcohol use during pregnancy and 8% binged when they were pregnant[70]. Prenatal exposure to alcohol has been associated with adverse birth outcomes, fetal alcohol syndrome and birth defects in newborns[71,72]. Even moderate amounts of alcohol consumed during pregnancy can cross the placental barrier and cause adverse effects on the development and migration of the cranial neural crest and other embryonic cells[73,74]. The greatest impact of alcohol on fetal development is when the blood alcohol levels are high, as in binge drinking. Binge drinking has been shown to inhibit fetal development[75] and has been significantly associated with fetal alcohol syndrome[76] and orofacial clefts[77-79]. These latter associations were further influenced by lack of folic acid use[80].

Caffeine

Exposure to caffeine during pregnancy has been associated with teratogenic outcomes[81]. Caffeine and its metabolites cross the placenta and affect fetal growth[82]. High intake of caffeine is also known to be a potential teratogen, which can cause vaso-constriction and impede placental circulation[20,83,84]. Caffeine has also been shown to increase serum noradrenalin levels, which in combination with decreased placental circulation, has been associated with the risk of birth defects[20,85,86]. When combined with

nicotine and alcohol exposure, even moderate doses of caffeine have been shown to significantly increase the risk of birth defects[81]. Positive, but nonsignificant, associations between maternal caffeine intake and orofacial clefts have been reported[87-89].

Nutrition

Sub-optimal nutrition during pregnancy is thought to contribute to the higher prevalence of birth defects among infants born to mothers from low socio-economic strata compared to mothers of other socio-economic strata[90]. Folic acid use one month before through the third month following conception (periconceptional) has been suggested to reduce the risk of orofacial clefts and neural tube defects[23,25,26]. Multivitamin supplementation from one month before the day of the last menstrual period through the third month following the day of the last menstrual period has been demonstrated to reduce the occurrence of orofacial clefts[91] and congenital heart defects[92]. Apart from the single vitamin and multivitamin supplements, nutrients acquired from food sources have also been suggested to have beneficial effects in prevention of birth defects. Periconceptional nutritional intake has been shown to reduce the risk of orofacial clefts[69,93-98]. Particularly, selected macro- and micro- nutrients present in fruits and vegetables such as vegetable protein, fiber, beta-carotene, ascorbic acid, alpha tocopherol, iron, and magnesium have shown protective effects against orofacial clefts[98].

Medications

Some prescription and over-the-counter medications have known teratogenic effects on the fetus[99-101]. One percent of all birth defects are estimated to be caused by

therapeutic drugs taken close to conception or during pregnancy[27]. The U.S. Food and Drug Administration (FDA) developed criteria for safety of medication use in pregnancy based on animal and human experimental trials. Unfortunately, about 91% of therapeutic drugs have not been tested for teratogenicity[100] and more than 55% of pregnant women are prescribed one or more medications[102]. Exposure to medications between the 3rd and 10th weeks of gestation is thought to be the critical time to produce structural deformities; however, certain medications taken pre-conception can metabolize at a slower rate and eventually influence the pregnancy in the first or later trimesters. Medication risk factors for orofacial clefts have been rather well-studied[103] and have shown an increased risk of clefting with periconceptional use of corticosteroids[104], anti-epileptic drugs[105], non-steroidal anti-inflammatory drugs[106] and folic acid antagonists[107].

Maternal use of anti-thyroidal medications (thionamides), including methimazole, during pregnancy was associated with choanal atresia in offspring in several hospital-based studies[108-112]. Maternal intake of carbimazole during pregnancy was shown to contribute to choanal atresia, hearing loss and developmental delay and developmental abnormalities of the gastrointestinal tract, nipples and the face, together termed as the 'carbimazole embryopathy'[40,113].

Summary and Significance

Because choanal atresia is a rare birth defect, potential risk factors for this defect are mostly unknown. Several hospital-based studies have examined clinical and surgical outcomes for this defect. Reported prevalence rates and clinical characteristics have varied due to small sample sizes, short follow-up periods and diagnostic

misclassification. Only one population-based study on epidemiology of choanal atresia has been published to date; however, many important risk factors could not be examined as data on pregnancy exposures were unavailable. The current study was the first population-based study to comprehensively investigate prevalence and descriptive epidemiology for both choanal atresia and choanal stenosis; as well as several risk factors for choanal atresia.

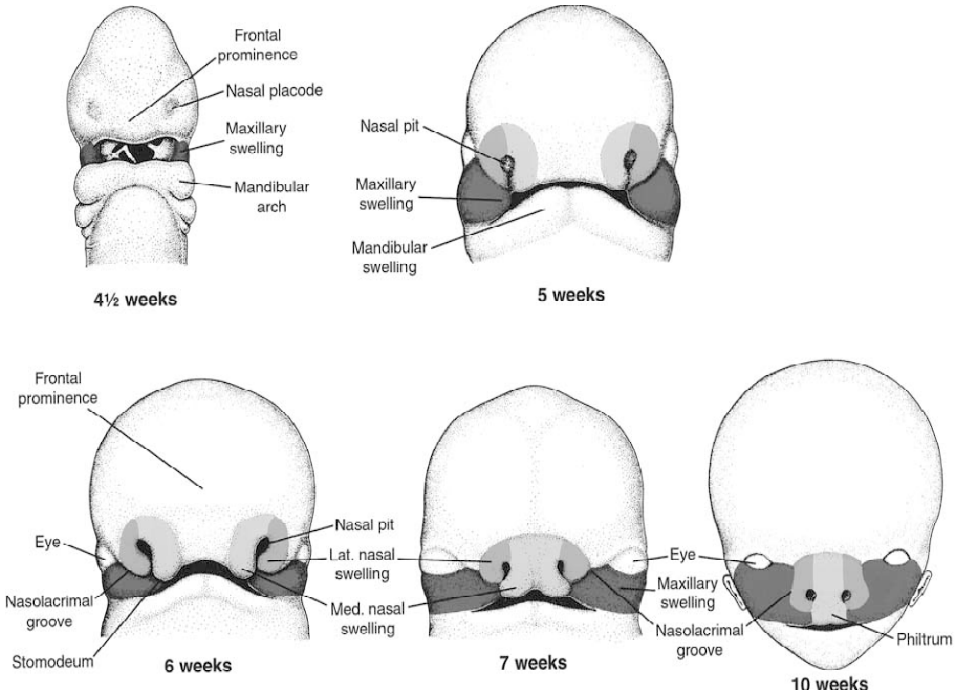


Figure 2.1. Midface Embryogenesis Between 4th and 11th Weeks of Gestation

Source: Lowe, L.H., Booth, T.N., Joglar, J.M., et al., (2000). Midface anomalies in children. *Radiographics*, *20*(4), 907-22; quiz 1106-7, 1112. Reprinted with permission from Elsevier publishers and Radiological Society of North America.

Table 2.1 Congenital Causes of Nasal Obstruction in the Newborn

Defect	Case Characteristics
Choanal atresia - Posterior	Partial to complete closure of nasal passage due to atresia of posterior nasal choanae
Choanal atresia - Anterior	Partial to complete closure of nasal passage due to atresia of anterior nasal choanae
Choanal stenosis – Posterior	Partial to complete closure of nasal passage due to stenosis or mucosal swelling
Nasal stenosis - Anterior	Shelf-like projections in the nasal cavity
Congenital nasal tumors	Growth of cranial and fibrous tissue in the nasal passage
Other nasal malformations	Other nasal deformities associated with the cleft lip and various degrees of nasal agenesis

Table 2.2 Published Hospital-Based Case-Series of Choanal Atresia

Author, Year of Publication	Study Period	No. of Cases	Source	Male: Female	Isolated: Multiple
Benjamin, 1978[114]	1966 - 1984	64	Royal Alexander Hospital for Children, Sydney	1:1.4	3:1
Black, 1983[115]	1976 - 1981	48	Great Ormond St Hospital for Sick Children, London	1:1	1.1:1
Bose, 1983[116]	1977 - 1983	8	Ahmadi Hospital, Kuwait	1:1	-
Coniglio, 1988[37]	1974 - 1986	24	University of Rochester Hospital, New York	-	1.7:1
Craig, 1959[117]	1952 - 1956	10	Otolaryngology Clinic, Belfast	2.3:1	2.3:1
Enriquez, 1983[118]	1969 - 1981	17	Children's Hospital of Barcelona, Italy	1:3.5	1:1.6
Flake, 1964[119]	(not stated)	40	Children's Hospital Medical Center, Boston	1:2.1	-
Freng, 1978[45]	1947 - 1974	82	National Hospital of Norway, Norway	1:2	-
Friedman, 2000[4]	1/1990 - 4/1998	65	Great Ormand St Hospital for Sick Children, London	2.4:1	1.5:1
Gosepath, 2007[120]	1980 - 2006	41	Hospital at University of Mainz, Germany	1:1.9	-
Hengerer, 2008[1]	1973 - 2005	73	University of Rochester Hospital, New York	1:1.2	1.6:1
Kawashiro, 1994[121]	1980 - 1992	8	National Children's Hospital, Tokyo	1.7:1	1:7
Mir, 1986[122]	7/1981 - 6/1983	11	Jamahira Maternity Hospital, Benghazi	1.2:1	1.2:1
Sadek, 1998[123]	1985 - 1996	8	Al-Qassimi Hospital, Sharjah	1:1	0:8
Samadi, 2003[2]	5/1979 - 1/1998	78	Children's Hospital of Philadelphia, Philadelphia	1:1.6	-
Schwartz, 1986[124]	1969 - 1985	21	Columbia Prysbytarian Medical Center, New York	1:1.3	1:1.6
Winther, 1978[125]	1963 - 1976	15	Kommune Hospitalet, Aarhus	1:2.8	-

CHAPTER III

RESEARCH DESIGN AND METHODS

Data Sources

To examine the prevalence and descriptive epidemiology of choanal atresia and choanal stenosis for study aim 1, data were obtained from the Iowa Registry for Congenital and Inherited Disorders (IRCID). To examine selected risk factors for choanal atresia for study aim 2, data were obtained from the National Birth Defects Prevention Study (NBDPS).

Iowa Registry for Congenital and Inherited Disorders

The IRCID is a program of the Iowa Department of Public Health (IDPH) and its operations are contracted to the College of Public Health at The University of Iowa (UI); thus, the UI is the bona fide agent of the IDPH for birth defect surveillance in Iowa. Each year, over 40,000 deliveries are monitored by the IRCID. These deliveries include live births, fetal deaths, and elective terminations occurring to Iowa residents. Trained field representatives review records in nurseries, maternity units, and pediatric wards of local hospitals, along with records in cytogenetic labs, prenatal diagnosis clinics, and genetic clinics. The diagnosis of a birth defect is confirmed by clinical geneticists using systematic review and classification. All information is stored in an electronic format. The IRCID also links abstracted data to the state vital records (birth certificates, death certificates, and fetal death certificates).

The National Birth Defects Prevention Study

The NBDPS was established by the Centers for Disease Control and Prevention (CDC) in 1996 as a multi-center case-control study of environmental (i.e., non-inherited) and genetic risk factors for over 30 major structural birth defects[126]. Case and control infants with an estimated date of delivery (EDD) on or after October 1, 1997 were included. Centers for the NBDPS include Arkansas (AR), California (CA), Iowa (IA), Massachusetts (MA), New Jersey (NJ), New York (NY), North Carolina (NC), Texas (TX), Utah (UT), and the CDC in Metropolitan Atlanta, Georgia. About 500,000 births annually are included in the catchment area for the NBDPS with inclusion of birth outcomes differing across centers (Table 3.1). Diagnosis of one or more eligible birth defects was assigned by clinical geneticists using systematic review of information abstracted from medical records[127]. Information abstracted included basic demographic information, diagnostic data, delivery events, cytogenetic data, birth complications, prenatal data, pregnancy history, family history, and selected risk factor information.

Subjects

For study aim 1, all deliveries (live births, stillbirths, and elective terminations) ascertained by the IRCID with choanal atresia or choanal stenosis with an EDD on or after January 1, 1998 and on or before December 31, 2005 were selected. For study aim 2, case deliveries diagnosed with choanal atresia with an EDD on or after October 1, 1997 (CA, CDC, IA, MA, NY, TX), January 1, 1998 (AR, NJ), or January 1, 2003 (NC, UT) and on or before December 31, 2002 (NJ) or December 31, 2005 (AR, CA, CDC, IA, MA, NC, NY, TX, UT) and whose mothers completed the NBDPS interview were

selected. Case deliveries identified included live births (all centers), fetal deaths or stillbirths (AR, CA, CDC, IA, MA, NY, TX [since year 2000], UT), and elective terminations (AR, CA, CDC, IA, NY, TX [since year 2000], UT) occurring to residents within the NBDPS catchment of each participating center (Table 3.1). Each case delivery had at least one eligible, major birth defect diagnosed within the first year of life. Cases that were a part of a known genetic syndrome or complex were excluded from the NBDPS

NBDPS control infants were a random sample of unaffected live births delivered in the same time frame and in the same catchment areas as case deliveries and selected from birth certificates (AR [2000-2005], CDC [2001-2005], IA, MA, NC, NJ, UT) or hospital delivery records (AR [1997-2000], CA, CDC [1997-2000], NY, TX).

As no individual case group exceeded 100 cases per year per center, recruitment of 100 controls per year maintained a minimum of 1:1 case:control ratio for each eligible defect. Overall, the participation rate among case and control mothers was approximately 70%. Case and control infants who were adopted or in foster care or whose biological mothers were deceased or did not speak English or Spanish were excluded.

Data Collection

IRCID

The IRCID abstracts various clinical and demographic data on each choanal atresia and choanal stenosis case. Clinical data include anthropomorphic measures, verbatim defect diagnosis, diagnostic test results, cytogenetic and molecular test results, family

history, and autopsy results. Demographic data include name of child, mother, and father, and residence.

NBDPS

State legislative rules or internal review board requirements guided initial recruitment with eligible case families. The IA, MA, and NY centers contacted the physician of the case infant (or the physician of the case mother for a stillbirth or elective termination) to inform the physician of the NBDPS and the intent to contact the mother of the infant. If a physician did not identify circumstances that would preclude contact with the mother or did not reply within 21 days, an introductory packet, including a fact sheet on rights as a research subject, a pregnancy calendar, food frequency item response list, study information pamphlet and $20 dollar check or money order was mailed to each eligible case mother. The remaining centers mailed introductory packets directly to each eligible case mother. Each center mailed introductory letters directly to eligible control mothers. Introductory mailings were in both English and Spanish languages. A systematic follow-up protocol was used to consent mothers to complete a computer-assisted telephone interview. The interviews were conducted no earlier than 6 weeks andno later than 24 months after the EDD of the case or control infant.

The NBDPS maternal interview consisted of sections on pregnancy history (including prenatal diagnostic history), maternal health and illnesses, family history, lifestyle and behavioral risk factors (including tobacco, alcohol, and substance abuse), nutrition, multivitamin use, and occupational exposures. Most exposures were assessed from three months before pregnancy through the end of the pregnancy; exposures related

to caffeine and nutritional intake were assessed for the one-year prior to the index pregnancy. The NBDPS maternal interview provided an automatic pregnancy calendar which calculated the time periods from the birth date or due date as provided by the mother. The advantage of using this tool was that it allowed easy reference throughout the interview to sequential months before and during pregnancy.

NBDPS Analytic Database

The NBDPS has assembled advanced technical systems and software tools which transport data from the telephone interviews to the analytical database as a SAS or SPSS data file for analysis. The NBDPS analytical database can be used to generate data sets for analysis of maternal interview responses linked to NBDPS clinical data.

Data management

Each center coded and stored complete electronic files for all interviews conducted in their respective center. All coded data were securely transmitted electronically to the CDC. Data transmitted to the CDC were de-identified and anonymous copies of all completed clinical and interview data for the previous month. The CDC uses Microsoft Access© to replicate and store data in a secure and confidential manner; thus, copies of all completed interviews and specimens are maintained and managed by the CDC Birth Defects and Pediatrics Genetics Branch (BDPGB). The BDPGB coordinates with the NBDPS at the CDC and facilitates release of data to each participating center. Each center obtained approval for human subjects research and took appropriate steps to protect the privacy and confidentiality of data collected.

Case-Control Study Design

The case-control study design was used for the NBDPS, as it provided a cost-effective design to study risk factors for rare outcomes. The case-control design permitted estimation of odds ratios and 95% confidence intervals. Parsimonious logistic regression models were fit to adjust for several covariables.

Case Selection

Choanal Atresia

For the NBDPS, choanal atresia was defined as a congenital obstruction of the posterior choana(e) (British Pediatric Association [BPA] codes 748.010, 748.011, 748.012, 748.013, or 748.014). Clinical geneticists at each center assigned the diagnosis of choanal atresia by review of abstracted information from medical records. All eligible choanal atresia cases were confirmed by a second clinical geneticist. Choanal atresia cases were classified as 'isolated' if the infant did not have an additional major birth defect. Alternately, if one or more major structural birth defects (not including the CHARGE syndrome - Coloboma, Heart defect, Choanal Atresia, Retarded growth and development, Genital anomaly and Ear defect) were present, the infant was classified as 'multiple, with no CHARGE.' If choanal atresia presented as part of the CHARGE syndrome, the infant was classified as 'multiple, with CHARGE.' Choanal atresia cases that were a part of a known genetic syndrome or complex were excluded from the NBDPS. Choanal stenosis, including pyriform aperture stenosis cases, was excluded from the NBDPS.

Choanal Stenosis

For study aim 1, choanal stenosis cases were defined as those with congenital obstruction of the choanae with a stenosis of the nasal passage and who were assigned a BPA diagnosis code of 748.000, 748.001, 748.002, 748.003, or 748.004. Choanal stenosis cases were not collected for the purpose of the NBDPS; hence, we obtained these cases from the IRCID.

Control Selection

Eligible controls were a random sample of non-malformed, live born infants from each participating center with EDDs during the same time period as cases. Overall, mothers of 6807 eligible control infants were identified and interviewed for the NBDPS.

Variable Selection

Clinical Variables

For study aim 1, the following variables were obtained from the IRCID for both choanal atresia and choanal stenosis deliveries. Infant characteristics examined were sex (male / female), birth date, birth weight in grams (< 2500 / ≥ 2500), plurality (singleton / multiple), gestational age in weeks (< 37 / ≥ 37), birth outcome (live birth / stillbirth / elective termination). Diagnostic and treatment characteristics included position (anterior / posterior), laterality (unilateral / bilateral), side (right / left), type (bony / membranous / mixed), CHARGE syndrome (yes / no), diagnostic method (nasal catheter / CT / endoscope + CT / other), cytogenetic testing (normal / abnormal / pending / not done), molecular testing (normal / abnormal / pending / not done), treatment (surgery / stenting

/ other non-surgical), and the type of surgery when cases were surgically repaired (transnasal / transpalatal / endoscopic / other). Information on family history of choanal atresia (yes / no / unknown), family history of choanal stenosis (yes / no / unknown), and other co-occurring defects (BPA codes and descriptions) was also obtained. Maternal variables examined were age at delivery in years (< 25 / 25 - 34 / ≥ 35), race / ethnicity (non-Hispanic white / non-Hispanic black / Hispanic / other), education in years (< 12 / 12 / > 12), and gravidity (0 / 1 - 2 / 3+)

Exposure Variables

Cigarette Smoking

Maternal exposure to cigarette smoking was assessed during 3 months prior to pregnancy (labeled B1, B2, and B3) and the duration of the pregnancy (labeled M1, M2, M3 for the first three months of pregnancy; T2 for second trimester; T3 for third trimester). Cigarette smoking was classified as ‘active’ if the mother reported to have smoked during the study period and ‘passive’ if the mother reported an indirect exposure. A positive response to either active or passive cigarette smoke exposure was followed by further inquiry about daily frequency of exposure. For active cigarette smoking, pre-specified categories of number of cigarettes or packs of cigarettes smoked per day were used. For passive cigarette smoking, exposure was categorized as ‘household’ or ‘workplace’ exposure, and mothers were asked to report specific month(s) during which passive exposure occurred. For our analyses, cigarette smoke exposure was restricted to the periconceptional period, which corresponded to the month prior to conception (B1) through the first three months of pregnancy (M1, M2, and M3).

Alcohol

Alcohol exposure was assessed by asking mothers if they had consumed beer, wine, mixed drinks, or shots of liquor during the year before the delivery of the child (B3 to T3). Similar to cigarette smoking, exposure was collected monthly for the three months prior to pregnancy (B3, B2, and B1) and the first three months of pregnancy (M1, M2, and M3) and by trimester (T2 and T3) for the remaining six months of pregnancy. Periconceptional average number of drinking days per month, average number of drinks per drinking day, and maximum number of drinks on one occasion per drinking month were assessed.

Caffeine

Caffeine consumption was assessed during the one-year period prior to the index pregnancy. Mothers were asked if they consumed caffeinated beverages, such as coffee, tea, and/or soda. Frequency of intake was measured by average number of cups per day (coffee, tea) or average number or cans, glasses or bottles per day (soda). A continuous measure of the total caffeine intake per day was estimated from the sum of reported intake of coffee, tea and caffeinated soda. Using this measure, total caffeine intake was categorized into four groups: none or very low (<100 mg/day), low (100 - <200 mg/day), moderate (200 - <300), and high or very high intake (≥ 300 mg/day).

Nutrition

Maternal diet was assessed using a shortened version of the Willett Food Frequency Questionnaire[128]. Fifty-eight food items including vegetables, meats, fruits,

cereals, and other foods were assessed for the year before pregnancy. The U.S. Department of Agriculture (USDA) version S19 nutrient database was used to calculate estimates of individual nutrient values from the reported food items[129]. Additionally, mothers reported daily intake of vitamins and mineral dietary supplements from three months before pregnancy through the end of the pregnancy. A combined folic acid intake value was calculated from individual intakes from prenatal multivitamins, mineral supplements, non-prenatal multivitamins, and other folic acid containing supplements.

Medications

Medication exposures were assessed by asking mothers if they had taken any medications three months prior to conception and any or all of the nine-months during pregnancy. Specific medications taken for diabetes, hypertension, seizures, respiratory illness, infections of kidney, bladder and urinary tract, pelvic inflammatory disease, other fevers or illnesses were queried. Although drug use for thyroid disease was not ascertained directly in the interview, mothers were able to report thyroid disease under 'other ailments suffered during pregnancy' and the use of thyroid medications during the study period. Open-ended questions were allowed for recording any other medications not listed in any of the pre-mentioned categories. For each reported medication, estimated dates of use, and frequency and duration of use were queried. Reported medications were then linked to their active ingredients using the Slone Epidemiology Center Drug Dictionary[130]. Medication exposures analyzed were restricted to those in the periconceptional period.

Covariables

Covariables examined were selected based on their relationship with both the exposure (cigarette smoking, alcohol, caffeine, nutrition, and medications) and choanal atresia. A bivariate analysis was conducted to examine the difference in the effect estimates with and without the covariable. Covariables were retained if they met the change-in-estimate criterion of 15%. Different covariables were selected for different statistical models based on their relationship to the exposure and outcome.

Covariables examined were infant sex (male / female), gestational age in months (< 37 / ≥ 37), birth weight in grams (≥ 2500 / < 2500), plurality (1 / 2 or more), maternal age (in years) (< 20 / 20 - 34 / ≥ 35), maternal race / ethnicity (white, non-Hispanic / black, non-Hispanic / Hispanic / other), parity (1 / 2 / > 2), maternal education in years (< 12 / 12 / > 12), parity (0 , 1 , ≥ 2), household income in dollars (before pregnancy) (< $20,000 / $20,000 - $50,000 / > $50,000), maternal body mass index (BMI) calculated as weight in kilograms per height in square meters (< 18.5 / 18.5 - < 25 / 25 - < 30 / ≥ 30), history of type 1 or 2 diabetes before index pregnancy (yes / no), history of hypertension (yes / no), nausea or vomiting during pregnancy (yes / no), fever during B1-T1 (yes / no), season of conception (summer / fall / winter / spring), and participating center (AR / CA / CDC / IA / MA / NJ / NY / NC / TX / UT).

Analytic Plan for Specific Aims

Aim 1

To determine the prevalence of choanal atresia and choanal stenosis and describe the characteristics of affected infants and their mothers

Prevalence of choanal atresia and choanal stenosis in Iowa was estimated as number of cases per 10,000 live births in Iowa during the study period (January 1, 1998 to December 31, 2005) along with 95% confidence intervals (CI)s using the Poisson distribution.

Prevalence per 10,000 livebirths in Iowa = p = $\frac{\text{Number of cases per year (n)} \times 10{,}000}{\text{Number of births per year (N)}}$

If $Np(1-p) \geq 5$; 95% confidence interval were calculated using the normal approximation, else if If $Np(1-p) < 5$, confidence intervals were calculated using an exact method.

$$95\%\ \text{CI} = p \pm Z_{0.975}\sqrt{\frac{p(1-p)}{N}}$$

Frequency distributions for selected infant and maternal characteristics were examined for choanal atresia and choanal stenosis and compared using the Pearson chi-square test or Fisher's exact test (when cell frequencies were less than 5).

Aim 2

To examine the associations between maternal exposure to cigarette smoke, alcohol, and medications during the month before through the third month following conception, and maternal exposure to caffeine and nutrients one-year before pregnancy in case and control mothers, and choanal atresia in the offspring

Selected infant characteristics (sex, gestational age at delivery, birth weight, plurality, family history), maternal characteristics (age at delivery, race/ethnicity, education, BMI, parity, nativity, household income, employment, folic acid use, prepregnancy diabetes, prepregnancy hypertension, and season of conception) and study center were compared between case and control families using the Pearson chi-square test or Fisher's exact test (when cell frequencies were less than 5). Crude odds ratios (OR)s

and 95% confidence intervals (CI)s were estimated to investigate the association of cigarette smoking, alcohol, caffeine, nutrition, and medications with choanal atresia. Each characteristic was examined for confounding by examining its association with the outcome and exposure. Effect modification was examined between active periconceptional cigarette smoking and alcohol using the Breslow-Day significance test. A backward logistic regression procedure was used in model selection. Covariables were retained in models if they met the change-in-estimate criterion of 15%. All caffeine models were examined by including any periconceptional smoking and drinking as covariables; however the two variables were excluded in the backward variable selection. A stratified Mantel-Haenszel method was used to calculate pooled dose-response effect (Cochran-Armitage test of trend) among selected exposures including cigarette smoking, alcohol, coffee, tea, soda, and total caffeine, adjusting for potential confounders. Exact methods were applied to trend test when cell frequencies were less than 5.

For nutritional analyses, ORs and 95% CIs were estimated using unconditional logistic regression. Quartiles were derived from the nutrient intake distribution among control mothers. ORs were compared between lower quartiles of intake (< 25 percentile) and middle quartiles (25 - 75 percentile); similarly, higher quartiles of intake (> 75 percentile) were compared to middle quartiles (25 - 75 percentile). All nutritional analyses were adjusted for total energy intake in kilocalories per day. Multivariable analyses for nutritional exposures were also performed using backward logistic regression. Potential confounding was evaluated using change-in-estimate criterion of 15% and covariates were selected based on this criterion. Medication classes for which at least one case mother reported use were included in the analyses (anti-infective agents,

anti-infective urinary tract infection (UTI) agents, anti-pyretics, anti-diarrheal, respiratory tract agents, anti-tussives, and expectorants). Because of low exposure frequencies that conferred low statistical power for the medication analyses, only crude ORs and 95% CIs were estimated for both isolated and all cases of choanal atresia combined. Exact ORs and 95% CIs were reported where cell frequencies were less than 5.

Analyses were conducted separately for all choanal atresia cases combined (isolated and multiple choanal atresia with or without the CHARGE syndrome) and for isolated choanal atresia cases only; analyses of multiple choanal atresia cases with or without the CHARGE syndrome were not examined separately due to small sample sizes. Multivariable analyses were also conducted using exact logistic regression where cell frequencies were less than 5. The magnitude and direction of the associations did not differ between non-parametric and parametric analyses. All statistical analyses were conducted using SAS, version 9.2 (SAS, Cary, NC).

Usual sample size guidelines recommend enrolling a similar number of cases and controls (1:1) for statistical precision. However, for rare diseases, the ratio of cases to controls is commonly increased to 1:4 to achieve statistical precision and sufficient power to test hypotheses. The NBDPS analytical database (version 7.04) includes 90 choanal atresia cases and 6703 controls with completed interviews. Of the 90 cases, there were 47 cases of isolated choanal atresia. Even though the ratio of cases to controls is considerably greater than 1:4, as the data were already available, we included all controls whose mothers completed the interview in our analysis. Minimum detectable odds ratios (OR)s for the bivariate associations were determined using PROC POWER in SAS, based on a statistical power of 80% (beta = 0.20), a two-sided test with type 1 error rate

of 5% (alpha = 0.05) (SAS, Cary, NC). Minimal detectable odds ratios were calculated separately for all cases of choanal atresia and isolated cases only for a range of exposure frequencies estimated from the control group (Table 3.2).

In summary, the available sample size provided sufficient power to detect an OR ranging from 1.8 to 3.2 for cigarette smoking and alcohol exposures in analyses that used all 90 cases; and between 2.3 to 4.3 in analyses using only isolated cases. For caffeine exposures, minimal detectable OR ranged between 2.2 and 2.9 among all cases of choanal atresia; and between 2.8 and 3.9 among isolated cases. For medication exposures with at least one reported exposure among cases, the minimal detectable OR range was very high (between 3.2 and 10.0 for all cases; and 3.4 and 15.9 for isolated cases). For sub-optimal nutrient intake, minimal detectable ORs were 1.9 and 2.4 for isolated and all cases of choanal atresia, respectively.

Protection of Human Research Subjects

Human subjects approval for study aim 1 was received from the Institutional Review Board at The University of Iowa. Human subjects approval for study aim 2, the NBDPS, was received from the institutional review boards at each participating center and the CDC.

Table 3.1 Centers for Birth Defects Research and Prevention

Centers	Years of enrollment	Delivery Outcomes
Arkansas	01/01/1998 - ongoing	Live births, Stillbirths, Elective terminations
California	10/01/1997 - ongoing	Live births, Stillbirths, Elective terminations
Centers for Disease Control and Prevention	10/01/1997 - ongoing	Live births, Stillbirths, Elective terminations
Iowa	10/01/1997 - ongoing	Live births, Stillbirths, Elective terminations
Massachusetts	10/01/1997 - ongoing	Live births, Stillbirths
New Jersey	01/01/1998 - 2002	Live births
New York	10/01/1997 - ongoing	Live births, Stillbirths, Elective terminations
North Carolina	01/01/2003 - ongoing	Live births
Texas	10/01/1997 - ongoing	Live births, Stillbirths, Elective terminations
Utah	01/01/2003 - ongoing	Live births, Stillbirths, Elective terminations

Table 3.2 Minimum Detectable Odds Ratios for Selected Risk Exposures for Choanal Atresia

Exposure	Exposure rate among controls – (%) (N=6703)	OR (All Cases) (N=90)	OR (Isolated Cases) (N=47)
Cigarette Smoking			
Lifetime exposure	(33.1)	1.83	2.28
Active exposure (B1-M3)	(10.0)	2.24	2.85
≥ 15 cig/day (B1-M3)	(5.7)	2.62	3.43
Alcohol			
Any exposure	(36.4)	1.82	2.28
Periconceptional exposure (B1-M3)	(3.3)	3.17	4.25
Drinking, ≥ 1 binge episodes (B1-M3)	(11.8)	2.15	2.73
Caffeine			
Coffee (≥ 3 cups per day)	(7.1)	2.46	3.18
Tea (≥ 3 cups per day)	(4.2)	2.90	3.85
Caffeinated soda (≥ 3 cups per day)	(11.5)	2.16	2.75
Total caffeine (≥ 300 mg/day)	(10.9)	2.19	2.79
Medications			
Anti-infective	(3.1)	3.24	4.37
Anti-infective (UTI) agents	(1.7)	4.15	5.77
Anti-pyretic	(0.4)	8.77	13.15
Anti-depressants	(2.9)	3.32	4.50
Anti-diarrheal	(0.3)	10.44	15.85
Respiratory tract agents	(0.5)	7.71	11.42
Anti-tussive	(2.8)	3.37	4.60
Expectorant	(5.7)	6.62	3.43
Nutrients	(25.0)	1.93	2.43

B1-M3 = periconceptional period; UTI=Urinary Tract Infection; OR=Odds Ratio

CHAPTER IV

RESULTS

Aim 1

To determine the prevalence of choanal atresia and choanal stenosis and describe the characteristics of affected infants and their mothers

For study aim 1, 304,008 live births were identified as delivered to Iowa residents from January 1, 1998 through December 31, 2005. Active surveillance by the IRCID identified 14 infants diagnosed with choanal atresia (British Pediatric Association [BPA] codes 748.010 - 748.014), and 54 infants diagnosed with choanal stenosis (BPA codes 748.000 - 748.004).

Fourteen infants, born to Iowa resident mothers from January 1, 1998 through December 31, 2005, were identified with choanal atresia by the IRCID. During the 8-year time period, the overall prevalence estimate of choanal atresia was 0.46 per 10,000 live births (95% confidence interval [CI] = 0.27, 0.78). No choanal atresia cases were identified during birth years 1999, 2002, or 2005, but the annual prevalence estimates for the remaining study years were relatively consistent and ranged between 0.54 and 0.80 per 10,000 live births (Table 4.1). By comparison, 54 infants with choanal stenosis were born to Iowa resident mothers from January 1, 1998 through December, 31 2005. Among the 54 infants with choanal stenosis, a preliminary diagnosis was reported as ‘possible’ or ‘suspected’ choanal atresia for two infants, and as ‘choanal atresia’ for six infants. A medical geneticist re-confirmed the diagnosis for these eight infants to be ‘choanal stenosis’ after reviewing supporting diagnostic and surgical evidence. Estimated prevalence of choanal stenosis during the 8-year study period for the 54 infants was 1.78

per 10,000 live births (95% CI = 1.36, 2.32) with annual prevalence estimates varying over the study period (Table 4.1).

Choanal atresia presented as a solitary (isolated) defect in 5 out of all 14 case infants. An additional five infants with choanal atresia had two or more major co-occurring birth defects of different organ systems (multiple) (Table 4.2). Another two of the 14 infants with choanal atresia had a single gene (Mendelian) defect and were classified as CHARGE (Coloboma, Heart defect, Choanal Atresia, Retarded growth and development, Genital anomaly and Ear defect) syndrome. Choanal atresia presented as a part of a chromosomal syndrome in two infants, one with a known etiology (DiGeorge Syndrome) and another with an unknown etiology (Table 4.2). Common, co-occurring defects among multiple choanal atresia cases were low set ears, bilateral anomalies of fingers, and micro- or macrognathia (Table 4.3). The prevalence of isolated or multiple choanal atresia (non-syndromic) was similar and estimated as 0.16 (95% CI= 0.07, 0.40) per 10,000 live births. Overall, one-half of all case infants had bilateral choanal atresia. Unilateral choanal atresia was predominantly right-sided (Table 4.2).

Similar to choanal atresia, about 35% of infants had isolated choanal stenosis, and 33% of infants had multiple choanal stenosis (Table 4.2). Nine out of the 54 infants with choanal stenosis had a chromosomal syndrome (Trisomy 21, 18, or 13; Wolf-Hirschorn Syndrome; Turner Phenotype; or deletion of short arm of 17 or 18), three had syndromes of unknown etiology; and five infants had a single gene (Mendelian) defect (Table 4.2). Common co-occurring defects among infants with multiple choanal stenosis included microcephalus, anomalies of the nose, micro- or macrognathia, low set ears or congenital nasal septum deviation (Table 4.4). Prevalence of isolated choanal stenosis was 0.62 per

10,000 live births (95% CI= 0.40, 0.98) and multiple choanal stenosis was 0.59 per 10,000 live births (95% CI= 0.37, 0.94). Among infants with a chromosomal syndrome, trisomy 18 and trisomy 21 were most common. Among choanal stenosis cases with known laterality (59%), one-half were bilateral, and unilateral cases were predominantly left-sided.

Qualitative data were abstracted from the IRCID to summarize diagnostic and treatment characteristics of choanal atresia and choanal stenosis. Commonly reported symptoms for choanal atresia and choanal stenosis were 'breathing difficulty or respiratory distress' and 'nasal congestion.' Other symptoms for choanal atresia included 'unable to hear air movement through the nares' or 'noisy breathing at rest.' A frequently reported clinical sign for both defects was 'inability to pass a catheter through one or both nares.' Respiratory distress was reported for most case infants, particularly those with bilateral choanal atresia, although location (anterior or posterior) was not provided**.**

Clinical notes abstracted for only two choanal atresia cases included mention of the quality of atresia, one being 'membranous' and the other 'bony.' The diagnostic method used most often to detect choanal atresia was computerized tomography (CT) with indicated treatment procedures being endoscopic stenting, transnasal surgery, mitomycin applications and myringotomy. Cytogenetic tests were reportedly conducted for five infants with the test results for each infant listed as normal. Among infants with choanal stenosis, 33 had cytogenetic test results (22 with normal results and 11 with abnormal results). There were no reports of other genetic testing of infants with either choanal atresia or choanal stenosis.

Selected characteristics differed little ($p>0.05$) between choanal atresia infants and those with choanal stenosis (Table 4.5). Among infants with choanal atresia, an equal proportion of females and males were affected, each was a singleton pregnancy, and most were live births. Four out of the 14 infants with choanal atresia were born preterm (<37 weeks of gestation) and were low birth weight (<2500 grams). Among infants with choanal stenosis, an excess was found for females, most were singleton pregnancies, and most were live births. About one-quarter of all infants with choanal stenosis were born preterm (<37 weeks of gestation) and were low birth weight (<2500 grams). Family history of choanal atresia was reported for one case infant, where the mother of the infant reported that she had undergone a choanal atresia repair at five years of age. Mothers of choanal atresia infants and those of choanal stenosis infants also differed little ($p>0.05$) (Table 4.5). Mothers of choanal atresia infants were most often between 25 and 34 years of age at delivery, were non-Hispanic white, and had some college education. Mothers of choanal stenosis infants were frequently over 35 years of age at delivery, predominantly non-Hispanic white, but less likely to have attended college.

Aim 2

To examine the associations between exposure to cigarette smoke, alcohol, and medications during the month before through the third month following conception, and exposure to caffeine and nutrients one-year before pregnancy in case and control mothers, and choanal atresia in the offspring

For study aim 2, a total of 102 eligible infants with choanal atresia (BPA codes 748.010 - 748.014) and 6807 eligible, unaffected healthy control infants with expected dates of delivery (EDD)s from October 1, 1997 through December 31, 2005 were identified from the NBDPS. Of the 102 infants with choanal atresia, 91 were classified as

definite cases after record review by clinical geneticists. Mothers of 90 infants completed the NBDPS telephone interview, and were included in the study. Similarly, 6703 of 6807 eligible control mothers who completed the telephone interview were included. Interview participation rates were 57% for case mothers and 69% for control mothers. The median time between EDD and interview was 8.5 months and 7.6 months for case and control mothers, respectively.

Data from the NBDPS were analyzed to produce descriptive statistics for selected characteristics of case and control infant-mother dyads (Table 4.6). Among case infants, the proportion of females was twice that of males. Compared to control infants, case infants had a significantly greater prevalence of preterm birth and multiple births. Two case mothers reported a first-degree relative affected with choanal atresia, whereas no control mother reported having a first-degree relative affected with choanal atresia.

Mothers of case infants were more likely to be older (greater or equal to 35 years), non-Hispanic white, a high school graduate, and have a household income from $10,000 - $50,000 US dollars, history of type 1 or 2 diabetes before the index pregnancy, history of hypertension, and fall or winter season of conception compared to those of control infants (Table 4.6). No significant differences were found between case and control mothers with regard to body mass index, parity, nativity, employment status, and periconceptional folic acid intake. Participating centers showed some differences in the proportion of cases and controls enrolled; however, these differences were not statistically significant.

Cigarette Smoking

Overall, 31 (34%) case mothers and 2219 (33%) control mothers reported periconceptional exposure to cigarette smoke (Table 4.7). Among isolated choanal atresia

cases, a greater proportion of mothers reported active smoking compared to control mothers. The frequency of exposure was the same (15 cigarettes/day) among mothers of isolated choanal atresia cases compared to control mothers (6%). No significant dose-response effect was noted with increased frequency of exposure (per day) for isolated choanal atresia, stratified by potential confounders such as gender, gestational age, type 1 or 2 diabetes before index pregnancy, history of hypertension, and season of conception. Similarly, No significant dose-response effect was noted with increased frequency of exposure (per day) for all cases of choanal atresia combined, stratified by potential confounders such as gender, gestational age, birth weight, plurality, maternal race and ethnicity, type 1 or 2 diabetes before index pregnancy, history of hypertension, and season of conception.

Odds ratios (OR)s for periconceptional smoking exposure were generally higher for isolated choanal atresia cases compared to all choanal atresia cases combined (Table 4.8). An elevated, but non-significant, association was noted between any periconceptional exposure to cigarette smoking and isolated choanal atresia (OR=1.4; 95% CI=0.8, 2.6), but not all choanal atresia cases combined (OR=1.0; 95% CI =0.6, 1.6) (Table 4.8).

Associations between choanal atresia and periconceptional exposure to cigarette smoke were further examined by 'active,' if the mother reported to have smoked, and 'passive' if the mother reported an indirect exposure at home or in the workplace (Table 4.8). Passive exposure during the periconceptional period yielded an elevated and marginally significant OR for isolated choanal atresia cases (OR=2.3; 95% CI=1.0, 5.3).

Alcohol

For alcohol exposure, 33 (37%) case mothers and 2442 (36%) control mothers reported any periconceptional consumption (Table 4.7). Little variation was found between case and control mothers with respect to frequency of average drinks consumed per month and number of binge episodes (4 or more drinks per occasion). Adjusted ORs for any maternal periconceptional exposure to alcohol and choanal atresia was not significant either isolated cases (OR=0.8; 95% CI=0.4, 1.6) or for all cases combined (OR=0.9; 95% CI=0.6, 1.5) (Table 4.8). Risk due to a high average number of drinks per month (≥ 16 drinks/month) could not be calculated for isolated cases because of insufficient cell size (n<5); however, maternal reports of 16 or more drinks per month did not show a significant increase in risk among all cases of choanal atresia. Further, a non-significant association was noted between binge drinking (4 or more drinks per occasion) and choanal atresia among both isolated and all choanal atresia cases combined. Use of 5 or more drinks to define a binge episode did not materially change the odds ratios (data not shown). No significant dose-response effect was noted for increased average number of drinks per month and isolated choanal atresia, stratified by potential confounders such as gender, gestational age, birth weight, any periconceptional active smoking, type 1 or 2 diabetes before index pregnancy, history of hypertension, and season of conception. No significant dose-response effect was noted for increased average number of drinks per month and all cases of choanal atresia combined, stratified by potential confounders such as gender, gestational age, birth weight, plurality, maternal race and ethnicity, type 1 or 2 diabetes before index pregnancy, history of hypertension, and season of conception.

An effect modification was observed between active periconceptional cigarette smoking and alcohol exposure. Mothers who reported active periconceptional smoking exposure, but no drinking, or mothers who reported any drinking, but no active periconceptional smoke exposure showed a higher risk of having an infant with choanal atresia; however, the same effect did not persist among mothers with both active periconceptional smoking and alcohol exposure. Excluding the interaction term from the multivariable model did not materially impact the estimates of the main effects for each exposure.

Caffeine

All case mothers and most (6688 or 99.8%) control mothers responded to items about caffeine use one-year prior to the index pregnancy (Table 4.9). Overall, 47 (52%) case mothers and 3116 (47%) control mothers reported any caffeine exposure (coffee, tea, or soda). Stratification by type of caffeinated beverage consumed produced elevated ORs for maternal reports of 3 or more cups of coffee per day and isolated choanal atresia (OR=2.89; 95% CI=1.30, 6.42). This association was attenuated for mothers of all choanal atresia cases combined. A significant dose-response effect was found when mothers reported increased intake of coffee (cups/day) for both isolated and all cases of choanal atresia compined (Cochran-Armitage test-Asymptomatic test p value=0.03). The dose-response effect was further stratified by gender, gestational age, birth weight, plurality, maternal race and ethnicity, type 1 or 2 diabetes before index pregnancy, history of hypertension, and season of conception, and maternal periconceptional smoking and alcohol, and the Mantel-Haenszel pooled trend test was significant (two-

sided $p<0.05$) for both isolated and all cases of choanal atresia combined. No significant association was found for higher intake of caffeinated tea (≥ 3 cups per day), although our analyses were somewhat limited due to small cell sizes among isolated choanal atresia cases ($n<5$), particularly for higher exposures (≥ 3 cups per day). Increased maternal intake of caffeinated soda also did not show a positive association with choanal atresia, either for isolated or all choanal atresia cases combined. In addition, cumulative exposure to caffeine from caffeinated beverages, coffee, tea, and soda showed an increased, but marginally significant, OR for isolated choanal atresia for mothers who reported consuming 300 or more mg/day of caffeine (OR=2.15; 95% CI=1.00, 4.73). Unlike coffee (cups/day) intake, no significant trend was noted between increasing levels of total caffeine (mg/day) before pregnancy and risk of choanal atresia in the offspring (both isolated or all cases of choanal atresia combined). Also, there was no significant interaction between caffeine intake and any active periconceptional tobacco exposure and alcohol use for both isolated and all cases of choanal atresia combined.

Nutrients

Macro-, micro-, and mineral nutrient intakes one-year prior to pregnancy were available for all case and control mothers. Quartiles of intake were created using control mother responses. No significant association was found for higher or lower intakes of macronutrients (Table 4.10) or minerals (Table 4.11). Among micro-nutrients, high intake of zinc for all choanal atresia cases combined was associated with an increased OR (OR=2.12; 95% CI=1.17, 3.86); for isolated choanal atresia cases only the OR was of a comparable magnitude but not statistically significant (OR=1.86; 95% CI=0.84, 4.13)

(Table 4.12). Interestingly, lower maternal intake of single vitamin A was associated with a reduced risk of isolated choanal atresia (OR=0.32; 95% CI=0.13, 0.79) after controlling for total energy intake and other covariables (Table 4.12). Overall, the nutritional analysis estimates were derived from exposure quartiles having small exposure frequencies among cases.

Medications

Very few case mothers (<6%) reported that they were exposed to medications during the periconceptional period of their index pregnancy (Table 4.13). A high frequency of exposure was noted for anti-infective (any), anti-infective urinary tract infection (UTI) agents, anti-pyretic, anti-depressant - selective serotonin reuptake inhibitors (SSRI)s, anti-diarrheal, respiratory tract agents, and anti-tussive medication among all choanal atresia cases combined and isolated choanal atresia case mothers compared to control mothers. Particularly, a greater proportion of all case mothers were exposed to anti-infective UTI agents compared to control mothers (5.6 vs. 1.7%). The frequency of exposure was also higher among mothers of isolated cases compared to control mothers for SSRI medications (6.4 vs. 2.9%) and anti-tussives (8.5 vs. 2.8%).

Adjusted analyses of maternal periconceptional medication exposures and choanal atresia could not be conducted due to insufficient cell sizes of exposed mothers (n<5); thus, unadjusted estimates were presented (Table 4.13). Elevated, but non-significant associations were noted between any periconceptional exposure to any anti-infective (OR=1.4, 95% CI=0.2,5.6), anti-infective urinary tract infection (UTI) agents (OR=2.5; 95% CI=0.3, 9.8), SSRIs (OR=2.3; 95% CI=0.5,7.3), and anti-tussive (OR=3.2; 95%

CI=0.8,8.9) and isolated choanal atresia; and between any periconceptional exposure to any anti-infective (OR=1.5; 95% CI=0.4,4.0); anti-pyretic (OR=2.9; 95% CI= 0.1,17.8), SSRIs (OR=1.6; 95% CI=0.4,4.2), anti-diarrheal (OR=3.6; 95% CI=0.1,22.7), respiratory tract agent (OR=2.3; 95% CI=0.1,14.3), and anti-tussive (OR=2.0; 95% CI=0.8,5.0) and all cases of choanal atresia combined.

Only one significant association was noted between periconceptional exposure to anti-infective UTI agents and all cases of choanal atresia (OR=3.3; 95% CI=1.3, 8.4). This association was also found among isolated cases of choanal atresia, but was not statistically significant (OR=2.5; 95% CI=0.3, 9.8). Frequently used anti-infective UTI drugs were examined including ampicillin, cephalexin, clotrimazole, diazepam, metronidazole, nitrofurantoin, and promethazin; however, case mothers reported no periconceptional exposure to any of the above stated drugs. Association between anti-thyroidal medication use during pregnancy and choanal atresia is well-reported in the literature; however, no case mother reported periconceptional use of anti-thyroid medications.

Summary of Findings

This is the first statewide, population-based study of choanal atresia and choanal stenosis and also the first population-based study of risk factors for choanal atresia. Overall prevalence estimates for choanal atresia and choanal stenosis among births to Iowa resident mothers from January 1, 1998 through December 31, 2005 were 0.46 and 1.78 per 10,000 live births, respectively. There were no significant differences in the infant and maternal characteristics between choanal atresia and choanal stenosis cases.

Review of IRCID clinical notes showed that some choanal stenosis cases were reported as choanal atresia before diagnostic confirmation, suggesting a potential for diagnostic misclassification between the two defects. Among the risk factors examined for choanal atresia using data from the NBDPS, maternal periconceptional exposure to smoking or alcohol did not increase risk; however, maternal intake of 3 or more cups of coffee a day, as well as high exposures to zinc in the diet at one-year before pregnancy, increased the risk of choanal atresia in the offspring. Periconceptional exposure to anti-infective UTI agents also showed a significant association with choanal atresia among all cases of choanal atresia combined.

Table 4.1 Prevalence of Choanal Atresia and Choanal Stenosis in Iowa, 1998-2005

Year	Total Population Live Births	Choanal Atresia		Choanal Stenosis	
		n	Prevalence per 10,000 live births (95% CI)	n	Prevalence per 10,000 live births (95% CI)
1998	37,262	2	0.54 (0.13, 2.15)	11	2.95 (1.63, 5.33)
1999	37,549	0	NC	10	2.66 (1.43, 4.95)
2000	38,250	3	0.78 (0.25, 2.43)	8	2.09 (1.05, 4.18)
2001	37,610	3	0.80 (0.26, 2.47)	2	0.53 (0.13, 2.13)
2002	37,555	0	NC	8	2.13 (1.07, 4.26)
2003	38,139	3	0.79 (0.25, 2.44)	4	1.05 (0.39, 2.80)
2004	38,368	3	0.78 (0.25, 2.42)	6	1.56 (0.70, 3.48)
2005	39,275	0	NC	5	1.27 (0.53, 3.06)
TOTAL	304,008	14	0.46 (0.27, 0.78)	54	1.78 (1.36, 2.32)

CI = Confidence Interval; n=Frequency; NC = Not Calculated

Table 4.2 Clinical Characteristics of Choanal Atresia and Choanal Stenosis in Iowa

	Choanal Atresia (N=14)		Choanal Stenosis (N=54)	
Characteristics	n	(%)	n	(%)
Classification				
Isolated	5	(35.7)	19	(35.2)
Multiple	5	(35.8)	18	(33.3)
Chromosomal syndrome	1	(7.1)	9	(16.7)
Known syndrome-unknown etiology	1	(7.1)	3	(5.5)
Single gene (Mendelian) defect	2	(14.3)	5	(9.3)
Laterality				
Bilateral	7	(50.0)	17	(31.5)
Unilateral, Right Sided	5	(35.6)	3	(5.6)
Unilateral, Left Sided	1	(7.2)	12	(22.2)
Laterality Unknown	1	(7.2)	22	(40.7)

Table 4.3 Common Co-occurring Defects in Multiple Choanal Atresia (N=5)

BPA code	Defect Description	n	(%)
744245	Low Set Ears	3	(60.0)
755504	Anomalies of fingers, Bilateral	3	(60.0)
524000	Micrognathia\Macrognathia	2	(40.0)
743804	Other specified anamolies of eye, Bilateral	2	(40.0)
744234	Other mishappen ear, Bilateral	2	(40.0)
744910	Congenital anamolies of face, NOS	2	(40.0)
745498	Ventricular Septal Defect, Probable	2	(40.0)
745500	Patent Formen Ovale	2	(40.0)

BPA=British Pediatric Association; n=Frequency; N=Total cases with multiple choanal atresia; NOS=Not Otherwise Specified

Table 4.4 Common Co-occurring Defects in Multiple Choanal Stenosis (N=18)

BPA code	Defect Description	n	(%)
742100	Microcephalus	5	(28.0)
748180	Other specified anomalies of nose	5	(28.0)
524000	Micrognathia\Macrognathia	4	(22.0)
744245	Low set ears	4	(22.0)
754020	Congenital deviation of nasal septum	4	(22.0)
743804	Other specified anomalies of eye, bilateral	3	(17.0)
744231	Other misshapen ear, left	3	(17.0)
744246	Posteriorly rotated ears	3	(17.0)
744910	Congenital anomalies of face, NOS	3	(17.0)
745510	Ostium (septum) secundum defect	3	(17.0)
752502	Undescended testicle, unilateral right	3	(17.0)
754050	Plagiocephaly	3	(17.0)

BPA=British Pediatric Association; n=Frequency; N=Total cases with multiple choanal stenosis; NOS=Not Otherwise Specified

Table 4.5 Infant and Maternal Characteristics of Choanal Atresia and Choanal Stenosis

	Choanal Atresia (N=14)		Choanal Stenosis (N=54)	
Characteristics	n	(%)	n	(%)
Infant				
Sex*				
Male	7	(50.0)	22	(40.7)
Female	7	(50.0)	31	(57.4)
Birth weight (grams)*				
<2500	4	(30.8)	16	(32.0)
≥ 2500	9	(69.2)	34	(68.0)
Gestational age at delivery (wks)				
<37	5	(35.7)	14	(25.9)
≥ 37	9	(64.3)	40	(74.1)
Plurality*				
Singleton	14	(100.0)	50	(92.6)
Multiple	0	(0)	3	(5.5)
Birth Outcome*				
Live	13	(92.7)	52	(96.3)
Elective Termination	1	(7.1)	1	(1.9)
Family history of same defect				
No	13	(92.7)	54	(100.0)
Yes	1	(7.1)	0	(0)
Maternal				
Age at delivery (years)*				
<25	3	(21.4)	19	(35.9)
25-34	10	(71.4)	21	(39.6)
≥ 35	1	(7.2)	13	(24.5)
Race/Ethnicity*				
Non-Hispanic White	14	(100)	49	(92.5)
Non-Hispanic Black	0	(0)	2	(3.8)
Hispanic	0	(0)	1	(1.9)
Other	0	(0)	1	(1.9)
Education*				
Elementary/High School	5	(35.7)	31	(58.5)
College (1-4 years)	7	(50.0)	20	(37.7)
College (5+ years)	2	(14.3)	2	(3.8)
Gravidity*				
1-2	8	(61.5)	28	(54.9)
≥ 3	5	(38.5)	23	(45.1)

* Frequency of cases and controls may vary because of missing data. Percentages may not total 100 because of rounding.

Table 4.6 Selected Characteristics of Choanal Atresia Infants and their Mothers

Characteristics	Controls (N=6703) n	(%)	Isolated Cases (N=47) n	(%)		All Cases (N=90) n	(%)	
Infant								
Sex								
Male	3389	(50.6)	15	(31.9)		33	(36.7)	*
Female	3309	(49.4)	32	(68.1)		57	(63.3)	
Birth weight (grams)								
≥ 2500	6286	(93.8)	41	(87.2)	*	62	(68.9)	*
< 2500	392	(5.9)	6	(12.8)		28	(1.1)	
Gestational age at delivery (wks)								
≥ 37	6067	(90.5)	37	(78.7)	*	62	(68.9)	*
< 37	635	(9.5)	10	(21.3)		28	(31.1)	
Plurality								
1	6492	(96.9)	44	(93.6)		85	(94.4)	
2 or more	205	(3.1)	3	(6.4)		5	(5.6)	
Family history-choanal atresia**								
No	6294	(93.9)	41	(87.2)		82	(91.1)	
Yes	0	(0)	1	(2.1)		2	(2.2)	
Maternal								
Age at delivery (years)								
< 25	2238	(33.4)	8	(17.0)	*	20	(22.2)	*
25-34	3519	(52.5)	27	(57.5)		51	(56.7)	
≥ 35	946	(14.1)	12	(25.5)		19	(21.1)	
Race/Ethnicity**								
Non-Hispanic white	4011	(59.8)	38	(80.9)	*	66	(73.3)	*
Non-Hispanic black	764	(11.4)	3	(6.4)		5	(5.6)	
Hispanic	1491	(22.2)	5	(10.6)		16	(17.8)	
Other	409	(6.1)	0	(0)		2	(2.2)	
Education (years)								
<12	1128	(16.8)	4	(8.5)		13	(14.4)	
12	1651	(24.6)	12	(25.5)		18	(20.0)	
>12	3915	(58.4)	31	(66.0)		59	(65.6)	
Body Mass Index (kg/m^2)**								
< 25	3949	(58.9)	25	(53.2)		51	(56.7)	
≥ 25	2491	(37.2)	20	(42.6)		33	(36.7)	
Parity								
0	1955	(29.2)	11	(23.4)		27	(30.0)	
1	1975	(29.5)	16	(34.0)		29	(32.2)	
≥ 2	2771	(41.4)	20	(42.6)		34	(37.8)	
Nativity								
United States	5410	(80.7)	42	(89.4)		72	(80.0)	
Other	1288	(19.2)	5	(10.6)		18	(20.0)	

Table 4.6 Continued

Characteristics	Controls (N=6703) n	(%)	Isolated Cases (N=47) n[b]	(%)		All Cases (N=90) n[b]	(%)	
Household Income (US dollars)**								
<10,000	1079	(17.7)	5	(10.6)	*	14	(15.6)	
10,000-50,000	2132	(35.3)	25	(53.2)		41	(45.6)	
>50,000	2885	(47.3)	17	(36.2)		32	(35.6)	
Employment								
No	1873	(27.9)	14	(19.8)		24	(26.7)	
Yes	4825	(72.0)	33	(70.2)		66	(73.3)	
Folic Acid[a]								
No	846	(12.6)	4	(8.5)		10	(11.1)	
Yes	5764	(86.0)	43	(91.5)		80	(88.9)	
Prepregnancy diabetes								
No	6653	(99.3)	45	(95.7)	*	88	(97.8)	*
Yes	39	(0.7)	2	(4.3)		2	(5.6)	
Prepregnancy hypertension								
No	5793	(86.4)	44	(93.6)		83	(92.2)	
Yes	901	(13.4)	3	(6.4)		7	(7.9)	
Season of conception								
Summer	1641	(24.5)	7	(14.9)		14	(15.6)	*
Fall	1759	(26.2)	16	(34.0)		33	(36.7)	
Winter	1654	(24.7)	17	(36.2)		28	(31.1)	
Spring	1649	(24.6)	7	(14.9)		15	(16.7)	
Study site								
Arkansas	838	(12.5)	3	(6.4)		6	(6.7)	
California	849	(12.7)	1	(2.1)		8	(8.9)	
Iowa	751	(11.2)	5	(10.6)		10	(11.1)	
Massachusetts	855	(12.8)	10	(21.3)		12	(13.3)	
New Jersey	573	(8.6)	6	(12.8)		14	(15.6)	
New York	591	(8.8)	9	(19.2)		11	(12.2)	
North Carolina	393	(5.9)	2	(4.3)		4	(4.4)	
CDC/Atlanta	724	(10.8)	5	(10.6)		11	(12.2)	
Texas	766	(11.4)	4	(8.5)		12	(13.3)	
Utah	363	(5.4)	2	(4.3)		2	(2.2)	

CDC=Centers for Disease Control and Prevention; n=Frequency; Kg=Kilograms; m=Meter; US=United States, wks=weeks

[a] Any intake from prenatal, multivitamin, or folic acid as a single vitamin

*$p<0.05$ for cases vs. controls

** Frequency of cases and controls may vary because of missing data. Percentages may not total 100 because of rounding.

Table 4.7 Maternal Periconceptional Cigarette Smoking or Alcohol and Choanal Atresia

	Controls (N=6703)		Isolated Cases (N=47)		All Cases (N=90)	
Exposure	n	(%)[a]	n	(%)[a]	n	(%)[a]
Cigarette Smoking						
Total reports	6700		47		90	
Any exposure	2219	(33.1)	19	40.4	31	(34.4)
Type of smoking						
None	4468	(66.7)	27	(57.5)	58	(64.4)
Active only	500	(7.5)	7	(14.9)	8	(8.9)
Passive only	946	(14.1)	7	(14.9)	15	(16.7)
Active and Passive	768	11.5)	5	(10.6)	8	(8.9)
Active smoking						
B1 only	150	(2.2)	0	(0)	0	(0)
B1 and M1	265	(4.0)	3	(6.4)	3	(3.3)
B1, M1, M2	151	(2.3)	0	(0)	0	(0)
B1, M1, M2, and M3	673	(10.0)	9	(19.2)	13	(14.4)
Other	33	(0.5)	0	(0)	0	(0)
Cigarettes/day						
1-14	888	(13.3)	9	(19.2)	11	(12.2)
≥ 15	380	(5.7)	3	(6.4)	5	(5.6)
Alcohol						
Total reports	6688		46		89	
Any exposure	2442	(36.4)	18	(38.3)	33	(36.7)
B1 only	916	(13.7)	7	(14.9)	13	(14.4)
B1 and M1	688	(10.3)	5	(10.6)	11	(12.2)
B1, M1, M2	154	(2.3)	3	(6.4)	3	(3.3)
B1, M1, M2, and M3	223	(3.3)	0	(0)	0	(0)
Other	461	(6.9)	3	(6.4)	6	(6.7)
Average drinks/month						
1-15	1901	(28.4)	14	(29.8)	28	(31.1)
≥ 16	523	(7.8)	4	(8.5)	5	(5.6)
Binge episodes (≥ 4 drinks)						
Drinking, no binge episodes	1626	(24.3)	12	(25.5)	25	(27.8)
Drinking, ≥ 1 binge episodes	793	(11.8)	6	(12.8)	8	(8.9)

[a] Percentage of completed reports

B1=One month before pregnancy; M1=First month of pregnancy; M2=Second month of pregnancy; M3=Third month of pregnancy; N=Frequency

Incomplete reports on smoking (3 control mothers) and alcohol exposures (15 control mothers, 1 case mother) were excluded from analyses.

Table 4.8 Multivariable Analyses for Maternal Periconceptional Cigarette Smoking or Alcohol and Choanal Atresia

	Controls (N=6703)	Isolated Cases (N=47)		All Cases (N=90)	
Exposure	n	n	aOR (95% CI)[a,b]	n	aOR (95% CI)[c]
Cigarette Smoking					
None	4468	27	Reference	58	Reference
Any exposure	2219	19	1.43 (0.79,2.59)	31	1.00 (0.63,1.57)
Type of smoking					
Active only	500	7	1.11 (0.42,2.90)	8	0.64 (0.30,1.39)
Passive only	946	7	2.30 (0.99,5.33)	15	1.05 (0.49,2.24)
Active and Passive	768	5	1.23 (0.53,2.86)	8	1.34 (0.74,2.42)
Cigarettes/day					
1-14	888	9	1.57 (0.75,3.29)	11	0.75 (0.39,1.45)
≥ 15	380	3	NC	5	0.74 (0.29,1.88)
Alcohol					
None	4205	28	Reference	56	Reference
Any exposure	2442	18	0.82 (0.44,1.55)	33	0.94 (0.60,1.48)
Average drinks/month					
1-15	1901	14	0.83 (0.42,1.63)	28	1.02 (0.64,1.64)
≥ 16	523	4	NC	5	0.69 (0.27,1.76)
Binge episodes (≥ 4 drinks)					
Drinking, no binge episodes	1626	12	0.86 (0.34,2.20)	25	1.05 (0.64,1.72)
Drinking, ≥ 1 binge episodes	793	6	0.81 (0.40,1.65)	8	0.73 (0.34,1.57)

aOR=Adjusted Odds Ratio; CI=Confidence Interval; N=Frequency; NC=Not Calculated (less than five mothers reported the exposure)

[a] Cigarette smoking variables only - adjusted for gender, gestational age, type 1 or 2 diabetes before index pregnancy, history of hypertension, and season of conception.

[b] Alcohol variables only - adjusted for gender, gestational age, birth weight, any periconceptional active smoking, type 1 or 2 diabetes before index pregnancy, history of hypertension, and season of conception.

[c] Adjusted for gender, gestational age, birth weight, plurality, maternal race and ethnicity, type 1 or 2 diabetes before index pregnancy, history of hypertension, and season of conception.

Table 4.9 Multivariable Analyses for Maternal Caffeine Intake One-Year Before Pregnancy and Choanal Atresia

	Controls (N=6703)	Isolated Cases (N=47)		All Cases (N=90)	
Exposure	n (%)*	n (%)*	aOR (95% CI)[a]	n (%)*	aOR (95% CI)[b]
Coffee (cups/day)**					
<1	4637 (65.6)	28 (59.6)	Reference	59 (65.6)	Reference
1-2	1588 (23.7)	11 (23.4)	1.17 (0.58,2.38)	21 (23.3)	0.99 (0.59,1.65)
≥ 3	473 (7.1)	8 (17.0)	2.89 (1.30,6.42)	10 (11.1)	1.41 (0.70,2.83)
Tea (cups/day)					
<1	5450 (81.3)	38 (80.9)	Reference	75 (83.3)	Reference
1-2	965 (14.4)	7 (14.9)	0.99 (0.44,2.24)	10 (11.1)	0.68 (0.35,1.34)
≥ 3	280 (4.2)	2 (4.3)	NC	5 (5.6)	1.18 (0.46,3.02)
Caffeinated Soda (cans, glasses or bottles/day)					
<1	4055 (60.5)	29 (61.7)	Reference	54 (60.0)	Reference
1-2	1867 (27.9)	12 (25.5)	0.88 (0.45,1.73)	22 (24.4)	0.82 (0.50,1.37)
≥ 3	771 (11.5)	6 (12.8)	1.04 (0.42,2.54)	14 (15.6)	1.16 (0.63,2.15)
Total caffeine (mg/day)					
<100 (none/low)	3572 (53.3)	21 (44.7)	Reference	43 (47.8)	Reference
100 - <200 (low)	1540 (23.0)	11 (23.4)	1.21 (0.58,2.52)	21 (23.3)	1.07 (0.62,1.83)
200 - <300 (moderate)	848 (12.7)	6 (12.8)	1.17 (0.47,2.92)	13 (14.4)	1.12 (0.59,2.12)
≥ 300 (high/very high)	728 (10.9)	9 (19.5)	2.15 (0.98,4.73)	13 (14.4)	1.21 (0.63,2.32)

*Frequency of cases and controls may vary because of missing data. Percents may not equal 100 because of missing data.
aOR=Adjusted Odds Ratio; CI=Confidence Interval; mg=milligrams; N=Frequency; NC=Not Calculated (less than five mothers reported the exposure)

**Cochran-Armitage Test for Trend Significant for Isoalted Cases (p=0.03)

[a] Adjusted for gender, gestational age, type 1 or 2 diabetes before index pregnancy, history of hypertension, and season of conception.

[b] Adjusted for gender, gestational age, birth weight, plurality, maternal race and ethnicity, type 1 or 2 diabetes before index pregnancy, history of hypertension, and season of conception

Table 4.10 Multivariable Analyses for Maternal Macronutrient Intake One-Year Before Pregnancy and Choanal Atresia

	Controls (N=6703)	Isolated Cases (N=47)		All Cases (N=90)	
Macronutrients	n (%)	n (%)	aOR (95% CI)[a]	n (%)	aOR (95% CI)[b]
Carbohydrate (g)					
Low	1675 (25.0)	13 (27.7)	0.85 (0.39,1.87)	26 (28.9)	0.87 (0.48,1.59)
Medium	3350 (50.0)	27 (57.4)	Reference	46 (51.1)	Reference
High	1676 (25.0)	7 (14.9)	0.56 (0.18,1.75)	18 (20.0)	1.25 (0.57,2.73)
Protein (g)					
Low	1675 (25.0)	10 (21.2)	0.53 (0.24,1.19)	26 (28.9)	0.99 (0.57,1.73)
Medium	3351 (50.0)	27 (57.5)	Reference	45 (50.0)	Reference
High	1675 (25.0)	10 (12.3)	1.29 (0.51,3.27)	19 (21.1)	1.35 (0.67,2.69)
Fat (g)					
Low	1673 (25.0)	11 (23.4)	0.63 (0.29,1.38)	27 (30.0)	1.04 (0.60,1.81)
Medium	3352 (50.0)	28 (59.6)	Reference	47 (52.2)	Reference
High	1676 (25.0)	8 (17.0)	0.77 (0.28,2.09)	16 (17.8)	0.84 (0.40,1.75)
Fiber (g)					
Low	1675 (25.0)	10 (21.3)	0.56 (0.26,1.22)	25 (27.8)	0.92 (0.54,1.58)
Medium	3350 (50.0)	29 (61.7)	Reference	47 (52.2)	Reference
High	1676 (25.0)	8 (17.0)	0.67 (0.27,1.62)	18 (20.0)	1.22 (0.62,2.40)

aOR=Adjusted Odds Ratio; CI=Confidence Interval; IU=International Units; Low=<25 percentile; Medium=25 - 75 percentile; High=>75 percentile

[a] Adjusted for gender, gestational age, type 1 or 2 diabetes before index pregnancy, history of hypertension, season of conception, and total energy intake in kilo calories.

[b] Adjusted for gender, gestational age, birth weight, plurality, maternal race/ethnicity, type 1 or 2 diabetes before index pregnancy, history of hypertension, season of conception, and total energy intake in kilo calories.

*Frequency of cases and controls may vary because of missing data. Percents may not equal 100 because of missing data

Table 4.11 Multivariable Analyses for Maternal Mineral Intake One-Year Before Pregnancy and Choanal Atresia

	Controls (N=6703)	Isolated Cases (N=47)		All Cases (N=90)	
Minerals	n (%)	n (%)	aOR (95% CI)[a]	n (%)	aOR (95% CI)[b]
Iron (mg)					
Low	1675 (25.0)	13 (27.7)	0.88 (0.43,1.81)	24 (26.7)	0.82 (0.48,1.41)
Medium	3350 (50.0)	26 (55.3)	Reference	50 (55.6)	Reference
High	1676 (25.0)	8 (17.0)	0.71 (0.29,1.73)	16 (17.8)	0.88 (0.47,1.65)
Magnesium (mg)					
Low	1675 (25.0)	10 (21.3)	0.57 (0.26,1.27)	25 (27.8)	0.94 (0.54,1.64)
Medium	3350 (50.0)	30 (63.8)	Reference	49 (54.4)	Reference
High	1676 (25.0)	7 (14.9)	0.57 (0.20,1.57)	16 (17.8)	0.98 (0.47,2.02)
Manganese (mg)					
Low	1675 (25.0)	12 (25.5)	0.82 (0.40,1.68)	19 (21.1)	0.65 (0.37,1.13)
Medium	3351 (50.0)	26 (55.3)	Reference	58 (64.4)	Reference
High	1675 (25.0)	9 (19.2)	0.81 (0.36,1.87)	13 (14.4)	0.59 (0.31,1.14)
Phosphorus (mg)					
Low	1675 (25.0)	11 (23.4)	0.58 (0.26,1.32)	26 (28.9)	1.01 (0.57,1.80)
Medium	3351 (50.0)	25 (53.2)	Reference	45 (50.0)	Reference
High	1675 (25.0)	11 (23.4)	1.60 (0.62,4.09)	19 (21.1)	1.20 (0.59,2.45)
Selenium (μg)					
Low	1676 (25.0)	9 (19.2)	0.48 (0.21,1.09)	24 (26.7)	0.82 (0.47,1.42)
Medium	3349 (50.0)	29 (61.7)	Reference	50 (55.6)	Reference
High	1676 (25.0)	9 (19.2)	0.93 (0.38,2.28)	16 (17.8)	0.94 (0.47,1.88)
Sodium (mg)					
Low	1675 (25.0)	16 (34.0)	1.28 (0.63,2.58)	31 (34.4)	1.47 (0.87,2.49)
Medium	3351 (50.0)	24 (51.1)	Reference	42 (46.7)	Reference
High	1675 (25.0)	7 (14.9)	0.54 (0.20,1.48)	17 (18.9)	1.00 (0.50,2.00)

aOR=Adjusted Odds Ratio; CI=Confidence Interval; IU=International Units; Low=<25 percentile; Medium=25 - 75 percentile; High=>75 percentile

[a] Adjusted for gender, gestational age, type 1 or 2 diabetes before index pregnancy, history of hypertension, season of conception,

Table 4.11 Continued

and total energy intake in kilo calories.

[b] Adjusted for gender, gestational age, birth weight, plurality, maternal race/ethnicity, type 1 or 2 diabetes before index pregnancy, history of hypertension, season of conception, and total energy intake in kilo calories.

*Frequency of cases and controls may vary because of missing data. Percents may not equal 100 because of missing data

Table 4.12 Multivariable Analyses for Maternal Micronutrient Intake One-Year Before Pregnancy and Choanal Atresia

	Controls (N=6703)	Isolated Cases (N=47)		All Cases (N=90)	
Micronutrients	n (%)	n (%)	aOR (95% CI)[a]	n (%)	aOR (95% CI)[b]
One-Carbon Compounds					
Betaine (mg)					
Low	1678 (25.0)	8 (17.0)	0.48 (0.22,1.07)	23 (25.6)	0.82 (0.48,1.38)
Medium	3349 (50.0)	30 (63.8)	Reference	49 (54.4)	Reference
High	1676 (25.0)	9 (19.2)	0.68 (0.31,1.46)	18 (20.0)	0.93 (0.53,1.64)
Choline (mg)					
Low	1678 (25.0)	10 (21.3)	0.53 (0.24,1.18)	22 (24.4)	0.68 (0.39,1.20)
Medium	3350 (50.0)	28 (59.6)	Reference	50 (55.6)	Reference
High	1675 (25.0)	9 (19.1)	0.97 (0.39,2.42)	18 (20.0)	1.24 (0.63,2.45)
Total Folate (µg)					
Low	1678 (25.0)	14 (29.8)	0.97 (0.48,1.94)	25 (27.8)	0.92 (0.54,1.57)
Medium	3350 (50.0)	26 (55.3)	Reference	46 (51.1)	Reference
High	1675 (25.0)	7 (14.9)	0.57 (0.23,1.43)	19 (21.1)	1.13 (0.63,2.05)
Methionine (g)					
Low	1678 (25.0)	11 (23.4)	0.67 (0.31,1.45)	29 (32.2)	1.28 (0.75,2.20)
Medium	3350 (50.0)	25 (53.2)	Reference	41 (45.6)	Reference
High	1675 (25.0)	11 (23.4)	1.32 (0.57,3.05)	20 (22.2)	1.20 (0.63,2.28)
Riboflavin (mg)					
Low	1676 (25.0)	11 (23.4)	0.70 (0.33,1.50)	23 (25.6)	0.86 (0.50,1.49)
Medium	3351 (50.0)	25 (53.2)	Reference	47 (52.2)	Reference
High	1676 (25.0)	11 (23.4)	1.25 (0.55,2.85)	20 (22.2)	1.20 (0.65,2.24)
Vitamin B-12 (mg)					
Low	1678 (25.0)	13 (27.7)	0.95 (0.46,1.95)	26 (28.9)	1.22 (0.71,2.11)
Medium	3349 (50.0)	23 (48.9)	Reference	37 (41.1)	Reference
High	1676 (25.0)	11 (23.4)	1.28 (0.57,2.84)	27 (30.0)	2.44 (1.38,4.33)

Table 4.12 Continued

Micronutrients	Controls (N=6703) n (%)	Isolated Cases (N=47) n (%)	Isolated Cases (N=47) aOR (95% CI)[a]	All Cases (N=90) n (%)	All Cases (N=90) aOR (95% CI)[b]
Vitamin B-6 (mg)					
Low	1677 (25.0)	11 (23.4)	0.68 (0.32,1.45)	22 (24.4)	0.76 (0.43,1.32)
Medium	3350 (50.0)	28 (59.6)	Reference	48 (53.3)	Reference
High	1676 (25.0)	8 (17.0)	0.72 (0.29,1.80)	20 (22.3)	1.45 (0.78,2.70)
Zinc (mg)					
Low	1678 (25.0)	12 (25.5)	0.79 (0.36,1.71)	25 (27.8)	1.02 (0.58,1.79)
Medium	3350 (50.0)	22 (46.8)	Reference	40 (44.4)	Reference
High	1675 (25.0)	13 (27.7)	1.86 (0.84,4.13)	25 (27.8)	2.12 (1.17,3.86)
Single Vitamins					
Niacin (mg)					
Low	1675 (25.0)	12 (25.5)	0.80 (0.38,1.68)	27 (30.0)	1.09 (0.64,1.85)
Medium	3350 (50.0)	26 (55.3)	Reference	42 (46.7)	Reference
High	1676 (25.0)	9 (19.2)	0.91 (0.38,2.18)	21 (23.3)	1.52 (0.82,2.82)
Pantothenic acid (mg)					
Low	1675 (25.0)	8 (17.0)	0.39 (0.17,0.90)	23 (25.6)	0.81 (0.47,1.41)
Medium	3350 (50.0)	30 (63.8)	Reference	50 (55.6)	Reference
High	1676 (25.0)	9 (19.2)	0.83 (0.36,1. 92)	17 (18.8)	0.86 (0.46,1.62)
Vitamin A (µg IU)					
Low	1675 (25.0)	6 (12.8)	0.32 (0.13,0.79)	20 (22.2)	0.75 (0.43,1.29)
Medium	3350 (50.0)	33 (70.2)	Reference	52 (57.8)	Reference
High	1676 (25.0)	8 (17.0)	0.58 (0.26,1.30)	18 (20.0)	0.96 (0.54,1.70)
Vitamin C (mg)					
Low	1675 (25.0)	13 (27.7)	0.87 (0.43,1.74)	25 (27.8)	0.93 (0.55,1.55)
Medium	3351 (50.0)	26 (55.3)	Reference	47 (52.2)	Reference
High	1675 (25.0)	8 (17.0)	0.73 (0.31,1.71)	18 (20.0)	1.13 (0.61,2.08)

Table 4.12 Continued

Micronutrients	Controls (N=6703)	Isolated Cases (N=47)		All Cases (N=90)	
	n (%)	n (%)	aOR (95% CI)[a]	n (%)	aOR (95% CI)[b]
Vitamin K (mg)					
Low	1675 (25.0)	11 (23.4)	0.84 (0.40,1.76)	23 (25.6)	0.84 (0.50,1.42)
Medium	3351 (50.0)	23 (48.9)	Reference	46 (51.1)	Reference
High	1675 (25.0)	13 (27.7)	1.27 (0.63,2.56)	21 (23.3)	1.12 (0.65,1.93)
Vitamin D (mg)					
Low	1673 (25.0)	14 (29.8)	1.40 (0.68,2.87)	28 (31.1)	1.42 (0.85,2.39)
Medium	3348 (50.0)	18 (38.3)	Reference	37 (41.1)	Reference
High	1673 (25.0)	15 (31.9)	2.04 (0.98,4.22)	25 (27.8)	1.54 (0.89,2.66)

aOR=Adjusted Odds Ratio; CI=Confidence Interval; IU=International Units; Low=<25 percentile; Medium=25 - 75 percentile; High=>75 percentile

[a] Adjusted for gender, gestational age, type 1 or 2 diabetes before index pregnancy, history of hypertension, season of conception, and total energy intake in kilo calories.

[b] Adjusted for gender, gestational age, birth weight, plurality, maternal race/ethnicity, type 1 or 2 diabetes before index pregnancy, history of hypertension, season of conception, and total energy intake in kilo calories.

*Frequency of cases and controls may vary because of missing data. Percents may not equal 100 because of missing data.

Table 4.13 Maternal Periconceptional Medication Use and Choanal Atresia

	Controls (N=6703)		Isolated Cases (N=47)			All Cases (N=90)		
Medication Class	n	(%)	n	(%)	cOR (95% CI)	n	(%)	cOR (95% CI)
Anti-infective (Any)								
No	6462	(96.9)	44	(95.7)	Reference	85	(95.5)	Reference
Yes	204	(3.1)	2	(4.4)	1.44 (0.17,5.59)	4	(4.5)	1.49 (0.39, 4.02)
Anti-infective (UTI agents)								
No	6568	(98.3)	45	(95.7)	Reference	85	(94.4)	Reference
Yes	116	(1.7)	2	(4.3)	2.52 (0.29,9.84)	5	(5.6)	3.33 (1.33, 8.36)
Anti-pyretic								
No	6677	(99.6)	47	(100.0)	Reference	89	(98.9)	Reference
Yes	26	(0.4)	0	0	NA	1	(1.1)	2.89 (0.07,17.79)
Anti-depressants (SSRI)								
No	6508	(97.1)	44	(93.6)	Reference	86	(95.6)	Reference
Yes	193	(2.9)	3	(6.4)	2.30 (0.45,7.27)	4	(4.4)	1.57 (0.41,4.23)
Anti-diarrheal								
No	6681	(99.7)	47	(100.0)	Reference	89	(98.9)	Reference
Yes	21	(0.3)	0	0	NA	1	(1.1)	3.57 (0.09,22.7)
Respiratory Tract Agent								
No	6664	(99.5)	47	(100.0)	Reference	89	(98.9)	Reference
Yes	32	(0.5)	0	0	NA	1	(1.1)	2.34 (0.06,14.3)
Anti-tussive								
No	6488	(95.2)	43	(91.5)	Reference	85	(94.4)	Reference
Yes	190	(2.8)	4	(8.5)	3.18 (0.82,8,87)	5	(5.6)	2.01 (0.81,5.01)
Expectorant								
No	6306	(94.3)	44	(93.6)	Reference	87	(96.7)	Reference
Yes	380	(5.7)	3	(6.4)	1.13 (0.22,3.56)	3	(3.3)	0.57 (0.12,1.74)

B1-P3=one month before and three months after conception; CI=Confidence Interval; cOR=Crude Odds Ratio; N=Frequency; SSRI=Selective Serotonin Reuptake Inhibitors; UTI=Urinary Tract Infection

CHAPTER V

DISCUSSION

Aim 1

To determine the prevalence of choanal atresia and choanal stenosis and describe the characteristics of affected infants and their mothers

The overall prevalence of choanal atresia in Iowa during the 8-year study period was 0.46 per 10,000 live births. This estimate was somewhat lower than the collective estimate (0.82 per 10,000 live births) as reported from a three-registry study (henceforth referred to as the tri-registry study) of more than 5 million live births in California, France, and Sweden[10]. Comparison of the current estimate identified in the Iowa population with those from each of the three registries individually showed similar prevalence among Iowa (0.46 per 10,000 live births), France (0.78 per 10,000 live births) and Sweden (0.54 per 10,000 live births), but not California (1.13 per 10,000 live births). Given that choanal atresia is thought to occur more often among non-Hispanic whites, the findings of lower prevalence in the populations comprised almost exclusively of non-Hispanic whites (Iowa, France, and Sweden) does not seem to explain the difference in estimates. More likely, the higher prevalence observed in California may be due to the inclusion of severe stenosis cases, which were excluded in the current study. Differences in other infant and maternal characteristics were also identified between the current and the tri-registry study with the finding of an excess of affected females and older maternal age at delivery not corroborated in the tri-registry study[10]. Variability in classification of case infants with chromosomal disorders may have contributed to the disparate findings.

For choanal stenosis, the overall prevalence in Iowa was estimated to be 1.78 per 10,000 live births. To date, no published, population-based studies of the prevalence of choanal stenosis were available for comparison; however, statewide data from the Utah Birth Defect Network (UBDN) suggested a much lower prevalence of choanal stenosis of 0.41 per 10,000 live births (personal communication from Dr. John Carey). Although the UBDN uses a surveillance methodology similar to that used by the Iowa Registry for Congenital and Inherited Disorders (IRCID) and each state's population has similar racial/ethnic distributions, the prevalence estimate from the UBDN was based on fewer cases (12 cases in UBDN vs. 54 cases in IRCID), potentially producing a less stable estimate. Additionally, co-occurrence of choanal stenosis with other major defects was consistent between the two registries for some associated defects (e.g., microcephaly), but not others (e.g., 8p deletion and partial 8q trisomy were noted in Utah but not in Iowa).

Strengths and Limitations of Aim 1

Findings were generated using data from a multi-source, active, population-based surveillance system. Cases identified were reviewed by a medical geneticist and those with an underlying single-gene or chromosomal etiology were also included. Although the case-series spanned 8 years, the longest evaluated to date, the numbers of cases identified were modest and prone to random fluctuation. Further, most cases were predominantly non-Hispanic white; thus, the findings can be generalized only to this population subgroup.

Aim 2

To examine the associations between choanal atresia and maternal exposure to cigarette smoke, alcohol, and medications during the month before through the third month following conception, and maternal exposure to caffeine and nutrients one-year before pregnancy

Several differences in infant and maternal characteristics were found between cases and controls. Specifically, case compared to control infants were predominantly female, preterm (<37 weeks gestation), and multiple births. Mothers of case infants were more likely to be older (≥ 35 years), non-Hispanic white, a high school graduate, and have a household income from $10,000 - $50,000 US dollars, history of type 1 or 2 diabetes before the index pregnancy, history of hypertension, and fall or winter season of conception compared to those of control infants.

The female excess observed in the current study suggests a possible genetic predisposition and is comparable to findings from two previously published studies of choanal atresia cases identified from tertiary hospitals[2,4]; but discordant from the tri-registry study as mentioned above[10]. The high prevalence of preterm births in choanal atresia has not been reported previously. Of the 28 case infants in the current study with low birth weight (<2500 grams), 23 (82%) were also preterm (<37 weeks gestation). An increased risk of choanal atresia among low birth weight infants was also reported in the tri-registry study[10]. With regard to multiple births, the tri-registry study reported 13 sets of twins (2.9% twinning rate excluding triplets) with one twin-pair concordant for choanal atresia. The current study reported twin births in 5 (5.6%) out of the 90 case infants with no concordance for choanal atresia among any of the twins. The rate of twinning among controls was 3%, similar to the rate of twin births in the U.S. population during the current study period [131]. Lastly, the finding of an increased risk of choanal

atresia among case infants who were conceived in fall or winter may be suggestive of an infectious agent (e.g., influenza virus) as a risk factor for choanal atresia. This finding is novel, but may be spurious, due to the modest number of case infants studied.

Few of the maternal characteristics examined in the current study could be compared to other previously published studies. The finding of an increased risk of choanal atresia among infants born to non-Hispanic white mothers was supported by results of the tri-registry study[10], whereas the finding of increased risk of choanal atresia due to advanced maternal age at delivery (≥ 35 years) was not.

In the current study, differences were also found in health behaviors between case and control mothers. An elevated, but non-significant, association was noted between any maternal periconceptional active cigarette smoking and all choanal atresia; maternal periconceptional passive exposure to cigarette smoke yielded an elevated and marginally significant risk for isolated choanal atresia only. Maternal periconceptional alcohol exposure was not associated with choanal atresia, either all cases combined or isolated case only, even among those mothers who reported high average monthly intake (≥ 16 drinks/month) or binge drinking (4 or more drinks per occasion). Also, maternal exposure to three or more cups of caffeinated coffee per day, and overall exposure to caffeine from coffee, tea, and/or soda (≥ 300 mg/day) showed an increased risk for isolated choanal atresia, with increased coffee intake showing a significant dose-response effect. High maternal intake of vitamin B-12 and zinc showed an increased risk for all choanal atresia combined, whereas low intake of pantothenic acid and vitamin A reduced the risk of isolated choanal atresia only. Additionally, maternal periconceptional exposure to selected medications including any anti-infective, anti-tussive, anti-pyretic, anti-diarrheal,

respiratory tract agents, and anti-depressants associated with selective Serotonin Reuptake Inhibitors (SSRIs) marginally increased the risk of all choanal atresia combined. Further, maternal periconceptional exposure to anti-infective urinary tract infection (UTI) agents was significantly associated with increased risk of all choanal atresia cases combined. Because of the few published studies on the etiology of choanal atresia, associations noted in the current study were also compared to published literature on maternal exposures to cigarette smoking, alcohol, caffeine, sub-optimal nutrition, and medications and orofacial clefts in the offspring. Orofacial clefts were chosen for comparison as they share a similar gestation period for development and embryological origin with choanal atresia[31].

Cigarette Smoking

The current study found no association with active smoking and choanal atresia, either all cases combined or isolated cases only, but a marginally elevated association with passive smoking among isolated choanal atresia cases. One explanation of this finding may be that case mothers who actively smoked during their pregnancies may not have disclosed or under-reported their use due to social reasons[132], but the same may not have been true for self-reported passive exposures. A study of NBDPS control mothers[11] found that a large proportion who reported smoking before pregnancy (53.3%) discontinued smoking just before or upon the knowledge of their pregnancy; however, reported passive smoking did not decline at a comparable rate (9.3% decline at home and 11.8% decline at work). Further evidence that the maternal reports of active cigarette smoking were underestimated in the NBDPS comes from data from the Pregnancy Risk

Assessment Survey (PRAMS) from 26 sites across the U.S. showing a combined estimate for smoking during pregnancy to be slightly higher (14.1%) between 2000 and 2005[133]. Alternatively, maternal reports of active cigarette smoking in the NBDPS might have been an accurate reflection of such behavior, and maternal reports of passive exposure to cigarette smoking may have been misclassified, which supports work by George et al. (2006), who validated self-reported maternal smoking both during early and late pregnancy in a prospective cohort study of 953 pregnant Swedish women between 1996 and 1998, and reported high reliability for active smoking compared to passive smoking[134].

Measurement error in self-reported active smoking has been well examined. A comprehensive study by Pickett et al. (2009) found high sensitivity and specificity of recalled smoking among low to moderate smokers when compared to both prospective self-reports and bioassay (cotinine) measures[135]; however, recall among heavy smokers was found to be less valid. Also, Klebanoff et al. (2001) reported that 85% of reports of active smoking and 95% of reports of non-smoking were validated using a cotinine bioassay in a prospective study[136]. In addition, validity and reliability of self-reported smoking was found to be higher among women when their exposures were queried in a systematic month by month approach including other modifiers such as frequency and duration, compared to an overall dichotomous measure (e.g., ever vs. never smoked during pregnancy)[137]. The NBDPS questionnaire queried mothers regarding their smoking behaviors using a systematic approach for each month spanning three months before conception and each month of first trimester, with an overall measure queried for the second trimester and an overall measure queried for last trimester. Unlike active

smoking, frequency and duration of passive smoking was not queried in detail for those reporting workplace and household exposures; thus it is hard to quantify the degree of passive smoking exposures among mothers who reported combined active and passive exposures or passive exposure only, compared to mothers who reported active exposure only.

No other published studies were found that examined the relation between maternal smoking during pregnancy and choanal atresia in the offspring. Published studies for orofacial clefts have shown that maternal smoking during pregnancy has been associated with an increased risk of orofacial clefts[77,96,138-141] and risk has been shown to be modified by specific genetic susceptibilities. Among these studies, associations differed by phenotype (e.g., cleft lip with or without cleft palate, cleft palate alone)[142]. A meta-analysis of 24 case-control and cohort studies found moderate and statistically significant associations between maternal smoking and cleft lip, with or without cleft palate (relative risk=1.34; 95% CI = 1.25, 1.44), and between maternal smoking and cleft palate alone (relative risk 1.22; 95% CI=1.10, 1.35), as well as with a modest dose-response effect for cleft lip with or without cleft palate[143]. Further, gene-environment interactions between smoking and polymorphisms in transforming growth factor-α *TaqI* polymorphism have been reported for orofacial clefts[144-145]. The current study did not support any association between maternal periconceptional smoking and choanal atresia in the offspring; analyses of gene-smoking interactions were beyond the scope of the study.

The role of nicotine on reproductive health is well studied[146]. Using in vivo experiments in animals, Huang et al (2009) showed that oxidative stress, telomere

shortening, apoptosis, and compromised embryo development due to tobacco exposure[147]. Genomic instabilities were also found to modify the effects of nicotine exposure. For example, short telomeres have been studied in both animal and human models and were attributed to inconclusive findings noted between congenital malformations in children of women who were exposed to nicotine during pregnancy[148-149]. The null association noted between active smoking and choanal atresia in the current study may in part be due to unmeasured genetic factors that interact with nicotine exposure.

Alcohol

No elevated association was identified between maternal periconceptional alcohol consumption or binge drinking and choanal atresia in the offspring. An interaction between maternal periconceptional exposure to smoking and alcohol was found, although with the modest sample size, may have been due to chance.

Use of self-reports of alcohol exposure has a potential for recall or reporting bias. Social stigma about drinking during pregnancy may have prevented some mothers from reporting true levels of consumption[150,151]. Drinking exposure in the current study was assessed from maternal reports of average number of days and average number of drinks per day consumed, and the maximum number of drinks consumed on one occasion during the study period. The frequency of binging was subsequently calculated from reported averages, and could have deviated from the true measure of exposure among mothers who drank infrequently and/or reported monthly average below a binge episode (4 or more drinks per occasion). Also, the volume of alcohol consumed per beverage type was

assumed to correspond to a standard serving volume for beer, wine, or liquor, and did not account for variability for serving volumes within beverage types. Overall, compared to other national studies on prevalence of drinking before and during pregnancy (Behavior Risk Factor Surveillance System, The National Survey on Drug Use and Health, the PRAMS, the National Maternal and Infant Health System), the NBDPS assessed alcohol exposures comprehensively using eight contiguous time spans, the three months immediately before conception; the three months during the first trimester, and trimesters two and three of pregnancy.

No previous studies examined maternal alcohol consumption during pregnancy and risk of choanal atresia in the offspring. Among studies of orofacial cleft, some studies reported an increased risk due to maternal alcohol exposure during pregnancy[77,79]. Using the NBDPS data, weak associations were reported between isolated clefts and all clefts combined and maternal alcohol consumption (adjusted odds ratio [OR]s were close to unity), and moderate associations for orofacial cleft defects with other co-occurring major defects (adjusted ORs ranged between 1.3 and 1.5 for cleft lip with cleft palate and cleft palate only, respectively)[80]. Binge drinking elevated the risk of multiple cleft lip with cleft palate (adjusted OR=1.9; 95% confidence interval (CI)=0.7,5.2), but the association was not statistically significant[80].

Rovasio and Battiato (2002) studied the role of ethanol on chick embryos and found developmental disruptions among exposed embryos that frequently resulted in cephalic and or facial anomalies[152]. Specifically, in vitro exposure to alcohol was associated with significant and permanent changes in neural crest cell shape, surface morphology, apoptotic cell death, cytoskeleton, distance and velocity traveled, as well as

with abnormal pattern of migration, all of which supports the molecular theory of abnormal neural crest cell patterns in the etiology of choanal atresia[1,7]. A more recent study[153] examined the role of gene-environment interactions associated with ethanol on cranial neural crest cell development and migration among ten-day old rat embryos. Findings showed that ethanol affected oxidative defense genes and genes involved in neural crest cell development and down-regulated cranial neural crest cell Hox genes; thus, the null association noted between alcohol exposure and choanal atresia may be further modified by genetic factors.

Caffeine

Maternal intake of 3 or more cups of coffe per day showed a modest, positive association with isolated choanal atresia. A significant dose-response relationship was identified for higher levels of coffee intake (cups/day) and risk of all choanal atresia cases combined and isolated choanal atresia cases only (Cochran-Armitage Test of Trend p value=0.03); however, the test of trend was based on a small number of cases. Examination of total caffeine intake from coffee, tea, and soda combined (300 mg per day or more) did not show an elevated association. Attenuation of risk from combined caffeine sources may in part be due to the anti-oxidants present in tea, but most coffee drinkers (24 out of 31) in the current study reported no exposure to tea. Risk of choanal atresia among tea-drinkers alone was difficult to estimate due to small number of reported tea drinkers who were not exposed to caffeinated coffee (n=8). Interactions between caffeine and periconceptional cigarette smoking and alcohol consumption were examined in the current study, with the limitation that detailed exposure for caffeine was

queried for the year before pregnancy with only difference in overall caffeine consumption (more, less, or same) collected for the periconceptional period.

Physiological changes during pregnancy may affect caffeine consumption (e.g. nausea and vomiting). A recent prospective cohort study of newly pregnant women from 2000 through 2004 found symptoms of nausea and vomiting were common in the first trimester, and are dependent on maternal age, race/ethnicity, and gravidity[154]. Second, women who planned their pregnancy may have reduced their caffeine intakes compared to their counterparts. In the current study, pregnancy intention was examined as a covariable in multivariable models, but was not retained in the final model as removing it did not change the effect estimates by more than 15%.

Exposure assessment for caffeine in the current study was from maternal self-reports, and the accuracy of the quantity of caffeine intake may have varied among case and control mothers given the retrospective nature of the interview. Questionnaire items on caffeine were unable to capture detailed information on the strength of the caffeinated beverages and specific times during the year these beverages were consumed. The serving size and caffeine content of the beverages reported to have been consumed may have varied, producing potential exposure misclassification. Also, caffeine from medications, weight loss supplements, and other dietary exposures (e.g., chocolate) were unable to be assessed due to the infrequent reports of each exposure.

No previous studies reported on the association between caffeine intake one-year before pregnancy and risk of choanal atresia in the offspring, but the association between maternal caffeine intake one-year before pregnancy and orofacial clefts was examined using NBDPS data[155]. A moderate level of caffeine (100-199 mg/day) intake from coffee,

tea, soda, and chocolate combined during the year before pregnancy was significantly associated with both isolated and multiple orofacial clefts (adjusted ORs ranged between 1.2 and 1.6), but not a higher level (≥ 300 mg/day). In contrast to the current study, a null association was found between isolated orofacial clefts and high (3 or more cups per day) intake of coffee (adjusted OR=0.9; 95% CI=0.7,1.1) for cleft lip with or without cleft palate and for cleft palate only (adjusted OR=0.7; 95% CI=0.5,1.1). Further, a positive association was found between orofacial clefts and high (3 or more cups per day) intake of tea and multiple cleft palate only (adjusted OR=2.4; 95% CI=1.3,4.6). Another study[89] using data from hospital, surgical, clinical and office records of participating institutions and physicians from selected regions of greater Boston, Philadelphia, and Toronto compared mothers of infants with orofacial clefts to mothers of infants with other birth defects, and reported an elevated but nonsignificant risk for cleft lip with or without cleft palate among mothers who consumed at least 400 mg/day of caffeine. The effect estimate for cleft lip with or without cleft palate (adjusted OR: 1.4; 95% CI = 0.7, 2.9) was similar to that found in the current study for all cases of choanal atresia combined (adjusted OR=1.21; 95% CI = 0.63, 2.32).

Caffeine is fat soluble and can readily cross the placenta[86]. The pharmacokinetics of caffeine are further affected by other factors such as exercise, altitude, menstrual cycles, contraceptive use, diet, and smoking[86]. A role of residual confounding from other unmeasured factors associated with high intake of coffee and choanal atresia cannot be ruled out. For example, high intake of coffee has also been associated with decreased iron absorption[156], but there was no significant association between iron and choanal atresia in

the current study. Higher intake of iron has been associated with a decreased risk for orofacial clefts[98].

Nutrition

No association between choanal atresia and intake of important macro-, micro-, and mineral nutrients was identified except for vitamin A, B-12, pantothenic acid, and zinc. Lower intakes of pantothenic acid and vitamin A were shown to confer reduced risk for isolated choanal atresia, whereas , high intakes of vitamin B-12 and zinc were found to significantly increase the risk for all choanal atresia cases combined, but not isolated choanal atresia. Further higher quartiles of intake of total folate were found to decrease the risk of choanal atresia among isolated choanal atresia cases only, although the association was statistically nonsignificant. The protection offered by folic acid through fortification of the U.S. Food supply during the study period is unknown.

A short-version of Willett Food Frequency Questionnaire (FFQ) was used to assess maternal dietary patterns one-year before pregnancy in the current study, but there were limitations in the FFQ[128,157-159]. The questionnaire instrument was not internally validated for NBDPS; however, it has been shown that the FFQ provides reasonably accurate estimates of dietary intake by women in the distant past[159]. Also, maternal reports can only reflect the intake levels and not the tissue-dose levels, as each individual mother may have absorbed and metabolized the nutrients differently. In addition, there could have been unmeasured variability in exchange of nutrients between the mother and fetus in the current study sample.

No previously published studies were identified that examined the relation between maternal nutritional intake and choanal atresia in the offspring, but the association has been well-examined in relation to orofacial clefts, as reviewed by Krapels et al. (2004)[98]. A population-based study found that higher intakes (over 95th percentile) of vitamin A protected against isolated cleft defects compared to moderate intakes (40th-60th percentile)[160]. Using data from the NBDPS, Shaw et al. (2006) found a null association between high intakes of zinc and cleft lip with or without cleft palate, or cleft palate alone[96]. Another study from Poland noted an increased risk of oralfacial clefts among mothers whose whole blood levels of zinc were close to deficient[161]. The current study results do not concur with any one of these above mentioned studies on orofacial clefts.

Nutrition is known to be associated with maternal immunity, as deficiencies of proteins, zinc, iron, copper, selenium, vitamin A, vitamin E, and vitamin C have been noted to predispose women to various infections during pregnancy[162]. Conversely, studies on orofacial clefts in the NBDPS found an increased intake of total protein, alanine, choline, methionine, cysteine, and a majority of single micronutrients to be associated with a decreased risk of cleft lip with or without cleft palate (crude analyses), and increased intake of iron (OR=0.52; 95% CI=0.18,1.54) and riboflavin (OR=0.45; 95% CI=0.15,1.33) was associated with a marginally decreased risk of cleft lip with or without cleft palate (adjusted analyses). Further, increased intakes of folate (OR=0.79; 95%CI=0.43,1.46), vitamin B-12 (OR=0.64; 95% CI=0.35,1.15), and zinc (OR=0.67; 95% CI=0.35,1.28) decreased the risk of cleft palate marginally (crude analyses) among infants delivered to women who did not use other vitamin supplements[96]. High intake of

zinc decreased the risk of isolated oral clefts (children without additional major defects or known syndromes) in a different study population reported by Tamura et al. (2005)[163].

A review of the effects of vitamin A on fetal development in animals and humans showed that both low and high intakes of vitamin A were associated with optimal fetal development[164]. In their review, Finnell et al. (2004) discussed the importance of nutritional factors, including endogenous retinoids, in normal craniofacial and neural morphogenesis[165]. Also, they suggested that the pathogenesis of retinoid teratogenesis was attributed to disturbances in one or more of several embryologic mechanisms to alterations in cell proliferation, pattern formation, cellular induction and differentiation, neural crest cell migration, apoptosis, or induced inflammation. Further, they concluded that these effects were further modified by select gene variants. Specifically, different retinoid compounds were shown to serve as ligands for several different receptor subtypes expressed in the developing embryo. An experimental study confirmed the occurrence of choanal atresia and other malformations of nasal cavities in knockout mice with suppressed retinoic acid synthesis[62]. The retinoic acid receptors (RARs) and retinoid X receptors (RXRs) are ligand-dependent transcription factors, which reportedly interact with retinoic acid intake and contribute to its teratogenicity[165,166]. A subtype of RAR, called RAR-γ was associated with posterior truncation anomalies and mediated cranial malformations in some animal models[167].

Active placental transport of zinc and other micronutrients from the mother to the fetus are well established[168,169]. Dietary zinc deficiency has been associated with sub-optimal fetal outcomes and disturbances in copper and iron metabolism, reduction in high density lipo-proteins, and overall immunity[170], but literature on teratogenicity associated

with high doses of zinc is limited. The homocysteine pathway has been associated with riboflavin, folate, pyridoxine, cobalamin, and zinc, where the stated nutrients act as cofactors or substrates (reviewed by Krapels et al. 2006)[171]. The potential influence of zinc deficiency on orofacial clefts has been proposed to be either direct or by de-regulation of genomic stability [172,173], which need to be examined in relation to high doses of zinc intake and choanal atresia.

Medications

Findings from the current study suggest a significant association between all choanal atresia cases combined and maternal use of anti-infective UTI agents. Methimazole, a thianomide taken as an anti-thyroidal medication during pregnancy, has been associated with an increased risk of choanal atresia in some hospital-based studies[39,111], but there were no larger population-based studies to further support this finding. The current study was unable to examine thianomides (e.g., propylthiouracil, methimazole, or carbimazole) and choanal atresia because no case mother reported periconceptional use of anti-thyroidal medications.

Medication exposures were self-reported, including timing, dosage, and frequency of use, and the NBDPS was unable to validate medication use using medical chart review. Maternal illness was obtained by chart reviews, which may not capture use of over-the-counter medications. Detailed information on infections was not assessed. For example, reports of UTIs may include specific conditions such as asymptomatic bacteriuria, acute cystitis, and pyelonephritis. UTI agents were further examined by anti-septic drugs (nitrofurantoin, nalidixic acid), antimicrobial drugs (ampicillin, cefalexin),

and drugs for genital infections (clotrimazole, metronidazole) but there were no exposed case mothers. No previously published studies have examined the role of UTI medications on the risk of choanal atresia. Those that have examined UTIs and birth defects have produced mixed results[174,175]. Additionally, medications associated with an increased risk of clefting, corticosteroids[104], anti-epileptic drugs[105], non-steroidal anti-inflammatory agents (NSAIDS)[106], and folic acid antagonists[107],were not reportedly used by mothers of choanal atresia cases in the current study.

Strengths and Limitations for Aim 2

The current study had several strengths. The NBDPS is the largest birth defects study covering almost 10% of births annually in the U.S.[126], offering a geographic advantage in identifying a rare birth defect like choanal atresia. Active surveillance approaches using multiple source ascertainment minimized referral-bias, a common problem reported in previous hospital-based studies of choanal atresia. Case specificity was established by systematic review for both choanal atresia and co-occurring birth defects[127], and risk factors could be examined in a homogenous sample of choanal atresia cases. Additionally, the NBDPS controls were representative of the live births in each catchment area[176].

With regard to exposure data collection, the study used a computer-assisted telephone interview that was developed and validated by experts in the field and pre-tested. Study response rates were improved through multiple attempts to reach and enroll eligible subjects. Standardized prompts and exposures specific to different time frames during pregnancy aided mothers to identify and recall risk factors. A large number of

exposures were measured, thus making it possible to statistically adjust for confounding in the analysis. In summary, the NBDPS database provided the largest and most comprehensive dataset available to study a rare birth defect such as choanal atresia. The ongoing nature of NBDPS will allow re-evaluation of risk factors.

There are some limitations to studying risk factors for choanal atresia using NBDPS data. Not all participating centers conducted active surveillance of stillbirths and elective terminations due to issues in tracking birth defects prenatally and state laws prohibiting release of such information; however, this should not have had considerable impact on study results as choanal atresia has not been associated with early fetal loss. Another limitation of this study was the retrospective nature of the case-control study design, which may have led to a potential for differential recall among case and control mothers; however, the median time from birth or expected date of delivery to interview was similar for case and control mothers. Mothers of both case and control infants who were in foster care were excluded. It could be that these mothers were more likely to be exposed to heavy smoking, drinking, and caffeine use and sub-optimal nutrition before and during pregnancy, but given the very small proportion of such mothers, the current study estimates were not expected to be materially influenced. Further, it was not possible to ascertain the reasons for foster placement of infants in NBDPS. Lastly, selection bias may have occurred if mothers who participated in the study had different health behaviors compared to mothers who elected not to participate in the study, although the demographic characteristics of the two groups did not differ significantly.

Conclusions

The prevalence of choanal atresia in Iowa was similar to that reported in other population-based studies among predominantly non-Hispanic white populations. Choanal stenosis was more prevalent in Iowa compared to choanal atresia. The two defects were sometimes misclassified in the absence of confirmatory computerized tomographic or endoscopy results. No significant differences were noted for infant and maternal characteristics between the two defects. Such population-based surveillance prevalence estimates will better quantify the burden of the two defects, and allow for resource allocation, focused counseling, and treatment options to affected families.

The current study is the largest and most comprehensive study on risk factors for choanal atresia to date. Screening for smoking, alcohol, and caffeine use among reproductive-age women can help to identify high-risk pregnancies and prevention strategies. Public health campaigns and health care providers already promote a well-balanced diet for women in the reproductive years, but can further increase awareness about optimal intake of folic acid, other vitamin supplements, and micronutrients. Finally, increasing awareness among both health care providers and reproductive-aged and pregnant women about restricting undue use of over-the-counter or prescription medications can help to reduce the risk of choanal atresia as well as other birth defects.

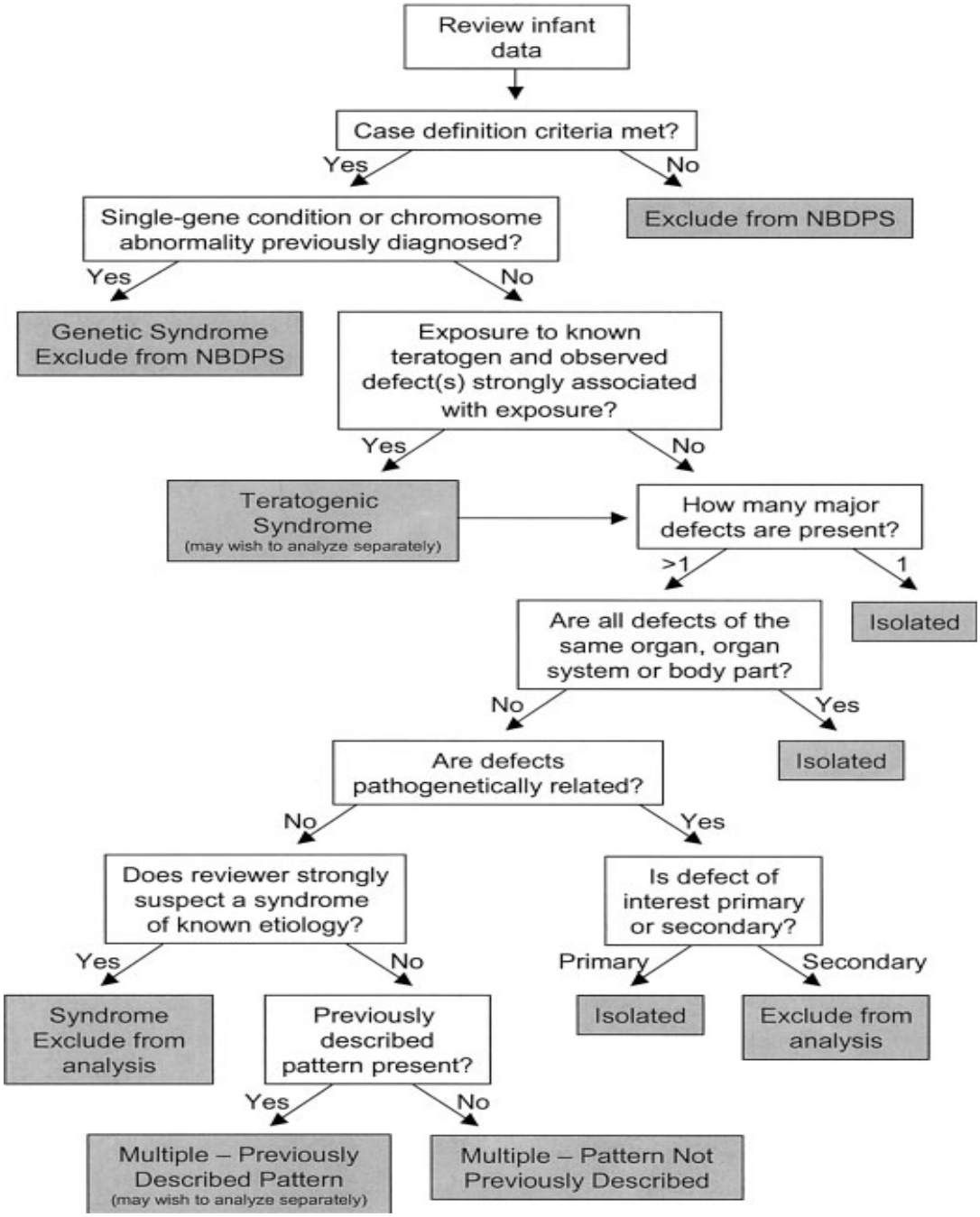
Recommendations

The results from the current study were exploratory, and the findings can be used to direct future hypotheses on the etiology of choanal atresia. There were limitations in the statistical power of the study findings due to the small sample of cases; and an even

smaller sample when restricted to isolated cases. This limitation can be addressed in coming years as more data become available within NBDPS or other registry-based studies. Re-analyses and confirmation of both significant and null associations noted in the current study are recommended in future population-based analyses. A detailed analysis of the effect modification between smoking and alcohol is also warranted in a larger sample of cases. Additionally, the association between maternal nutrient intake and their interactions with folate and vitamin supplements and the risk of choanal atresia in the offspring needs further evaluation as does evaluation of genetic polymorphisms in association with smoking, alcohol, caffeine, and nutrition need to be assessed in choanal atresia etiology in future studies. Lastly, there is a need to further explore the association between individual anti-infective UTI drugs and choanal atresia.

APPENDIX A

NBDPS CLINICAL CLASSIFICATION FOR BIRTH DEFECTS



Summary of the process of determining whether an infant has the defect of interest as an isolated defect, as one of multiple congenital anomalies, or as component of a syndrome, The National Birth Defects Prevention Study.

APPENDIX B

NBDPS QUESTIONNAIRE SECTIONS

ID #.....

RECORD.....01 SUBRECORD #.....00

BLANK .....

FORM.....01 VERSION.....3.11

National Birth Defects Prevention Study

Mother Questionnaire CATI Version 3.11

Centers for Disease Control and Prevention
U.S. Department of Health and Human Services
Public Health Service

November 2, 2001

Information contained on this form which could permit identification of any individual or establishment has been collected with an assurance that it will be held in strict confidence by the contractor and CDC, will be used only for purposes stated in this study, and will not be disclosed or released to anyone other than authorized staff of CDC without the consent of the participant in accordance with Section 308(d) of the Public Health Service Act (42 U.S.C. 242m).

SECTION E: TOBACCO-MOTHER

E1. The next questions are about tobacco use. Did you ever smoke cigarettes?

YES 1
NO ..... (SKIP TO E5) 2
DK ..... (SKIP TO E5) 8

E2. At any time from (-3) to (DOIB), did you smoke cigarettes?

YES 1
NO ..... (SKIP TO E5) 2
DK ..... (SKIP TO E5) 8

E3. During which months did you smoke?
CIRCLE FOR EACH MONTH. DO NOT CODE SHADED AREA.

MO	YES (ASK E4)	NO	DK	E4. During (SPECIFY MONTH) about how many cigarettes did you smoke a day?/Did you continue to smoke that many cigarettes through (LAST MONTH STATED)?
B3	1	2	8	<1/DAY ..... 01 1/DAY ..... 02 2-4/DAY ..... 03 ½ PACK (5-14) ..... 04 1 PACK(15-24) ..... 05 1 ½ PACK (25-34) ..... 06 2 PACK (35-44) ..... 07 >2 PACK ..... 08 DK 98
B2	1	2	8	<1/DAY ..... 01 1/DAY ..... 02 2-4/DAY ..... 03 ½ PACK (5-14) ..... 04 1 PACK(15-24) ..... 05 1 ½ PACK (25-34) ..... 06 2 PACK (35-44) ..... 07 >2 PACK ..... 08 DK 98
B1	1	2	8	<1/DAY ..... 01 1/DAY ..... 02 2-4/DAY ..... 03 ½ PACK (5-14) ..... 04 1 PACK(15-24) ..... 05 1 ½ PACK (25-34) ..... 06 2 PACK (35-44) ..... 07 >2 PACK ..... 08 DK 98
P1	1	2	8	<1/DAY ..... 01 1/DAY ..... 02 2-4/DAY ..... 03 ½ PACK (5-14) ..... 04 1 PACK(15-24) ..... 05 1 ½ PACK (25-34) ..... 06 2 PACK (35-44) ..... 07 >2 PACK ..... 08 DK 98
P2	1	2	8	<1/DAY ..... 01 1/DAY ..... 02 2-4/DAY ..... 03 ½ PACK (5-14) ..... 04 1 PACK(15-24) ..... 05 1 ½ PACK (25-34) ..... 06 2 PACK (35-44) ..... 07 >2 PACK ..... 08 DK 98

	MO	YES (ASK E4)	NO	DK	E4. During (SPECIFY MONTH) about how many cigarettes did you smoke a day?/Did you continue to smoke that many cigarettes through (LAST MONTH STATED)?
	P3	1	2	8	<1/DAY .....01 1/DAY .....02 2-4/DAY .....03 ½ PACK (5-14) .....04 1 PACK(15-24) .....05 1 ½ PACK (25-34) .....06 2 PACK (35-44) .....07 >2 PACK.....06 DK.....96
	T2	1	2	8	<1/DAY .....01 1/DAY .....02 2-4/DAY .....03 ½ PACK (5-14) .....04 1 PACK(15-24) .....05 1 ½ PACK (25-34) .....06 2 PACK (35-44) .....07 >2 PACK.....06 DK.....96
	T3	1	2	8	<1/DAY .....01 1/DAY .....02 2-4/DAY .....03 ½ PACK (5-14) .....04 1 PACK(15-24) .....05 1 ½ PACK (25-34) .....06 2 PACK (35-44) .....07 >2 PACK.....06 DK.....96

TOBACCO-HOUSEHOLD

E5. Did anyone in your household smoke cigarettes in your home between (-3) and (DOIB)?

YES 1
NO .....(SKIP TO E7) 2
DK .....(SKIP TO E7) 8

E6. During which months did someone smoke in your home? CIRCLE FOR EACH MONTH. DO NOT CODE SHADED AREA.

MO	YES	NO	DK
B3	1	2	8
B2	1	2	8
B1	1	2	8
P1	1	2	8
P2	1	2	8
P3	1	2	8
T2	1	2	8
T3	1	2	8

TOBACCO-WORKPLACE

E7. Did anyone smoke cigarettes near you at a workplace or school you may have attended during that year?

YES 1
NO .....(SKIP TO F1) 2
DK .....(SKIP TO F1) 8

E8. During which months from (-3) to (DOIB) did someone smoke near you at work/school? CIRCLE FOR EACH MONTH. DO NOT CODE SHADED AREA.

MO	YES	NO	DK
B3	1	2	8
B2	1	2	8
B1	1	2	8
P1	1	2	8
P2	1	2	8
P3	1	2	8
T2	1	2	8
T3	1	2	8

SECTION F: ALCOHOL

F1. Now I'm going to ask you some questions about drinking alcoholic beverages. We define an alcoholic drink as one beer, one glass of wine, one mixed drink, or one shot of liquor. Between (-3) and (DOIB), did you drink any wine, beer, mixed drinks or shots of liquor?

YES 1
NO ..... (SKIP TO G1) 2
DK ..... (SKIP TO G1) 8
RF ..... (SKIP TO G1) 7

MO	F2. During which months did you drink any alcoholic beverages? CIRCLE FOR EACH MONTH. DO NOT CODE SHADED AREA. YES (ASK F3-F5)	NO (NXT)	DK (NXT)	F3. In the (3rd/2nd/1st month before pregnancy, 1st/2nd/3rd…9th month of pregnancy), on average, how many days did you drink alcoholic beverages? (DK = 98) (RF = 97) # DAYS	F4. On those days that you drank alcoholic beverages, on average, how many drinks did you have per day? (DK = 98) (RF = 97) # DRINKS	F5. What was the greatest number of drinks you had on one occasion in (MONTH)? (DK = 98) (RF = 97) # DRINKS
B3	1	2	8	___	___	___
B2	1	2	8	___	___	___
B1	1	2	8	___	___	___
P1	1	2	8	___	___	___
P2	1	2	8	___	___	___
P3	1	2	8	___	___	___
T2	1	2	8	___	___	___
T3	1	2	8	___	___	___

F6. On the days that you drank alcohol, what type(s) of alcohol did you usually drink? READ CHOICES.

		YES	NO	RF	DK
a.	Beer	1	2	7	8
b.	Wine	1	2	7	8
c.	Mixed drink	1	2	7	8
d.	Shot liquor	1	2	7	8
e.	Other alcohol	1	2	7	8

SPECIFY: _____

CAFFEINE

The next questions are about caffeine. We will be asking you about your average use of coffee, tea and soda during the year before you became pregnant with (NOIB).

D19. How many cups of caffeinated or regular coffee did you usually drink?

NEVER OR < ONCE PER MONTH	0
1 PER MONTH	1M
2 PER MONTH	2M
3 PER MONTH	3M
1 PER WEEK	1W
2 PER WEEK	2W
3 PER WEEK	3W
4 PER WEEK	4W
5 PER WEEK	5W
6 PER WEEK	6W
1 PER DAY	1D
2 PER DAY	2D
3 PER DAY	3D
4 PER DAY	4D
5 PER DAY	5D
6+ PER DAY	6D
RF	97
DK	98

D20. How many cups of caffeinated or regular tea did you usually drink?

NEVER OR < ONCE PER MONTH	0
1 PER MONTH	1M
2 PER MONTH	2M
3 PER MONTH	3M
1 PER WEEK	1W
2 PER WEEK	2W
3 PER WEEK	3W
4 PER WEEK	4W
5 PER WEEK	5W
6 PER WEEK	6W
1 PER DAY	1D
2 PER DAY	2D
3 PER DAY	3D
4 PER DAY	4D
5 PER DAY	5D
6+ PER DAY	6D
RF	97
DK	98

D21. Did you drink sodas or soft drinks?

YES 1
NO ..... (SKIP TO D26) 2
DK ..... (SKIP TO D26) 8

FOR EVERY BRAND ANSWERED IN D22, ASK D23 AND D24, UNLESS SKIPPED BY PRECODES:

7 up (SKIP D24) = 01 A&W cream soda (SKIP D24) = 02 A&W root beer (SKIP D24) = 03 After the Fall spritzers (SKIP D24) = 04 Barq's root beer (SKIP D24) = 05 Black cherry soda = 06 Cheerwine (SKIP D24) = 07 Cherry 7-up (SKIP D24) = 08 Cherry coke (SKIP D24) = 09 Cherry soda = 10 Clearly Canadian (SKIP D24) = 11 Club soda (SKIP D23 & D24) = 12 Coke = 13 Cola , NOS = 14 Cranberry ginger ale (SKIP D24) = 15 Cream soda, NOS = 16 Diet Rite cola(SKIP D23 & D24) = 17 Diet Rite (fruit flavors) (SKIP D23 & D24) = 18 Dr. Brown's(all flavors) (SKIP D24) = 19	Dr. Pepper (SKIP D24) = 20 Fanta (all flavors) (SKIP D24) = 21 Fresca (SKIP D23 & D24) = 22 Ginger ale (SKIP D24) = 23 Ginger beer soda, NOS = 24 Grapefruit soda, NOS = 25 Hires root beer (SKIP D24) = 26 IBC black cherry (SKIP D24) = 27 IBC cherry cola (SKIP D24) = 28 IBC cream soda (SKIP D24) = 29 IBC root beer (SKIP D24) = 30 Jarritos sodas (all flavors) (SKIP D24) = 31 Jolt cola (SKIP D24) = 32 Josta = 33 Knudsen sparkling juices (SKIP D24) = 34 Lemon/lime soda, NOS = 35 Mellow yellow (SKIP D24) = 36 Mountain dew = 37 Mr. Pibb (SKIP D24) = 38 Nugrape (SKIP D24) = 39 Orange crush (SKIP D24) = 40	Orange soda, NOS = 41 Pepsi = 42 Quinine water (SKIP D23 & D24) = 43 RC cola (SKIP D24) = 44 Root beer , NOS = 45 Slice (SKIP D24) = 46 Sparkling water flavors (SKIP D24) = 47 Sprite (SKIP D24) = 48 Squirt (both flavors) (SKIP D24) = 49 Strawberry soda = 50 Sun-drop (SKIP D24) = 51 Sunkist fruit punch (SKIP D24) = 52 Sunkist orange (SKIP D24) = 53 Surge = 54 Tab (SKIP D23 & D24) = 55 Tahitian treat = 56 Tonic water (SKIP D23 & D24) = 57 Wild cherry Pepsi (SKIP D24) = 58 Wink (SKIP D24) = 59 Yoohoo Choc. (SKIP D24) = 60 Other, specify = 61

	D22. What brand(s) or types did you usually drink?/Anything else? LIST ALL. USE PRECODED SODA LIST TO PROBE. IF TYPE ON LIST IS KNOWN TO HAVE CAFFEINE OR BE A DIET DRINK, THOSE SELECTIONS WILL SKIP OVER D23 AND/OR D24	D23. Is (BRAND) diet?	D24. Is (BRAND) caffeine free?	D25. How many (cans/glasses/ bottles) of (BRANDS) did you usually drink?
A.	_____ DK = 98	YES ..... 1 NO ..... 2 DK 8	YES ..... 1 NO ..... 2 DK 8	NEVER OR LESS THAN 1 PER MONTH ..... 0 1 PER MONTH ..... 1M 2 PER MONTH ..... 2M 3 PER MONTH ..... 3M 1 PER WEEK ..... 1W 2 PER WEEK ..... 2W 3 PER WEEK ..... 3W 4 PER WEEK ..... 4W 5 PER WEEK ..... 5W 6 PER WEEK ..... 6W 1 PER DAY ..... 1D 2 PER DAY ..... 2D 3 PER DAY ..... 3D 4 PER DAY ..... 4D 5 PER DAY ..... 5D 6+ PER DAY ..... 6D RF ..... 97 DK 98

	D22. What brand(s) or types did you usually drink?//Anything else? LIST ALL. USE PRECODED SODA LIST TO PROBE. IF TYPE ON LIST IS KNOWN TO HAVE CAFFEINE OR BE A DIET DRINK, THOSE SELECTIONS WILL SKIP OVER D23 AND/OR D24	D23. Is (BRAND) diet?	D24. Is (BRAND) caffeine free?	D25. How many (cans/glasses/ bottles) of (BRANDS) did you usually drink?
B.	_____ DK = 98	YES.....1 NO.....2 DK.....8	YES.....1 NO.....2 DK.....8	NEVER OR LESS THAN 1 PER MONTH.....0 1 PER MONTH.....1M 2 PER MONTH.....2M 3 PER MONTH.....3M 1 PER WEEK.....1W 2 PER WEEK.....2W 3 PER WEEK.....3W 4 PER WEEK.....4W 5 PER WEEK.....5W 6 PER WEEK.....6W 1 PER DAY.....1D 2 PER DAY.....2D 3 PER DAY.....3D 4 PER DAY.....4D 5 PER DAY.....5D 6+ PER DAY.....6D RF.....97 DK.....98
C.	_____ DK = 98	YES.....1 NO.....2 DK.....8	YES.....1 NO.....2 DK.....8	NEVER OR LESS THAN 1 PER MONTH.....0 1 PER MONTH.....1M 2 PER MONTH.....2M 3 PER MONTH.....3M 1 PER WEEK.....1W 2 PER WEEK.....2W 3 PER WEEK.....3W 4 PER WEEK.....4W 5 PER WEEK.....5W 6 PER WEEK.....6W 1 PER DAY.....1D 2 PER DAY.....2D 3 PER DAY.....3D 4 PER DAY.....4D 5 PER DAY.....5D 6+ PER DAY.....6D RF.....97 DK.....98

D26. When you were pregnant with (NOIB) did you drink more, the same, less, or no caffeinated coffee?

More 1
Same 2
Less 3
None 4
DK 8

D27. When you were pregnant with (NOIB) did you drink more, the same, less, or no caffeinated tea?

More 1
Same 2
Less 3
None 4
DK 8

D28. When you were pregnant with (NOIB) did you drink more, the same, less, or no caffeinated sodas?

More 1
Same 2
Less 3
None 4
DK 8

SECTION D: PRENATAL VITAMINS

D1. Between (-3) and (DOIB), did you take any prenatal vitamins?

YES 1
NO ..... (SKIP TO D2) 2
DK ..... (SKIP TO D2) 8

FOR EACH VITAMIN ASK D1bb TO D1ee. IF YOU GET EXACT DATES IN D1bb **AND** D1cc, SKIP D1dd.

	D1aa. What did you take? / Anything else? PROBE WITH CHART BELOW. LIST ALL.	D1bb. Between (-3) and DOIB, when did you start using (PRENATAL VITAMIN)?	D1cc. When did you stop using (PRENATAL VITAMIN)? OR-	D1dd. How long did you take it? DURATION	D1ee. How often did you use the prenatal vitamin? FREQUENCY
A.	_____ DK ..... ❑	MM DD YYYY	MM DD YYYY	DK = 93 DAY(S) ..... 1 WEEK(S) ..... 2 MONTH(S) 3	DK = 98 PER DAY ..... 1 PER WEEK ..... 2 PER MONTH ..... 3 PER YEAR 4
B.	DK ..... ❑	MM DD YYYY	MM DD YYYY	DK = 93 DAY(S) ..... 1 WEEK(S) ..... 2 MONTH(S) 3	DK = 98 PER DAY ..... 1 PER WEEK ..... 2 PER MONTH ..... 3 PER YEAR 4
C.	_____ DK ..... ❑	MM DD YYYY	MM DD YYYY	DK = 93 DAY(S) ..... 1 WEEK(S) ..... 2 MONTH(S) 3	DK = 98 PER DAY ..... 1 PER WEEK ..... 2 PER MONTH ..... 3 PER YEAR 4

PROBE FOR D1aa:

IF CANNOT RECALL, READ LIST: Was it (READ LIST)?

- Fampren Forte
- Materna
- Natalins
- Natalins Rx
- Niferex PN
- Prenate 90
- Stuart Natal 1 + 1
- Stuart Natal Plus
- Zenate

PRENATAL VITAMIN, NOS

SINGLE VITAMINS

D2. Now I want to ask you about some single vitamins and minerals. Between (-3) and (DOIB), did you take any of the following single vitamins or minerals?

	YES	NO	DK
a. Vitamin A	1	2	8
b. Retinol	1	2	8
c. Beta carotene	1	2	8
d. B complexes	1	2	8
e. B6	1	2	8
f. B12	1	2	8
g. Folic acid	1	2	8
h. Vitamin C	1	2	8
i. Vitamin D	1	2	8
j. Vitamin E	1	2	8
k. Iron	1	2	8
l. Calcium	1	2	8
m. Zinc	1	2	8
n. Selenium	1	2	8

FOR EACH YES, ASK D2aa-D2dd. IF ALL NO OR DK, SKIP TO D3.

IF GET EXACT DATES TO D2aa **AND** D2bb, SKIP D2cc.

	D2aa. Between (-3) and DOIB, when did you start using (VITAMIN)?	D2bb. When did you stop using (VITAMIN)? OR:	D2cc. How long did you take it? DURATION	D2dd. How often did you use the vitamin? FREQUENCY
_____ FIRST VITAMIN	MM DD YYYY	MM DD YYYY	DK = 98 DAY(S)..... 1 WEEK(S) ..... 2 MONTH(S)..... 3	DK = 98 PER DAY ..... 1 PER WEEK ..... 2 PER MONTH..... 3 PER YEAR..... 4
_____ SECOND VITAMIN	MM DD YYYY	MM DD YYYY	DK = 98 DAY(S)..... 1 WEEK(S) ..... 2 MONTH(S)..... 3	DK = 98 PER DAY ..... 1 PER WEEK ..... 2 PER MONTH..... 3 PER YEAR..... 4
_____ THIRD VITAMIN	MM DD YYYY	MM DD YYYY	DK = 98 DAY(S)..... 1 WEEK(S) ..... 2 MONTH(S)..... 3	DK = 98 PER DAY ..... 1 PER WEEK ..... 2 PER MONTH..... 3 PER YEAR..... 4
_____ FOURTH VITAMIN	MM DD YYYY	MM DD YYYY	DK = 98 DAY(S)..... 1 WEEK(S) ..... 2 MONTH(S)..... 3	DK = 98 PER DAY ..... 1 PER WEEK ..... 2 PER MONTH..... 3 PER YEAR..... 4

MULTIVITAMINS

D3. Other than prenatal or single vitamins, between (-3) and (DOIB), did you take any multivitamins or vitamin complexes?

YES 1
NO ..... (SKIP TO D9) 2
DK ..... (SKIP TO D9) 8

FOR EACH VITAMIN ASK D5 TO D8. IF GET EXACT DATES IN D5 **AND** D6, SKIP D7.

	D4. What did you take? PROBE: Anything else? Do you remember the brand name? LIST ALL IN CHART.	D5. Between (-3) and DOIB, when did you start using (VITAMIN)?	D6. When did you stop using (VITAMIN)? OR:	D7. How long did you take it? DURATION	D8. How often did you use the vitamin? FREQUENCY
A.	_____ DK ..... ❑	MM DD Y'YYY	MM DD YYYY	DK = 98 DAY(S) ..... 1 WEEK(S) ..... 2 MONTH(S) 3	DK = 98 PER DAY ..... 1 PER WEEK ..... 2 PER MONTH ..... 3 PER YEAR 4
B.	_____ DK ..... ❑	MM DD Y'YYY	MM DD YYYY	DK = 98 DAY(S) ..... 1 WEEK(S) ..... 2 MONTH(S) 3	DK = 98 PER DAY ..... 1 PER WEEK ..... 2 PER MONTH ..... 3 PER YEAR 4
C.	_____ DK ..... ❑	MM DD Y'YYY	MM DD YYYY	DK = 98 DAY(S) ..... 1 WEEK(S) ..... 2 MONTH(S) 3	DK = 98 PER DAY ..... 1 PER WEEK ..... 2 PER MONTH ..... 3 PER YEAR 4

DIETARY ASSESSMENT-INTRODUCTION

Next I will read a list of food items, and for each one I would like to know how often you ate that food on average during the year before you became pregnant with (NOIB). You may use the list of Food Frequency Responses that was sent to you in the mail to help you answer these questions. You do not have to remember exactly what you ate, we are only trying to determine what your usual diet was like before you were pregnant. For seasonal foods, such as fruits and vegetables, you can average over the six months prior to pregnancy. For foods that you ate less than once a month, you can report as never or none.

D18. How often, on average, did you use (READ LIST)?

		0 NEVER OR < 1 PER MONTH	1M 1 PER MONTH	2M 2 PER MONTH	3M 3 PER MONTH	1W 1 PER WEEK	2W 2 PER WEEK	3W 3 PER WEEK	4W 4 PER WEEK	5W 5 PER WEEK	6W 6 PER WEEK	1D 1 PER DAY	2D 2 PER DAY	3D 3 PER DAY	4D 4 PER DAY	5D 5 PER DAY	6D 6+ PER DAY	97 RF	98 DK
a.	Skim or lowfat milk (8 oz glass)	0	1M	2M	3M	1W	2W	3W	4W	5W	6W	1D	2D	3D	4D	5D	6D	97	98
b.	Whole milk (8 oz glass)	0	1M	2M	3M	1W	2W	3W	4W	5W	6W	1D	2D	3D	4D	5D	6D	97	98
c.	Yogurt (1 cup)	0	1M	2M	3M	1W	2W	3W	4W	5W	6W	1D	2D	3D	4D	5D	6D	97	98
d.	Ice cream (1/2 cup)	0	1M	2M	3M	1W	2W	3W	4W	5W	6W	1D	2D	3D	4D	5D	6D	97	98
e.	Cottage or Ricotta cheese	0	1M	2M	3M	1W	2W	3W	4W	5W	6W	1D	2D	3D	4D	5D	6D	97	98
f.	Other cheese e.g., American, cheddar, etc., plain or part of a dish (1 slice or 1 oz serving)	0	1M	2M	3M	1W	2W	3W	4W	5W	6W	1D	2D	3D	4D	5D	6D	97	98
g.	Margarine (pat), added to food or bread; exclude use in cooking	0	1M	2M	3M	1W	2W	3W	4W	5W	6W	1D	2D	3D	4D	5D	6D	97	98
h.	Butter (pat), added to food or bread; exclude use in cooking	0	1M	2M	3M	1W	2W	3W	4W	5W	6W	1D	2D	3D	4D	5D	6D	97	98
i.	Fresh apples or pears (1)	0	1M	2M	3M	1W	2W	3W	4W	5W	6W	1D	2D	3D	4D	5D	6D	97	98
j.	Oranges (1)	0	1M	2M	3M	1W	2W	3W	4W	5W	6W	1D	2D	3D	4D	5D	6D	97	98
k.	Orange juice (small glass)	0	1M	2M	3M	1W	2W	3W	4W	5W	6W	1D	2D	3D	4D	5D	6D	97	98
l.	Peaches, apricots, plums, or nectarines (1 fresh or ½ cup canned)	0	1M	2M	3M	1W	2W	3W	4W	5W	6W	1D	2D	3D	4D	5D	6D	97	98
m.	Bananas (1)	0	1M	2M	3M	1W	2W	3W	4W	5W	6W	1D	2D	3D	4D	5D	6D	97	98
n.	Cantaloupe (1/4 melon)	0	1M	2M	3M	1W	2W	3W	4W	5W	6W	1D	2D	3D	4D	5D	6D	97	98
o.	Avocado (1) or guacamole (1 cup)	0	1M	2M	3M	1W	2W	3W	4W	5W	6W	1D	2D	3D	4D	5D	6D	97	98
p.	Other fruits fresh, frozen, or canned (1/2 cup)	0	1M	2M	3M	1W	2W	3W	4W	5W	6W	1D	2D	3D	4D	5D	6D	97	98
q.	Tomatoes (1) or tomato juice (small glass)	0	1M	2M	3M	1W	2W	3W	4W	5W	6W	1D	2D	3D	4D	5D	6D	97	98
r.	String beans (1/2 cup)	0	1M	2M	3M	1W	2W	3W	4W	5W	6W	1D	2D	3D	4D	5D	6D	97	98
s.	Broccoli (1/2 cup)	0	1M	2M	3M	1W	2W	3W	4W	5W	6W	1D	2D	3D	4D	5D	6D	97	98
t.	Cabbage, cauliflower, or brussel sprouts (1/2 cup	0	1M	2M	3M	1W	2W	3W	4W	5W	6W	1D	2D	3D	4D	5D	6D	97	98

D18. How often, on average, did you use (READ LIST)?

		0 NEVER OR < 1 PER MONTH	1M 1 PER MONTH	2M 2 PER MONTH	3M 3 PER MONTH	1W 1 PER WEEK	2W 2 PER WEEK	3W 3 PER WEEK	4W 4 PER WEEK	5W 5 PER WEEK	6W 6 PER WEEK	1D 1 PER DAY	2D 2 PER DAY	3D 3 PER DAY	4D 4 PER DAY	5D 5 PER DAY	6D 6+ PER DAY	97 RF	98 DK
u.	Carrots, raw (1/2 carrot or 2-4 sticks)	0	1M	2M	3M	1W	2W	3W	4W	5W	6W	1D	2D	3D	4D	5D	6D	97	98
v.	Carrots, cooked (1/2 cup)	0	1M	2M	3M	1W	2W	3W	4W	5W	6W	1D	2D	3D	4D	5D	6D	97	98
w.	Corn (1 ear or ½ cup frozen, canned)	0	1M	2M	3M	1W	2W	3W	4W	5W	6W	1D	2D	3D	4D	5D	6D	97	98
x.	Peas or lima beans (1/2 cup frozen, canned)	0	1M	2M	3M	1W	2W	3W	4W	5W	6W	1D	2D	3D	4D	5D	6D	97	98
y.	Yams or sweet potatoes (1/2 cup)	0	1M	2M	3M	1W	2W	3W	4W	5W	6W	1D	2D	3D	4D	5D	6D	97	98
z.	Spinach or collard greens, cooked (1/2 cup)	0	1M	2M	3M	1W	2W	3W	4W	5W	6W	1D	2D	3D	4D	5D	6D	97	98
aa.	Refried beans (1 cup)	0	1M	2M	3M	1W	2W	3W	4W	5W	6W	1D	2D	3D	4D	5D	6D	97	98
bb.	Beans or lentils, baked or dried (1/2 cup)	0	1M	2M	3M	1W	2W	3W	4W	5W	6W	1D	2D	3D	4D	5D	6D	97	98
cc.	Yellow (winter) squash (1/2 cup)	0	1M	2M	3M	1W	2W	3W	4W	5W	6W	1D	2D	3D	4D	5D	6D	97	98
dd.	Raw Chile peppers, Jalopeño (1)	0	1M	2M	3M	1W	2W	3W	4W	5W	6W	1D	2D	3D	4D	5D	6D	97	98
ee.	Salsa (1 cup)	0	1M	2M	3M	1W	2W	3W	4W	5W	6W	1D	2D	3D	4D	5D	6D	97	98
ff.	Eggs (1)	0	1M	2M	3M	1W	2W	3W	4W	5W	6W	1D	2D	3D	4D	5D	6D	97	98
gg.	Chicken or Turkey (4-6 oz)	0	1M	2M	3M	1W	2W	3W	4W	5W	6W	1D	2D	3D	4D	5D	6D	97	98
hh.	Bacon (2 slices)	0	1M	2M	3M	1W	2W	3W	4W	5W	6W	1D	2D	3D	4D	5D	6D	97	98
ii.	Hot dogs (1)	0	1M	2M	3M	1W	2W	3W	4W	5W	6W	1D	2D	3D	4D	5D	6D	97	98
jj.	Processed meats, e.g. sausage, salami, bologna, chorizo, etc. (piece or slice)	0	1M	2M	3M	1W	2W	3W	4W	5W	6W	1D	2D	3D	4D	5D	6D	97	98
kk.	Liver (3-4 oz)	0	1M	2M	3M	1W	2W	3W	4W	5W	6W	1D	2D	3D	4D	5D	6D	97	98
ll.	Chicken livers (1 oz)	0	1M	2M	3M	1W	2W	3W	4W	5W	6W	1D	2D	3D	4D	5D	6D	97	98
mm.	Organ meats Barbacoa, Menudo, sweetbreads, tongue, intestines (3-4 oz)	0	1M	2M	3M	1W	2W	3W	4W	5W	6W	1D	2D	3D	4D	5D	6D	97	98
nn.	Hamburger (1 patty)	0	1M	2M	3M	1W	2W	3W	4W	5W	6W	1D	2D	3D	4D	5D	6D	97	98
oo.	Beef, pork, lamb or cabrito as as a sandwich or mixed dish, e.g. stew, casserole, lasagna, etc.	0	1M	2M	3M	1W	2W	3W	4W	5W	6W	1D	2D	3D	4D	5D	6D	97	98
pp.	Beef, pork, lamb or cabrito as a main dish, e.g. Steak, roast, ham, etc. (4-6 oz)	0	1M	2M	3M	1W	2W	3W	4W	5W	6W	1D	2D	3D	4D	5D	6D	97	98

D18. How often, on average, did you use (READ LIST)?

	0 NEVER OR < 1 PER MONTH	1M 1 PER MONTH	2M 2 PER MONTH	3M 3 PER MONTH	1W 1 PER WEEK	2W 2 PER WEEK	3W 3 PER WEEK	4W 4 PER WEEK	5W 5 PER WEEK	6W 6 PER WEEK	1D 1 PER DAY	2D 2 PER DAY	3D 3 PER DAY	4D 4 PER DAY	5D 5 PER DAY	6D 6+ PER DAY	97	98
qq. Fish (3-5 oz)	0	1M	2M	3M	1W	2W	3W	4W	5W	6W	1D	2D	3D	4D	5D	6D	97	98
rr. Chocolate (1 oz)	0	1M	2M	3M	1W	2W	3W	4W	5W	6W	1D	2D	3D	4D	5D	6D	97	98
ss. Candy without chocolate (1 oz)	0	1M	2M	3M	1W	2W	3W	4W	5W	6W	1D	2D	3D	4D	5D	6D	97	98
tt. Pie (slice)	0	1M	2M	3M	1W	2W	3W	4W	5W	6W	1D	2D	3D	4D	5D	6D	97	98
uu. Cake (slice)	0	1M	2M	3M	1W	2W	3W	4W	5W	6W	1D	2D	3D	4D	5D	6D		98
vv. Cookies (1)	0	1M	2M	3M	1W	2W	3W	4W	5W	6W	1D	2D	3D	4D	5D	6D	97	98
ww. White bread (slice), including pita bread	0	1M	2M	3M	1W	2W	3W	4W	5W	6W	1D	2D	3D	4D	5D	6D	97	98
xx. Dark bread (slice) including wheat pita bread	0	1M	2M	3M	1W	2W	3W	4W	5W	6W	1D	2D	3D	4D	5D	6D	97	98
yy. French fried potatoes (4 oz)	0	1M	2M	3M	1W	2W	3W	4W	5W	6W	1D	2D	3D	4D	5D	6D	97	98
zz. Potatoes baked, boiled (1) or mashed (1 cup)	0	1M	2M	3M	1W	2W	3W	4W	5W	6W	1D	2D	3D	4D	5D	6D	97	98
aaa. Rice or pasta e.g. Spanish rice, spaghetti, noodles, etc. (1 cup)	0	1M	2M	3M	1W	2W	3W	4W	5W	6W	1D	2D	3D	4D	5D	6D	97	98
bbb. Tortilla (1)	0	1M	2M	3M	1W	2W	3W	4W	5W	6W	1D	2D	3D	4D	5D	6D	97	98
ccc. Potato chips or corn Chips (small bag or 1 oz)	0	1M	2M	3M	1W	2W	3W	4W	5W	6W	1D	2D	3D	4D	5D	6D	97	98
ddd. Nuts (small packet or 1 oz)	0	1M	2M	3M	1W	2W	3W	4W	5W	6W	1D	2D	3D	4D	5D	6D	97	98
eee. Peanut butter (1tbs)	0	1M	2M	3M	1W	2W	3W	4W	5W	6W	1D	2D	3D	4D	5D	6D	97	98
fff. Oil and vinegar Dressing e.g., Italian(1 tbs)	0	1M	2M	3M	1W	2W	3W	4W	5W	6W	1D	2D	3D	4D	5D	6D	97	98

MEDICATION

B105. We are interested in some medicines that you may have taken between (-3) and (DOIB)/(DOPT). These would include prescription and nonprescription medicines. Some of these medicines we may have already discussed.

Between (-3) and (DOIB)/(DOIPT), did you take any of the following medications? READ CHOICES.
(IF NO OR DK TO ALL, SKIP TO B110).

		YES	NO	DK
a.	Tylenol, or	1	2	8
b.	Darril, or	1	2	8
c.	Acetaminophen	1	2	8
d.	Advil, or	1	2	8
e.	Motrin, or	1	2	8
f.	Nuprin, or	1	2	8
g.	Ibuprofin	1	2	8
h.	Wellbutrin, or	1	2	8
i.	Zyban	1	2	8
j.	Dilantin, or	1	2	8
k.	Phenytoin	1	2	8
l.	Pondimin, or	1	2	8
m.	Redux	1	2	8
n.	Cytotec, or	1	2	8
o.	Misoprostol	1	2	8
p.	Aspirin	1	2	8
q.	Aleve	1	2	8
r.	Amoxicillin	1	2	8
s.	Augmentin	1	2	8
t.	Bactrim	1	2	8
u.	Septra	1	2	8
v.	Cipro	1	2	8
w.	Prozac	1	2	8
x.	Zoloft	1	2	8
y.	Paxil	1	2	8
z.	Nicotine Patch NOS	1	2	8
aa.	Nicotine Gum NOS	1	2	8
bb.	Valproic Acid	1	2	8
cc.	Dexatrim	1	2	8
dd.	Methotrexate	1	2	8
ee.	Between (-3) and DOIB did you take any medications, remedies, or treatments that we haven't already talked about? For example, medications for asthma, allergies, STDs, or HIV/AIDS? SPECIFY	1	2	8

1. _____

2. _____

TYPE OF MEDICINE	B106. Between (-3) and (DOIB), when did you start using (MEDICINE)? IF A MEDICINE ON THE LIST WAS ALREADY REPORTED, ASK: at what other times besides (USE DATE TO DATE) did you use (MEDICINE)?	B107. When did you stop using (MEDICINE)? OR: IF GET EXACT DATES IN B106 AND B107, SKIP B108.	B108. How long did you take it? DURATION	B109. How often did you use (MEDICATION)? FREQUENCY
1. _____	MM DD YYYY	MM DD YYYY	DK = 98 DAY(S) ..... 1 WEEK(S) ..... 2 MONTH(S) 3	DK = 98 PER DAY ..... 1 PER WEEK ..... 2 PER MONTH ..... 3 PER YEAR 4
2. _____	MM DD YYYY	MM DD YYYY	DK = 98 DAY(S) ..... 1 WEEK(S) ..... 2 MONTH(S) 3	DK = 98 PER DAY ..... 1 PER WEEK ..... 2 PER MONTH ..... 3 PER YEAR 4
3. _____	MM DD YYYY	MM DD YYYY	DK = 98 DAY(S) ..... 1 WEEK(S) ..... 2 MONTH(S) 3	DK = 98 PER DAY ..... 1 PER WEEK ..... 2 PER MONTH ..... 3 PER YEAR 4
4. _____	MM DD YYYY	MM DD YYYY	DK = 98 DAY(S) ..... 1 WEEK(S) ..... 2 MONTH(S) 3	DK = 98 PER DAY ..... 1 PER WEEK ..... 2 PER MONTH ..... 3 PER YEAR 4
5. _____	MM DD YYYY	MM DD YYYY	DK = 98 DAY(S) ..... 1 WEEK(S) ..... 2 MONTH(S) 3	DK = 98 PER DAY ..... 1 PER WEEK ..... 2 PER MONTH ..... 3 PER YEAR 4
6. _____	MM DD YYYY	MM DD YYYY	DK = 98 DAY(S) ..... 1 WEEK(S) ..... 2 MONTH(S) 3	DK = 98 PER DAY ..... 1 PER WEEK ..... 2 PER MONTH ..... 3 PER YEAR 4

TYPE OF MEDICINE	B106. Between (-3) and (DOIB), when did you start using (MEDICINE)? IF A MEDICINE ON THE LIST WAS ALREADY REPORTED, ASK: at what other times besides (USE DATE TO DATE) did you use (MEDICINE)?	B107. When did you stop using (MEDICINE)? OR: IF GET EXACT DATES IN B106 AND B107, SKIP B108.	B108. How long did you take it? DURATION	B109. How often did you use (MEDICATION)? FREQUENCY
7. _____	MM DD YYYY	MM DD YYYY	DK = 98 DAY(S) ..... 1 WEEK(S) ..... 2 MONTH(S) 3	DK = 98 PER DAY ..... 1 PER WEEK ..... 2 PER MONTH ..... 3 PER YEAR 4
8. _____	MM DD YYYY	MM DD YYYY	DK = 98 DAY(S) ..... 1 WEEK(S) ..... 2 MONTH(S) 3	DK = 98 PER DAY ..... 1 PER WEEK ..... 2 PER MONTH ..... 3 PER YEAR 4
9. _____	MM DD YYYY	MM DD YYYY	DK = 98 DAY(S) ..... 1 WEEK(S) ..... 2 MONTH(S) 3	DK = 98 PER DAY ..... 1 PER WEEK ..... 2 PER MONTH ..... 3 PER YEAR 4

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