5-2006

Smoking, Anemia, and Risk of Oral Clefts in Utah

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SMOKING, ANEMIA, AND RISK OF ORAL CLEFTS IN UTAH

by

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A thesis submitted in partial fulfillment
of the requirements for the degree of

MASTER OF SCIENCE

in

Nutrition and Food Sciences

Approved:

UTAH STATE UNIVERSITY
Logan, Utah

2006

ABSTRACT

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Utah State University, 2006

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Cigarette smoke contains sufficient carbon monoxide to induce maternal and fetal hypoxia. Hypoxia is a known teratogen, and consequently maternal smoking has been the focus of many studies on adverse birth outcomes, including cleft lip and palate. Current literature of epidemiological studies on smoking and clefts suggests a modest but statistically significant increase in risk of clefting associated with maternal smoking. A biological condition that may also contribute to hypoxia is anemia. Data from the Utah Child and Family Health Study was used to assess the effects of hypoxia-inducing conditions, maternal smoking, anemia, and their interaction, on the risk of having a child with a cleft. Smoking during the first trimester and hemoglobin levels of less than 12.0 g/dL were the defined risk exposures and logistic regression modeling was used to test the hypotheses. Smoking during the first trimester of pregnancy was associated with increased risk of clefting; however, anemia did not appear to be associated with clefting

in this population, and there was also no apparent additional increase in risk for those mothers who both smoked and were anemic. Prospects for future studies include using populations that have higher rates of anemia and smoking to gain more statistical power, and using more sensitive measures of red blood cell health other than hemoglobin. From a public health perspective, evidence from this study would suggest that efforts to promote smoking cessation in women of child-bearing years is of considerable importance.

(196 pages)

ACKNOWLEDGMENTS

Words cannot express how grateful I am to the many people who helped bring this manuscript to fruition. I want to thank my committee members for their feedback, and the staff members at the Center for Epidemiological Studies for their tireless efforts in collecting and managing the data for this study. I am especially grateful for the love and support my family members and friends have offered me, particularly over the years it took me to complete this work.

The Utah Child and Family Health Study was funded by a grant from the U.S. National Institutes of Health (R01-HD 39061), with Ronald Munger as the principal investigator.

Melinda M. Moss

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CHAPTER 1

INTRODUCTION AND BACKGROUND

Abstract

Identifying risk factors associated with cleft lip and palate abnormalities may help reduce the incidence of these conditions, thus eliminating a tremendous medical burden for many families worldwide. Data from the Utah Child and Family Health study will be used to determine the risk of clefting associated with smoking and anemia, two conditions that contribute to hypoxia, a known teratogen. General data collection procedures, analysis techniques and hypothesis are set forth herein.

Introduction

Despite an increased understanding of disease etiology in recent years, cleft lip and cleft palate abnormalities continue to be a burden on society. The World Health Organization estimates that 1 out of every 500 to 700 live births involves a cleft of the lip or palate, making cleft defects one of the most prevalent birth defects, not only in the U.S., but also across the globe (1). Based on both environmental and genetic factors, cleft palate rates differ across geographic regions and within ethnic groups (2, 3). Reported rates range from a low of 0.18 to 1.67 per 1,000 live births among blacks, to a high of 1.45 to 4.04 per 1,000 live births among Chinese populations (2). Unfortunately, it is developing countries with populations of low socioeconomic status that seem to suffer most from increased risk of cleft lip and cleft palate (4). For example, in the

Philippines where the GDP is less than 5,000 U.S. dollars per year, the rate of cleft lip and cleft palate is 1.94 per 1,000 live births (4, 5).

Children born with cleft lip and palate have increased difficulty in feeding, speaking, and social development. One Vietnamese mother laments, "It is impossible for my baby to eat. She only drinks milk and porridge and vomits frequently. Social norms dictate that many of these children enter adulthood without having experienced many of the societal benefits that many of us take for granted: education, marriage and friendship for example. In some cultures, children with cleft lip and palate are thought to be "cursed" (5). Economic studies estimated that in 1992 cleft palate birth defects cost the U.S. over 697 million dollars (6). Part of that estimate is related to direct medical costs, while a significant portion is loss due to decreased productivity, special education and other considerations (6). In places where access to medical technology is limited and funds for medical care are scarce, at any cost, there is little hope that these birth defects can be corrected. While the cost of corrective surgery in these countries is comparatively low, as little as $ 750 per patient, many people still cannot afford medical treatment (5).

Background

Morphology

Palate formation in the human fetus begins around day 45 when mesenchymal cells migrate from the neural crest to the oral cavity (7). Rapid cell proliferation occurs and two shelves are formed at the surface of the maxillary processes (7). The palate shelves are composed of mesechymal cells surrounded by a matrix of extracellular gycosaminoglycans and collagen and encompassed by two to three layers of epithelial

cells (7, 8). Due in part to the relatively fast growth of the palate shelves in relation to the growth rate of the headspace, as well as the hydration and electrostatic repulsion of the gycosaminoglycans, as the shelves form they move from a vertical position on either side of the tongue to a horizontal position above the tongue (7). Once the shelves come in contact above the tongue, surface epithelial cells from the opposing shelves adhere to each other via surface carbohydrates and proteins (7, 8). The epithelial cells then undergo an epithelial to mesenchyme transition and mesenchymal continuity is achieved across the seam (7, 9).

Ferguson et al. categorizes the mechanisms that may be responsible for incorrect palate formation into five basic groups: 1) inhibition of cell division or migration 2) failure of palate shelf to elevate at the correct time 3) excessive head width 4) failure of shelf fusion and 5) post fusion rupture (7). Gene expression, hormone levels and environmental factors, such as toxins, can affect one or more of these processes causing the formation of a cleft (7, 10). The most common form of orofacial clefts is cleft lip with or without cleft palate CL(P), and is categorized by a cleft in the lip with or without a cleft in the primary or hard palate. CL(P) is further classified by the site of the lip cleft: left, right, or midline unilateral lip clefts are possible, as well as bilateral clefts.

Several syndromes can be associated with clefting, including those associated with chromosomal syndromes, teratogens and other uncategorized syndromes (11, 12). In studying cleft deformities, a distinction needs to be made between the different types in order to account for their different pathologies. The difference between CL(P) and cleft of the soft palate alone, CP, needs particular consideration (12). Epidemiological data would suggest a different mechanism for clefts of the soft, or secondary palate alone

and accordingly, statistical analysis are typically performed looking at CL(P) and CP separately (13).

Genetics

Recently a great deal of research has focused on genetic factors and their contribution to palate malformation. Loci scanning projects in humans have found some 30 genes that appear to be associated with palate formation (14). Genes for extracellular matrix proteins, transcription factors and cell signaling molecules have been linked to cleft palate phenotypes (14). While these studies show an increased risk for cleft palate with certain genetic variation, many studies also focus on how genes and environment interact to induce clefting (10, 12, 15).

Environment

Migrant studies are an excellent example of how the environment can interact with, or over-ride genetics to induce clefting. For example, hospitals in Manila report different rates of clefting dependant on the socioeconomic status of the patient population (16). Other studies show that Philippino people who have immigrated to the United States, either to California or Hawaii, have cleft rates similar to other people in those areas and not their home countries (14, 17, 18). These patterns would suggest that altering environmental factors could help reduce the incidence of cleft lip and cleft palate birth defects.

Animal Models

Animal models have shown that cleft palate formation can be induced in several species by altering various environmental factors (19-23). Folate antagonists, corticosteroid, and anticonvulsive drugs are among the known teratogens that cause oral clefts in animals (24, 25). Some plant based poisons are also suspect (23). Several nutritional deficiencies or toxicities have likewise been shown to induce clefts in animal models, including vitamin A, riboflavin, folic acid, pantothenic acid, vitamins B-12 and B-6 and zinc (24, 26-28).

Hypoxia

Blood oxygen levels are one particular environmental factor of interest that has been associated with cleft lip and palate in mice (21). Oxygen is essential in virtually all metabolic processes and hypoxia has been shown to be a powerful, nonspecific teratogen (21, 29). Induced hypoxia in mice studies have conclusively shown that reduced levels of blood oxygen can increase the risk and severity of cleft palate (20, 21). A study done by Millicovsky and published in the Proceeding of the National Academy of Science, goes even further to show a dose response relationship between hypoxia and incidence of cleft lip in mice (19). While there is not a complete understanding of how oxygen, or a lack thereof affects palate formation, one specific mechanism of interest is the inhibition of the enzyme lysyl oxidase. Lysyl oxidase is responsible for oxidizing lysine residues in collagen thus forming stabilized crosslinks (30). While teratogens can inhibit this enzyme, basic bioenergetics also dictate that low levels of O_2 will retard enzyme function. Regardless of the specific mechanism involved, oxygen is essential for proper

fetal growth and development and two significant environmental factors that can alter the availability of oxygen are maternal smoking and anemia.

Smoking

The World Health Organization lists smoking as one of the major risks to public health (31). Smoking has been associated with a myriad of health concerns such as coronary heart failure, cancer, emphysema, stroke, etc (31). Due in large part to targeted advertising by tobacco companies, tobacco use among developing nations has been steadily increasing, and while developed nations show a decrease in smoking rates amongst males, smoking rates for females continue to rise (32, 33). According to the CDC, Utah has one of the lowest reported smoking rates in the nation, with only approximately 13% of women smoking (34), which is good news in terms of public health as smoking may account for a higher incidence of certain diseases including cleft lip and palate, especially in areas where tobacco use is wide spread and on the increase.

In fact, an ever-increasing body of research has implicated cigarette smoke as a risk factor for spontaneous abortions and certain birth complications including orofacial clefts (35). While the epidemiological data are not conclusive, a significant number of studies show that maternal smoking can influence cleft palate rates (15, 36-48). There have been two meta-analysis published in recent years and both show an overall increased risk of cleft palate formation in association with maternal smoking. Wyszynski et al. (49) looked at 9 different studies in 1997 and Little et al. (50) looked at 15 studies in 2004. Both studies show an over-all increase in risk for clefts in conjunction with smoking. For those studies that reported the number of cigarettes smoked, Little et al.

(50) indicates a marginal, though not significant, dose-response relationship. Studies that failed to show such an association had very little statistical power due to small sample sizes, which may explain an apparent lack of association. It is also unclear as of yet, exactly what components of tobacco smoke contribute to the physiological effects of smoking on the fetus. Certainly, more work needs to be done in order to verify or refute an association between maternal smoking and cleft palate rates, and identify the many pathways by which tobacco smoke could be a contributor to palate malformation.

Anemia

Anemia is defined by the World Health Organization as, "a condition in which the hemoglobin content of the blood is lower than normal as a result of a deficiency of one or more essential nutrients regardless of the cause of such deficiency" (51). As this statement indicates, a number of different nutritional factors can contribute to anemia, including vitamin A, vitamin B 12, riboflavin, zinc and iron (52-55). In fact, iron deficiency anemia is the most prevalent source of anemia worldwide (55). UNICEF estimates that globally 4-5 billion people have some level of iron deficiency and two billion are anemic (56). Iron deficiency anemia rates in developed countries are lower than those in developing nations, but pregnant women are more at risk than the general population irrespective of geography.

Anemia can be diagnosed based on the World Health Organization standards of hemoglobin levels greater than or equal to 11g/dL (57). Hemoglobin levels correspond with long-term dietary iron intake and are usually a good indicator of late stage anemia (29). However, some concern has been expressed over the universal application of the

WHO standard of diagnosis because of inherent differences in hemoglobin levels in various populations (57). For example, one study that focused on anemia in pregnant Philippina women suggests that a standard of 10.4 g/dL for determining anemia status would be more appropriate for that population (57). Certain factors can also artificially elevate hemoglobin levels without actually increasing oxygen availability in the body. This may inhibit proper screening of anemia using hemoglobin values. Smoking is a particular concern because smoking can raise hemoglobin levels significantly without any reduction in tissue hypoxia (58). The increase is in fact just a reflection of increased levels of hemoglobin bound with carbon monoxide.

The Utah Child and Family Health Study

Study Objectives

Anemia and smoking have been independently suspected as risk factors for adverse birth outcomes, however, the combined effect of smoking and anemia on cleft rates have not been thoroughly explored. Based on the animal models for hypoxia and the propensity of anemia and smoking to decrease oxygen availability in the body, it seems likely that there is an interaction between the two that may be responsible for an increased risk of cleft lip and cleft palate birth defects. Consequently, the objectives of this study are to:

1. Ascertain the prevalence of anemia and smoking in the Utah Child and Family Health Study, and the associated odds ratio for each in relation to cleft risk, and,

2. Determine the combined effect of smoking and anemia on the risk (OR) of having a child with CL(P) independent of other confounding factors including the mother's age, weight, height, alcohol use and parity.

Hypothesis

Women who smoke and are anemic are subject to a combined physiological reduction in oxygen transport that can synergistically increase the risk of cleft lip and palate in their offspring.

Study Design

The low incidence of cleft lip and palate dictates that the design for this study must be of the case-control nature. Data from the Utah Family and Child Health Study used to test this hypothesis included over 800 cases with both interview and lab data, and were collected thanks to funding from the NIH. All of the study methods and materials for this particular study have been reviewed and approved by the Utah State University IRB.

Data Collection

All study subjects, including both cases and controls, were identified and informed of the purposes and procedures involved with the study through the Utah Department of Health (UDOH), which monitors the Utah Birth Defect Network. UDOH officials issued letters of invitation to qualified families to participate in the study, and then actively pursued a response from each household if necessary. Once written consents were obtained, participant information was released to researchers at Utah State

University. Trained staff at the center for epidemiological studies at USU were responsible for providing participating mothers with information regarding the study, including providing materials to help the mothers prepare for the interviews, and conducting the phone interviews. These interviews lasted for approximately one hour and consisted of questions regarding demographic, anthropometric, diet and other lifestyle characteristics of the mothers, as well as specific information about the site and classification of the cleft for case children. Case classification was verified by official department of health records.

A 20 ml blood sample was later collected from each mother that consented to donate a biological sample. Hemoglobin levels were determined on site using the Hemocue System, produced by the HemoCue corporation in Sweden. Blood analyzed with the HemoCue machine must be used within 24 hours and use EDTA as an anticoagulant. It is recommended that samples be mixed for at least ten minutes and be at room temperature before using. Accordingly, during the course of data collection for this study the exact time of blood draw was recorded as well as the centrifuge time in order to ensure that no spurious results were generated due to differences in processing time.

The chemical basis on which the HemoCue operates is as follows: A small drop of blood is placed in a HemoCue cuvette and inserted into the machine. Inside the machine, sodium deoxycholate hemolyzes the erythrocytes and hemoglobin is released. Sodium nitrite then converts hemoglobin to methemogobin, which reacts with sodium azide to produce axide methemoglobin. Azide methemoglobin can be detected photometrically at 570nm and 880nm. The HemoCue has an optimal range of 0.0-23.5 g/dL and expected values from 11.0-16.0 g/dL. The HemoCue has its own control

cuvette that should be used daily, and the acceptable deviance from the control cuvette value is $\pm$ 0.3 g/dL.

Data Management

Data management for this project was multifaceted. The consent forms and questionnaire data were obtained prior to collection of biological samples. All forms were subjected to quality assurance checks, including coding for variables, followed by data entry. A double entry method was used as a measure of data entry quality control. All data was stored as Microsoft Access files.

Statistical Analysis

SPSS 12.0 statistical analysis software will be used for exploratory analysis, followed by SAS 9.1 software for generation of logistic regression models. Hemoglobin levels and smoking status will be looked at as individual risk factors and then associations will be explored. Separate analysis will be conducted for cleft palate alone CP and cleft lip with or without cleft palate CL(P). Confounding factors that need to be controlled for include: mother's age, height and weight, parity, and alcohol usage.

Summary

In order to reduce the incidence of cleft lip and cleft palate birth defects there needs to be a greater understanding of the risk factors involved in the disease etiology. Based on animal models, there is strong evidence that hypoxia could be a major risk factor. Because smoking and anemia both reduce oxygen availability in the body, it needs to be determined what effect, if any, those risk factors have on cleft rates. While

there is already a large push for pregnant women to stop smoking, further evidence of adverse pregnancy outcomes may help to boost anti-tobacco efforts. A greater understanding of maternal nutrition and its importance in birth outcomes is also desirable.

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CHAPTER 2

SMOKING, ANEMIA, AND ORAL CLEFTS: A REVIEW

Abstract

Oral clefts engender economic, health and social burdens for a substantial number of children and their families worldwide. Genetic and environmental risk factors are thought to play a role in cleft malformation, a process that is biologically complex and not completely understood. Maternal smoking is one of the most widely studied exposures associated with clefting and despite considerable amounts of data, there is not a consensus among researchers as to how much of a risk maternal smoking truly imposes. Animal models target carbonmonoxide-induced hypoxia as the mechanism by which cigarette smoke might alter fetal development of the palate. Maternal anemia is also a widely researched as a risk factor for poor birth outcomes, although not necessarily clefts. Since both conditions, smoking and anemia, have the capacity to exacerbate hypoxia in the developing fetus it is reasonable to consider that there may be an association between the two conditions and oral clefts. Current health recommendations are for anyone, but particularly women of childbearing age, to quite smoking. Several programs in this country and worldwide are designed to increase the overall nutritional health of women and children. Hopefully, progress in these endeavors will have an impact on the rate of clefting globally. Further research regarding smoking and nutrition and their relationship to palate formation will help resolve some of the many questions left unanswered by the current literature.

Introduction

Despite an increased understanding of disease etiology in recent years, cleft lip and cleft palate abnormalities continue to be a burden on society. The worldwide incidence of clefting is 1 out of every 500 to 700 live births, making it one of the most prevalent forms of birth defects not only in the US, but also across the globe (1, 2). Based on both environmental and genetic factors, cleft palate rates differ across geographic regions and within ethnic groups (3, 4). Reported rates range from a low of 0.18 to 1.67 per 1,000 live births among blacks, to a high of 1.45 to 4.04 per 1,000 live births among Chinese populations (3) . In the US, it is estimated that there is a child born with a cleft lip or palate every two minutes (5).

The physical and emotional burden of cleft palate birth defects is immeasurable. Children born with cleft lip and palate have increased difficulty in feeding, speaking and social development. One Vietnamese mother laments, "It is impossible for my baby to eat. She only drinks milk and porridge and vomits frequently (6)." In excess of physical restraints, social norms dictate that many of these children enter adulthood without having experienced many of the societal benefits that many of us take for granted including education, marriage and friendship. One father in Iraq, whose daughter had a severe cleft defect, was told not to let her come back to school because she frightened the other children. In some cultures, children with cleft lip and palate are even thought to be "cursed" (6). In places where access to medical technology is limited and funds for medical care are scarce, there is little hope that these birth defects can be corrected.

While the cost of corrective surgery in these countries is comparatively low, as little as $750 per patient, many people still cannot afford medical treatment (6).

In the United States where more advanced medical options exist, the lifetime cost of treating a cleft can be monumental. Economic studies estimated that in 1992 cleft palate birth defects cost the US over 697 million dollars (7, 8), which is nearly 101,000 US dollars for *each* child born with a cleft (5). Part of that estimate is related to direct medical costs, while a significant portion is loss due to decreased productivity, special education and other considerations (7). Congenital malformations, especially those clearly exposed like cleft lip and palate, are also not exempt from social stigma in the U.S and other developed countries.

For several decades now, researchers have sought to understand the complex developmental processes that contribute to the formation of the human palate in hopes of finding a way to prohibit its malformation and consequently eliminate a measure of human suffering. There is in existence a large body of knowledge to draw from, and yet there is no shortage of questions for which to find answers. Data for the Utah Family and Child Health Study used to test the hypothesis set forth in this manuscript was generated thanks to funding from the NIH and collected by the Center for Epidemiological Studies at Utah State University, with Dr. Ronald Munger as the center director and principal investigator.

Background

Morphology

Palate formation in the human fetus begins around day 45 when mesenchymal cells migrate from the neural crest to the oral cavity (9). Rapid cell proliferation occurs and two shelves are formed at the surface of the maxillary processes (9). The palate shelves are composed of mesenchymal cells surrounded by a matrix of extracellular glycosaminoglycans and collagen and encompassed by two to three layers of epithelial cells (9, 10). Due in part to the relatively fast growth of the palate shelves in relation to the growth rate of the headspace, as well as the hydration and electrostatic repulsion of the glycosaminoglycans, as the shelves form they move from a vertical position on either side of the tongue to a horizontal position above the tongue (9). Once the shelves come in contact above the tongue, surface epithelial cells from the opposing shelves adhere to each other via surface carbohydrates and proteins (9, 10). The epithelial cells then undergo an epithelial-mesenchymal transition and mesenchymal continuity is achieved across the seam (11, 12). Figure 2-1 is a series of time-lapse pictures of in vitro mice palates that clearly illustrate the process of cell adhesion and fusion.

The palate forming process is complicated at best and requires the timely participation of a number of hormones and other signaling molecules; utilizing a myriad of signaling pathways that are not yet completely undisclosed (11). Some of the hormones that are purportedly involved in the adhesion of opposing shelves and the conversion of midline epithelial cells into mesenchyme include epidermal growth factor (EGF) and transforming growth factor beta (TFG-b) (13-16). EGF regulates "cellular

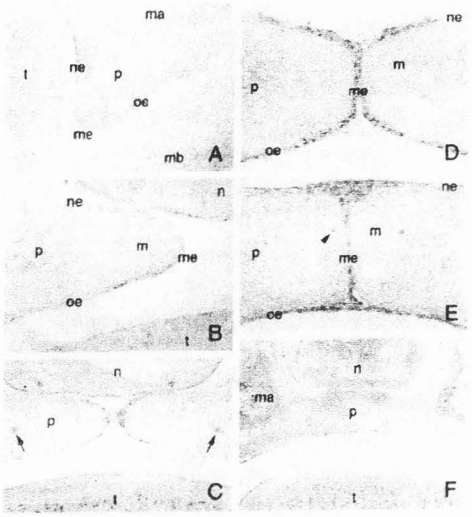

Figure 2-1: Palate formation in mice. (t)tongue, (p)palatal shelf, (n)nasal septum, (me) midline epithelium, (ne)nasal epithelium, (oe) oral epithelium, (m)palatal mesenchyme, (ma)maxilla, (mb)mandible

Source: (14)

proliferation, extracellular matrix synthesis and cellular differentiation" (13). Increased EGF corresponds with the phosphorylation of extracellular receptor kinases 1 and 2 (ERK1/2) indicating a very common method of cell signaling (13). TFG-b3 is crucial to palate formation as shown by cell culture methods with mice, and may work by activating matrix metaloproteinases that play a role in altering the extracellular matrix (16-18). TGF-b also inhibits nuclear DNA synthesis and promulgates the synthesis of gelatinase enzymes, which are key to palate formation (13, 17). Some researchers have demonstrated that the phosphorylation of SMAD proteins via TGF-b is responsible for the inhibition of DNA synthesis in epithelial cells during palate fusion (14).

Improper hormone balance can likewise inhibit proper palate fusion. A few retinoic acid derivatives including trans-retinoic acid and 13-cis-retinoic acid, have been shown to reverse the process of normal palate fusion (19). They block the programmed

cell death of midline epithelials and can, in some combination with EGF, persuade some epithelial cells to even acquire nasal cell characteristics (19). These results have been confirmed in both animal and human organ culture models (20). The compound effects of these and other regulatory molecules are clearly very complex, and as of yet have not been extremely well charted. Fortunately, there is a great deal of active research in this area and more complete information will likely aide in uncovering exactly how these compounds work conjointly to influence embryonic development.

Ferguson et al. (9) categorizes the mechanisms that may be responsible for incorrect palate formation into five basic groups: 1) inhibition of cell division or migration 2) failure of palate shelf to elevate at the correct time 3) excessive head width 4) failure of shelf fusion and 5) post fusion rupture. Gene expression, hormone levels and environmental factors, such as toxins, can affect one or more of these processes causing the formation of a cleft (9, 21).

Types of clefts

The most common form of orofacial clefts is cleft lip with or without cleft palate CL(P), or more specifically, a cleft in the lip with or without a cleft in the primary or hard palate. CL(P) is further classified by the site of the lip cleft: left, right, or midline unilateral lip clefts are possible, as well as bilateral clefts. A graphic graciously provided by the Cleft Lip and Palate Association illustrates these different cleft types. See figure 2-2.

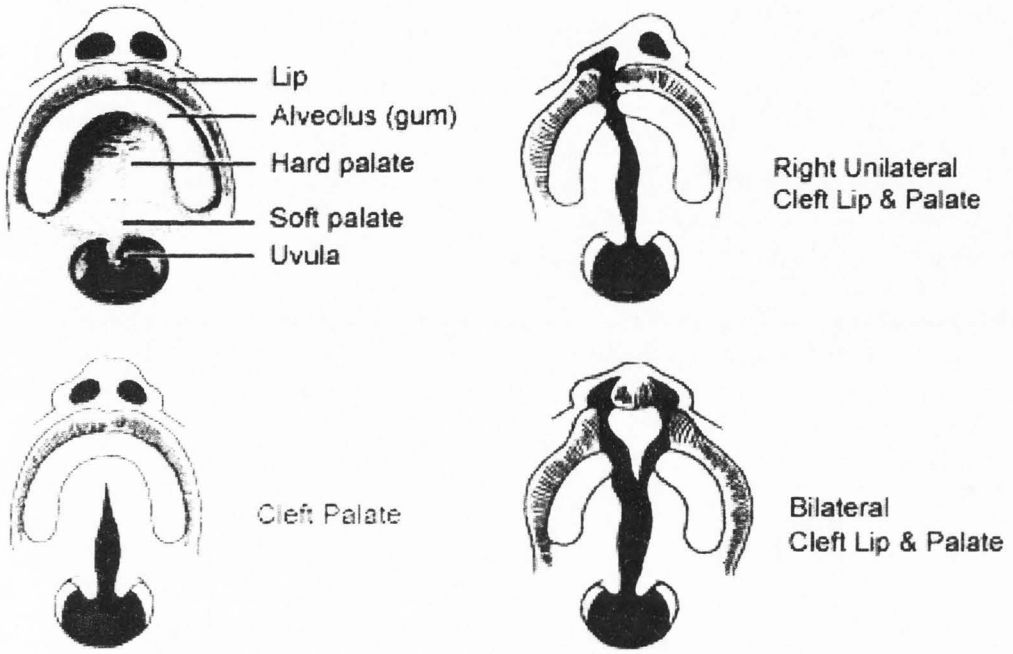

Figure 2-2. Types and positions of oral clefts.

Source: (22)

The morphology and pathology of clefts other than CL(P) are less well understood. Median, oblique, versal, nasoocular and oroocular clefts, while less frequent, are also known to occur and several syndromes can be associated with clefting, including those associated with chromosomal syndromes, teratogens and other uncategorized syndromes, the most well studied of which is probably the Van der Woude syndrome (23, 24). In studying cleft deformities, a distinction needs to be made between the different types in order to account for their different etiologies. The difference between CL(P) and cleft of the soft palate alone, CP, needs particular consideration (24). Epidemiological data would suggest a different mechanism for clefts of the soft, or secondary palate alone and accordingly, statistical analysis are typically performed looking at CL(P) and CP separately (25-27).

Cleft Rates

Descriptive analyses of clefting among the world's populations are not in short supply. In fact, they are so numerous as to become almost daunting. One journal reviewer made the comment, "I must confess that when this manuscript came to my attention for review and comment, my first reaction was, 'Oh no! Not another epidemiological study of clefts!'" (28). Indeed, defining even the vary most basic epidemiological parameters for cleft lip and cleft palate has proven to be easier said than done. Consequently, many studies have been published throughout the literature and reported incidence and prevalence rates for similar populations vary considerably. Tremendous effort has been put forth by researchers in order to compile and make sense of these data, and several others have recently published reviews of clefting rates, including Tolarova, Vanderas and the WHO (3, 29). In fact, it has been estimated that a review article on cleft rates appears about every five years (30). For example, the comprehensive book Cleft Lip and Palate edited by Diego Wysyzyski, contains *12 pages* of charts that summarize cleft rates around the world (31)! Two are presented herein: one to illustrate the considerable length of time that cleft research has been going on, and the other to provide a visual representation of how drastically cleft rates vary across the globe. The first summary, presented as Figure 2-3, is a portion of a table from Tolarova et al. (29) and catalogs over 70 studies starting from 1864. Figure 2-4 (page 28) is a series of maps found in the WHO's 2002 publication on craniofacial anamolies and presents average clefting rates for several nations where data is available. According to the WHO, rates per 10,000 range from 1.35 to 25.31, although it seems that after only 4

years there is a considerable amount of new data that needs to be added to the maps (32-35).

TABLE X. Prevalence of Orofacial Clefts Per 1,000 Newborns

Date	Author	Population	CL	CLP	CL±P	CP	All types
1864	Frobelius[a]	Petersburg—Leningrad	—	—	—	—	0.66
1929	Peron[a]	France—Paris	—	—	—	—	1.06
1934	Grothkopp[a]	Germany—Hamburg	—	—	—	—	1.57
1939	Edgerb[b]	Sweden	—	—	—	—	1.00
1939	Rubaskina[b]	USSR	—	—	—	—	1.00
1940	Conway[b]	USA—New York	—	—	—	—	1.40
1940	Faltin[b]	Finland	—	—	—	—	1.00
1940	Sanvenero Rosseli[b]	Italy	—	—	—	—	1.00
1940	Vaughan[b]	USA—Philadelphia	—	—	—	—	0.80
1942	Fogh-Anderson	Denmark (1934–1941)	—	—	1.10	—	—
1942	Lindswoop[b]	USA-Pennsylvania	—	—	—	—	1.20
1943	Grace	USA—Pennsylvania	—	—	—	—	1.20
1944	Mueller et al.[b]	USA—Wisconsin	—	—	—	—	1.30
1949	Litmanovic[b]	USSR	—	—	—	—	1.20
1949	Oldfield[b]	England	—	—	—	—	1.60
1950	Ivy[b]	USA—Pennsylvania	—	—	—	—	1.30
1951	Wallace et al.[b]	USA—New York	—	—	—	—	0.80
1953	Wallace et al.[b]	USA—New York	—	—	—	—	0.80
1954	Douglas[b]	USA—Tennessee	—	—	—	—	0.60
1955	Haym[b]	West Germany	—	—	—	—	1.00
1955	Lending et al.[b]	USA—New York	—	—	—	—	0.70
1955	Loretz et al.[b]	USA—California	—	—	—	—	1.20
1958	Neel	Japan (1948–1954)	—	—	2.14	0.57	2.71
1960	Ivy[b]	USA—Pennsylvania	—	—	—	—	1.10

Figure 2-3: Reported clefting rates staring in 1864.

Source: (29)

While rate discrepancies may be due to real differences between populations, they are also due, at least in part, to differences in study design. Some of these descriptive studies rely solely on birth certificate data to ascertain cleft cases (30). This may lead to certain types of bias based on incomplete data medical geneticist or similar form of verification. Some studies include fetal deaths and abortions in calculating incidence rates, although this does not seem to be the standard (3, 30). Earlier studies fail to recognize the difference between varied types of clefts or the now known genetic disorders that exhibit cleft phenotypes (30). That being said, it is easy to understand why there is difficulty comparing separate studies, and the subsequently urgent need to update and standardize methodologies. Dr. Oka of the Plastic and Reconstructive Surgery

Figure 3b: Cleft lip with or without cleft palate
Asia, Middle East, Australia & Oceania, Africa

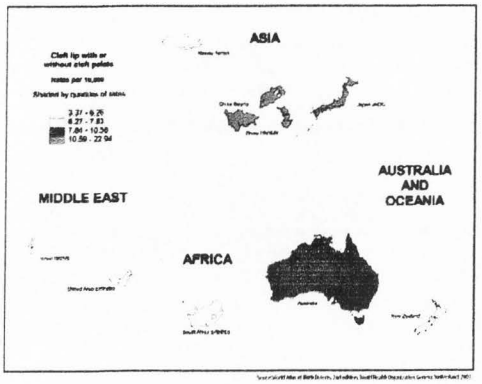

Figure 4b: Cleft palate without cleft lip
Asia, Middle East, Australia & Oceania, Africa

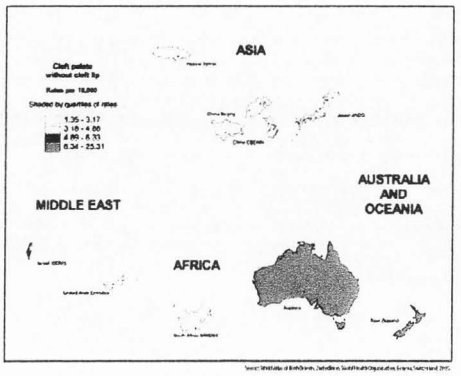

Figure 3c: Cleft lip with or without cleft palate
Europe

Figure 4c Cleft palate without cleft lip
Europe

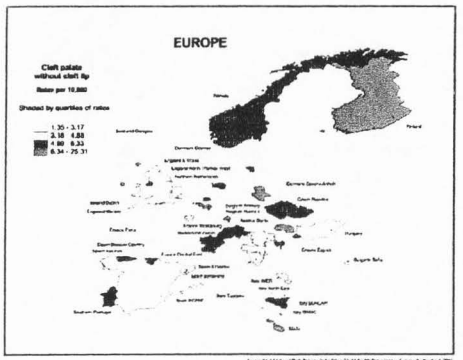

Figure 3a: Cleft lip with or without cleft palate
The Americas

Figure 4a: Cleft palate without cleft lip
The Americas

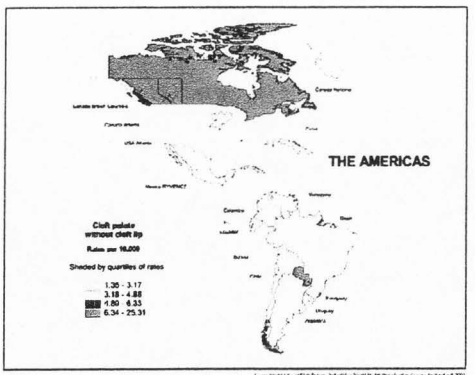

Figure 2-4. Rates of clefting around the world.

Source: (1)

Department at Penn State reports that only 4 things have remained consistent with each review of the epidemiology of clefts: 1) a distinct racial gradient exists (asians>whites>blacks) 2) difference in the incidence of congenital defects by sex 3) a higher incidence of isolated cleft lip or cleft lip and palate occurs on the left side and, 4) clefts are often associated with other congenital anomalies and are frequently a part of a distinct syndrome (30). One thing that Tolarova notes rather or misclassification (30). Other studies have more active methods of discovery and diagnosis, including certification of all cases by a unfortunately, is that despite the vast amount of data that has been collected and analyzed, as well as the many important advances in medical care and changes in public health policy in recent decades, cleft rates "remain largely unchanged since 1942" (29).

Genetics

Clefting shows strong patterns of familial inheritance and fairly consistent rates within races, early indicators of a genetic component of this disease (36, 37). For instance, in a study conducted in the United States comparing the risk for clefts in offspring of mixed racial couples, it was found that even with controlling for fathers race, white women were more at risk for having a child with a cleft than their black counterparts (OR=2.33, p=0.0001) (4). Some environmental exposures have also been shown to be modified dependent on certain genoptypes, such as in the case of maternal smoking and transforming growth factor alpha (TGFa) where the rare C2 geneotype and light smoking (less than 10 cigarettes per day) together raised the risk of having a child with cleft palate (CP) over six fold, (OR=6.16) and the same allele with heavy smoking

(greater than 10 cigarettes per day) raised the risk of CP by over eight fold (OR=8.69) (38). With the advent and development of molecular biological technologies, the amount of genetic available in conjunction to cleft lip and cleft palate research has burgeoned as of late and these studies are barely the tip of the iceberg. Certainly the wealth of accumulated data in that area is well beyond the scope of review for this study. However, there are a few crucial aspects of cleft genetics worth mentioning here.

Like many other aspects of cleft research, animal models have proven an invaluable tool for genetic explorations. Martyn Cobourne, a researcher with Division of Orthodontics and Craniofacial Development at the Dental Institute in London, summarizes the contributions that animal models have made in identifying the roles of certain genes in cleft formation in his 2004 publication. These include several transcription factors, signaling peptides, vasoactive peptides and the like. Many of the knock-out studies performed using mice models have helped to solidify the idea that one or a few genes of interest may lead to cleft phenotypes (24).

As a preliminary step in identifying genes of interest related to cleft formation in humans, several gene-scanning projects have been performed using samples from a number of different populations. These scans have located some thirty genes that seem to warrant further investigation (5, 24, 39) (Table 2-1). Genes for extracellular matrix proteins, transcription factors and cell signaling molecules have been linked to cleft palate phenotypes (40). While some genes are identified based on parametric parameters, such as LOD scores greater than 1, others have been singled out based on biological plausibility (38, 40-42). TGFa has been the focus of a good deal of genetic research in various populations, so much so that there is even a meta-analyses of gene-environment

interaction studies TGF and smoking in relation to clefting (18, 38, 43, 44). Because of the interest in maternal folic acid metabolism and the risk of birth defects, most especially neural tube defects, genes involved with folate transport and function have also been extensively investigated candidate genes. Included in these studies are genes for methylenetetrahydrafolate reductase (MTHFR) (15, 45), reduced folate carrier gene (RFC1) (46), methionine synthase reductase (MTRR), cystathionine ß-synthase (CBS), and methionine synthase (MS) (47). Members of the folate receptor family, FOLR1 and FOLR2, have also been studied and appear to have no association with clefting (48). As pointed out by Marazita and others, these studies show that susceptible loci differ from population to population, making it difficult to come to any concrete conclusions about the role of any particular loci or genes (49). Particularly, it seems that there is a difference between Asian populations and the more widely studied Caucasian populations (50).

Table 2-1: Genes regulating the development of the head, in particular of the lip and palate

Gene	Type[a]	Feature[b]	Mouse[c]	Human[d]	Key references
TGFA	GF	L/P		LD	23,24
END1	SF	M	KO	Linkage	88
RARA	SF	P/M	TG/EXP	LD, linkage	27
TGFB	GF	L/P	KO, EXP	LD	26,28
SKI	GF	L/P/M	KO, EXP		89
MSX1	HD	L/P	KO, EXP	LD, linkage	26
DLX 1/2	HD	P/M	EXP		90
PITX2	HD	P/M	KO, EXP	Rieger	91
PAX9	HD	P/M	KO, EXP		92
AP2	TF	L/P/M	KO, EXP	Linkage	42
TTF2	TF	P	KO	Thyroid dysgenesis	93,94

[a]GF, growth factor; HD, homeodomain; SF, signaling factor; TF, transcription factor.
[b]L, lip; P, palate; M, maxilla and/or mandible.
[c]KO, knockout; TG, transgene; EXP, expression.
[d]LD, linkage disequilibrium.

Source: (39)

Despite a lack of consistent results, one overall result of these studies has been to help narrow down on the most probable model of inheritance for cleft lip and palate. At one point, the genetic model of preference was the multifactorial threshold model that supported the idea that there were several genes, each of miniscule importance individually, that that when taken together resulted in palate malformation (49). Increasingly however, it seems more likely that a single loci model, or major loci with as few as four susceptible sites is more likely the case (49, 51). In reviewing three separate scanning projects with populations in China, England, and Denmark, Marazita et al. (50) concluded that: 1) there is insufficient evidence to support the previously popular multifactorial threshold model, 2) based on data currently available, a single autosomal recessive gene is most likely the major gene responsible for cleft phenotypes, and 3) there may not be genetic homogeneity for CLP as English, Chinese and Danish populations do not support the same probable inheritance model (50). The most promising candidate genes under consideration include: TGF-a, RARA, BCL3, 6p23, MSX1, MTHFR and TGF-b3 (24, 43). "Is there a major gene determination of liability to cleft lip with or without cleft palate?" asks Marazita et al, " we would answer an unqualified 'Probably (50).'" Obviously there will be more work done in this area.

Environmental Risk Factors

Several different study designs have been used to show that environmental factors can interact with genetic factors to induce clefting and may even be the predominant risk type associated with clefting. Maternal exposure to toxins like cigarette smoke and pesticides, as well as diet deficiencies have profound effects on pregnancy outcomes. For

example, migrant studies report that Philippinos and Japanese who have immigrated to the United States, either to California or Hawaii, have cleft rates similar to other people in those areas and not their home countries, a clear indication of environmental impact on cleft rates (39, 52-54). Hospitals in Manila have different rates of clefting dependant on the socioeconomic status of the patient population. Animal models have shown that cleft palate formation can be induced in several species by altering various external factors (55-59). Folate antagonists, corticosteroids, and anticonvulsive drugs are among the known teratogens that cause oral clefts in animals (60-62). Some plant based poisons are also suspect (59). Several nutritional deficiencies or toxicities have likewise been shown to induce clefts in animal models, including vitamin A, riboflavin, folic acid, vitamins B-12 and B-6 and zinc (63-65).

Maternal Nutrition

Maternal nutrition has been a focal point of birth defect studies since the early 1940's and the first animal models that showed that vitamin deficiencies could induce clefting were reported as early as 1914 (66). Josef Warkany was instrumental in pioneering these animal models, and his studies confirmed that riboflavin and other B vitamin deficiencies are teratogenic in rats (66). Since then, work done by various research groups has also established that vitamin B-6, zinc, and folate deficiencies, as well as vitamin A toxicity, cause clefting in a variety of animals including rats, mice, chickens, rabbits, dogs, sheep, pigs and primates. An concise overview of animal models related to nutrition and clefts is available in the chapter authored by Ronald Munger in the text edited by Diego Wyszynski (66).

Human studies in nutrition have largely been limited to observational methods, as clinical trials have proven economically and ethically cumbersome. The early attempts at vitamin supplementation trials for clefting have been subject to a number of methodological follies and the results are therefore un-interpretable (29, 67). There is however an impressive amount of data available in the form of case- control and cohort studies that show reduction in fetal abnormalities including cleft lip and palate with maternal vitamin supplementation (68-71). A number of specific nutrients have been singled out for their biological roles in fetal development, however interactions between different nutrients have also become increasingly important.

Folic acid

Folic acid is a nutrient found in abundance in many leafy green vegetables, grains, legumes and liver (72). Folic acid is used biologically in different forms of tetrahydrofolate, which facilitates the metabolism of 1- carbon units in various metabolic pathways, most particularly to facilitate methylation of DNA and signaling proteins (70). Additionally, folate is used to methylate homocysteine in order to form the amino acid methionine. Folate levels therefore are directly related to homocysteine concentrations in the body and excess homocystiene has been associated with increased risk for cleft lip and palate. Hyperhomocystemia may cause apoptosis in cells and disrupt the normal course of palatogenesis (73).

The history of folic acid and birth defect studies started with the publication of the1947 edition of the Proceedings of the Society for Experimental Biology and Medicine, in which Nelson reported that the folate antagonist succinylsulfathiazole was

teratogenic to developing fetal rats (66). Shortly afterwards she also published the results of other rat models regarding folate deficient diets in relation to congenital abnormalities. Studies with dogs, chickens and mice confirmed this relationship (66).

Folic acid has been the most extensively considered nutrient in birth defects studies due mainly to its relation to neural tube defects. In 1997 the United States started supplementing processed cereal grains with folic acid because there was convincing evidence that pregnant woman who are folate deficient are at increased risk for having children with neural tube defects. In fact, prenatal supplementation with 400 ug of folic acid per day can significantly reduce neural tube defects (74). Because neural crest cells give rise to most craniofacial structures during fetal development, it has been hypothesized that folic acid may also be a factor dictating the incidence of orofacial clefts. Animal models have also confirm this relationship and show that folate supplementation in mice can reduce the risk of clefting from 40% to 10% (75). There are some observational studies that have detected an association with maternal folate levels. As recently as 2004 researchers in the Netherlands found that women who reported using even low levels of folate have a decreased risk of having children with clefts – as much as a 47% less chance (76) . For one California population, a case control study showed that taking multivitamins periconceptually did in fact produce a protective defense against clefts (OR=0.5-0.7) (71). Itikala et al. (68) found a protective effect of folic acid supplementation for CLP (OR= 0.52, 95% CI=0.34 –0.80) corresponding to 48% reduction in risk (68), and Loffredo (77) likewise found a significant rate of folate supplementation for CP cases and controls (RR=0.60) (77).

Vitamin B-6

Otherwise known as pyridoxal phosphate, or PLP, vitamin B-6 is linked to many of the same biological processes as folate. Also a contributor of carbon units during metabolism, PLP is needed for transamination reactions, decarboxylation reactions, steroid hormone action and as a coenzyme for other reactions (5, 78). Like folate, a lack of B-6 can cause a build up of homocysteine in the tissue. Vitamin B-6 is found in whole grains, and meat products as well as fruits and vegetables and soybeans (72).

The protective effects of B-6 have been confirmed using a number of different study designs. Animal models have shown that B-6 supplementation can protect against corticosteroid , valproic acid, B-amino-proprionitrile, and vitamin A induced clefts (65). In a recently published article, vitamin B-6 was linked to folate levels and risk of cleft lip in humans as well. Munger et al. (79) found that case mothers in the Philippines who were both B-6 deficient and had low blood folate levels had a significantly increased risk of having children with cleft palate. Trend data was also significant over quartiles of consumption for B-6 ($p=0.003$) in a population in Europe (80).

Riboflavin Vitamin B-2

Riboflavin is also one of the B vitamins. In the body it contributes the flavin portion of flavin adenine dinucleotide, FADH, which is a reducing agent imperative to central metabolism (78). By accepting electrons from metabolites in the citric acid cycle, it facilitates the catabolism of sugars and provides reducing equivalents for the production of ATP (78). Like other B vitamins, it is found naturally in meat and dairy products and in the US it is also found in fortified cereal products (72).

Aside from the initial studies done by Warkany and others in the early 40's, work done with riboflavin and orofacial clefts has been limited. A 2004 study looking at B vitamins and clefts did report a difference in dietary intake of B-12 between cases and controls, but that did not hold true for riboflavin (81). Interestingly enough, the rat study done by Warkany was very specific in showing that pregnant rats on a deficient diet that were supplemented with only riboflavin before day 13 of gestation gave birth to normal babies (82). This is certainly supportive evidence that more work should be done with this particular nutrient.

Vitamin B-12

Vitamin B-12 is involved largely for DNA synthesis (70, 78). B-12 is mostly available from animal sources, including dairy products (72). B-12 deficiencies are most often associated with impairment of neural function (70). Supplementing with folate can often mask a B-12 deficiency, so it is important to ascertain the status of both in the event of clinical or observational studies (83). One study did find a significant difference in B-12 between smokers and nonsmokers (84), however that Wong et al. (85) did not find a significant difference in B-12 between cases and controls.

Vitamin A

As one of the fat-soluble vitamins, vitamin A has the capacity to be stored in the body more readily than the water soluble vitamins and consequently it exhibits teratogenic effects with both dietary insufficiencies and toxicities. Retinoid compounds like beta carotene are the dietary precursors for vitamin A and are found abundantly in colorful vegetables: carrots, broccoli, liver, and leafy greens. Vitamin A plays a role in

both cell differentiation and maintenance of epithelial tissue- both important factors in palatogenesis. Endocrinology studies confirm that retinoic acid alters the efficacy of other signaling molecules like EGF during palate formation. Animal studies show that supplementing with folic acid can prevent clefts caused by retinoic acid toxicity (63).

Zinc

Zinc is a metal ion found almost exclusively in red meat products (72). In the nucleus of living cells, intercellular receptors that regulate DNA translation attach to both DNA and steroid hormones by means of a "zinc finger" (78). Thus, zinc can directly affect the binding capacity of hormones and regulation of DNA expression. This is critical in rapidly developing cells such as fetal cells. Another venue that zinc levels may influence the development of the palate is in conjunction with matrix metaloproteinases (17). This family of enzymes includes nine proteins each with a highly conserved zinc-binding site (86). These enzymes are responsible for the degradation and alteration of many proteins in the extracellular matrix including collagen, elastin, and fibrin during many developmental phases, in many parts of the body (86). Some studies have shown that production of different matrix metaloproteinases can be induced by hormones like TGF, EGF and others, many of which are also hormones known to play a part in palatogenesis. Certain genetic polymorphisms of those hormones have also been associated with clefting (18). A study by Laurence Blavier links metaloproteinases, induced by TGF-b3 expression, with palate formation by showing that metaloproteinases were expressed both in midline epithelial and adjacent mesenchymal cells during regular

fusion and that inhibition of the MMP resulted in the failure of opposing shelf palates to fuse (17).

New methods of handling blood samples in order to preserve the integrity of the micronutrient profile are very important. This includes holding blood samples on dry ice immediately after collection until analysis can be completed, which proves difficult in the field where sampling conditions are not always optimal (87). Utilizing these techniques, and recent case-control study conducted in the Philippines showed that higher plasma zinc was indeed associated with reduced risk of oral clefts (88).

Food Frequency Questionnaires (FFQ) and dietary recall

There are many tools used by researchers to determine nutritional status in human populations. Clinical lab data is often considered the gold standard for such information, and there is certainly no scarcity of available assays for generating such data (89). Many would argue that relying heavily on lab data is both expensive and naïve (90). Biological samples, while not subject to recall biases, are not exempt from other types of downfalls. Blood samples alone for instance do not shed light on the differences between nutrients consumed and nutrients absorbed and blood samples are rarely if ever collected during the critical time period- typically blood samples are not even collected until many years after the birth of an affected child. Often times, cheaper, less appropriate lab data is used as an indicator of disease rather than a more expensive but more accurate disease marker such as is the case of always using hemoglobin values to assess anemia instead of serum ferritin or cobalamin levels (83). Lab work is also subject to human error. Some alternative methods of determining nutrient status and food consumption patterns are

cheaper and have been shown to render precise, validated results (89). These methods include 24 hour recall, food record, diet history and FFQ's.

It is not unusual for epidemiological studies to rely on FFQ's to assess a mother's diet before or during a pregnancy (91). FFQ's have several distinct advantages over other assessment methods for these types of studies. First, they are relatively inexpensive to perform. Usually FFQ's can be filled out without the help of trained dieticians or nurses. This is a useful feature for any assessment method when considering the large numbers of participants in most studies, as more complex methods may be cumbersome financially, as well as logistically inconvenient. Two, FFQ's are versatile. Many previously developed FFQ's from studies like the Nurses' Health Study can be modified to fit a certain population. Third, food frequency questionaries can be developed to focus on relevant time periods (91) and still report dietary patterns with acceptable accuracy. This is an essential element of FFQ's in that it can match more closely the proper exposure time for nutrition related diseases (92). In the Utah Child and Family Health Study for instance, the mother is provided with a pregnancy calendar that references her to the index pregnancy in order to help increase the accuracy of her dietary recall. FFQ's show correlation rates with actually dietary intake (as determined by other dietary assessment methods) of up to 0.70 (89), a correlation that parallels other measures of biological functions including blood pressure, physical activity and skinfold tests (93).

Oxygen Transport

The concept for this particular study was initially conceived after reviewing some intriguing animal models that clearly show that the offspring of genetically susceptible

mice will be born with clefts palates if there is a lack of available oxygen during the critical embryonic period. Environmental factors that decrease the availability of oxygen or impede its transport then become suspect as risk factors for cleft palate formation. In human studies this translates into two major factors that effect blood oxygen levels and distribution efficiency –smoking and anemia. While both of these conditions have been studied extensively as risk factors for undesirable birth outcomes, the combination of the two, and their relationship with palate formation, is left largely undefined. Therefore, the goal of this study is to determine what relationship exists, if any, between oxygen depletion via smoking and nutritional anemia, and cleft palate formation.

Oxygen is transported through the body by means of hemoglobin proteins (94-96). Hemeoglobin is a tetrameric protein composed of two alpha and two beta subgroups (96). Each subgroup is associated with one heme group, and each heme group has the capacity to bond with one molecule of oxygen, for a total of four oxygen molecules per hemoglobin protein (78). Hemoglobin is particularly well designed to deliver oxygen to the tissues for several reasons. First, hemoglobin is an allosteric protein that exhibits a sigmoidal bonding curve (96). When one oxygen molecule attaches to a heme group the protein undergoes a conformational change that makes it successively easier for the other heme groups on that protein to bind with oxygen molecules. This type of cooperative binding makes hemoglobin very sensitive to differences in the concentration of oxygen available in the lungs and in the tissues. Secondly, in the tissues where the pH is lower than in the lungs, hemoglobin has a much lower affinity for oxygen and will readily release the oxygen into the tissue (96). In order to facilitate the removal of waste from the tissues, hemoglobin will also bind to carbon dioxide at physiological pH and release it

in the higher pH of the lungs conversely to the binding and releasing of oxygen in peripheral tissues. In patients that smoke it is estimated that nearly 10% of their blood hemoglobin is saturated with carbon dioxide- significantly more than that of non-smokers, the effects of which will be discussed more fully in a later section.

It is interesting to note that hemoglobin is again well suited to transport oxygen in fetal tissue. Hemoglobin in fetal tissue is composed of two alpha and two *gamma* subunits. This increases its affinity for oxygen above that of the hemoglobin in the mother's blood and thus allows the fetus to extract oxygen from the mother. Blood oxygen levels in fetal tissue are proportionly related to the blood oxygen of the mother, and smoking likewise effects oxygen transport and availability in fetal tissue (97). In fact, fetal tissue in pregnant women who smoke exhibits as much as 1.8 times more carboxy hemoglobin than in those that don't smoke (98).

Hypoxia

In studies of mice, it has been fairly straightforward to produce evidence linking blood oxygen availability and cleft palate formation (57). Oxygen is essential in virtually all metabolic processes and hypoxia has been shown to be a powerful, nonspecific teratogen (57, 96). Induced hypoxia in mice studies have conclusively shown that reduced levels of blood oxygen can increase the risk and severity of cleft palate (56, 57). A study done by Millicovsky and published in the Proceeding of the National Academy of Science, goes even further to show a dose response relationship between hypoxia and incidence of cleft lip in mice (55). A 1972 study in Denmark showed that as little as 8% carboxyhemoglobin in rabbits adversely affected fetal development and which correlated

well with their concurrent findings of decreased birth weight of children born to mother's who smoked (99). While not directly related to clefting, a study in Colorado lends support to hypoxia as a risk factor for adverse decreased birth weight (100).

While there is not a complete understanding of how oxygen, or a lack thereof affects palate formation, one specific mechanism of interest is the inhibition of the enzyme lysyl oxidase (LO). Lysal oxidase is an enzyme that has been isolated from a number of different animal and human tissues. While some of the important enzymatic functions of LO have been uncovered, there is still a great deal of information about the enzyme that is unknown. The basic role of LO is the oxidation of lysine residues during the formation of cross-links between collagen and elastin fibrils in extra cellular matrix proteins (101). New evidence suggests that LO may also act intracellularly to oxidize other enzymes, including certain growth factors (TGF-b), a hormone previously identified as having a role in palatogenesis (101). Because the catalytic activity of the enzyme is dependant on the availability of molecular oxygen, it is reasonable to assume that a lack of oxygen would impair the LO efficiency.

Smoking

The correlation between smoking and disease has been a widely studied controversy in the public health arena for decades. Smoking has been associated with a myriad of health concerns such as coronary heart failure, cancer, emphysema, stroke, etc. (102). The World Health Organization lists smoking as one of the major risks to public health (102). The May 2002 World Health Organization Factsheet on smoking sites a number of starteling statistics that help illuminate the extent of tobacco damage:

- More than 4,000 toxic or carcinogenic chemicals have been found in tobacco smoke.
- Smoking related-diseases kill one in 10 adults globally, or cause four million deaths.
- Smoking is the single largest preventable cause of disease and premature death. It is a prime factor in heart disease, stroke, and chronic lung disease. It can cause cancer of the lungs, larynx, esophagus, mouth and bladder and contributes to cancer of the cervix, pancreas and kidneys.
- Cigarettes cause more than one in five American deaths

A full one third of smokers in the United States and other developed countries are women (103). Social and psychological factors contribute to women's smoking status and their success rates with cessation (103). Smoking can significantly alter economic status of women, as smoking is expensive and often cigarette purchasing is a priority over adequate nutrition (103). Politics, of course, always plays a significant role in health and economic matters, and as quickly as lawmakers pass new regulations, tobacco companies have managed to avert many of those political boundaries.

Besides the normal health concerns, cigarette smoke is of particular concern for pregnant women because it has also been associated with birth defects like coronary heart disease, spontaneous abortions, and low birth weight, as well as post-birth complications like Sudden Infant Death Syndrome (42, 104). This is not surprising considering the chemical make-up of cigarette smoke, which includes physiologically detrimental levels of nicotine, tar and carbon monoxide and a host of other toxic chemicals, the most toxic of which are most likely the combustible toxins (104). This may account for a higher

incidence of certain diseases including cleft lip and palate, especially in countries where tobacco use is wide spread and on the increase. Carbon monoxide intoxications in pregnant women have been shown to cause deformities in children as well as increase still births (104).

Epidemiological Studies of Smoking and Clefts

Nearly forty years ago, Senator Walter Mondale expressed the following concern regarding the state of research in the field of education, "…For every study, statistical or theoretical, that contains a proposed solution or recommendation, there is always another equally well-documented study, challenging the assumptions or conclusions of the first. No one seems to agree with anyone else's approach. But more distressing: no one seems to know what works" (105). Such is the case in many fields of research and Senator Mondale may as well have been referring to the many studies regarding clefts and smoking. An ever-increasing body of research has implicated cigarette smoke as a risk factor for spontaneous abortions and certain birth complications, including orofacial clefts (106). However, the epidemiological data are not entirely conclusive: a significant number of the studies show that maternal smoking can influence cleft palate rates (107) (27, 108-119), and a number found no association at all (38, 108, 118). Table 2-2 is a summary of those studies.

Table 2-2: Review of studies on smoking and clefts

Author	Year	Place	Data	Case	Control	Control Type	Cleft Categories	Point Estimate	95% CI	Confounding	Notes
Kullander (106)	1971	Malmo	Total Malformations Prospective	NA	NA		Any malformation	X2=1.7 p=0.7-0.5	NA	1 year survival	No association between smoking and malformations
Andrew (26)	1972	UK-Cardiff	Cardiff birth survey Prospective	30	18,631		Cleft palate and/or hair lip	X2=5.36 p=0.05-0.01	NA		Study notes sig. Anemia*smoking interaction
Saxen (110)	1974	Finland	Finnish Registry of Congenital Malformations	599		Matched (time/place birth)	CP CL(P) Clefts mult.	All clefts vs controls P<0.05 X2	NA		Anemia 4.4(%) control 5.7(%) cases NS
Kelsey (111)	1978	Conneticut	Hospitals/Private Clinics	1370 malformed	2968	Birth date ±1year	(N=40) Cleft palate and cleft lip	1-20=1.0 20+=1.7	NA	Trends considered	Smoking assessed during Third month of pregnancy
Ericson (112)	1979	Sweden	National Board of Health Records	66	130	Matched (place/time birth, Maternal age, parity)	CLP (desc. Chart includes CP, CL, and CLP) X2 is all together	X2=13.1 p<0.01	NA	Not stated Non-smoking <10 cig/day 10-19 20+	Non-smoking includes a few cig a week
Christianson (120)	1980	San Francisco	Child Health and Development Study	24	14,735	Cohort	Anomalies of ear, face and neck	X2=NS			Bad design
Hemminki (114)	1983	Finland	Finnish Registry of Congenital Malformations	3300 total malformations	NR	Previous birth in district	Oral clefts	1)1.28 (1.24) 2) 1.28 (1.25) 3)1.25 (1.48) adjusted (crude)	Smoking: 1Sporadically 2Regularly 3>5 cig/day	(13)Age, parity,alcohol, coffee,abortion rooms in house occup pregnancy conditions, medical treatments etc	First tri exposures
Shiono (108)	1986	Maryland	Kaiser Permanente &Collaborative Perinatal Project	KP- 32 CLP 24 CP	KP 33,536 CPP 53512	Prospective	CP CLP	0.7 1.1 NS	0.3-1.8 0.5-2.4	Smoking 0,10,20,30 trend is considered=findings not corroborated in CPP	CPP used to corroborate KP findings
Khoury (116)	1987	USA	Maryland Birth Defects Reporting &Info Systems	CLP(28) CP(26)	198	Controls are other malformed births	CLP CP Other (cont)	2.56 2.39	1.13-5.78 1.04-5.45	Smoking groups 0,1-10,11-20,21+ Odds ratios	

										reported (see article)	
Koury (116)	1989	USA	Atlanta Birth Defects	CLP (238) CP(107)	2809	Matched race, birth area, hospital	All cases CP All cases CLP Isolated CP Isolated CLP Multiple CP Multiple CLP	1.39 (1.50) 1.48(1.47) 1.87(1.96) 1.59(1.55) 0.88(1.01) 1.17(1.30) (adjusted)	(0.97-2.32) (1.09-1.97) (1.10-3.50) (1.10-2.18) (0.45-2.26) (0.69-2.44) (adjusted)	Smoking:<14, 15-24,25+ Trend not sig across any groups	Population based First reference to Pierre Robin no syndromic cases
Malloy (117)	1989	Missouri	Missouri Center for Health Statistics	451	288,067	Matched time/place birth, child's sex, race	CLP	0.84	0.68-1.05	Parity, marital status, edu, age	Large # cases
VanDenEeden (121)	1990	Washington	Washington State Birth Records	173 total clefts	4500	Random	CLP (N=105) CLP mult(N=22) CP (N=37) CP mult (N=15 All Cleft	1.5 1.1 1.2 1.9 1.4	1.0-2.3 0.4-2.9 0.6-2.5 0.6-5.6 1.0-2.0	Maternal age Parity	
Werler (118)	1990	Boston (Multiple Centers)	Sloan Epi Birth Defects Study	CP 215 CLP 400	2710	Other malformed births	CP(N=136) CP mult(N=79) CLP(N=292) CLP mult(N=108)	Non significant across all smoking categories	Never smoked, exsmoker, 1-14, 15-24, 25+	Age, Edu, race, alcohol, vit A suppl	First three months pregnancy
McDonald (122)	1992	Montreal	Survey	96	89317		Cleft of Lip or Palate			Maternal age, education, ethnic	Dose
Zhang (123)	1992	Shanghai, China	Random, 29 hospitals in Shanghai	CLP(48) CP(10)	419	Birth before or after case	CLP CP	Non smoking, 1-9, 10-19, 20+	All odds ratios non-sig for each smoking group	No adjustments made	Paternal Smoke exposure (based on mother's reports)
Hwang (38)	1995	Maryland	Maryland Birth Defects Reporting and Information System	69 (CP) 114 (CLP)	284	Malformed controls matched time & place of birth, maternal age	CLP CP	0.79 0.75	0.49-1.29 0.42-1.33	Cig/day <10,>10 significant association with smoking and Taq1 (TFGa) polymorphism	TFGA, family history
Shaw (107)	1996	California	California Birth Defects Monitoring System	731	734		CLP (N=348) CP (N=141) CLP mult(N=99) CP mult(N=74)	**CLP** (1-19 cig) 1.2-2.3 **CLP**(20+cig) 1.3-3.6 **CP** (20+cig) 1.1-4.5	Smoking groups:0,1-19, 20+ Paternal smoke-sig for Cp and CLP at	Age, race, edu, gravidity, alcohol, diabetes, folate TFGa	Good review of what is wrong with the other studies HYPOXIA

									20+cig	Exposure from 1 month before conception & 1st tri	
Beaty (124)	1997	Maryland	Maryland Health Dept Birth Defects Registry	121	86	Random Hospital Based	Bilateral CL/P (15) Unilateral CL (22) Unilateral CLP (38) CP (46) All All	2.67 1.58 1.51 1.73 1.75 X2=4.09 (p=0.04)	0.78-9.17 0.49-5.07 0.57-6.03 0.71-2.46 1.02-3.02	Family History	TGFα
Wyszynski (125)	1997	NA	See references	NA	NA	Meta-Analysis	CL/P CP	1.29 1.32	1.18-1.42 1.10-1.62		1st Trimester Exposure
Kallen (109)	1997	Sweden Power analysis	Swedish Registry of Malformations & Medical Birth Registries	1834	1,002,742	Population based – same data as Keels '91 but more data	CLP CP PR All clefts CLP mult CP mult PR mult All clefts mult	1.13 1.35 0.79 1.18 1.43 0.88 0.94 1.19	0.99-1.29 1.12-1.63 0.47-1.33 1.06-1.31 0.98-2.09 0.51-1.54 0.28-3.19 0.88-1.62	This is the same data as Keels (1991) but more data SEI (socioeco status based on edu and 'workers' Smoking:>10 or <10	Maternal age, parity, year of birth
Christensen (126)	1999	Denmark	Population based Centralized treatment centers	302	567	Hospital, preceding 2 births	CLP CP CLP(10-19 cig) CLP(10-19 cig)adj	1.4 0.87 1.56 1.62	0.99-2.0 0.50-1.52 1.07-2.27 1.06-2.49	Infant TGFA, alcohol, vitamin: cig/day 0, 1-9,10-19,>20	First trimester exposure Dose non sig
Lieff (127)	1999	USA Boston Philli Iowa	Slone Epi Unit Birth Defects Study	1479 (total clefts)	2295	Malformed controls	CL (N=334) CLP(N=494) CP (N=244) CL mult (N=58) CLP(N=140) CP mult (N=209)	Analysis 1-out of 32 OR's only 7 had CI that do not include 1 Analysis 2- not enough smokers to divide into catagories	Smoking:1-14, 15-24, 25+ Dose Response sig for CLP mult	Analysis 2- has more precise data on smoking time and dose	Whites only, geographic area, sex, age, edu, family history, miscarriage, abortion folate etc.
Chung (128)	2000	USA	1996 US Natality Database	2207	4414	Healthy controls And malformed controls- results not different between control	CLP: (Smoking) 1-10 1.32 11-20 1.28 21+ 1.69 adjusted			Smoking: 0,1-10,11-20,21+ ANEMIA, edu, race, diabetes, hypertension, Sex, age , birth weight,	Dose response positive

						groups					
Lorente (129)	2000	France, UK, Italy, Netherlands	European Registration of Congenital Anomalies	161	1134	Hospital Based- &birth records	CL(P) (N=109) CP (N=52)	1.79 0.86	1.07-3.04 0.40-1.87	Alcohol use Socio-economic	Dose
Beaty (130)	2001	Maryland	Treatment Centers/ Craniofacial Clinic of Washington DC/ BDRIS	171	182	Random Hospital Based	CL/P CL/P (adj) CP CP (adj)	1.36 1.04 1.74 1.05	0.68-2.72 0.44-2.43 0.75-4.02 0.23-2.92	Maternal Age & Education	No dose response 0-3 month critical time
Honein (131)	2001	USA 45 States NYC, Dist COlumbia	National Vital Statistics system	2,632 (1997) 2,606 (1998)	3 mill (1997) 3.1 mill (1998)	Cohort	Oral Clefts (1997) Oral Clefts (1998) Oral Clefts (97-98)	1.32 1.38 1.35	1.19-1.46 1.24-1.53 1.25-1.45	Maternal age, education,race Cig/day: 1-5,6-10,11-21,21+	Dose NS
Woods (132)	2001	Cincinnati	TriHealth Hospitals	75	18,076	Retrospective Cohort	Head,ear,nose, and throat anomalies	0.7	0.30-1.63	Age, race, diabetes	Not cleft – Analysis for head, ear, nose and throat
Wyszynski (133)	2002	USA	1997 US Natality Database	2029	4050	Matched race, sex, time& place birth	CLP	1.16	1.01-1.33	Alcohol	Dose-slightly sig
DeRoo (95)	2003	Washington State	Birth Cerificates (WA state birth defects registry)	298,138	608	Matched time birth ; healthy	Isolated CLP Isolated CP Non Iso- CLP Non Iso CP	1.1 1.4 1.2 1.1	0.8-1.5 0.9-2.3 0.7-2.1 0.7-1.5	Maternal age, ethnicity, marital status, infant sex	
Little (69)	2004		Meta –Analysis	15 studies			CLP CP	1.34 1.22	1.25-1.44 1.1-1.35	No evidence of publication bias	CLP (7 of 10 studies have sig dose-response) CP (3 of8 studies have sig dose-response)
Meyer (134)	2004	Sweden	Swedish Medical Birth Registry	128,688	CP (678) CLP (1175)	Control births restricted to at least 2 births	CP CLP	All combined 1.1(1.0-1.3) 1.3(1.1-1.5)	1-9 cig/day 10+ cig/day	age, year of birth, diabetes, marital status, history, parity	Case crossover, case control, case-time crossover, bi-directional study design
Zeiger (44)	2004	Meta-Analysis	TGFa and Smoking	5 Studies			Smoking alone Smoking + Cs allele	1.64 1.1.95	1.33-2.02 1.22-3.10		
Lammer (135)	2005	CA, USA	CA Birth defects monitoring syatem	423	294	Non-malformed controls	Matched by county	Genotype dependent OR's 2.9 6.3	1.2-7.2 1.3-42	GSTT1 (-/-) GSTT1&GSTM1 (-/-)	These genes are for enzymes that detox tobacco chemicals

Included in the summary table are the principle investigators, the publication date and reference as well as a number of specifics about the study design. Table 1 is similar in design with the review published as part of a recent meta-analysis by Julian Little (69), but includes a number of addition pieces of information not in that review that are helpful for the current author! By considering the epidemiological data on smoking and clefts chronologically it is possible to see the evolution of study design that has served to eliminate some of the methodological shortcomings of earlier studies. These shortcomings are diverse and generate various difficulties when trying to interpret any inferential statistics. They include everything from case misclassification, to limited sample size and everything in between. In fact, it has only been in the last few decades, a short fraction of the history of cleft research, that studies have been shaped to eliminate some of these research flaws.

One of the problems with the earliest studies regards the classification of cases. Kullander et al's paper published in 1971 is a good representative study where clefts are considered only as a minor subgroup of birth defects if considered separately at all (106). The fact that many of these studies fail to find any significant association between smoking and malformations is no surprise considering the wide variety of malformations clumped together in the study. Everything from diseases of the central nervous system to minor defects like club foot are considered with little or no regard to differences in disease etiology. Such gross classification of malformations overlooks disease specific exposures not to mention genetic components of certain birth defects. Even in the studies where clefts are separated from other malformations, there is no separate analysis of CLP and CP, not to mention multiple malformations that include cleft phenotypes that are of

known genetic etiology. One illustration that clearly shows how this type of general grouping of malformations limits research productivity has been previously mentioned- that of neural tube defects and clefts. Due to the fact that the human palate is morphologically derived from the same tissue as the neural tube, it has been hypothesized that the two malformations may have similar etiologies. However, supplementation trials with folic acid in both the US and China that have significantly reduced NTD's have failed to elicit similar reductions in cleft rates (136). Considering clefts and cleft sub-groups independently from other malformations has been a tremendous help in accurately estimating attributable risk for disease specific risk factors.

A shift in focus from malformations in general to individual birth defects has necessitated a significant change in study design. Cohort studies rely on birth registries for data and can include information from millions of live births. However, while cleft rates are high enough to procure a steady and significant public health burden, they are not great enough to produce case numbers sufficient enough to provide the statistical power needed for analysis, unless large population sizes are considered. A power analysis conducted by Karin Kallen (109) estimated that in order to identify any significant risk difference due to smoking between cases and controls there would need to be at least 1,000 cases- and that is an estimate based on having a 40 % smoking rate and with a 20% true risk increase, not a very conservative estimate for some populations, like Utah where smoking rates are lowest in the nation (109, 137). Early studies did not have the kinds of numbers needed to produce meaningful analyses. Kallen et al. (109) had to track data for over a million live births in Sweden over the course of nine years in order to get a total of 1834 cleft cases, a sufficient amount, but the time and resources required

to establish such extensive registries is tremendous (109). Case control studies can elicit similar numbers of study participants much quicker while significantly decreasing the burden of data collection and effectively providing data from afflicted children. For example, Susan Lieff et al. (127) had only to collect data from a total of 3774 participants, 1479 cases and 2295 controls, in order to get enough data to effectively test the same hypotheses as Kallen.

Case control studies offer additional benefits over other designs for studies of low frequency diseases like clefts. Cohort studies that rely on birth registries are limited to retrospective data that often do not have data specific enough to pinpoint certain exposures. Palate formation is completed before the end of the first trimester and smoking exposure during that critical time-period is difficult to determine when birth registry data on smoking is limited to a simple yes or no question without regard to the time frame or degree of exposure. Case-control studies also rely on retrospective data for information, but these studies also utilize data collected via interviews with questionnaires carefully designed to precisely determine a temporal relationship between exposure and disease status. The questionnaires include vast amounts of information-enough to test numerous hypotheses within each population while also providing much needed information about specific confounding factors that may not be taken into consideration with birth registries. The Hungarian Case-Control Surveillance of Congenital Anomoalies recently published a paper supporting their position that retrospective, self reported smoking status is highly unreliable (138). While that may be the case, randomized controlled trials for smoking will never be an option, and though retrospective reporting falls short of providing any concrete causation, using

questionnaires specific to cleft research and relevant exposure times undoubtedly adds a measure of decisive information over general registry data. Population based case and control studies, where controls are randomly picked as representative samples of a larger population, can still be used to define prevalence rates within the population and thus serve dually as descriptive studies as well. Case crossover, case-time-control and bi-directional studies have been investigated as optional study designs for cleft research. By utilizing the same mother at two or more different times with differing levels of exposures, they eliminate sources of bias in traditional case control designs and can be an increased source for risk assessment (134).

Another important piece of information that has not been routinely collected for cleft studies over the years is the number of cigarettes smoked. Some of the birth registries do include this information irrespective of the date of publication – it appears to be based solely on registry specifications. For studies that do include this data, trend tests for dose-response relationships have varied results. Each researcher has a different way to categorize the number of cigarettes smoked, which makes between study comparisons somewhat. A further limitation to some studies is that while active smoking on the part of the mother is recorded, any exposure to second hand smoke is not jointly considered. Those studies that scrutinize exposure to second hand smoke do not concurrently assess active smoking. The fashionable design for current studies is to look at some measure of both active and passive smoking as a better indicator of degree of exposure.

The criteria used to identify acceptable controls for each case-control study are another area where each study maintains its own priori. Hospital controls are a popular choice, as well as healthy, population-based controls obtained from various sources such

as drivers license information. Controls are frequently matched for place and time of birth. Other matching criteria differ from one study to the next. One frequent complaint about case-control studies is their propensity for recall bias. It is not unusual for study participants to underreport exposures, but it is also not unusual for there to be a greater measure of under-reporting exposures for mothers with affected children versus mother's with unaffected children. One method used to remedy this problem is to use children with malformations other than clefts as controls. A few of the studies listed in table 1 opted to utilized malformed controls for their analysis. Chung et al. (128) computed odds ratios for their data twice - once with healthy controls and once with malformed controls. The results were fairly similar, enough so to warrant the continued use of healthy controls in cleft research.

Taken together these studies look like a patchwork quilt with nearly every possible idea and combination of ideas represented in some form or another. Consequently, it is difficult to interpret the collective results embodied by the years of dedicated research and untold amounts of financial investment represented here. This is not an uncommon problem in research today and as such meta-analyses are taking more of a spotlight role in interpreting data. Meta analyses utilize odds ratios and other inferential statistics from numerous studies and combine them to generate an overall odds ratio and confidence intervals for the accumulated data. To date there have been two meta-analyses for clefts and smoking.

Published in 1997 Wyszynski et al. (125) looked at 9 different studies for their meta-analysis. The group odds ratio represented by this group of studies is 1.29 with a confidence interval of 1.18-142 for CLP and 1.32 and 1.10-1.62 for CP. Wyszynski

cautiously summarizes that there is a higher risk associated with smoking. In 2004 Little et al. (69) looked at over 15 different studies and also concluded that there is an increased risk of cleft lip and cleft palate associated with maternal smoking based on a combined relative risk of 1.34 and confidence interval of 1.25-1.44 for CLP and a relative risk of 1.22 with a confidence interval of 1.1-1.35 for CP (69). The authors were very thorough and also included formal tests for publication bias (results were not significant) and analysis of dose- response relationships where possible (69). Four of the studies that provided dose response data had positive, but not significant dose effects, while four did not support a dose response pattern. Certainly, more work needs to be done in order to substantiate these findings and help estimate the impact of smoking on fetal palate formation. The data as it currently stands suggests that 11 to 12% of all clefts could be avoided if women of reproductive age quite smoking (43).

Andrews and McGarry published an interesting finding that links both smoking exposure and anemia in human populations (27). In 1972 they analyzed data from the Cardiff birth survey, which included 18,631 births. While their primary goal was not to assess risk factors for clefts, they were looking at "the habit of smoking during pregnancy in relation to maternal and fetal well being," the implications for the study currently at hand are remarkable. In their report they found that for the parameters of premature birth and infant weight, not only were the chi-square values for both anemia and smoking significant, so was the interaction between the two. This is further evidence that these two conditions may work conjointly to effect fetal growth, and possibly cleft malformation (27). Saxen also investigated anemia in a study published two years later

in 1974 (110). They reported a small difference in anemic status between cases and controls, but this difference did not reach significance (110).

Anemia

Anemia is defined by the World Health Organization as "a condition in which the hemoglobin content of the blood is lower than normal as a result of a deficiency of one or more essential nutrients regardless of the cause of such deficiency" (139). As this statement indicates, a number of different nutritional factors can contribute to anemia, including many of the same nutrients that are already under investigation in relation to oral clefts: vitamin A, folate, vitamin B-12, riboflavin, and zinc (140-144). However, different nutrient deficiencies that lead to anemia are not physiologically equivalent. While iron deficiency leads to a reduction in red blood cell production; untimely destruction of red cells and swelling of red cells due to other nutrient insufficiencies are also of significant health consequence (141). Many researchers use instead a broader definition of anemia, "a decrease in the oxygen carrying capacity of the blood," to incorporate anemias that do not directly relate to hemoglobin levels, but nonetheless alter oxygen transport through the blood (144). Studies have supported the idea that maternal anemia may in fact decrease oxygen transfer to the fetus (145).

Iron deficiency anemia, or microcytic anemia is the most prevalent source of anemia worldwide, due quite heavily to iron deficiencies in developing nations (143). And, like other risk factors, anemia has been associated with clefts in mice (36) and some evidence suggests association in humans (81). UNICEF estimates that globally 4-5 billion people have some level of iron deficiency and two billion are anemic (146). This

translates into a significant risk for women of reproductive age and children as iron deficiency has been shown to extensively increase mortality and illness. Those high rates for iron deficiency anemia do not seem to hold true for more developed nations however where studies like NHANES attribute as few as 2% of anemia to iron deficiency, and instead attribute other factors for the anemia (147).

Anemia is typically diagnosed based on the World Health Organization standards of hemoglobin levels less than 11g/dL (148). Hemoglobin levels correspond with long-term dietary iron intake and are usually a good indicator of late stage microcytic anemia (96). However, hemoglobin only accounts for 65% of total iron stores and so a more complete account of iron stores includes ferritin and hemosiderin (94). Iron deficiency develops in stages. The first stage is characterized by a depletion of iron stores and a subsequent decrease in serum ferritin. The second stage is transient, and accounts for a decrease in transport iron and continued decreases in serum iron. The final stage, or anemic stage, results when iron stores are depleted enough to cause a marked decrease in hemoglobin production (94).

Anemia due to vitamin B-12 and folate deficiencies, megoblastic or macrocytic anemia, occurs when red blood cells grow too large inhibiting the transport of oxygen in the body (144). Megoblastic and macrocytic anemia can not diagnosed based on hemoglobin alone, but instead are determined by mean corpuscular volume (MCV), and mean corpuscular hemoglobin (MCH) values (83, 144, 149). An alternative lab test, the mean corpuscular hemoglobin concentration (MCHC), can also be used to determine the concentration of hemoglobin proteins within red cells (150). In developed countries it

has been observed that folate and B-12 responsive anemias are more prevalent than iron deficiency anemia.

Some concern has been expressed over the universal application of the WHO standard of diagnosis for anemia because of inherent differences in hemoglobin levels in various populations (141, 148). For instance, one study that focused on anemia in pregnant Philippina women (141) suggests that a standard of 10.4 g/dL for determining anemia status would be more appropriate for that population (148). The CDC uses 12g/dL as a cutoff point for anemia in the United States. Hemoglobin and hematocrit levels are also very insensitive measures of total iron. Other more accurate measures of iron status include, "serum ferritin, mean corpuscular volume (MCV), transferrin saturation, free erythrocyte protoporphyrin, and hemoglobin response to therapeutic trial with iron" (147). Certain factors can also artificially elevate hemoglobin levels without actually increasing oxygen availability in the body (37). This may inhibit proper screening of anemia using hemoglobin values. Smoking is of particular concern because smoking can raise hemoglobin levels significantly without any reduction in tissue hypoxia (37). The increase is in fact just a reflection of increased levels of hemoglobin bound with carbon monoxide.

During the course of a normal pregnancy it is typical for hemoglobin levels to fluctuate, and many women develop anemia due to decreased blood iron levels before delivery (151). Third term development of anemia is considered a normal result of the necessary increase in blood volume and the subsequent dilution of blood proteins (151). Scholl (152)also reports that women who showed poor nutritional status earlier in pregnancy not only had higher rates of iron deficiency anemia, but also had serum ferritin

and red cell folate were also lower (147). A current meta analysis has linked maternal anemia during early pregnancy with poor pregnancy outcomes including low birth weight, perinatal mortality and preterm birth (153-156). These findings hold true in a number of independent studies. Even after controlling for age, ethnicity, education, marital status, and smoking, a study from the Kaiser-Permanete Birth Defects Study Cohort showed that anemia diagnosed during the second and third trimester using hemoglobin concentrations, was a statistically significant risk factor for preterm birth (OR=1.9). Populations from Tanzania and India also both showed that low birth weights are attributable to iron deficiency anemia (149, 155). And again with a population from Camden New Jersey, a statistically significant odds ratio of 3.10 for low birth rates was seen for those mothers starting prenatal care with iron deficiency anemia (147). Using an energy adjusted FFQ, researchers in the Netherlands found significant a differences in iron consumption between cleft cases and controls (80). Just like other research fields however, these studies cannot conclusively prove causality and there are plenty of observational studies that found no association between iron deficiency and or anemia and birth outcomes.

The Utah Child and Family Health Study

Utah

Although exposure to low iron levels and nutritional anemias are limited in the United States, NHANES data attribute as few as 2% of anemia to iron deficiency in the US, (152) Utah has several other demographic and cultural features that make it a good option for conducting population based studies on birth defects. Utah is situated in the

Great Basin in the northwestern part of the country. The landscape is diverse and ranges from the arid, minimalistic beauty of the red rock canyons in the south, to the bitter cold snow lands and rocky-mountains in the north. Utah was home to several Native Americans, including Ute, Shoshone, Anasazi and Navajo tribes, long before any western settlers came to the area. The first European settlers to make the trek across the plains to settle in the area were religious refugees from The Church of Jesus of Christ of Latter-Day Saints, LDS or Mormons, who had been driven from Missouri and Illinois by mobs on account of their religious convictions. A good portion of the population in Utah today are descendants of the early Mormons

Members of the LDS church are counseled by their leaders to observe strict health standards, which include not drinking or smoking, and abstaining from coffee and tea consumption. The large numbers of faithful observers of these religious ethics provide a large, very clean, non-smoking group for analysis. CDC data list Utah as the state with the smallest proportion of active smokers, 13.7% as compared with the national average of 23.3% (41). Conveniently, the majority of those who have had neither active nor passive smoking exposure also do not drink alcohol, which is usually considered a confounding variable in cleft research. Access to such a discrete control group with very limited exposure to tobacco and alcohol should help make more exact distinctions between risk exposure groups and result in more precise odds ratios.

Like other religious groups, members of the church of Jesus Christ of Latter Day Saints cherish family relationships and many tend to have large families. It is not unusual to find families with 10 or more children in fact. Preliminary looks at the Utah Child and Family Health Data show women with up to14 children. Women who have had

numerous live births provide much needed information about the role of nutrient depletion due to excessive parity and short pregnancy intervals (95, 157). The LDS church also fosters a deep interest and respect for their ancestry, and most Mormon families have access to family genealogical records for several generations. This can be a great resource for determining family history of a disease and the Utah State University Center for Epidemiological Research hopes to join their databases with the other genetic data available in the state, which will likely prove to be a tremendous asset for research.

Conclusion

"Assuming 15,000 children are born per hour worldwide (United States Bureau of the Census, 2001), a child is born with a cleft somewhere in the world approximately every 2 minutes (5)." This is indeed motivation enough to drive researchers to look for some insight into the intricate process of palate formation and search for ways to preclude developmental shortcomings. Perhaps at this juncture in time it is most advisable, while not disregarding the historical data on hand, to do as Dr. Bixler suggests and shift the focus of research from descriptive analyses to testable hypotheses (28).

On that note, a panel of cleft experts has established some guidelines to help streamline cleft research and make independent research efforts more valuable to the collective research efforts. Published in 2002, they make the following recommendations to maximize data collection for hypotheses testing:

1) Description of the cleft. At a minimum, the side and extent of the cleft should be documented as should palatal involvement.

2) Pregnancy history. The results of prenatal testing, identification of maternal illness, medication (e.g., anticonvulsants and retinoic acid derivatives), vitamin (before and after conception) and tobacco use, ethanol intake, and other maternal exposures should be documented.

3) Birth history. Birth weight and length and the need for any additional medical services not related to the cleft should also be documented. Specific attention should be given to a history of feeding disorders (beyond early cleft feeding difficulties) and infections. Additional information, such as Apgar scores, may be helpful but is not essential.

4) Developmental history. The identification of developmental disabilities, including learning disabilities and attention deficits, hearing impairment, and speech deficits or abnormalities may be the first indication of an underlying genetic disorder.

5) Family history. At a minimum, a three-generation pedigree should be obtained. The family history should emphasize orofacial clefts and related conditions, including any additional major associated anomalies (e.g., cardiac defects and eye and brain anomalies). The family history should also identify spontaneous abortions, stillbirths, and infant deaths. There should be special attention to identification of developmental disabilities because theses may indicate the presence of specific predisposing disorders(25).

Other study design recommendations have also been set forth by the World Health Organization. They call for a "standardization of research" that includes universal data collection methods for: nutrition, lifestyle, occupational, medical, obstetric, drug, genetic, biochemical, and family history factors, as well as classification for cases and

controls (1). Field experts across the globe are pushing in particular for the creation of a centralized registry for all craniofacial anomalies worldwide (1). Successful reorganization of existing cleft research and standardizing of future studies will greatly facilitate the application of any public health initiatives that may arise from such a collaborative effort. For a detailed set of universal guidelines, see the Global Registry and Database on Craniofacial Anomalies, Chapter 5 (1).

The Utah Child and Family Health Study follows quite closely these guidelines and is a prime example of the direction in which cleft research is headed. With access to both biological samples for DNA and nutritional biomarker data and detailed interview data including a food frequency questionaire, the Utah study has a wealth of information and an excellent database from which to draw some conclusions about the role of smoking and anemia in palatogenesis.

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CHAPTER 3

MATERNAL SMOKING AND ANEMIA AND THE RISK OF ORAL CLEFTS IN UTAH

Abstract

Hypoxia is a known teratogen, and animal models have shown that maternal hypoxia can in fact contribute to oral clefting (1, 2). In humans, maternal smoking and anemia are likely the two biggest factors that can lead to hypoxia during pregnancy. Data from the Utah Child and Family Health Study were used to test the hypothesis that maternal smoking and anemia may synergistically increase the risk of oral facial clefting. In this population-based study there was a total of 838 women who completed both detailed interviews and donated biological samples for analysis. Overall, 12.7% of the mothers smoked during the periconceptional period and 17% were anemic based on CDC standards of 12 g/dL for non-smokers and 12.3g/dL for smokers (3). There was a strong pattern of smoking cessation over the course of pregnancy for both cases and controls, but there was a significantly larger portion of case mother's who reported smoking at each time point as compared to the percentage of control mother's who were smoking. There was an increased risk of clefting associated with smoking across all cleft subgroups (N=358) that remained significant after adjusting for education, income, race, alcohol consumption, vitamin supplementation, mother's age, parity, hemoglobin quartile and child's gender (OR=1.82, 95% CI=1.02-3.24). The impact of maternal anemia, as determined by hemoglobin levels, did not contribute any additional risk itself, nor as an interactive term with smoking. Based on the current findings and other published

literature, it is reasonable to single out smoking as a public health concern of consequence in relation to maternal health and oral clefting (4).

Introduction

Oral clefts affect one out of every five to seven hundred births worldwide (5). Years of research have yielded significant amounts of data regarding possible causes for clefting, which include a large range of genetic factors and environmental factors including diet, drug and behavioral exposures (4-12). Some syndromic conditions with known genetic origins include cleft lip or palate (13), but thus far no single exposure, genetic or otherwise, has been identified as the principal exposure responsible for the majority of isolated clefts, although, there has been an increased understanding of the disease in recent decades. It is now known that the etiology of cleft lip with or without cleft palate (CLP) is somewhat different than that of cleft palate only (CP) (14) and that the formation of the palate occurs in early embryogenesis, around day 45 in humans and entails a complex sequence of cell signaling and morphological changes in the facial tissue (15-18). Clefting rates differ between ethnic groups, with Asians and Native Americans consistently having the highest rates, and blacks the lowest (19, 20). Among the numerous environmental exposures that have been shown in animal and human studies to be associated with clefting are: plant toxins(6), epileptic drugs (11), nutritional factors such as folate, B12, B6, and zinc deficiencies and vitamin A toxicities (7), alcohol consumption (21, 22), and smoking (4, 21, 23-44).

The detrimental effects of smoking on fetal growth are most largely attributed to the development of hypoxia (45, 46). In smokers, a portion of hemoglobin in the blood is bound to carbon monoxide and the consequential increase in carboxyhemoglobin in the blood decreases the oxygen carrying capacity of the blood (1, 47, 48). The decrease in available oxygen is even more pronounced in fetal tissue and in animal models a dose-dependant effect has been confirmed between blood carboxyhemoglobin levels and clefting (46). Human epidemiological studies have produced inconsistent results with regard to smoking and clefts, however, much of the inconsistency may be due to insufficient study designs, including small sample sizes, and insufficient detail about case-status and exposure levels. A recently published review in the form of a meta-analyses did show a marginal increase in clefting in cases over controls, an indication that smoking does indeed contribute to a portion of isolated clefts (4).

The purpose of this study is to investigate the effects of smoking in addition to another factor that contributes to hypoxia , anemia, and their relationship to oral facial clefting. Iron deficiency anemia is clinically diagnosed as hemoglobin values less than 11g/dl according to WHO standards (49) or 12 g/dL for non-smokers and 12.3g/dL for smokers based on CDC standards (3). Iron deficiency is common throughout the world although iron deficiency, or microcytic anemia, is more common in developing nations (49, 50). Anemia in general is characterized by a decrease in oxygen transport in the body, a condition undoubtedly found when hemoglobin production decreases due to iron deficiency (51), however other nutritional deficiencies can also induce anemia (52, 53). These other types of anemia also inhibit the efficiency of oxygen transport in the body, but without affecting hemoglobin level (54). They include folate and vitamin B12

responsive anemia, or macrocytic anemia, and these are characterized by a swelling of red bloods and a decreased in red blood cell transport through capillaries (55).

Materials and Methods

Participants

All study procedures for were reviewed and approved by the institutional review boards at Utah State University, University of Utah and the Utah Department of Health. Participants for the Utah Child and Family Health Study were identified with the help of the Utah Department of Health (UDOH), which oversees the Utah Birth Defect Network. Mothers of children with clefts born in Utah between 1996 –2005 were contacted by the UDOH via a letter explaining the study objectives. UDOH workers used telephone follow-ups to confirm or elicit a response to the initial introductory letter. Every possible option was exhausted in recruiting participants by the UDOH, including field tracing and home visits if necessary. This active method of recruitment ensured the highest possible participation rates, thus eliminating participation bias. 560 case mothers and 630 control mothers agreed to participate in the study after being contacted by the Utah Department of Health, for participation rates of 79.4% and 58.9% respectively. After the 1190 mothers who consented to participate in the study returned a consent form to the health department, their information was then released to the Center for Epidemilogical studies at Utah State University. Eligibility for the study was determined for cases based on the following criteria: 1) the mother was English or Spanish speaking, and 2) there was a confirmed cleft birth. Those not included in the study were those mothers who had died previous to the study, did not speak English and were therefore unable to complete an

interview, the address of the potential participant was unknown or was outside of Utah, and refusals. Controls were also identified through the UDOH birth certificate information and were matched to cases by month of birth and gender of the child.

Interviews

After release of participant information to USU, an initial phone contact was carried out by trained interviewers from the Center for Epidemiological Studies to assess the eligibility of participants. Verification was obtained for contact information as well as the date of birth for the index child, the child's gender and the corroboration of cleft status for case participants. Information was also obtained regarding the timing of the pregnancy in question, including whether or not the pregnancy ended early. At this time, as well as throughout the entire study, mothers were given the opportunity to refuse participation in the study. At the conclusion of the initial phone contact, a future appointment was made to conduct the full telephone interview.

Before the full telephone interview commenced, each mother was mailed a packet of information to help them prepare for the interview, including another summary of the study for their consideration. Central to the preparation materials provided were pregnancy calendars constructed by the USU team from UDOH birth records and corroborated pregnancy information from the previous phone contact. The calendar was specific for each participant, and supplied a referent time frame to help them more readily and accurately recall any relevant exposures during the pregnancy in question. In addition to the pregnancy calendar, an example of which can be found in appendix A, all participating mothers were given a list of helpful items to collect and have on hand to

help them during their phone interview, including the index child's birth certificate, health records and scrapbook and the mother's prenatal health records, nutritional supplement bottles and medication bottles. Diet interview cards were also supplied. They provided information on how respondents were to answer the food frequency questionnaire- including what time periods they were to focus on, and how to determine the average amount of each food consumed. The phone interviews lasted approximately 1 hour. Data collection included information for demographic variables, self-reported anthropometric data, cigarette, alcohol and drug exposure and dietary assessment via a food frequency questionnaire. Relevant paternal exposures were also determined as reported by the mothers. See appendix A for a complete copy of the Utah Child and Family Health Questionnaire (Appendix A).

Particulars about the type and site of cleft for each case child were self-reported by the mothers during the phone interviews. Mothers were probed for specific information as to whether the cleft was bilateral or unilateral, and on what side of the face, or if the child had a cleft of the soft palate only. Questions regarding any further malformations of the index child were also addressed. This data was later corroborated by UDOH health records, and the official UDOH coding was used to classify cleft subgroups for further analysis.

Blood Samples

Mother's who consented to donate biological samples during the phone interview were visited at a later date by a staff phlebotomist. Besides collecting blood samples from the mothers and buccal swabs from the children and fathers, the phlebotomists also

collected additional information on currently used, and measured the mother's height and weight. At the time of biological sample collection, written consent was obtained for each facet of the study individually: the interview, height and weight measurements, blood sample collection, storage of blood samples, labeling of the blood samples, and future contact. Similar consents were obtained for the collection and storage of cheek swabs for the children and fathers. Hemoglobin assays were done as part of the preparation and analysis of the blood samples following the recommendations and procedures for the HemoCue system, (HemoCue, Sweden). A total of 835 women, 70% of those that consented to participate in the study, had donated blood samples at the time this data was analyzed.

Statistical Analysis

As described in the literature, factors that may be associated with clefting were compared between cases and controls from the Utah Child and Family Health Study using statistical software SPSS 12.0 (SPSS,Chicago, IL,USA) and SAS 9.1 (SAS Institute, Cary, NC, USA). For the continuous variables, differences in the means were evaluated using t-tests, after appropriately applying transformations to meet the assumptions of normality, however the non-transformed means are reported in Table 1 for ease of analysis. All categorical variables were collapsed from the interview data into groups appropriate for analysis based on their individual distributions, and associations between them were assessed using chi-squared tests.

Height and weight information was collected both during the phone interview as self-reported data, and during the phlebotomist visit as a measured parameter. Because

there were several participants who volunteered interview data but did not consent to giving blood, there was more data available from the self- reported questionnaire. Correlation rates between the self-reported and measured data showed consistent results and in order to be more consistent with the retrospective nature of other exposure variables considered in the study, the self reported data was used to calculate body mass index (BMI, kg/M^2).

BMI, hemoglobin, and parity showed non-linear risk association with clefts and so they were included in adjusted models as categorical variables, as quartiles of BMI and hemoglobin, and parity from 1 through 5 (Appendix C). This is consistent with available literature that indicates higher risk for low infant birth weight with extremely high and extremely low BMI (56, 57). Maternal age did show a linear risk relationship and was kept as a continuous variable in the models. Normality of maternal age at birth of the index child was not particularly improved with the application of transformations, and so the data was used in modeling without any (Appendix C). Comparisons of log likelihood values for models with and without quadratic terms for maternal age showed no indication of a quadratic effect.

Early exploratory analyses included six measures of smoking exposure for consideration. Active smoking during the preconception period and during first trimester, passive smoking during the preconception period and during the first trimester, and both active and passive smoking during the preconception period and during the first trimester. Exposure to passive smoke was highly correlated to active smoking. Out of the 109 subjects who reported exposure to second hand smoke pre-conceptually, 97 or 88.9% of those also actively smoked. During the first trimester that dropped to 82%, still

a substantial amount. All of the participants who reported smoking during the first trimester had also smoked pre-conceptionally, and so subsequent analyses were conducted classifying smokers only based on first trimester, or periconceptional exposure.

Results

Characteristics of the study population are listed in Table 3-1. Education, alcohol consumption, vitamin supplementation, religion, smoking, and child's gender all showed significant differences between some subgroups of cases and controls, although active smoking is the only variable that shows an association with all clefts grouped together (Table 3-1). Many of the variables were also associated with smoking, as evidenced by highly significant chi-squared values or ANOVA p-values. These differences between smokers and non-smokers are highlighted in Table 3-2. Marital status and religion were not included as covariates in adjusted models because marital status showed no association with clefting and religion only showed association with clefting in two of the smallest subgroups. Any risk associated with these two descriptors is most likely due to their relationship to smoking and other lifestyle factors, like alcohol consumption, ethnicity, education and income with which they are also highly correlated. (Appendix B).

Self-reported smoking data from the Utah Child and Family Health study shows that 152 out of 1189 participants that had completed interviews as of September of 2005

Table 3-1: Characteristics of Cases and Controls for the Utah Child and Family Health Study

Covariates	Controls [1] N=637	CLP iso N=306	CP iso N=98	All Iso N=405	CLP mult N=59	CP mult N=89	All mult N=149	All Clefts N=552
Mean (SD)								
Age [2]	26.23 (5.2)	26.29 (5.6)	26.67 (5.6)	26.38 (5.6)	26.42 (6.0)	26.98 (6.2)	26.77 (6.1)	26.48 (5.7)
Height [3]	65.19 (2.6)	65.15 (2.64)	64.68 (2.57)	65.04 (2.83)	64.92 (2.90)	65.14 (2.64)	65.05 (2.7)	65.04 (2.81)
Weight [4]	146.46 (32.67)	147.83 (34.09)	145.96 (35.41)	147.38 (34.38)	145.53 (33.32)	142.91 (25.71)	143.96 (28.93)	146.46 (33.02)
BMI [5]	24.3 (5.32)	24.45 (4.89)	24.62 (5.88)	24.49 (5.15)	24.31 (5.31)	23.74 (4.32)	23.97 (4.74)	24.35 (5.04)
Hemoglobin	13.04 (1.26)	13.08 (1.31)	13.29 (0.88)	13.13 (1.22)	13.17 (1.10)	13.23 (1.03)	13.20 (1.05)	13.148 (1.18)
No. (%)								
Education								
0-11 years	38 (6.0)	32 (10.4)**	3 (3.1)	35 (8.6)**	2 (3.4)*	6 (6.7)	8 (5.4)*	43 (7.8)**
HS grad/votec	137 (21.5)	87 (28.3)	29 (29.6)	116 (28.6)	23 (39.0)	26 (29.2)	49 (33.1)	165 (29.8)
Some College	265 (41.6)	115 (37.5)	33 (33.7)	148 (36.5)	16 (27.1)	30 (33.7)	46 (31.1)	194 (35.1)
BS/BA/MS	197 (30.9)	73 (23.8)	33 (33.7)	106 (26.2)	18 (30.5)	27 (30.3)	45 (30.4)	151 (27.3)
Ethnic Group								
White	572 (89.8)	267 (87)	87 (88.8)	354 (87.4)	49 (83.1)	81 (91.0)	130 (87.8)	484 (87.5)
Hispanic	33 (5.2)	24 (7.8)	4 (4.1)	28 (6.9)	7 (11.9)	3 (3.4)	10 (6.8)	38 (6.9)
Other	32 (5.0)	16 (5.2)	7 (7.1)	23 (5.7)	3 (5.1)	5 (5.6)	8 (5.4)	31 (5.6)
Income								
$0-20,000	122 (19.6)	79 (26.7)	18 (19.0)	97 (24.01)	11 (19.6)	18 (21.4)	29 (20.7)	126 (23.7)
$20-30,000	155 (24.9)	73 (24.7)	18 (19.0)	91 (22.52)	17 (30.4)	17 (20.2)	34 (24.3)	125 (23.5)
$30-40,000	115 (18.5)	46 (15.5)	20 (21.1)	66 (16.34)	10 (17.9)	18 (21.4)	28 (20.0)	94 (17.7)
$40-50,000	78 (12.5)	32 (10.8)	17 (17.9)	49 (12.13)	6 (10.71)	9 (10.7)	15 (10.7)	64 (12.1)
$50,000+	153 (24.6)	66 (22.3)	22 (23.2)	88 (21.78)	12 (21.4)	22 (26.2)	34 (24.3)	122 (23.0)
Alcohol Exposure [6]								
Yes	40 (6.3)	19 (6.2)	7 (7.1)	26 (6.4)	4 (6.8)	12 (13.5)*	16 (10.8)*	42 (7.6)
No	597 (93.7)	287 (93.8)	91 (92.9)	378 (93.6)	55 (93.2)	77 (86.5)	132 (89.2)	510 (92.4)
Active Smoking [7]	49 (8.4)	40 (14.3)**	13 (13.7)	53 (14.1)**	9 (17.6)*	12 (14.8)	21 (15.9)**	74 (14.6)***
Passive Smoking [7]	69 (11.4)	45 (15.8)	11 (11.8)	56 (14.8)	10 (19.2)	12 (14.8)	22 (16.5)	78 (15.3)
Religion								
LDS	508 (79.7)	237 (77.2)	80 (81.6)	317 (78.3)	41 (69.5)	65 (73)**	106 (71.6)**	423 (76.5)
Other	76 (11.9)	47 (15.3)	10 (10.2)	19 (4.69)	8 (13.6)	9 (10.1)	17 (11.5)	74 (13.4)
None	53 (8.3)	23 (7.5)	8 (8.2)	31 (7.7)	10 (16.9)	14 (15.7)	24 (16.2)	55 (9.9)
Marital Status								
Married/Live In	603 (94.7)	282 (91.9)	93 (94.9)	375 (92.6)	54 (91.5)	82 (92.1)	136 (91.9)	511 (92.4)
Other	34 (5.3)	25 (8.1)	5 (5.1)	30 (7.4)	5 (8.5)	7 (7.9)	12 (8.1)	42 (7.6)
Vitamin Supplementation [8]								
Yes	578 (90.7)	278 (90.8)	90 (91.8)	36 (8.9)	55 (93.2)	87 (97.8)*	142 (95.9)*	510 (92.4)
No	59 (9.3)	28 (9.2)	8 (8.2)	368 (91.1)	4 (6.8)	2 (2.2)	6 (4.1)	42 (7.6)
Child's gender								
Male	391 (61.4)	194 (63.2)	45 (45.9)**	239 (59.0)	44 (74.6)***	50 (56.2)	94 (63.5)	333 (60.2)
Female	246 (38.6)	113 (36.8)	53 (54.1)	166 (41.0)	14 (23.7)	39 (43.8)	53 (35.8)	219 (39.6)
Parity [9]								
1	198 (31.1)	87 (28.3)	37 (37.8)	124 (30.6)	21 (35.6)	32 (36.0)	53 (35.8)	177 (32.0)
2	136 (21.4)	80 (26.1)	21 (21.4)	101 (24.9)	12 (20.3)	19 (21.3)	31 (20.9)	132 (23.9)
3	138 (21.7)	56 (18.2)	19 (19.4)	75 (18..5)	8 (13.6)	10 (11.2)	18 (12.2)	93 (16.8)
4	76 (11.9)	38 (12.4)	13 (13.3)	51 (12.6)	9 (15.3)	13 (14.6)	22 (14.9)	73 (13.2)
5+	89 (14.0)	46 (15.0)	8 (8.2)	54 (13.3)	9 (15.3)	15 (16.9)	24(16.2)	78 (14.1)

*p≤0.05 **p≤0.01 ***p≤0.001

1. N may differ slightly due to missing data; percentages may not add to 100 due to rounding
2. Maternal age at time of birth of index child
3. Self-reported height
4. Self-reported weight at birth of the index child (p-values calculated with inverse log transformation)
5. BMI calculated from self-reported height/weight data (p-values calculated with inverse transformation)
6. Any alcohol exposure during the first trimester
7. Periconceptional smoke exposure
8. Multivitamin and/or folate use during pregnancy
9. Parity at birth of the index child

Table 3-2: Characteristics of active smokers vs. non-smokers separate by case and control status[1]

	All Clefts		Controls	
	Smoker	Non-smoker	Smoker	Non-Smoker
Mean (SD)				
Age [2]	22.97 (5.88)***	27.32 (5.38)	22.43 (4.21)***	26.83 (5.07)
Height [3]	64.65 (2.83)	65.18 (2.72)	64.5 (2.47)*	65.34 (2.67)
Weight [4]	143.69 (30.4)	148.19 (33.68)	147.83 (35.73)	145.57 (30.58)
BMI [5]	24.23 (5.03)	24.53 (5.13)	25.09 (6.38)	24.04 (4.96)
Hemoglobin	13.42 (1.22)**	13.09 (1.18)	13.49 (1.23)**	13.01 (1.21)
(%)				
Education				
0-11 years	25.7***	3.2	28.6***	3.0
HS grad/votec	56.8***	23.1	49.0***	17.0
Some College	16.2***	40.0	16.3***	44.1
BS/BA/MS	1.4***	33.7	6.1***	35.9
Ethnic Group				
White	85.1	89.8	83.7	90.8
Hispanic	5.4	5.5	6.1	4.7
Other	9.5	4.6	10.2	4.5
Income				
$0-20,000	45.16***	19.16	44.68***	16.35
$20-30,000	30.65***	21.73	40.43***	24.33
$30-40,000	9.68***	19.16	6.38***	19.01
$40-50,000	6.45***	13.08	4.26***	13.30
$50,000+	8.06***	26.87	4.26***	26.81
Alcohol Exposure [6]				
Yes	29.7***	3.07	38.8***	2.6
No	70.3***	97.0	61.2***	97.4
Religion				
LDS	48.6***	84.3	38.8***	86.4
Other	16.2***	11.1	34.7***	8.8
None	35.1***	4.6	26.5***	4.9
Marital Status				
Married/Live In	75.7***	97.9	73.5***	97.9
Other	24.3***	2.1	26.5***	2.1
Vitamin Supplementation [7]				
Yes	91.9	93.1	83.7	91.4
No	8.1	6.9	16.3	8.6
Child's gender				
Male	47.3*	61.8	55.1	62.2
Female	52.7*	38.2	44.9	37.8
Parity [8]	2.0	2.0	2.0	2.0

*p≤0.05 **p≤0.01 ***p≤0.001

1. Active smoking exposure during the periconceptional period
2. Age of the mother at the birth of the index child
3. Self reported height data
4. Self reported weight data at the time of the birth of the index child (p-values calculated with inverse log transformation)
5. BMI calculated from self-reported height/weight data (p-values calculated with inverse transformation)
6. Any alcohol exposure during the first trimester
7. Multivitamin and/or folate use during pregnancy
8. Median parity at birth of the index child

reported smoking. That is 12.7% of the study population and corresponds well with the CDC's reported smoking rates for Utah of 13% (58). Fortunately, the data also show a strong pattern of smoking cessation over the course of pregnancy. This is true of the study population as a whole, as smoking rates declining from 12.7 pre-conceptually, to 10.5%, 5.03%, and 4.61% during the first, second, and third trimesters respectively. Interestingly enough, the biggest decline in smoking rates among the participants is between the first and second trimesters. Unfortunately since the relevant exposure period for cleft formation is during the early period of fetal development, well before the second trimester, the huge decline in smoking rates between months 3 and 4 may be too late during the pregnancy to effectively protect against the physiological effects of smoking. Figure 3-1 illuminates an even more important finding regarding smoking exposure among participants, namely that there is a difference in smoking rates between cases and control. For all cleft cases considered together the smoking rates were: 15.2% pre-conceptually, 13.4% during the first semester, 6.52% for the second trimester and 5.79% for the third trimester. This pattern can also be seen when comparing controls to all the subgroups of clefts (Figure 3-1).

Table 3-3 shows the unadjusted and adjusted odds ratios for active, passive, and both active and passive smoking during the first trimester by oral cleft subgroups. Odds ratios ranged from 1.73 for isolated CP to 2.34 for CLP multiple. For active smokers, all of the lower bounds of the 95% confidence intervals are well above 1.0, except in the cases of isolated and multiple CP, indicating that the increased risk from smoking before adjustment is significant. Risk attribuTable to passive smoking was insignificant, and for

those with both active and passive exposure, the odds ratios changed little from the active smoking exposure alone, however, the confidence intervals did change slightly.

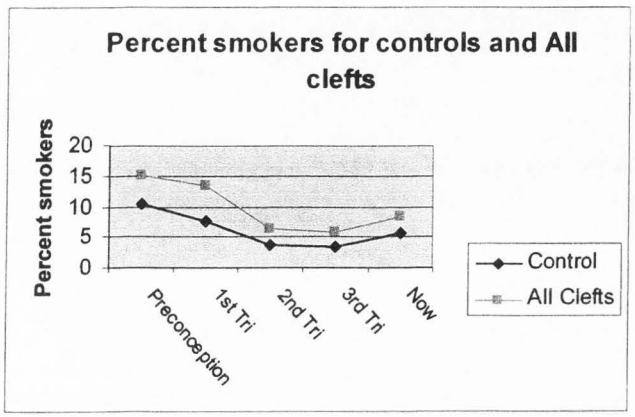

Figure 3-1. Percentages of case mothers (all cleft types) and control mother's who reported smoking over the course of pregnancy, Utah Child and Family Health Study.

After adjusting for mother's age, BMI, race, education, income, alcohol consumption, vitamin supplementation, hemoglobin quartiles and child's gender, the odds ratios did change slightly, but the overall risk trends were the same as for the unadjusted risk estimates. These results are also found in Table 3-3 (page 94). Some caution should be used when comparing the two sets of odds ratios however, as controlling for hemoglobin, the only model parameter to come from the lab data, reduced the sample size considerably, approximately 30% for cases overall. Nonetheless, active smoking during the periconceptional period is significantly associated with all cleft groups combined with an odds ratio of 1.82 and confidence interval 1.02 to 3.24.

Literature exploring the dangers of smoking during pregnancy clearly indicates that smokers have elevated hemoglobin levels. This is a physiological response on part of the body to compensate for the decreased oxygen transport due to the build up of

Table 3-3: Adjusted[1] and unadjusted odds ratios for cleft type by first trimester smoking

	Unadjusted Odds Ratios									Adjusted Odds Ratios								
	Active Smoking			Passive Smoking			Active and Passive Smoking			Active Smoking			Passive Smoking			Active and Passive Smoking		
	N	OR	95% CI	N	OR	95% CI	N	OR	95% CI	N	OR	95% CI	N	OR	95% CI	N	OR	95% CI
CLP	280	1.82	1.17-2.8	285	1.45	0.97-2.18	267	1.82	1.07-3.1	203	1.75	0.88-3.47	206	1.07	0.58-1.97	194	1.13	0.75-1.7
CP	95	1.73	0.90-3.3	93	1.04	0.53-2.05	91	1.78	0.82-3.9	67	2.23	0.77-6.46	65	1.14	0.397-3.28	64	1.47	0.78-2.8
Isolated	375	1.79	1.19-2.7	378	1.35	0.92-1.9	358	1.81	1.1-2.9	270	1.71	0.915-3.21	271	1.05	0.595-1.85	258	1.15	0.79-1.7
CLP mult[2]	51	2.34	1.08-5.09	52	1.85	0.89-3.8	48	2.32	0.92-5.8	33	5.33	1.31-21.62	31	1.31	0.34-4.49	30	1.828	0.76-4.4
CP mult	81	1.89	0.96-3.7	81	1.35	0.69-2.6	76	1.65	0.7-3.9	55	2.13	0.64-7.14	53	0.85	0.256-2.82	51	1.034	0.47-2.3
Multiple	716	2.06	1.2-3.6	133	1.54	0.91-2.6	124	1.89	0.97-3.7	88	2.65	1.04-6.78	84	1.04	.415-2.59	81	1.23	0.68-2.2
All Clefts	507	1.86	1.27-2.7	511	1.39	0.99-1.98	482	1.84	1.16-2.9	358	1.82	1.02-3.24	355	1.04	.611-1.76	339	1.15	0.81-1.6

1- Adjusted for maternal BMI, education, ethnicity, alcohol consumption, income, vitamin supplementation, parity and gender of child.
2- Mult refers to non-isolated clefts cases or cases with multiple birth defects

carboxyhemoglobin in the blood. This phenomenon can be seen in the present data as well. Table 3-2 clearly shows that there is a substantial and significant association between smoking and hemoglobin levels and that relationship holds true for both cases and controls. Table 3-1 does show an increase in hemoglobin among cases versus controls, although not enough of a difference is detected to obtain statistical significance. Table 3-4 shows the percentage of study participants who were clinically anemic based on the CDC standards of anemia with cutoffs at 12.0g/dl for non-smokers, and 12.3 g/dl for smokers. As would be expected for a population in a developed country, anemia levels are low, with approximately 7 to 10 percent of women with clinical anemia. The difference in anemia rates between cases and controls was not significant. After controlling for anemia status, smoking still produced an increased risk of clefting as shown in Table 3-5, and Breslow-Day statistics show no significant difference between odds ratios for smoking and clefts after stratification for anemia.

Table 3-4: Percentage of smokers and non-smokers by cases and control status, stratified by anemia [1]

	Anemic		Non-Anemic	
	Control	Case	Control	Case
Non-Smoker	67 (93.06%)	53 (89.83%)	306 (90.8%)	265 (84.9%)
Smoker	5 (6.94%)	6 (10.17%)	31 (9.2%%)	47 (15.06%)[2]

1 Anemia is defined according to the CDC criteria of 12 g/dL for non-smokers and 12.3 g/dL for smokers
2 Chi square p-value <0.05 between cases and controls within anemia

Table 3-5: Risk of Clefts (all types combined) associated with smoking, stratified by anemia status[1]

	Cases	Controls	Odds Ratios (95% CI)
Anemic	59	72	1.5 (0.44-5.24)
Non-Anemic	312	337	1.75 (1.08-2.8)[2]

1 Anemia is defined according to the CDC criteria of 12 g/dL for non-smokers and 12.3 g/dL for smokers
2 Breslow Day test for homogeneity of odds ratios is insignificant (p=0.833)

Assesment of clefting risk based on quartiles of hemoglobin also did not show any indication of an effect of maternal hemoglobin levels on the risk of having a child with a cleft. Table 3-6 shows the distribution of hemoglobin levels for cases and controls and Table 3-7 shows odds ratios associated with those levels of hemoglobin both crude and adjusted. With all point estimates and confidence intervals centrally located around 1, it is safe to conclude that there is no discernable risk increase for clefting with maternal hemoglobin.

Table 3-6: Percentage of cases and controls by hemoglobin quartiles, stratified by active smoking status[1]

	Non-smokers		Smokers	
	Cases	Controls	Cases	Controls
Quartile 1	80 (25.16%)	105 (28.15%)	9 (16.98%)	6 (16.7%)
Quartile 2	80 (25.16%)	89 (23.86%)	12 (22.6%)	7 (19.4%)
Quartile 3	84 (26.42%)	101 (27.08%)	14 (26.4%)	9 (25.0%)
Quartile 4	74 (23.27%)	78 (20.91%)	18 (33.96%)	14 (38.9%)

1 All chi squared p-values for Tables (2x4) are insignificant

Table 3-7: Adjusted and unadjusted odds ratios and 95% confidence intervals for risk of cleft (all types combined) by level of hemoglobin

	All Clefts	Controls	Odds Ratios (95% Confidence Interval)		
	N	N	Crude	Adjusted [1]	Adjusted[2]
Quartile 1	89	111	.78 (.53-1.14)	.808 (.53-1.24)	.843 (.56-1.26)
Quartile 2	92	96	0.99 (0.67-1.46)	.988 (.64-1.52)	.995 (.66-1.49)
Quartile 3	98	110	0.88 (0.60-1.29)	.938 (.62-1.43)	.920 (.62-1.37)
Quartile 4	92	92	1.0 (ref)	1.0 (ref)	1.0 (ref)

1 Controlling for active smoking only
2 Adjusted for education, income, ethnicity, BMI, mother's age, vitamin supplementation, parity, alcohol consumption, child's gender and active smoking

Log likelihood test statistics were used to compare logistic regression models both with and without smoking and hemoglobin interaction terms. These comparisons showed

little to no added benefit to having interaction terms in the model, another indication that hemoglobin and anemia have little if any bearing on clefting in this population (Appendix B).

Discussion

There is a considerable amount of literature on smoking and cleft birth defects, however a number of those studies have had methodological flaws, which makes interpretation difficult (4, 5). Some of the studies were looking for associations between smoking and clefts, with clefts being only a small subgroup of general birth defects- these studies did not distinguish between different types of birth defects, and diseases that may have no pathological similarities were lumped together (25, 26, 43, 44). Other studies were of a prospective nature and even with huge amounts of available births to consider, there were only small numbers of cleft cases available for analysis. Also, many studies rely on birth certificate or registry data to assess smoking status, which leaves smoking exposure uncertain, both as to relevance of exposure times and exposure amount ie. number of cigarettes smoked (4). Data from birth registries may also lack imperative information about possible confounding factors that need to be controlled for in statistical analyses.

The design used in the Utah Child and Family Health Study follows closely the World Health Organization's recommendations for studies of clefts, which adds a measure of validity to the results that are presented herein (14, 59). First, the study is population-based with participation rates in the 70th percentile for cases and close to the 60th percentile for controls. Case status was confirmed using official Department of

Health records using standardized coding and detailed information was obtained to categorize cases as comprehensively as possible. All initial analysis was done with cleft groups separately so as to account for the cited differences in etiologies between different cleft subgroups. Only after finding consistent results for smoking across all groups were the cases combined to achieve higher statistical power. Detailed information was also obtained from participants regarding smoking behaviors during each trimester individually, including the number of cigarettes smoked. Information regarding a number of other factors pertinent for modeling purposes was also collected, including maternal education, income, race, alcohol consumption, vitamin supplementation, age and child's gender. The study also utilized materials like the birth calendar that may have helped the participants recall their diet and exposures more accurately. And although not used in this analysis, the study also has data from a food frequency questionnaire to help assess the participants' diets in future studies. Biological samples also contribute a tremendous amount of information about each participant for future analysis. The study also benefits from the availability of a large control group that, due to lifestyle habits, have lower exposure to cigarette and alcohol consumption than many other populations in the United States. Overall, this study is very comprehensive and since clinical trials to test the effects smoking and other exposures are undoubtedly out of the question due to ethical concerns, the Utah study has a lot to offer in terms of a comprehensive observational study.

With that in mind, data from the Utah Study of Child and Family Health confirms an increased risk of smoking and oral clefts across all subgroups of clefts. The current data supports the findings of the most recent meta-analysis for smoking and cleft

research, which also indicates that smoking is related to cleft formation and may have a corresponding attributable risk of up to 30% (4). The public health implications seem obvious. Especially, since smoking is highly correlated with education, it seems that educating women, especially those of child bearing age, is a crucial step in reducing maternal smoking and the risk of clefting. Hopefully, being aware that smoking may increase the risk of having a child with an oral cleft may serve as an incentive for younger women not to smoke. This is even more crucial considering the trends found in this study, indicating that even though there is a pattern of smoking cessation over the course of pregnancy, the most significant reduction in percent of smokers who quite happens between the first and second trimesters, after the critical embryonic growth period for the cleft region.

The addition of hemoglobin and anemia as cofactors in the current analysis is a new component in the arena of smoking and cleft research. One early study on birth defects did report significant effects of anemia, smoking and the interaction between the two on birth weight, but no further analysis has looked specifically at that combination of risk factors for clefts (25, 26). While the current results do not show any significant signs of anemia contributing to cleft risk, there may be reason not to dismiss the hypothesis in its entirety. First, the hypothesis has a firm foundation on biological plausibility. The role of hypoxia has been confirmed in several animal studies and carboxyhemoglobin levels have a dose dependent effect on clefting (46). Other populations that exhibit higher rates and more extreme cases of anemia among women of childbearing age may be better suited to assess those risks (50, 60, 61). Smoking also confounds the levels of hemoglobin in the blood, and other more sensitive measures of red blood cell health may

be needed to determine a more complete picture of how the two are related and to tease out the effects of inflated levels of hemoglobin in smokers (54). On that note, the smoking trends in this study show that the percent of women who were smoking at the time of the interviews was lower than the percent of women smoking before pregnancy and during the first trimester. The negative results of anemia in relation to clefting would be much more convincing if lab data was available for the participants at the time of pregnancy- not something that is practically possible even for future studies. Anemia itself is also a very complex physiological state, and a number of things contribute to the health and efficacy of the red blood cells besides iron, not a few of which are nutritional factors that are also suspected risk factors for clefts, like vitamin B6 and folate (62-68). Deficiencies in these and other nutritional biomarkers may be responsible for megoblastic anemia, and may decrease the oxygen distributing capacity of red blood cells without altering hemoglobin values (54). It could be argued that the biological mechanism by which folate, B12 and B6 contribute to cleft risk is by means of compromised oxygen carrying capacity of red blood cells. Without a doubt there will be many more years of research in both smoking and nutritional factors associated with clefts and hopefully there will be more in-depth research looking at the two in conjunction with one another.

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CHAPTER 4

SUMMARY AND CONCLUSION

Summary

Many factors, both genetic and environmental, are thought to be responsible for the formation of clefts in the lip or palate. The current database of literature is extensive, therefore the first objective of the research at hand was to review as thoroughly as possible the available information regarding cleft lip and palate birth defects (Chapter 2). Of particular interest were the many epidemiological studies on smoking and clefts, which is a main focus of this study, and a comprehensive review of those studies is presented in Table 2-2. Factors affecting nutritional anemias were also reviewed, as well as the relationship that anemia has with smoking status. An alternative mechanism by which smoking and anemia may contribute to cleft formation was set forth based on how the two conditions contribute to hypoxia, a known risk factor for clefts. Data from the population-based case control study of clefts in Utah, the Utah Child and Family Health Study, was analyzed to test for associations between maternal smoking, anemia and risk of cleft. The data suggests that smoking does indeed increase the risk of clefts across all subgroups, which coincides with the most current meta-analysis. In Utah where the prevalence of anemia is low, there was no indication of any association between anemia and clefting, and anemia did not have an effect on the risk estimates associated with smoking.

Conclusion

The findings from the Utah Child and Family Health Study bring to light several important public health issues regarding the prevention of cleft lip and palate. The analysis presented herein confirms that maternal smoking is a risk factor for clefts. The data also indicates that smoking rates are highest among women who are less educated, which makes educating women, particularly of low education and income status, a top priority for prevention programs. This is even more pertinent considering the startling data that shows that even when women stop smoking during pregnancy, many of them are not quitting early enough in the pregnancy to eliminate cigarette smoke as a risk factor for having a child with a cleft. Targeting young women and other women of child bearing age for prevention education will be an important step in stopping increasing numbers of women from taking up smoking.

Due to the fact that smoking also significantly alters nutritional status, and that poor nutrition can likewise have detrimental effect on birth outcome, monitoring the two conditions together should continue to gain importance. More sensitive and specific measures of nutritional health can be used in future studies to gain a more complete and accurate picture of how red blood cell health and the development of hypoxia in fetal tissue may contribute cleft formation.

APPENDICES

APPENDIX A: Utah Child and Family Health Study Interview Booklet

Utah State
UNIVERSITY

The Utah Child and Family Health Study

Telephone Interview Questionnaire

NIH Grant Number R01 HD39061

Center for Epidemiologic Studies
Department of Nutrition and Food Sciences
Utah State University
Logan, UT 84322-4450

May 6, 2002

INTRODUCTION TO THE INTERVIEW

1. Hello, (MOTHER'S NAME), this is (INTERVIEWER'S NAME) from Utah State University. This is the time we arranged for me to call you to do the telephone interview for the Utah Child and Family Health Study.

2. Do you have the materials that we sent to you? Those include the

Summary of the Utah Child and Family Health Study	YES	NO
Pregnancy Calendar	YES	NO
Diet Interview Card	YES	NO

3. REVIEW OF SUMMARY OF THE STUDY

   We'll talk more about the Pregnancy Calendar and Diet Interview Card later. Let's talk about the Summary of the study now. Did you have a chance to read the Summary of the study? Before we begin, I need to review the study procedures with you.

   As you know, at this time, I'd like to interview you about your pregnancy history and your diet and health shortly before and during your pregnancy (with CHILD'S NAME/that ended on DATE). After you complete the interview, we'll make arrangements to measure your height and weight and to obtain a small blood sample, equal to the amount in about one tablespoon, from your arm. We'll measure the levels of several vitamins and other nutrients and study the DNA in your blood sample.

   To make our genetic studies complete, we'd like to obtain samples of cells from inside your child's and his/her biological father's cheek to collect a small amount of genetic material or DNA. The cheek cells will be collected by gently brushing the inside of the cheek with a soft brush.

   Your participation in this study will contribute to knowledge that may lead to the prevention of cleft lip, cleft palate and related birth defects and improve the health of children in the future. There is no immediate health benefit to you or your family; however, your family will receive $50 for full participation in the study to compensate you for your time and effort. "Full participation" means that you've completed the interview and we've collected a blood sample from you and we've obtained cheek cell samples from (CHILD'S NAME) and (his/her) biological father. You'll receive less than $50 for less than full participation in the study.

   All research information will be treated in a confidential manner and you will not be personally identified in the reporting of results. Members of the research team will have access to study information only after they have signed an Agreement of Confidentiality.

   Your participation is entirely voluntary. If you refuse to participate there will be no penalty or loss of benefits to which you're otherwise entitled. You may stop your participation at any time.

4. Do you have questions about any of the information in the Summary? (ANSWER ANY QUESTIONS)

5. Do you agree to participate in this interview?

   YES..... 1
   NO..... 2

   Signature of Interviewer _____

6. Your responses in this interview will make a great contribution to our knowledge about the causes of cleft birth defects. Because of this, it's important that your answers be as accurate as possible. Let me explain a few things about the structure of the interview.

- All of the questions have been carefully designed and, for research purposes, must be asked exactly as they're written. Some questions may not seem applicable based on answers you've given previously but just answer them as best you can.

- If you don't understand a question, make sure that you ask me for clarification.

- If you're not sure about your answer, we'd like your best judgment. If you really can't remember something or you just don't know, it's OK to say, "I don't know."

- We'll be talking mostly about your pregnancy (with CHILD'S NAME/that ended on DATE) and I'll make sure you know what time period we're referring to before I ask a question.

- The interview should last about an hour. If you need to take a break before we're finished, just tell me and we'll work it out.

7. Do you have any other questions before we start?

B. MOTHER'S DEMOGRAPHICS First I'd like to ask you a few questions about your background.	
B1. In what state were you born?	UTAH ..... 1 OTHER (SPECIFY) ..... 2 SPECIFY STATE OR COUNTRY: _____ ☐☐☐
B2. What is your birth date?	MONTH ☐☐ DAY ☐☐ YEAR ☐☐☐☐
B3. What is your maiden name?	_____
B4a. At the time (CHILD'S NAME was born/ that your pregnancy ended), what was the total number of years you had lived in Utah?	NUMBER OF YEARS ..... ☐☐
B4b. Are you now a permanent resident of Utah?	YES ..... 1 NO 2
B4c. How long have you lived in your current residence?	LESS THAN 1 YEAR ..... 1 1-3 YEARS ..... 2 4-6 YEARS ..... 3 7-9 YEARS ..... 4 10 YEARS OR MORE 5
B5. What was your marital status when (CHILD'S NAME was born/your pregnancy ended) Were you ...	Married ..... 1 Living with someone as married ..... 2 Separated or divorced ..... 3 Widow/widower ..... 4 Never married ..... 5 RF 7

B6.	What racial or ethnic group do you consider yourself a part of? You may tell me more than one group. Would you consider yourself ... (IF MOTHER REPORTS MORE THAN ONE RACIAL GROUP, CODE "OTHER" AND SPECIFY GROUPS , I.E. BLACK AND HISPANIC) IF NEEDED, SEE DEFINITIONS ON PAGE 6.	American Indian or Alaska Native ..... 1 TRIBE _____ ☐☐ Asian ..... 2 COUNTRY _____ ☐☐ Black or African American ..... 3 Native Hawaiian or Other Pacific Islander ..... 4 GROUP _____ ☐☐ White ..... 5 Hispanic or Latino ..... 6 GROUP _____ ☐☐ Other ..... 7 SPECIFY _____ ☐☐ RF ..... 97 DK 98
B7.	At the time that (CHILD'S NAME was born/ your pregnancy ended), how many years of school had you completed?	NO FORMAL SCHOOLING ..... 1 1-6 YEARS ..... 2 7-8 YEARS ..... 3 9-11 YEARS ..... 4 HIGH SCHOOL GRADUATE OR GED ..... 5 VOCATIONAL ED. AFTER H.S. DIPLOMA OR GED ..... 6 SOME COLLEGE (INCLUDES AA DEGREE) ..... 7 COLLEGE GRADUATE (BS, BA) ..... 8 GRADUATE DEGREE (MS, MA, PH.D, MD, JD, DVM) ..... 9 RF ..... 97 DK 98
B8.	At the time that (CHILD'S NAME was born/ your pregnancy ended), what was your religious preference, if any? *NOTE: PROTESTANT INCLUDES METHODIST, BAPTIST, EPISCOPALIAN, LUTHERAN, PRESBYTERIAN, AND CHURCH OF ENGLAND	CATHOLIC ..... 1 EASTERN ORTHODOX (GREEK OR RUSSIAN) ..... 2 ISLAM ..... 3 JEWISH ..... 4 LDS (MORMON) ..... 5 PROTESTANT* ..... 6 SEVENTH DAY ADVENTIST ..... 7 OTHER (SPECIFY) ..... 8 SPECIFY: _____ ☐☐ NO RELIGIOUS PREFERENCE ..... 9 RF 97

B9.	Did <u>you</u> have a congenital anomaly or malformation at birth or a birth defect that was found at any time thereafter? This doesn't include minor conditions like birthmarks, skin tags, postnatal jaundice, etc. (SEE LIST BELOW FOR EXAMPLES OF SOME MINOR BIRTH DEFECTS)	YES (LIST BELOW).....1 NO .....2 DK .....8 SPECIFY: _____ ☐☐☐.☐☐☐ _____ ☐☐☐.☐☐☐ _____ ☐☐☐.☐☐☐

MINOR BIRTH DEFECTS – DO NOT LIST IN B9

Umbilical hernia
Café au lait spots
Birthmark
Port wine stain (Flammus Nevus)
Tongue tie
Lop ear

DEFINITIONS OF RACIAL AND ETHNIC CATEGORIES (Question B6)

American or Alaska Native: A person having origins in any of the original peoples of North, Central, or South America, and who maintains tribal affiliation or community attachment.

Asian: A person having origins in any of the original peoples of the Far East, Southeast Asia, or the Indian subcontinent including, for example, Cambodia, China, India, Japan, Korea, Malaysia, Pakistan, the Philippine Islands, Thailand, and Vietnam. (Note: Individuals from the Philippine Islands have been recorded as Pacific Islanders in previous data collection strategies).

Black or African American: A person having origins in any of the black racial groups of Africa. Terms such as "Haitian" or "Negro" can be used in addition to "Black" or "African American."

Native Hawaiian or Other Pacific Islander: A person having origins in any of the original people of Hawaii, Guam, Samoa, or other Pacific Islands (including Australian Aborigine and New Zealand Maori).

White: A person having origins in any of the original peoples of Europe, the Middle East, or North Africa.

Hispanic or Latino: A person of Cuban, Mexican, Puerto Rican, South or Central American, or other Spanish culture or origin, regardless of race. The term, "Spanish origin," can be used in addition to "Hispanic or Latino".

C. BIOLOGICAL FATHER'S DEMOGRAPHICS

LIVE BIRTH/STILLBIRTH: Now I'd like to ask you a few questions about (CHILD's NAME/ the baby)'s biological father's background.

MISCARRIAGE/ABORTION: Now I'd like to ask you a few questions about the biological father involved in your pregnancy that ended on (DATE).

C1.	In what state was (the /CHILD'S NAME's) biological father born?	UTAH.....1 OTHER (SPECIFY).....2 RF.....7 DK.....8 SPECIFY STATE OR COUNTRY: _____ ☐☐☐
C2.	What is his birth date?	MONTH ☐☐ DAY ☐☐ YEAR ☐☐☐☐ RF.....7 DK.....8
C3a.	At the time that (CHILD'S NAME was born/ your pregnancy ended), what was the total number of years he had lived in Utah?	NUMBER OF YEARS..... ☐☐ RF.....7 DK.....8
C3b.	Is he now a permanent resident of Utah?	YES.....1 NO.....2 RF.....7 DK.....8
C3c.	How long has he lived in his current residence?	LESS THAN 1 YEAR.....1 1-3 YEARS.....2 4-6 YEARS.....3 7-9 YEARS.....4 10 YEARS OR MORE.....5 RF.....7 DK.....8
C4.	What was the father's marital status when (CHILD'S NAME was born/ your pregnancy ended)? Was he...	Married .....1 Living with someone as married.....2 Separated or divorced.....3 Widow/widower.....4 Never married .....5 RF.....7 DK.....8

C5.	What racial group or racial groups does he consider himself a part of? You may tell me more than one group. (IF FATHER REPORTS MORE THAN ONE RACIAL GROUP, I.E. BLACK AND HISPANIC, CODE "OTHER" AND SPECIFY GROUPS) IF NEEDED SEE DEFINITIONS ON PAGE 9.	American Indian or Alaska Native ..... 1 TRIBE _____ ☐☐ Asian ..... 2 COUNTRY _____ ☐☐ Black or African American ..... 3 Native Hawaiian or Other Pacific Islander ..... 4 GROUP _____ ☐☐ White ..... 5 Hispanic or Latino ..... 6 GROUP _____ ☐☐ Other ..... 7 SPECIFY _____ ☐☐ RF ..... 97 DK 98
C6.	At the time that (CHILD'S NAME was born/ your pregnancy ended), how many years of school had the father completed?	NO FORMAL SCHOOLING ..... 1 1-6 YEARS ..... 2 7-8 YEARS ..... 3 9-11 YEARS ..... 4 HIGH SCHOOL GRADUATE OR GED ..... 5 VOCATIONAL ED. AFTER H.S. DIPLOMA OR GED ..... 6 SOME COLLEGE (INCLUDES AA DEGREE) ..... 7 COLLEGE GRADUATE (BS, BA) ..... 8 GRADUATE DEGREE (MS, MA, PH.D, MD, JD, DVM) ..... 9 RF ..... 97 DK 98
C7.	At the time that (CHILD'S NAME was born/ your pregnancy ended) what was the father's religious preference, if any? *NOTE: PROTESTANT INCLUDES METHODIST, BAPTIST, EPISCOPALIAN, LUTHERAN, PRESBYTERIAN, AND CHURCH OF ENGLAND	CATHOLIC ..... 1 EASTERN ORTHODOX (GREEK OR RUSSIAN) ..... 2 ISLAM ..... 3 JEWISH ..... 4 LDS (MORMON) ..... 5 PROTESTANT* ..... 6 SEVENTH DAY ADVENTIST ..... 7 OTHER (SPECIFY) ..... 8 SPECIFY: _____ ☐☐ NO RELIGIOUS PREFERENCE ..... 9 RF ..... 97 DK 98

C8.	Did the father have a congenital anomaly or malformation at birth or a birth defect that was found at any time thereafter? This doesn't include minor conditions like birthmarks, skin tags, postnatal jaundice, etc. (SEE LIST BELOW FOR EXAMPLES OF MINOR BIRTH DEFECTS)	YES (LIST BELOW) ..... 1 NO ..... 2 DK ..... 8 SPECIFY: _____ ☐☐☐.☐☐☐ _____ ☐☐☐.☐☐☐ _____ ☐☐☐.☐☐☐

MINOR BIRTH DEFECTS – DO NOT LIST IN C8

Umbilical hernia
Café au lait spots
Birthmark
Port wine stain (Flammus Nevus)
Tongue tie
Lop ear

DEFINITIONS OF RACIAL AND ETHNIC CATEGORIES (Question C5)

American or Alaska Native: A person having origins in any of the original peoples of North, Central, or South America, and who maintains tribal affiliation or community attachment.

Asian: A person having origins in any of the original peoples of the Far East, Southeast Asia, or the Indian subcontinent including, for example, Cambodia, China, India, Japan, Korea, Malaysia, Pakistan, the Philippine Islands, Thailand, and Vietnam. (Note: Individuals from the Philippine Islands have been recorded as Pacific Islanders in previous data collection strategies).

Black or African American: A person having origins in any of the black racial groups of Africa. Terms such as "Haitian" or "Negro" can be used in addition to "Black" or "African American."

Native Hawaiian or Other Pacific Islander: A person having origins in any of the original people of Hawaii, Guam, Samoa, or other Pacific Islands (including Australian Aborigine or New Zealand Maori).

White: A person having origins in any of the original peoples of Europe, the Middle East, or North Africa.

Hispanic or Latino: A person of Cuban, Mexican, Puerto Rican, South or Central American, or other Spanish culture or origin, regardless of race. The term, "Spanish origin, " can be used in addition to "Hispanic or Latino".

INTERVIEWER INFORMATION
DEFINITIONS OF PREGNANCY RESULTS

STILLBIRTH:	PREGNANCY LASTED MORE THAN 4 MONTHS; INFANT DIED BEFORE BIRTH.
MISCARRIAGE:	PREGNANCY LASTED 4 MONTHS OR LESS.
ABORTION/TERMINATION:	PREGNANCY INTENTIONALLY TERMINATED.
TUBAL/ECTOPIC:	FERTILIZED EGG DEVELOPED OUTSIDE OF THE WOMB (IN STOMACH, OVARY, TUBES, ETC.).
MOLAR PREGNANCY:	RESULT OF AN ABNORMAL PREGNANCY WHEN THE FERTILIZED EGG DOESN'T DEVELOP AND THE PLACENTA OVERGROWS AND THICKENS, SOMETIMES BECOMING A TUMOR.

D. PREGNANCY HISTORY
The next questions are about your pregnancy history and fertility.

D1.	How old were you when you had your first menstrual period?	AGE IN YEARS ☐☐ DK 98
D2.	How many times have you been pregnant in the past, including all pregnancies that may have ended in a live birth, miscarriage, stillbirth, termination, abortion, or a tubal or molar pregnancy? If you're pregnant now, don't count that pregnancy in this number.	TOTAL NUMBER OF PAST PREGNANCIES ☐☐
D3.	Which one was the pregnancy (with CHILD'S NAME/ that ended on DATE)?	NUMBER OF INDEX PREGNANCY ☐☐

FIRST PREGNANCY:
Now I'm going to ask you some questions about each of your pregnancies, starting with your first one.

D4.	How old were you when you became pregnant for the first time?	AGE IN YEARS ⌊__⌋
D5.	What was the result of your first pregnancy? By this I mean was it a live birth, stillbirth or some other outcome?	LIVE BIRTH ..... 1 STILLBIRTH (GO TO D7) ..... 2 MISCARRIAGE (GO TO D7) ..... 3 ABORTION (GO TO D7) ..... 4 TUBAL/ECTOPIC (GO TO D7) ..... 5 MOLAR PREGNANCY (GO TO D7) ..... 6 OTHER ..... 7 SPECIFY _____ RF (GO TO CHECKPOINT #2, PG. 13) ..... 97 DK (GO TO CHECKPOINT #2, PG. 13) 98
D6a.	LIVE BIRTH: Was the pregnancy full-term or did it end early or late? (IF EARLY OR LATE, ASK AND RECORD NUMBER OF WEEKS)	Full term (ON TIME)..... 1 Early (PREMATURE)..... 2 Late (OVER-DUE)..... 3 BY ⌊__⌋ WEEKS
D6b.	LIVE BIRTH: What date did your first pregnancy end?	MONTH ⌊__⌋ DAY ⌊__⌋ YEAR ⌊__⌋⌊__⌋
D6c.	LIVE BIRTH: How many babies did you carry in your first pregnancy?	TOTAL NUMBER OF BABIES ⌊__⌋
D6d.	LIVE BIRTH: What was the (first) baby's name?	PRINT FULL NAME: _____
D7.	INDEX BABY? (LIVE BIRTH GO TO D9)	YES ..... 1 NO 2
D8a.	STILL/MIS/AB/TUB/MOL: How far along were you when the pregnancy ended? PROBE TO GET BEST ESTIMATE OF # WEEKS (CONVERT MONTHS TO WEEKS IF NECESSARY).	⌊__⌋ WEEKS
D8b.	STIL/MIS/AB/TUB/MOL: Do you know how many babies you were carrying?	TOTAL NUMBER OF BABIES ⌊__⌋
D8c.	STILL/MIS/AB/TUB/MOL: Were you told anything about the baby you lost, such as its sex or if there were any apparent health problems?	YES ..... 1 NO (GO TO CHECKPOINT # 1) 2

D9.	What (is/was) (CHILD'S NAME/ the baby)'s sex?	MALE ..... 1 FEMALE ..... 2 UNKNOWN 3
D10.	Did this baby have a cleft lip or cleft palate?	YES ..... 1 NO (GO TO D11) ..... 2 DK (GO TO D11) 8
D10a.	Please tell me the structures that were involved in the baby's cleft. Did the baby have a cleft lip?	YES ..... 1 NO (GO TO D10c) ..... 2 DK (GO TO D10c) 8
D10b.	Was the cleft on one side, both sides or in the middle of the lip? (IF ONE SIDE REPORTED, ASK WHETHER RIGHT OR LEFT.)	RIGHT SIDE ONLY ..... 1 LEFT SIDE ONLY ..... 2 BOTH SIDES (BILATERAL) ..... 3 MIDLINE ..... 4 DK 8
D10c.	Was there a cleft of the palate, that is the roof of the mouth?	YES ..... 1 NO (GO TO D10e) ..... 2 DK (GO TO D10e) 8
D10d.	Did it involve only the soft part at the rear of the palate or both the soft and hard parts of the palate?	SOFT PALATE ONLY ..... 1 SOFT AND HARD PALATE ..... 2 DK 8
D10e.	Did the cleft involve the gums and teeth?	YES ..... 1 NO ..... 2 DK 8
D11.	Did this baby have any other birth defects?	YES (LIST BELOW) ..... 1 NO ..... 2 DK ..... 8 SPECIFY: _____ ☐☐☐.☐☐☐ _____ ☐☐☐.☐☐☐ _____ ☐☐☐.☐☐☐

#1	INTERVIEWER CHECKPOINT:	SINGLE BABY: ONE PREGNANCY ONLY (GO TO D74, PG. 26) ..... 1 SINGLE BABY: ADDITIONAL PREGNANCIES (GO TO 2ND PREGNANCY, PG. 14) ..... 2 MULTIPLE BABIES IN THIS PREGNANCY (CONTINUE) 3

Now I'd like to ask some questions about the second baby in this pregnancy.

D12.	LIVE BIRTH: What was the second baby's name?	PRINT FULL NAME: _____
D13.	INDEX BABY? (LIVE BIRTH GO TO D15)	YES ..... 1 NO 2

D14.	STILL/MIS/AB/TUB/MOL: Were you told anything about the baby you lost, such as its sex or if there were any apparent health problems?	YES ..... 1 NO (GO TO CHECKPOINT # 2) 2
D15.	What (is/was) (CHILD'S NAME/ the second baby)'s sex?	MALE ..... 1 FEMALE ..... 2 UNKNOWN 3
D16.	Did this baby have a cleft lip or cleft palate?	YES ..... 1 NO (GO TO D17) ..... 2 DK (GO TO D17) 8
D16a.	Please tell me the structures that were involved in the baby's cleft. Did the baby have a cleft lip?	YES ..... 1 NO (GO TO D16c) ..... 2 DK (GO TO D16c) 8
D16b.	Was the cleft on one side, both sides or in the middle of the lip? (PROBE IF ONE SIDE REPORTED, ASK WHETHER RIGHT OR LEFT.)	RIGHT SIDE ONLY ..... 1 LEFT SIDE ONLY ..... 2 BOTH SIDES (BILATERAL) ..... 3 MIDLINE ..... 4 DK 8
D16c.	Was there a cleft of the palate, that is the roof of the mouth?	YES ..... 1 NO (GO TO D16e) ..... 2 DK (GO TO D16e) 8
D16d.	Did it involve only the soft part at the rear of the palate or both the soft and hard parts of the palate?	SOFT PALATE ONLY ..... 1 SOFT AND HARD PALATE ..... 2 DK 8
D16e.	Did the cleft involve the gums and teeth?	YES ..... 1 NO ..... 2 DK 8
D17.	Did this baby have any other birth defects?	YES (LIST BELOW) ..... 1 NO ..... 2 DK ..... 8 SPECIFY: _____ ☐☐☐.☐☐☐ _____ ☐☐☐.☐☐☐ _____ ☐☐☐.☐☐☐

#2	INTERVIEWER CHECKPOINT:	2 BABIES IN THIS PREGNANCY; 1 PREGNANCY ONLY (GO TO D74, PG. 26) ..... 1 2 BABIES IN THIS PREGNANCY; ADD'L PREGNANCIES (CONTINUE) ..... 2 3+ BABIES IN THIS PREGNANCY (GO TO PREG. CONTINUATION FORM) 3

SECOND PREGNANCY: Now I'd like to ask you a few questions about your second pregnancy.		
D18.	How old were you when you became pregnant for the second time?	AGE IN YEARS ⌊__⌋
D19.	What was the result of your second pregnancy? By this I mean was it a live birth, stillbirth or some other outcome?	LIVE BIRTH ..... 1 STILLBIRTH (GO TO D21) ..... 2 MISCARRIAGE (GO TO D21) ..... 3 ABORTION (GO TO D21) ..... 4 TUBAL/ECTOPIC (GO TO D21) ..... 5 MOLAR PREG (GO TO D21) ..... 6 OTHER (SPECIFY) ..... 7 SPECIFY _____ RF (GO TO CHECKPOINT #4 PG. 16) ..... 97 DK (GO TO CHECKPOINT #4 PG.16) 98
D20a.	LIVE BIRTH: Was the pregnancy full-term or did it end early or late? (IF EARLY OR LATE, ASK AND RECORD NUMBER OF WEEKS)	On time (FULL TERM) ..... 1 Early (PREMATURE) ..... 2 Late (OVER-DUE) ..... 3 BY ⌊__⌋ WEEKS
D20b.	LIVE BIRTH: What date did your second pregnancy end?	MONTH ⌊__⌋ DAY ⌊__⌋ YEAR ⌊__⌋⌊__⌋
D20c.	LIVE BIRTH: How many babies did you carry in your second pregnancy?	TOTAL NUMBER OF BABIES ⌊__⌋
D20d.	LIVE BIRTH: What was the (first) baby's name?	PRINT FULL NAME: _____
D21.	INDEX BABY? (LIVE BIRTH GO TO D23)	YES ..... 1 NO 2
D22a.	STILL/MIS/AB/TUB/MOL: How far along were you when the pregnancy ended? PROBE TO GET BEST ESTIMATE OF # WEEKS (CONVERT MONTHS TO WEEKS IF NECESSARY).	⌊__⌋ WEEKS
D22b.	STIL/MIS/AB/TUB/MOL: Do you know how many babies you were carrying?	TOTAL NUMBER OF BABIES ⌊__⌋
D22c.	STILL/MIS/AB/TUB/MOL: Were you told anything about the baby you lost, such as its sex or if there were any apparent health problems?	YES ..... 1 NO (GO TO CHECKPOINT # 3) 2
D23.	What (is/was) (CHILD'S NAME/ the baby)'s sex?	MALE ..... 1 FEMALE 2

D24.	Did this baby have a cleft lip or cleft palate?	YES ..... 1 NO (GO TO D25) ..... 2 DK (GO TO D25) 8
D24a.	Please tell me the structures that were involved in the baby's cleft. Did the baby have a cleft lip?	YES ..... 1 NO (GO TO D24c) ..... 2 DK (GO TO D24c) 8
D24b.	Was the cleft on one side or both sides of the lip? (PROBE IF ONE SIDE REPORTED, ASK WHETHER RIGHT OR LEFT.)	RIGHT SIDE ONLY ..... 1 LEFT SIDE ONLY ..... 2 BOTH SIDES (BILATERAL) ..... 3 MIDLINE ..... 4 DK 8
D24c.	Was there a cleft of the palate, that is the roof of the mouth?	YES ..... 1 NO (GO TO D24e) ..... 2 DK (GO TO D24e) 8
D24d.	Did it involve only the soft part at the rear of the palate or both the soft and hard parts of the palate?	SOFT PALATE ONLY ..... 1 SOFT AND HARD PALATE ..... 2 DK 8
D24e.	Did the cleft involve the gums and teeth?	YES ..... 1 NO ..... 2 DK 8
D25.	Did this baby have any other birth defects?	YES (LIST BELOW) ..... 1 NO ..... 2 DK ..... 8 SPECIFY: _____ ☐☐☐.☐☐☐ _____ ☐☐☐.☐☐☐ _____ ☐☐☐.☐☐☐

#3	INTERVIEWER CHECKPOINT:	SINGLE BABY: TWO PREGNANCIES ONLY (GO TO D74, PG. 26) ..... 1 SINGLE BABY: ADDITIONAL PREGNANCIES (GO TO 3RD PREGNANCY, PG. 17) ..... 2 MULTIPLE BABIES IN THIS PREGNANCY (CONTINUE) 3

Now I'd like to ask some questions about the second baby in this pregnancy.

D26.	LIVE BIRTH: What was the second baby's name?	PRINT FULL NAME: _____
D27.	INDEX BABY? (LIVE BIRTH GO TO D29)	YES ..... 1 NO 2

D28.	STILL/MIS/AB/TUB/MOL: Were you told anything about the baby you lost, such as its sex or if there were any apparent health problems?	YES ..... 1 NO (GO TO CHECKPOINT # 4) 2
D29.	What (is/was) (CHILD'S NAME/ the second baby)'s sex?	MALE ..... 1 FEMALE ..... 2 UNKNOWN 3
D30.	Did this baby have a cleft lip or cleft palate?	YES ..... 1 NO (GO TO D31) ..... 2 DK (GO TO D31) 8
D30a.	Please tell me the structures that were involved in the baby's cleft. Did the baby have a cleft lip?	YES ..... 1 NO (GO TO D30c) ..... 2 DK (GO TO D30c) 8
D30b.	Was the cleft on one side or both sides of the lip? (PROBE IF ONE SIDE REPORTED, ASK WHETHER RIGHT OR LEFT.)	RIGHT SIDE ONLY ..... 1 LEFT SIDE ONLY ..... 2 BOTH SIDES (BILATERAL) ..... 3 MIDLINE ..... 4 DK 8
D30c.	Was there a cleft of the palate, that is the roof of the mouth?	YES ..... 1 NO (GO TO D30e) ..... 2 DK (GO TO D30e) 8
D30d.	Did it involve only the soft part at the rear of the palate or both the soft and hard parts of the palate?	SOFT PALATE ONLY ..... 1 SOFT AND HARD PALATE ..... 2 DK 8
D30e.	Did the cleft involve the gums and teeth?	YES ..... 1 NO ..... 2 DK 8
D31.	Did this baby have any other birth defects?	YES (LIST BELOW) ..... 1 NO ..... 2 DK ..... 8 SPECIFY: _____ ☐☐☐.☐☐☐ _____ ☐☐☐.☐☐☐ _____ ☐☐☐.☐☐☐

#4	INTERVIEWER CHECKPOINT:	2 BABIES IN THIS PREGNANCY; 2 PREGNANCIES ONLY (GO TO D74, PG. 26) .... 1 2 BABIES IN THIS PREGNANCY; ADD'L PREGNANCIES (CONTINUE) ..... 2 3+ BABIES IN THIS PREGNANCY (GO TO PREG. CONTINUATION FORM) 3

THIRD PREGNANCY: Now I'd like to ask you a few questions about your third pregnancy.		
D32.	How old were you when you became pregnant for the third time?	AGE IN YEARS ☐☐
D33.	What was the result of your third pregnancy?	LIVE BIRTH ..... 1 STILLBIRTH (GO TO D35) ..... 2 MISCARRIAGE (GO TO D35) ..... 3 ABORTION (GO TO D35) ..... 4 TUBAL/ECTOPIC (GO TO D35) ..... 5 MOLAR PREG (GO TO D35) ..... 6 OTHER (SPECIFY) ..... 7 SPECIFY _____ RF (GO TO CHECKPOINT #6 PG. 19) ..... 97 DK (GO TO CHECKPOINT #6 PG. 19) 98
D34a.	LIVE BIRTH: Was the pregnancy full-term or did it end early or late? (IF EARLY OR LATE, ASK AND RECORD NUMBER OF WEEKS)	On time (FULL TERM) .. 1 Early (PREMATURE) .... 2 Late (OVER-DUE) ..... 3 BY ☐☐ WEEKS
D34b.	What date did your third pregnancy end?	MONTH ☐☐ DAY ☐☐ YEAR ☐☐☐☐
D34c.	LIVE BIRTH: How many babies did you carry in your third pregnancy?	TOTAL NUMBER OF BABIES ☐☐
D34d.	LIVE BIRTH: What was the (first) baby's name?	PRINT FULL NAME: _____
D35.	INDEX BABY? (LIVE BIRTH GO TO D37)	YES ..... 1 NO 2
D36a.	STILL/MIS/AB/TUB/MOL: How far along were you when the pregnancy ended? PROBE TO GET BEST ESTIMATE OF # WEEKS (CONVERT MONTHS TO WEEKS IF NECESSARY).	☐☐ WEEKS
D36b.	STIL/MIS/AB/TUB/MOL: Do you know how many babies you were carrying?	TOTAL NUMBER OF BABIES ☐☐
D36c.	STILL/MIS/AB/TUB/MOL: Were you told anything about the baby you lost, such as its sex or if there were any apparent health problems?	YES ..... 1 NO (GO TO CHECKPOINT # 5) 2

D37.	What (is/was) (CHILD'S NAME/ the baby)'s sex?	MALE ..... 1 FEMALE ..... 2 UNKNOWN 3
D38.	Did this baby have a cleft lip or cleft palate?	YES ..... 1 NO (GO TO D39) ..... 2 DK (GO TO D39) 8
D38a.	Please tell me the structures that were involved in the baby's cleft. Did the baby have a cleft lip?	YES ..... 1 NO (GO TO D38c) ..... 2 DK (GO TO D38c 8
D38b.	Was the cleft on one side or both sides of the lip? (PROBE IF ONE SIDE REPORTED, ASK WHETHER RIGHT OR LEFT.)	RIGHT SIDE ONLY ..... 1 LEFT SIDE ONLY ..... 2 BOTH SIDES (BILATERAL) ..... 3 MIDLINE ..... 4 DK 8
D38c.	Was there a cleft of the palate, that is the roof of the mouth?	YES ..... 1 NO (GO TO D38e) ..... 2 DK (GO TO D38e) 8
D38d.	Did it involve only the soft part at the rear of the palate or both the soft and hard parts of the palate?	SOFT PALATE ONLY ..... 1 SOFT AND HARD PALATE ..... 2 DK 8
D38e.	Did the cleft involve the gums and teeth?	YES ..... 1 NO ..... 2 DK 8
D39.	Did this baby have any other birth defects?	YES (LIST BELOW) ..... 1 NO ..... 2 DK ..... 8 SPECIFY: _____ ☐☐☐.☐☐☐ _____ ☐☐☐.☐☐☐ _____ ☐☐☐.☐☐☐

#5	INTERVIEWER CHECKPOINT:	SINGLE BABY: THREE PREGNANCIES ONLY (GO TO D74, PG. 26) ..... 1 SINGLE BABY: ADDITIONAL PREGNANCIES (GO TO 4TH PREGNANCY, PG. 20) ..... 2 MULTIPLE BABIES IN THIS PREGNANCY (CONTINUE) 3

Now I'd like to ask some questions about the second baby in this pregnancy.

D40.	LIVE BIRTH: What was the second baby's name?	PRINT FULL NAME: _____
D41.	INDEX BABY? (LIVE BIRTH GO TO D43)	YES ..... 1 NO 2

D42.	STILL/MIS/AB/TUB/MOL: Were you told anything about the baby you lost, such as its sex or if there were any apparent health problems?	YES ..... 1 NO (GO TO CHECKPOINT # 6) 2
D43.	What (is/was) (CHILD'S NAME/ the second baby)'s sex?	MALE ..... 1 FEMALE ..... 2 UNKNOWN 3
D44.	Did this baby have a cleft lip or cleft palate?	YES ..... 1 NO (GO TO D45) ..... 2 DK (GO TO D45 8
D44a.	Please tell me the structures that were involved in the baby's cleft. Did the baby have a cleft lip?	YES ..... 1 NO (GO TO D44c) ..... 2 DK (GO TO D44c) 8
D44b.	Was the cleft on one side or both sides of the lip? (PROBE IF ONE SIDE REPORTED, ASK WHETHER RIGHT OR LEFT.)	RIGHT SIDE ONLY ..... 1 LEFT SIDE ONLY ..... 2 BOTH SIDES (BILATERAL) ..... 3 MIDLINE ..... 4 DK 8
D44c.	Was there a cleft of the palate, that is the roof of the mouth?	YES ..... 1 NO (GO TO D44e) ..... 2 DK(GO TO D44e) 8
D44d.	Did it involve only the soft part at the rear of the palate or both the soft and hard parts of the palate?	SOFT PALATE ONLY ..... 1 SOFT AND HARD PALATE ..... 2 DK 8
D44e.	Did the cleft involve the gums and teeth?	YES ..... 1 NO ..... 2 DK 8
D45.	Did this baby have any other birth defects?	YES (LIST BELOW) ..... 1 NO ..... 2 DK ..... 8 SPECIFY: _____ ☐☐☐.☐☐☐ _____ ☐☐☐.☐☐☐ _____ ☐☐☐.☐☐☐

#6	INTERVIEWER CHECKPOINT:	2 BABIES IN THIS PREGNANCY; 3 PREGNANCIES ONLY (GO TO D74, PG. 26) ..... 1 2 BABIES IN THIS PREGNANCY; ADD'L PREGNANCIES (CONTINUE) ..... 2 3+ BABIES IN THIS PREGNANCY (GO TO PREG. CONTINUATION FORM) 3

FOURTH PREGNANCY:
Now I'd like to ask you a few questions about your fourth pregnancy.

D46.	How old were you when you became pregnant for the fourth time?	AGE IN YEARS ☐☐
D47.	What was the result of your fourth pregnancy?	LIVE BIRTH ..... 1 STILLBIRTH (GO TO D49) ..... 2 MISCARRIAGE (GO TO D49) ..... 3 ABORTION (GO TO D49) ..... 4 TUBAL/ECTOPIC (GO TO D49) ..... 5 MOLAR PREG (GO TO D49) ..... 6 OTHER (SPECIFY) ..... 7 SPECIFY _____ RF (GO TO CHECKPOINT #8, PG. 22) ..... 97 DK (GO TO CHECKPOINT #8, PG. 22) 98
D48a.	LIVE BIRTH: Was the pregnancy full-term or did it end early or late? (IF EARLY OR LATE, ASK AND RECORD NUMBER OF WEEKS)	On time (FULL TERM) .. 1 Early (PREMATURE) .... 2 Late (OVER-DUE) ..... 3 BY ☐☐ WEEKS
D48b.	LIVE BIRTH: What date did your fourth pregnancy end?	MONTH ☐☐ DAY ☐☐ YEAR ☐☐☐☐
D48c.	LIVE BIRTH: How many babies did you carry in your fourth pregnancy?	TOTAL NUMBER OF BABIES ☐☐
D48d.	LIVE BIRTH: What was the (first) baby's name?	PRINT FULL NAME: _____
D49.	INDEX BABY? (LIVE BIRTH GO TO D51)	YES ..... 1 NO 2
D50a.	STILL/MIS/AB/TUB/MOL: How far along were you when the pregnancy ended? PROBE TO GET BEST ESTIMATE OF # WEEKS (CONVERT MONTHS TO WEEKS IF NECESSARY).	☐☐ WEEKS
D50b.	STIL/MIS/AB/TUB/MOL: Do you know how many babies you were carrying?	TOTAL NUMBER OF BABIES ☐☐
D50c.	STILL/MIS/AB/TUB/MOL: Were you told anything about the baby you lost, such as its sex or if there were any apparent health problems?	YES ..... 1 NO (GO TO CHECKPOINT # 7) 2
D51.	What (is/was) (CHILD'S NAME/ the baby)'s sex?	MALE ..... 1 FEMALE ..... 2 UNKNOWN 3

D52.	Did this baby have a cleft lip or cleft palate?	YES ..... 1 NO (GO TO D53) ..... 2 DK (GO TO D53) 8
D52a.	Please tell me the structures that were involved in the baby's cleft. Did the baby have a cleft lip?	YES ..... 1 NO (GO TO D52c) ..... 2 DK (GO TO D52c) 8
D52b.	Was the cleft on one side or both sides of the lip? (PROBE IF ONE SIDE REPORTED, ASK WHETHER RIGHT OR LEFT.)	RIGHT SIDE ONLY ..... 1 LEFT SIDE ONLY ..... 2 BOTH SIDES (BILATERAL) ..... 3 MIDLINE ..... 4 DK 8
D52c.	Was there a cleft of the palate, that is the roof of the mouth?	YES ..... 1 NO (GO TO D52e) ..... 2 DK (GO TO D52e) 8
D52d.	Did it involve only the soft part at the rear of the palate or both the soft and hard parts of the palate?	SOFT PALATE ONLY ..... 1 SOFT AND HARD PALATE ..... 2 DK 8
D52e.	Did the cleft involve the gums and teeth?	YES ..... 1 NO ..... 2 DK 8
D53.	Did this baby have any other birth defects?	YES (LIST BELOW) ..... 1 NO ..... 2 DK ..... 8 SPECIFY: _____ ☐☐☐.☐☐☐ _____ ☐☐☐.☐☐☐ _____ ☐☐☐.☐☐☐

#7	INTERVIEWER CHECKPOINT:	
		SINGLE BABY: FOUR PREGNANCIES ONLY (GO TO D74, PG. 26) ..... 1 SINGLE BABY: ADDITIONAL PREGNANCIES (GO TO 5TH PREGNANCY, PG. 23) ..... 2 MULTIPLE BABIES IN THIS PREGNANCY (CONTINUE) 3

Now I'd like to ask some questions about the second baby in this pregnancy.

D54.	LIVE BIRTHS ONLY: What was the second baby's name?	PRINT FULL NAME: _____
D55.	INDEX BABY? (LIVE BIRTH GO TO D57)	YES ..... 1 NO 2
D56.	STILL/MIS/AB/TUB/MOL: Were you told anything about the baby you lost, such as its sex or if there were any apparent	YES ..... 1 NO (GO TO CHECKPOINT # 8) 2

D57.	What (is/was) (CHILD'S NAME/ the baby)'s sex?	MALE ..... 1 FEMALE ..... 2 UNKNOWN 3
D58.	Did this baby have a cleft lip or cleft palate?	YES ..... 1 NO (GO TO D59) ..... 2 DK (GO TO D59) 8
D58a.	Please tell me the structures that were involved in the baby's cleft. Did the baby have a cleft lip?	YES ..... 1 NO (GO TO D58c) ..... 2 DK (GO TO D58c) 8
D58b.	Was the cleft on one side or both sides of the lip? (PROBE IF ONE SIDE REPORTED, ASK WHETHER RIGHT OR LEFT.)	RIGHT SIDE ONLY ..... 1 LEFT SIDE ONLY ..... 2 BOTH SIDES (BILATERAL) ..... 3 MIDLINE ..... 4 DK 8
D58c.	Was there a cleft of the palate, that is the roof of the mouth?	YES ..... 1 NO (GO TO D58e) ..... 2 DK (GO TO D58e) 8
D58d.	Did it involve only the soft part at the rear of the palate or both the soft and hard parts of the palate?	SOFT PALATE ONLY ..... 1 SOFT AND HARD PALATE ..... 2 DK 8
D58e.	Did the cleft involve the gums and teeth?	YES ..... 1 NO ..... 2 DK 8
D59.	Did this baby have any other birth defects?	YES (LIST BELOW) ..... 1 NO ..... 2 DK ..... 8 SPECIFY: _____ ☐☐☐.☐☐☐ _____ ☐☐☐.☐☐☐ _____ ☐☐☐.☐☐☐

#8	INTERVIEWER CHECKPOINT:	2 BABIES IN THIS PREGNANCY; 4 PREGNANCIES ONLY (GO TO D74, PG. 26) ..... 1 2 BABIES IN THIS PREGNANCY; ADD'L PREGNANCIES (CONTINUE) ..... 2 ADD'L BABIES IN THIS PREGNANCY (GO TO PREG. CONTINUATION FORM) 3

FIFTH PREGNANCY:
Now I'd like to ask you a few questions about your fifth pregnancy.

D60.	How old were you when you became pregnant for the fifth time?	AGE IN YEARS ☐☐
D61.	What was the result of your fifth pregnancy?	LIVE BIRTH ..... 1 STILLBIRTH (GO TO D63) ..... 2 MISCARRIAGE (GO TO D63) ..... 3 ABORTION (GO TO D63) ..... 4 TUBAL/ECTOPIC (GO TO D63) ..... 5 MOLAR PREG (GO TO D63) ..... 6 OTHER (SPECIFY) ..... 7 SPECIFY _____ RF (GO TO CHECKPOINT #10, PG. 25) ..... 97 DK (GO TO CHECKPOINT #10, PG. 25) 98
D62a.	LIVE BIRTH: Was pregnancy full-term or did it end early or late? (IF EARLY OR LATE, ASK AND RECORD NUMBER OF WEEKS)	On time (FULL TERM) ... 1 Early (PREMATURE) ..... 2 Late (OVER-DUE) ..... 3 BY ☐☐ WEEKS
D62b.	LIVE BIRTH: What date did your fifth pregnancy end?	MONTH ☐☐ DAY ☐☐ YEAR ☐☐☐☐
D62c.	LIVE BIRTH: How many babies did you carry in your fifth pregnancy?	TOTAL NUMBER OF BABIES ☐☐
D62d.	LIVE BIRTH: What was the (first) baby's name?	PRINT FULL NAME: _____
D63.	INDEX BABY? (LIVE BIRTH GO TO D65)	YES ..... 1 NO 2
D64a.	STILL/MIS/AB/TUB/MOL: How far along were you when the pregnancy ended? PROBE TO GET BEST ESTIMATE OF # WEEKS (CONVERT MONTHS TO WEEKS IF NECESSARY).	☐☐ WEEKS
D64b.	STIL/MIS/AB/TUB/MOL: Do you know how many babies you were carrying?	TOTAL NUMBER OF BABIES ☐☐
D64c.	STILL/MIS/AB/TUB/MOL: Were you told anything about the baby you lost, such as its sex or if there were any apparent health problems?	YES ..... 1 NO (GO TO CHECKPOINT # 9) 2

D65.	What (is/was) (CHILD'S NAME/ the baby)'s sex?	MALE ..... 1 FEMALE ..... 2 UNKNOWN 3
D66.	Did this baby have a cleft lip or cleft palate?	YES ..... 1 NO (GO TO D67) ..... 2 DK (GO TO D67) 8
D66a.	Please tell me the structures that were involved in the baby's cleft. Did the baby have a cleft lip?	YES ..... 1 NO (GO TO D66c) ..... 2 DK (GO TO D66c) 8
D66b.	Was the cleft on one side or both sides of the lip? (PROBE IF ONE SIDE REPORTED, ASK WHETHER RIGHT OR LEFT.)	RIGHT SIDE ONLY ..... 1 LEFT SIDE ONLY ..... 2 BOTH SIDES (BILATERAL) ..... 3 MIDLINE ..... 4 DK 8
D66c.	Was there a cleft of the palate, that is the roof of the mouth?	YES ..... 1 NO (GO TO D66e) ..... 2 DK (GO TO D66e) 8
D66d.	Did it involve only the soft part at the rear of the palate or both the soft and hard parts of the palate?	SOFT PALATE ONLY ..... 1 SOFT AND HARD PALATE ..... 2 DK 8
D66e.	Did the cleft involve the gums and teeth?	YES ..... 1 NO ..... 2 DK 8
D67.	Did this baby have any other birth defects?	YES (LIST BELOW) ..... 1 NO ..... 2 DK ..... 8 SPECIFY: _____ ☐☐☐.☐☐☐ _____ ☐☐☐.☐☐☐ _____ ☐☐☐.☐☐☐

#9	INTERVIEWER CHECKPOINT:	SINGLE BABY; FIVE PREGNANCIES ONLY (GO TO D74, PG. 26) ..... 1 SINGLE BABY; ADDITIONAL PREGNANCIES (GO TO PREG HISTORY SUPPL.) ..... 2 MULTIPLE BABIES IN THIS PREGNANCY (CONTINUE) 3

Now I'd like to ask some questions about the second baby in this pregnancy.

D68.	LIVE BIRTH: What was the second baby's name?	PRINT FULL NAME: _____
D69.	INDEX BABY? (LIVE BIRTH GO TO D71)	YES ..... 1 NO 2

D70.	STILL/MIS/AB/TUB/MOL: Were you told anything about the baby you lost, such as its sex or if there were any apparent health problems?	YES ..... 1 NO (GO TO CHECKPOINT # 10) 2
D71.	What (is/was) (CHILD'S NAME/ the baby)'s sex?	MALE ..... 1 FEMALE ..... 2 UNKNOWN 3
D72.	Did this baby have a cleft lip or cleft palate?	YES ..... 1 NO (GO TO D73) ..... 2 DK (GO TO D73) 8
D72a.	Please tell me the structures that were involved in the baby's cleft. Did the baby have a cleft lip?	YES ..... 1 NO (GO TO D72c) ..... 2 DK (GO TO D72c) 8
D72b.	Was the cleft on one side or both sides of the lip? (PROBE IF ONE SIDE REPORTED, ASK WHETHER RIGHT OR LEFT.)	RIGHT SIDE ONLY ..... 1 LEFT SIDE ONLY ..... 2 BOTH SIDES (BILATERAL) ..... 3 MIDLINE ..... 4 DK 8
D72c.	Was there a cleft of the palate, that is the roof of the mouth?	YES ..... 1 NO (GO TO D72e) ..... 2 DK (GO TO D72e) 8
D72d.	Did it involve only the soft part at the rear of the palate or both the soft and hard parts of the palate?	SOFT PALATE ONLY ..... 1 SOFT AND HARD PALATE ..... 2 DK 8
D72e.	Did the cleft involve the gums and teeth?	YES ..... 1 NO ..... 2 DK 8
D73.	Did this baby have any other birth defects?	YES (LIST BELOW) ..... 1 NO ..... 2 DK ..... 8 SPECIFY: _____ ☐☐☐.☐☐☐ _____ ☐☐☐.☐☐☐ _____ ☐☐☐.☐☐☐

#10	INTERVIEWER CHECKPOINT:	2 BABIES IN THIS PREGNACY; 5 PREGNANCIES ONLY (CONTINUE) ..... 1 2 BABIES IN THIS PREGNANCY; ADD'L PREGNANCIES (GO TO PREG. HISTORY SUPPLEMENT) ..... 2 3+ BABIES IN THIS PREGNANCY (GO TO PREG. CONTINUATION FORM) 3

D74.	Are you currently pregnant?	YES ..... 1 NO (GO TO CHECKPOINT #11) ..... 2 RF (GO TO CHECKPOINT #11) ..... 7 DK (GO TO CHECKPOINT #11) 8
D74a.	What is your due date?	MONTH ☐☐ DAY ☐☐ YEAR ☐☐☐☐
D74b.	What was the date of the first day of your last menstrual period before you became pregnant?	MONTH ☐☐ DAY ☐☐ YEAR ☐☐☐☐
#11	INTERVIEWER CHECKPOINT:	THE NUMBER OF PREGNANCIES REPORTED IN D2 (PAGE 10) SHOULD EQUAL THE NUMBER OF PREGNANCIES COVERED IN THE PREGNANCY HISTORY, STARTING WITH D4. YES ..... 1 NO 2
D75.	Have any of your biological relatives, not including your children, had a cleft lip or palate? This would include your parents, sisters or brothers.	YES (SPECIFY BELOW) ..... 1 NO ..... 2 RF ..... 7 DK ..... 8 _____ ☐☐ _____ ☐☐
D76.	Have any of (INDEX CHILD)'s biological <u>father's</u> relatives had a cleft lip or palate? This would include his parents, sisters or brothers.	YES (SPECIFY BELOW) ..... 1 NO ..... 2 RF ..... 7 DK ..... 8 _____ ☐☐ _____ ☐☐

INTRODUCTION TO INDEX PREGNANCY AND PREGNANCY CALENDAR

For the rest of the interview, I'll be asking you about things you did during the period shortly before and during your pregnancy (with CHILD'S NAME/ that ended on DATE).

Please take out the Pregnancy Calendar that we sent you and let's review it together.

(INTERVIEWER SHOULD HAVE A COPY OF MOTHER'S PREGNANCY CALENDAR FOR REFERENCE)

The calendar is based on (CHILD'S NAME's birth date/ the date your pregnancy ended) and the length of your pregnancy. The important dates and time periods I'll be asking about are:

- the three months before you became pregnant, from (DATE) to (DATE), which are shown in green on the calendar;
- the estimated date you became pregnant, (DATE), shown in a red box;
- the first trimester of your pregnancy, from (DATE) to (DATE), shown in yellow;
- the second trimester of your pregnancy, from (DATE) to (DATE), shown in pink;
- the third trimester of your pregnancy, from (DATE) to (DATE), shown in blue; and
- (CHILD'S NAME's date of birth (DATE)/ the date your pregnancy ended) is shown in a red box.

Sometimes it's helpful to recall other things that happened during a time period to help give a clearer picture when you're answering questions. Try to write down a few things about each time period now, for instance:

- Did you take any trips or vacations just before or while you were pregnant?
- Were there any big family events like weddings, deaths, other births?
- What was your employment situation at the time – (WHERE APPLICABLE) both yours and your spouse's?
- Were there any serious illnesses or health problems among family members?
- Did you buy a new house, move or otherwise change your living situation?
- Anything else that will help you recall that time period?

(ALLOW TIME FOR MOTHER TO RECALL AND MAKE NOTES ON HER CALENDAR. THERE IS NO NEED FOR HER TO RESPOND TO THE QUESTIONS VERBALLY)

If you're ready to continue, we'll start with some questions about your weight and height and then go on to questions about your diet before and during your pregnancy (with CHILD'S NAME).

E. WEIGHT AND HEIGHT HISTORY		
E1.	About how much did you weigh at the time you became pregnant (with CHILD'S NAME)? This would be about ☐☐ of ☐☐ Month Year	POUNDS ☐☐☐
E2.	How much weight did you gain or lose from the beginning to the end of your pregnancy (with CHILD'S NAME)?	POUNDS ☐☐☐ GAINED..... 1 LOST..... 2
E3.	What is your current height without shoes?	FEET ☐☐ INCHES ☐☐ .☐
E4.	What is your current weight?	POUNDS ☐☐☐
E5.	LIVE BIRTH ONLY: What was (CHILD'S NAME)'s weight at birth?	POUNDS ☐☐ OUNCES ☐☐
E6.	LIVE BIRTH ONLY: What was (CHILD'S NAME)'s length at birth?	INCHES ☐☐ .☐

F. DIET INTERVIEW

Now I'd like to ask you about your typical diet during the period three months before you became pregnant (with CHILD'S NAME) and the first three months of your pregnancy. This would be from (DATE) to (DATE) which is shown in the green and yellow sections of your pregnancy calendar.

I'll read through a list of foods one at a time. After I read each food, please tell me how often, on average, you had the food during that period. This should include your total intake from meals and snacks.

We want to know your average intake over the whole six month time period, including the months before you became pregnant. So if there were foods you ate only before or only after you became pregnant, we'll need to figure out your average. I'll help you do that. Here is an example:

- If you ate fish four times a month before you became pregnant but never ate it in the first three months after you became pregnant, then your average over the six months would be two times per month.

If you didn't change your frequency of eating a food during the six month time period we're interested in, then just report your average frequency. For example:

- If you ate eggs once a week both before and after you were pregnant, then your average frequency would be once a week for the whole six months.

The response choices are listed on the back of the card we sent you marked "Diet Interview Card." Let's review them. They are:

- Never or less than 1 time per month
- 1-3 times per month
- 1 time per week
- 2-4 times per week
- 5-6 times per week
- 1 time per day
- 2-3 times per day
- 4-5 times per day
- 6 or more times per day

Let's begin with ...	**DAIRY FOODS**	
F1. Skim or fat free milk (8 oz. glass)	**F2. 1% or 2% milk** (8 oz. glass)	**F3. Whole milk** (8 oz. glass)
Never or < 1 per month 1	Never or < 1 per month 1	Never or < 1 per month 1
1-3 per month 2	1-3 per month 2	1-3 per month 2
1 per week 3	1 per week 3	1 per week 3
2-4 per week 4	2-4 per week 4	2-4 per week 4
5-6 per week 5	5-6 per week 5	5-6 per week 5
1 per day 6	1 per day 6	1 per day 6
2-3 per day 7	2-3 per day 7	2-3 per day 7
4-5 per day 8	4-5 per day 8	4-5 per day 8
6 + per day 9	6 + per day 9	6 + per day 9

F4. Cream, e.g., coffee, whipped or sour cream (1Tbs)	**F5. Non-dairy coffee whitener** (1 tsp)	**F6. Frozen yogurt, sherbet or non-fat ice cream** (1/2 cup)
Never or < 1 per month 1	Never or < 1 per month 1	Never or < 1 per month 1
1-3 per month..... 2	1-3 per month..... 2	1-3 per month.....2
1 per week..... 3	1 per week..... 3	1 per week 3
2-4 per week 4	2-4 per week 4	2-4 per week 4
5-6 per week 5	5-6 per week 5	5-6 per week 5
1 per day 6	1 per day 6	1 per day.....6
2-3 per day 7	2-3 per day 7	2-3 per day.....7
4-5 per day 8	4-5 per day 8	4-5 per day.....8
6 + per day 9	6 + per day..... 9	6 + per day.....9

F7. Ice cream (1/2 cup)	**F8. Yogurt** (1 cup)	**F9. Did you usually eat regular, low fat or non-fat yogurt?**
Never or < 1 per month..... 1	Never ;< 1 per month (TO F11). 1	Regular 1
1-3 per month 2	1-3 per month 2	Low fat.....2
1 per week 3	1 per week 3	Nonfat.....3
2-4 per week..... 4	2-4 per week..... 4	
5-6 per week..... 5	5-6 per week..... 5	
1 per day..... 6	1 per day..... 6	
2-3 per day 7	2-3 per day 7	
4-5 per day..... 8	4-5 per day 8	
6 + per day..... 9	6 + per day 9	

F10. Did you usually eat unsweetened or plain yogurt, yogurt sweetened with fruit or sugar, or artificially sweetened yogurt?	**F11. Cottage or ricotta cheese** (1/2 cup)	**F12. Cream cheese** (1 oz.)
Unsweetened (plain)..... 1	Never or < 1 per month..... 1	Never or < 1 per month.....1
Sweetened with fruit or sugar 2	1-3 per month 2	1-3 per month.....2
Artificially sweetened..... 3	1 per week..... 3	1 per week.....3
	2-4 per week..... 4	2-4 per week.....4
	5-6 per week..... 5	5-6 per week.....5
	1 per day..... 6	1 per day 6
	2-3 per day 7	2-3 per day 7
	4-5 per day 8	4-5 per day 8
	6 + per day 9	6 + per day.....9

F13. Other cheese, e.g., American, Swiss, cheddar, etc. by itself or as part of a sandwich or dish (a slice or 1 oz. serving)	**F14. Did you usually eat regular, low fat or nonfat cheese?**	**F15. Butter** (small pat or tsp.), **added to food or bread; exclude use in cooking**
Never ; < 1 per month (TO F15). 1	Regular..... 1	Never or < 1 per month.....1
1-3 per month..... 2	Low fat or lite..... 2	1-3 per month.....2
1 per week..... 3	Nonfat..... 3	1 per week.....3
2-4 per week..... 4		2-4 per week.....4
5-6 per week..... 5		5-6 per week.....5
1 per day..... 6		1 per day 6
2-3 per day 7		2-3 per day 7
4-5 per day..... 8		4-5 per day 8
6 + per day..... 9		6 + per day.....9

F16. Margarine (small pat or tsp.), **added to food or bread; exclude use in cooking or use of spray margarine**	**F17a. What form of margarine did you usually use?**	**F17b. What type of margarine did you usually use?**
Never;< 1 per month (TO F18).. 1	Stick..... 1	Regular 1
1-3 per month..... 2	Tub..... 2	Light spread.....2
1 per week..... 3	Squeeze (liquid)..... 3	Extra light spread.....3
2-4 per week..... 4		Nonfat.....4
5-6 per week..... 5		**F17c. What specific brand and type was it (e.g., Land O'Lakes Country Morning Blend Light)?**
1 per day..... 6		_____
2-3 per day 7		
4-5 per day..... 8		_____ ☐☐☐
6 + per day..... 9		

Next I'll ask you about

FRUITS

F18. Raisins (1 oz. or small pack) **or grapes**	**F19. Prunes** (1/2 cup or 7 prunes)	**F20. Bananas** (1)
Never or < 1 per month..... 1	Never or < 1 per month..... 1	Never or < 1 per month.....1
1-3 per month..... 2	1-3 per month..... 2	1-3 per month.....2
1 per week..... 3	1 per week..... 3	1 per week.....3
2-4 per week..... 4	2-4 per week..... 4	2-4 per week.....4
5-6 per week..... 5	5-6 per week..... 5	5-6 per week.....5
1 per day..... 6	1 per day..... 6	1 per day 6
2-3 per day 7	2-3 per day 7	2-3 per day 7
4-5 per day..... 8	4-5 per day 8	4-5 per day 8
6 + per day..... 9	6 + per day..... 9	6 + per day.....9

F21. Cantaloupe (1/4 melon)	**F22. Avocado** (½ fruit or ½ cup)	**F23. Applesauce** (1/2 cup)
Never or < 1 per month 1	Never or < 1 per month 1	Never or < 1 per month 1
1-3 per month 2	1-3 per month 2	1-3 per month 2
1 per week 3	1 per week 3	1 per week 3
2-4 per week 4	2-4 per week 4	2-4 per week 4
5-6 per week 5	5-6 per week 5	5-6 per week 5
1 per day 6	1 per day 6	1 per day 6
2-3 per day 7	2-3 per day 7	2-3 per day 7
4-5 per day 8	4-5 per day 8	4-5 per day 8
6 + per day 9	6 + per day 9	6 + per day 9
F24. Fresh apples or pears (1)	**F25. Apple juice or cider** (small glass)	**F26. Oranges** (1)
Never or < 1 per month 1	Never or < 1 per month 1	Never or < 1 per month 1
1-3 per month 2	1-3 per month 2	1-3 per month 2
1 per week 3	1 per week 3	1 per week 3
2-4 per week 4	2-4 per week 4	2-4 per week 4
5-6 per week 5	5-6 per week 5	5-6 per week 5
1 per day 6	1 per day 6	1 per day 6
2-3 per day 7	2-3 per day 7	2-3 per day 7
4-5 per day 8	4-5 per day 8	4-5 per day 8
6 + per day 9	6 + per day 9	6 + per day 9
F27. Orange juice (small glass)	**F28. Grapefruit** (1/2)	**F29. Grapefruit juice** (small glass)
Never or < 1 per month 1	Never or < 1 per month 1	Never or < 1 per month 1
1-3 per month 2	1-3 per month 2	1-3 per month 2
1 per week 3	1 per week 3	1 per week 3
2-4 per week 4	2-4 per week 4	2-4 per week 4
5-6 per week 5	5-6 per week 5	5-6 per week 5
1 per day 6	1 per day 6	1 per day 6
2-3 per day 7	2-3 per day 7	2-3 per day 7
4-5 per day 8	4-5 per day 8	4-5 per day 8
6 + per day 9	6 + per day 9	6 + per day 9

F30. Other fruit juices (small glass)	F31. Strawberries, fresh, frozen or canned (1/2 cup)	F32. Blueberries, fresh, frozen or canned (1/2 cup)
Never or < 1 per month 1	Never or < 1 per month 1	Never or < 1 per month 1
1-3 per month 2	1-3 per month 2	1-3 per month 2
1 per week 3	1 per week 3	1 per week 3
2-4 per week 4	2-4 per week 4	2-4 per week 4
5-6 per week 5	5-6 per week 5	5-6 per week 5
1 per day 6	1 per day 6	1 per day 6
2-3 per day 7	2-3 per day 7	2-3 per day 7
4-5 per day 8	4-5 per day 8	4-5 per day 8
6 + per day 9	6 + per day 9	6 + per day 9

F33. Peaches, apricots or plums (1 fresh, or ½ cup canned)
Never or < 1 per month 1
1-3 per month 2
1 per week 3
2-4 per week 4
5-6 per week 5
1 per day 6
2-3 per day 7
4-5 per day 8
6 + per day 9

Next I'll ask you about

VEGETABLES

F34. Fresh tomatoes (1)	F35. Tomato or V8 juice (small glass)	F36. Canned tomatoes or tomato sauce (1/2 cup) e.g., spaghetti sauce
Never or < 1 per month 1	Never or < 1 per month 1	Never or < 1 per month 1
1-3 per month 2	1-3 per month 2	1-3 per month 2
1 per week 3	1 per week 3	1 per week 3
2-4 per week 4	2-4 per week 4	2-4 per week 4
5-6 per week 5	5-6 per week 5	5-6 per week 5
1 per day 6	1 per day 6	1 per day 6
2-3 per day 7	2-3 per day 7	2-3 per day 7
4-5 per day 8	4-5 per day 8	4-5 per day 8
6 + per day 9	6 + per day 9	6 + per day 9

F37. Salsa, picante or taco sauce (1/4 cup)	**F38. Tofu or soybeans** (3-4 oz.)	**F39. String beans** (1/2 cup)
Never or < 1 per month 1	Never or < 1 per month 1	Never or < 1 per month 1
1-3 per month 2	1-3 per month 2	1-3 per month 2
1 per week 3	1 per week 3	1 per week 3
2-4 per week 4	2-4 per week 4	2-4 per week 4
5-6 per week 5	5-6 per week 5	5-6 per week 5
1 per day 6	1 per day 6	1 per day 6
2-3 per day 7	2-3 per day 7	2-3 per day 7
4-5 per day 8	4-5 per day 8	4-5 per day 8
6 + per day 9	6 + per day 9	6 + per day 9
F40. Broccoli (1/2 cup)	**F41. Cabbage or cole slaw** (1/2 cup)	**F42. Cauliflower** (1/2 cup)
Never or < 1 per month 1	Never or < 1 per month 1	Never or < 1 per month 1
1-3 per month 2	1-3 per month 2	1-3 per month 2
1 per week 3	1 per week 3	1 per week 3
2-4 per week 4	2-4 per week 4	2-4 per week 4
5-6 per week 5	5-6 per week 5	5-6 per week 5
1 per day 6	1 per day 6	1 per day 6
2-3 per day 7	2-3 per day 7	2-3 per day 7
4-5 per day 8	4-5 per day 8	4-5 per day 8
6 + per day 9	6 + per day 9	6 + per day 9
F43. Brussels sprouts (1/2 cup)	**F44. Carrots, raw** (1/2 carrot or 2-4 sticks)	**F45. Carrots cooked** (1/2 cup)
Never or < 1 per month 1	Never or < 1 per month 1	Never or < 1 per month 1
1-3 per month 2	1-3 per month 2	1-3 per month 2
1 per week 3	1 per week 3	1 per week 3
2-4 per week 4	2-4 per week 4	2-4 per week 4
5-6 per week 5	5-6 per week 5	5-6 per week 5
1 per day 6	1 per day 6	1 per day 6
2-3 per day 7	2-3 per day 7	2-3 per day 7
4-5 per day 8	4-5 per day 8	4-5 per day 8
6 + per day 9	6 + per day 9	6 + per day 9

F46. Corn (1 ear or ½ cup frozen or canned)	**F47. Peas, or lima beans** (1/2 cup fresh, frozen, canned)	**F48. Mixed vegetables** (1/2 cup); **any type of mixture**
Never or < 1 per month 1	Never or < 1 per month 1	Never or < 1 per month 1
1-3 per month 2	1-3 per month 2	1-3 per month 2
1 per week 3	1 per week 3	1 per week 3
2-4 per week 4	2-4 per week 4	2-4 per week 4
5-6 per week 5	5-6 per week 5	5-6 per week 5
1 per day 6	1 per day 6	1 per day 6
2-3 per day 7	2-3 per day 7	2-3 per day 7
4-5 per day 8	4-5 per day 8	4-5 per day 8
6 + per day 9	6 + per day 9	6 + per day 9
F49. Beans or lentils, baked or dried (1/2 cup)	**F50. Dark orange (winter) squash** (1/2 cup)	**F51. Eggplant, zucchini, or other summer squash** (1/2 cup)
Never or < 1 per month 1	Never or < 1 per month 1	Never or < 1 per month 1
1-3 per month 2	1-3 per month 2	1-3 per month 2
1 per week 3	1 per week 3	1 per week 3
2-4 per week 4	2-4 per week 4	2-4 per week 4
5-6 per week 5	5-6 per week 5	5-6 per week 5
1 per day 6	1 per day 6	1 per day 6
2-3 per day 7	2-3 per day 7	2-3 per day 7
4-5 per day 8	4-5 per day 8	4-5 per day 8
6 + per day 9	6 + per day 9	6 + per day 9
F52. Yams or sweet potatoes (1/2 cup)	**F53. Spinach, cooked** (½ cup)	**F54. Spinach, raw as in salad** (1 cup)
Never or < 1 per month 1	Never or < 1 per month 1	Never or < 1 per month 1
1-3 per month 2	1-3 per month 2	1-3 per month 2
1 per week 3	1 per week 3	1 per week 3
2-4 per week 4	2-4 per week 4	2-4 per week 4
5-6 per week 5	5-6 per week 5	5-6 per week 5
1 per day 6	1 per day 6	1 per day 6
2-3 per day 7	2-3 per day 7	2-3 per day 7
4-5 per day 8	4-5 per day 8	4-5 per day 8
6 + per day 9	6 + per day 9	6 + per day 9

F55. Kale, mustard, collard or chard greens (1/2 cup)	**F56. Iceberg or head lettuce** (1 cup)	**F57. Romaine or leaf lettuce (1 cup)**
Never or < 1 per month 1	Never or < 1 per month 1	Never or < 1 per month 1
1-3 per month 2	1-3 per month 2	1-3 per month 2
1 per week 3	1 per week 3	1 per week 3
2-4 per week 4	2-4 per week 4	2-4 per week 4
5-6 per week 5	5-6 per week 5	5-6 per week 5
1 per day 6	1 per day 6	1 per day 6
2-3 per day 7	2-3 per day 7	2-3 per day 7
4-5 per day 8	4-5 per day 8	4-5 per day 8
6 + per day 9	6 + per day 9	6 + per day 9

F58. Celery (4" stick)	**F59. Green, red or yellow sweet peppers** (3 slices or ½ pepper)	**F60. Onions as a garnish or in a salad** (1 slice)
Never or < 1 per month 1	Never or < 1 per month 1	Never or < 1 per month 1
1-3 per month 2	1-3 per month 2	1-3 per month 2
1 per week 3	1 per week 3	1 per week 3
2-4 per week 4	2-4 per week 4	2-4 per week 4
5-6 per week 5	5-6 per week 5	5-6 per week 5
1 per day 6	1 per day 6	1 per day 6
2-3 per day 7	2-3 per day 7	2-3 per day 7
4-5 per day 8	4-5 per day 8	4-5 per day 8
6 + per day 9	6 + per day 9	6 + per day 9

F61. Onions as a vegetable, rings or soup (1 onion)
Never or < 1 per month 1
1-3 per month 2
1 per week 3
2-4 per week 4
5-6 per week 5
1 per day 6
2-3 per day 7
4-5 per day 8
6 + per day 9

Next I'll ask you about

EGGS, MEAT, ETC.

F62. Egg beaters or egg whites only (1/2 cup or 1 egg white)	**F63. Eggs whole, with yolk** (1)	**F64. Bacon** (2 slices)
Never or < 1 per month 1	Never or < 1 per month 1	Never or < 1 per month 1
1-3 per month 2	1-3 per month 2	1-3 per month 2
1 per week 3	1 per week 3	1 per week 3
2-4 per week 4	2-4 per week 4	2-4 per week 4
5-6 per week 5	5-6 per week 5	5-6 per week 5
1 per day 6	1 per day 6	1 per day 6
2-3 per day 7	2-3 per day 7	2-3 per day 7
4-5 per day 8	4-5 per day 8	4-5 per day 8
6 + per day 9	6 + per day 9	6 + per day 9
F65. Chicken or turkey sandwich	**F66. Other chicken or turkey, with skin** (4-6 oz.)	**F67. Other chicken or turkey, without skin** (4-6 oz.)
Never or < 1 per month 1	Never or < 1 per month 1	Never or < 1 per month 1
1-3 per month 2	1-3 per month 2	1-3 per month 2
1 per week 3	1 per week 3	1 per week 3
2-4 per week 4	2-4 per week 4	2-4 per week 4
5-6 per week 5	5-6 per week 5	5-6 per week 5
1 per day 6	1 per day 6	1 per day 6
2-3 per day 7	2-3 per day 7	2-3 per day 7
4-5 per day 8	4-5 per day 8	4-5 per day 8
6 + per day 9	6 + per day 9	6 + per day 9
F68. Beef or pork hot dogs (1)	**F69. Chicken or turkey hot dogs** (1)	**F70. Salami, bologna or other processed meat sandwiches** (1 piece or slice)
Never or < 1 per month 1	Never or < 1 per month 1	Never or < 1 per month 1
1-3 per month 2	1-3 per month 2	1-3 per month 2
1 per week 3	1 per week 3	1 per week 3
2-4 per week 4	2-4 per week 4	2-4 per week 4
5-6 per week 5	5-6 per week 5	5-6 per week 5
1 per day 6	1 per day 6	1 per day 6
2-3 per day 7	2-3 per day 7	2-3 per day 7
4-5 per day 8	4-5 per day 8	4-5 per day 8
6 + per day 9	6 + per day 9	6 + per day 9

F71. Processed meats, e.g., sausage, kielbasa, etc. (2 oz. or 2 small links)	**F72. Hamburger, lean or extra lean** (1 patty)	**F73. Hamburger, regular** (1 patty)
Never or < 1 per month 1	Never or < 1 per month 1	Never or < 1 per month 1
1-3 per month 2	1-3 per month 2	1-3 per month 2
1 per week 3	1 per week 3	1 per week 3
2-4 per week 4	2-4 per week 4	2-4 per week 4
5-6 per week 5	5-6 per week 5	5-6 per week 5
1 per day 6	1 per day 6	1 per day 6
2-3 per day 7	2-3 per day 7	2-3 per day 7
4-5 per day 8	4-5 per day 8	4-5 per day 8
6 + per day 9	6 + per day 9	6 + per day 9
F74. Beef, pork, or lamb as a sandwich or mixed dish, e.g., stew, casserole, lasagna, etc.	**F75. Pork as a main dish, e.g., ham or chops** (4-6 oz.)	**F76. Beef or lamb as a main dish, e.g., steak, roast** (4-6 oz.)
Never or < 1 per month 1	Never or < 1 per month 1	Never or < 1 per month 1
1-3 per month 2	1-3 per month 2	1-3 per month 2
1 per week 3	1 per week 3	1 per week 3
2-4 per week 4	2-4 per week 4	2-4 per week 4
5-6 per week 5	5-6 per week 5	5-6 per week 5
1 per day 6	1 per day 6	1 per day 6
2-3 per day 7	2-3 per day 7	2-3 per day 7
4-5 per day 8	4-5 per day 8	4-5 per day 8
6 + per day 9	6 + per day 9	6 + per day 9
F77. Liver: beef, calf or pork (4 oz.)	**F78. Liver: chicken or turkey** (1 oz.)	**F79. Canned tuna fish** (3-4 oz.)
Never or < 1 per month 1	Never or < 1 per month 1	Never or < 1 per month 1
1-3 per month 2	1-3 per month 2	1-3 per month 2
1 per week 3	1 per week 3	1 per week 3
2-4 per week 4	2-4 per week 4	2-4 per week 4
5-6 per week 5	5-6 per week 5	5-6 per week 5
1 per day 6	1 per day 6	1 per day 6
2-3 per day 7	2-3 per day 7	2-3 per day 7
4-5 per day 8	4-5 per day 8	4-5 per day 8
6 + per day 9	6 + per day 9	6 + per day 9

F80. Breaded fish cakes, pieces, or fish sticks (1 serving, store bought)

Never or < 1 per month 1
1-3 per month 2
1 per week 3
2-4 per week 4
5-6 per week 5
1 per day 6
2-3 per day 7
4-5 per day 8
6 + per day 9

F81. Shrimp, lobster, scallops, or clams as a main dish (1 serving)

Never or < 1 per month 1
1-3 per month 2
1 per week 3
2-4 per week 4
5-6 per week 5
1 per day 6
2-3 per day 7
4-5 per day 8
6 + per day 9

F82. Dark meat fish, e.g., mackerel, salmon, trout, sardines, bluefish, swordfish (3-5 oz.)

Never or < 1 per month 1
1-3 per month 2
1 per week 3
2-4 per week 4
5-6 per week 5
1 per day 6
2-3 per day 7
4-5 per day 8
6 + per day 9

F83. Other fish (3-5 oz.)

Never or < 1 per month 1
1-3 per month 2
1 per week 3
2-4 per week 4
5-6 per week 5
1 per day 6
2-3 per day 7
4-5 per day 8
6 + per day 9

Next I'll ask you about

BREADS, CEREALS, STARCHES

F84. Cold breakfast cereal (1 cup)

Never; < 1 per month (TO F85) .. 1
1-3 per month 2
1 per week 3
2-4 per week 4
5-6 per week 5
1 per day 6
2-3 per day 7
4-5 per day 8
6 + per day 9

F84a. What brand and type of cold breakfast cereal did you usually eat (e.g., Ralston Rice Chex)?

_____

_____

☐☐☐☐

_____

_____

☐☐☐☐

F85. Cooked oatmeal/cooked oat bran (1 cup)

Never or < 1 per month 1
1-3 per month 2
1 per week 3
2-4 per week 4
5-6 per week 5
1 per day 6
2-3 per day 7
4-5 per day 8
6 + per day 9

F86. Other cooked breakfast cereal (1 cup)	**F87. White bread** (slice), **including pita bread**	**F88. Dark bread** (slice), **including wheat pita bread**
Never or < 1 per month 1	Never or < 1 per month 1	Never or < 1 per month 1
1-3 per month 2	1-3 per month 2	1-3 per month 2
1 per week 3	1 per week 3	1 per week 3
2-4 per week 4	2-4 per week 4	2-4 per week 4
5-6 per week 5	5-6 per week 5	5-6 per week 5
1 per day 6	1 per day 6	1 per day 6
2-3 per day 7	2-3 per day 7	2-3 per day 7
4-5 per day 8	4-5 per day 8	4-5 per day 8
6 + per day 9	6 + per day 9	6 + per day 9
F89. Bagels, English muffins or rolls (1 whole)	**F90. Muffins** (regular) **or biscuits** (1)	**F91. Brown rice** (1 cup)
Never or < 1 per month 1	Never or < 1 per month 1	Never or < 1 per month 1
1-3 per month 2	1-3 per month 2	1-3 per month 2
1 per week 3	1 per week 3	1 per week 3
2-4 per week 4	2-4 per week 4	2-4 per week 4
5-6 per week 5	5-6 per week 5	5-6 per week 5
1 per day 6	1 per day 6	1 per day 6
2-3 per day 7	2-3 per day 7	2-3 per day 7
4-5 per day 8	4-5 per day 8	4-5 per day 8
6 + per day 9	6 + per day 9	6 + per day 9
F92. White rice (1 cup)	**F93. Pasta, e.g., spaghetti, noodles, etc.** (1 cup)	**F94. Tortillas** (1) **or foods with tortillas like burritos, fajitas or sandwich wraps**
Never or < 1 per month 1	Never or < 1 per month 1	Never or < 1 per month 1
1-3 per month 2	1-3 per month 2	1-3 per month 2
1 per week 3	1 per week 3	1 per week 3
2-4 per week 4	2-4 per week 4	2-4 per week 4
5-6 per week 5	5-6 per week 5	5-6 per week 5
1 per day 6	1 per day 6	1 per day 6
2-3 per day 7	2-3 per day 7	2-3 per day 7
4-5 per day 8	4-5 per day 8	4-5 per day 8
6 + per day 9	6 + per day 9	6 + per day 9

F95. Other grains, e.g., bulgur, kasha, couscous, etc. (1 cup)

Never or < 1 per month 1
1-3 per month 2
1 per week 3
2-4 per week 4
5-6 per week 5
1 per day 6
2-3 per day 7
4-5 per day 8
6 + per day 9

F96. Pancakes or waffles (3 pieces)

Never or < 1 per month 1
1-3 per month 2
1 per week 3
2-4 per week 4
5-6 per week 5
1 per day 6
2-3 per day 7
4-5 per day 8
6 + per day 9

F97. French fried potatoes (small order or ½ cup)

Never or < 1 per month 1
1-3 per month 2
1 per week 3
2-4 per week 4
5-6 per week 5
1 per day 6
2-3 per day 7
4-5 per day 8
6 + per day 9

F98. Potatoes, baked, boiled (1) or mashed (1 cup)

Never or < 1 per month 1
1-3 per month 2
1 per week 3
2-4 per week 4
5-6 per week 5
1 per day 6
2-3 per day 7
4-5 per day 8
6 + per day 9

F99. Potato chips or corn chips (small bag or 1 oz.)

Never or < 1 per month 1
1-3 per month 2
1 per week 3
2-4 per week 4
5-6 per week 5
1 per day 6
2-3 per day 7
4-5 per day 8
6 + per day 9

F100. Crackers, Triscuits, Wheat Thins (5)

Never or < 1 per month 1
1-3 per month 2
1 per week 3
2-4 per week 4
5-6 per week 5
1 per day 6
2-3 per day 7
4-5 per day 8
6 + per day 9

F101. Pizza (2 slices)

Never or < 1 per month 1
1-3 per month 2
1 per week 3
2-4 per week 4
5-6 per week 5
1 per day 6
2-3 per day 7
4-5 per day 8
6 + per day 9

Next I'll ask you about

BEVERAGES

F102. Low-calorie cola, e.g., Diet coke with caffeine (1 glass, bottle, can)	**F103. Low-calorie caffeine-free cola** (1 glass, bottle, can)	**F104. Other low-calorie carbonated beverage, e.g., Diet 7-Up, Fresca, diet ginger ale** (1 glass, bottle, can)
Never or < 1 per month 1	Never or < 1 per month 1	Never or < 1 per month 1
1-3 per month 2	1-3 per month 2	1-3 per month 2
1 per week 3	1 per week 3	1 per week 3
2-4 per week 4	2-4 per week 4	2-4 per week 4
5-6 per week 5	5-6 per week 5	5-6 per week 5
1 per day 6	1 per day 6	1 per day 6
2-3 per day 7	2-3 per day 7	2-3 per day 7
4-5 per day 8	4-5 per day 8	4-5 per day 8
6 + per day 9	6 + per day 9	6 + per day 9
F105. Coke, Pepsi, or other cola with sugar (1 glass, bottle, can)	**F106. Caffeine Free Coke, Pepsi, or other colas with sugar** (1 glass, bottle, can)	**F107. Other carbonated beverage with sugar, e.g., 7-Up** (1 glass, bottle, can)
Never or < 1 per month 1	Never or < 1 per month 1	Never or < 1 per month 1
1-3 per month 2	1-3 per month 2	1-3 per month 2
1 per week 3	1 per week 3	1 per week 3
2-4 per week 4	2-4 per week 4	2-4 per week 4
5-6 per week 5	5-6 per week 5	5-6 per week 5
1 per day 6	1 per day 6	1 per day 6
2-3 per day 7	2-3 per day 7	2-3 per day 7
4-5 per day 8	4-5 per day 8	4-5 per day 8
6 + per day 9	6 + per day 9	6 + per day 9
F108. Hawaiian Punch, lemonade, Kool Aid or other non-carbonated drinks (1 glass, bottle, can)	**F109. Regular beer** (1 glass, bottle, can)	**F110. Light beer e.g., Bud Light** (1 glass, bottle, can)
Never or < 1 per month 1	Never or < 1 per month 1	Never or < 1 per month 1
1-3 per month 2	1-3 per month 2	1-3 per month 2
1 per week 3	1 per week 3	1 per week 3
2-4 per week 4	2-4 per week 4	2-4 per week 4
5-6 per week 5	5-6 per week 5	5-6 per week 5
1 per day 6	1 per day 6	1 per day 6
2-3 per day 7	2-3 per day 7	2-3 per day 7
4-5 per day 8	4-5 per day 8	4-5 per day 8
6 + per day 9	6 + per day 9	6 + per day 9

F111. Red wine (4 oz. glass)	**F112. White wine** (4 oz. glass)	**F113. Liquor, e.g., whiskey, gin, etc.** (1 drink or 1 oz. shot)
Never or < 1 per month 1	Never or < 1 per month 1	Never or < 1 per month 1
1-3 per month 2	1-3 per month 2	1-3 per month 2
1 per week 3	1 per week 3	1 per week 3
2-4 per week 4	2-4 per week 4	2-4 per week 4
5-6 per week 5	5-6 per week 5	5-6 per week 5
1 per day 6	1 per day 6	1 per day 6
2-3 per day 7	2-3 per day 7	2-3 per day 7
4-5 per day 8	4-5 per day 8	4-5 per day 8
6 + per day 9	6 + per day 9	6 + per day 9

F114. Plain water, bottled or tap including mineral water and soda water (1 cup or glass)	**F115. Herbal tea, brewed; cold or hot** (1 cup)	**F116. Regular Tea; brewed, cold or hot** (1 cup)
Never or < 1 per month 1	Never or < 1 per month 1	Never or < 1 per month 1
1-3 per month 2	1-3 per month 2	1-3 per month 2
1 per week 3	1 per week 3	1 per week 3
2-4 per week 4	2-4 per week 4	2-4 per week 4
5-6 per week 5	5-6 per week 5	5-6 per week 5
1 per day 6	1 per day 6	1 per day 6
2-3 per day 7	2-3 per day 7	2-3 per day 7
4-5 per day 8	4-5 per day 8	4-5 per day 8
6 + per day 9	6 + per day 9	6 + per day 9

F117. Decaffeinated coffee (1 cup)	**F118. Coffee with caffeine** (1 cup)
Never or < 1 per month 1	Never or < 1 per month 1
1-3 per month 2	1-3 per month 2
1 per week 3	1 per week 3
2-4 per week 4	2-4 per week 4
5-6 per week 5	5-6 per week 5
1 per day 6	1 per day 6
2-3 per day 7	2-3 per day 7
4-5 per day 8	4-5 per day 8
6 + per day 9	6 + per day 9

Next I'll ask you about

SWEETS, BAKED GOODS, MISCELLANEOUS

F119. Pure chocolate candy bar or packet **(e.g., Hershey's, M&M's)**

Never or < 1 per month 1
1-3 per month 2
1 per week 3
2-4 per week 4
5-6 per week 5
1 per day 6
2-3 per day 7
4-5 per day 8
6 + per day 9

F120. Other mixed candy bars, **(e.g., Snickers, Milky Way, Reeses)**

Never or < 1 per month 1
1-3 per month 2
1 per week 3
2-4 per week 4
5-6 per week 5
1 per day 6
2-3 per day 7
4-5 per day 8
6 + per day 9

F121. Candy without chocolate (e.g., 1 pack mints, Lifesavers)

Never or < 1 per month 1
1-3 per month 2
1 per week 3
2-4 per week 4
5-6 per week 5
1 per day 6
2-3 per day 7
4-5 per day 8
6 + per day 9

F122. Jams, jellies, preserves, syrup, or honey (1 Tbs.)

Never or < 1 per month 1
1-3 per month 2
1 per week 3
2-4 per week 4
5-6 per week 5
1 per day 6
2-3 per day 7
4-5 per day 8
6 + per day 9

F123. Peanut butter (1 Tbs.)

Never or < 1 per month 1
1-3 per month 2
1 per week 3
2-4 per week 4
5-6 per week 5
1 per day 6
2-3 per day 7
4-5 per day 8
6 + per day 9

F124. Popcorn (1 cup)

Never or < 1 per month 1
1-3 per month 2
1 per week 3
2-4 per week 4
5-6 per week 5
1 per day 6
2-3 per day 7
4-5 per day 8
6 + per day 9

F125. Pretzels (1 oz., or small bag)

Never or < 1 per month 1
1-3 per month 2
1 per week 3
2-4 per week 4
5-6 per week 5
1 per day 6
2-3 per day 7
4-5 per day 8
6 + per day 9

F126. Cookies, home baked (1)

Never or < 1 per month 1
1-3 per month 2
1 per week 3
2-4 per week 4
5-6 per week 5
1 per day 6
2-3 per day 7
4-5 per day 8
6 + per day 9

F127. Cookies, ready made (1)

Never or < 1 per month 1
1-3 per month 2
1 per week 3
2-4 per week 4
5-6 per week 5
1 per day 6
2-3 per day 7
4-5 per day 8
6 + per day 9

F128. Brownies (1)	F129. Doughnuts (1)	F130. Cake, homemade (slice)
Never or < 1 per month 1	Never or < 1 per month 1	Never or < 1 per month 1
1-3 per month 2	1-3 per month 2	1-3 per month 2
1 per week 3	1 per week 3	1 per week 3
2-4 per week 4	2-4 per week 4	2-4 per week 4
5-6 per week 5	5-6 per week 5	5-6 per week 5
1 per day 6	1 per day 6	1 per day 6
2-3 per day 7	2-3 per day 7	2-3 per day 7
4-5 per day 8	4-5 per day 8	4-5 per day 8
6 + per day 9	6 + per day 9	6 + per day 9
F131. Cake, ready made (slice)	**F132. Pie, homemade (slice)**	**F133. Pie, ready made (slice)**
Never or < 1 per month 1	Never or < 1 per month 1	Never or < 1 per month 1
1-3 per month 2	1-3 per month 2	1-3 per month 2
1 per week 3	1 per week 3	1 per week 3
2-4 per week 4	2-4 per week 4	2-4 per week 4
5-6 per week 5	5-6 per week 5	5-6 per week 5
1 per day 6	1 per day 6	1 per day 6
2-3 per day 7	2-3 per day 7	2-3 per day 7
4-5 per day 8	4-5 per day 8	4-5 per day 8
6 + per day 9	6 + per day 9	6 + per day 9
F134. Sweet roll, coffee cake or other pastry, homemade (serving)	**F135. Sweet roll, coffee cake or other pastry, ready made (serving)**	**F136. Peanuts (small packet or 1 oz.)**
Never or < 1 per month 1	Never or < 1 per month 1	Never or < 1 per month 1
1-3 per month 2	1-3 per month 2	1-3 per month 2
1 per week 3	1 per week 3	1 per week 3
2-4 per week 4	2-4 per week 4	2-4 per week 4
5-6 per week 5	5-6 per week 5	5-6 per week 5
1 per day 6	1 per day 6	1 per day 6
2-3 per day 7	2-3 per day 7	2-3 per day 7
4-5 per day 8	4-5 per day 8	4-5 per day 8
6 + per day 9	6 + per day 9	6 + per day 9

F137. Other nuts (small packet or 1 oz.)	**F138. Oat bran, added to food** (1 Tbs.)	**F139. Other bran, added to food** (1 Tbs.)
Never or < 1 per month 1	Never or < 1 per month 1	Never or < 1 per month 1
1-3 per month 2	1-3 per month 2	1-3 per month 2
1 per week 3	1 per week 3	1 per week 3
2-4 per week 4	2-4 per week 4	2-4 per week 4
5-6 per week 5	5-6 per week 5	5-6 per week 5
1 per day 6	1 per day 6	1 per day 6
2-3 per day 7	2-3 per day 7	2-3 per day 7
4-5 per day 8	4-5 per day 8	4-5 per day 8
6 + per day 9	6 + per day 9	6 + per day 9
F140. Wheat germ (1 Tbs.)	**F141. Chowder or cream soup** (1 cup)	**F142. Ketchup or red chili sauce** (1 Tbs.)
Never or < 1 per month 1	Never or < 1 per month 1	Never or < 1 per month 1
1-3 per month 2	1-3 per month 2	1-3 per month 2
1 per week 3	1 per week 3	1 per week 3
2-4 per week 4	2-4 per week 4	2-4 per week 4
5-6 per week 5	5-6 per week 5	5-6 per week 5
1 per day 6	1 per day 6	1 per day 6
2-3 per day 7	2-3 per day 7	2-3 per day 7
4-5 per day 8	4-5 per day 8	4-5 per day 8
6 + per day 9	6 + per day 9	6 + per day 9
F143. Salt added at table (1 shake)	**F144. If you added sugar to your beverages or food, how many teaspoons of sugar did you add each day?**	**F145. NutraSweet or Equal** (1 packet) **NOT Sweet 'N Low**
Never or < 1 per month 1		Never or < 1 per month 1
1-3 per month 2		1-3 per month 2
1 per week 3		1 per week 3
2-4 per week 4	Teaspoons ⌊⌋⌋⌋	2-4 per week 4
5-6 per week 5		5-6 per week 5
1 per day 6	IF NONE, CODE 000	1 per day 6
2-3 per day 7		2-3 per day 7
4-5 per day 8		4-5 per day 8
6 + per day 9		6 + per day 9

F146. Garlic (1 clove or 4 shakes)

Never or < 1 per month 1
1-3 per month 2
1 per week 3
2-4 per week 4
5-6 per week 5
1 per day 6
2-3 per day 7
4-5 per day 8
6 + per day 9

F147. Low fat mayonnaise/ fat free mayonnaise (2 Tbs.)

Never or < 1 per month 1
1-3 per month 2
1 per week 3
2-4 per week 4
5-6 per week 5
1 per day 6
2-3 per day 7
4-5 per day 8
6 + per day 9

F148. Regular mayonnaise (2 Tbs.)

Never or < 1 per month 1
1-3 per month 2
1 per week 3
2-4 per week 4
5-6 per week 5
1 per day 6
2-3 per day 7
4-5 per day 8
6 + per day 9

F149. Salad dressing (2 Tbs.)

Never;< 1 per month (TO F150) 1
1-3 per month 2
1 per week 3
2-4 per week 4
5-6 per week 5
1 per day 6
2-3 per day 7
4-5 per day 8
6 + per day 9

F149a. Did you usually use nonfat, low fat or regular salad dressing?

Nonfat 1
Low fat 2
Regular 3

149b. What brand and type of salad dressing did you usually use (e.g. Kraft Thousand Island)?

_____

_____ ☐☐☐

F150. Olive oil added to food or bread (1 Tbs.); **exclude use in cooking**

Never or < 1 per month 1
1-3 per month 2
1 per week 3
2-4 per week 4
5-6 per week 5
1 per day 6
2-3 per day 7
4-5 per day 8
6 + per day 9

F151. How much of the visible fat on your beef, pork or lamb did you remove before eating?

Don't eat meat 1
Remove all visible fat 2
Remove most 3
Remove small part of fat 4
Remove none 5

F152. How often did you eat food fried, stir-fried in oil, or sautéed at home?

Never (GO TO F154) 1
Less than once a week 2
Once per week 3
2-4 times per week 4
5-6 times per week 5
Daily 6

F153. What kind of fat or oil did you usually use for frying, stir-frying or sautéing at home?

Real butter 1
Margarine (GO TO 153a) 2
Olive oil 3
Vegetable oil (GO TO 153a) 4
Vegetable shortening 5
Lard/bacon fat 6
Pam type spray 7

153a. What brand and type of (margarine/vegetable oil) did you use for frying, stir-frying or sautéing (e.g. Wesson Canola Oil)?

_____

_____ ☐☐☐

F154. What kind of fat or oil was usually used for baking at home?

Don't bake 1
Real butter 2
Margarine (GO TO 154a) 3
Olive oil 4
Vegetable oil (GO TO 154a) 5
Vegetable shortening 6
Lard/bacon fat 7
Pam type spray 8

154a. What brand and type of (margarine/vegetable oil) did you use for baking?

_____

_____ ☐☐☐

F155. How often did you eat deep fried food away from home or as take out (e.g., french fries, fried chicken, fish, clams, shrimp, etc.)?

Never 1
Less than once a week 2
Once per week 3
2-4 times per week 4
5-6 times per week 5
Daily 6

F156. Are there any other foods not mentioned up to now that you usually ate at least once per week?
Include for example: Pâté, cream sauce, custard, radishes, fava beans, coconut, mango, horseradish, parsnips, rhubarb, papaya, dried apricots, dates, figs. (Do not include dry spices and do not list something that has been listed in the previous sections.)

YES..... 1

NO (GO TO F157)..... 2

What were the other foods that you usually ate at least once per week?	What was your usual serving size for (name of food)?	How many times per week did you eat it?
a. ☐☐☐	☐☐ _____ ☐☐☐	☐☐
b. ☐☐☐	☐☐ _____ ☐☐☐	☐☐
c. ☐☐☐	☐☐ _____ ☐☐☐	☐☐

F157. Did you follow a special diet?

YES..... 1

NO (GO TO SECTION G)..... 2

F157a. Was the diet prescribed by a nurse, physician or dietician or was it chosen by you?

PHYSICIAN..... 1

NURSE..... 2

DIETICIAN..... 3

SELF-PRESCRIBED..... 4

F157b. For how many years had you been following this diet?

Number of years ☐☐

F157c. What kind of diet did you follow?	Yes	No
Weight reduction (low calorie)	1	2
Low cholesterol	1	2
Low sodium	1	2
Diabetic	1	2
Low fat	1	2
Low triglyceride	1	2
Ulcer	1	2
High Potassium	1	2
Other Specify ☐☐☐ _____	1	2

G. NUTRITIONAL SUPPLEMENTS

I'd like to ask you about your use of nutritional and dietary supplements including vitamins and minerals. I'd like to know about any supplements you took during the period three months before you became pregnant (with CHILD'S NAME) and during your entire pregnancy. This would be the period from (DATE) to (DATE) or the entire time period shown on your pregnancy calendar.

If you have any vitamins, minerals or other supplements you took during that time period available in the house now, please refer to the information on their bottles for these questions. During this period did you take...

G1. ...multivitamins?	YES (GO TO SUPPLEMENT FORM) ..... 1 NO ..... 2 RF ..... 7 DK 8
G2. ...any other combination of vitamins and minerals in the same tablet?	YES (GO TO SUPPLEMENT FORM) ..... 1 NO ..... 2 RF ..... 7 DK 8
G3. The rest would be vitamins and minerals taken alone and not part of a multivitamin or other combination you already told me about. During this period did you take any Vitamin A or retinol?	YES (GO TO SUPPLEMENT FORM) ..... 1 NO ..... 2 RF ..... 7 DK 8
G4. ...Beta-carotene?	YES (GO TO SUPPLEMENT FORM) ..... 1 NO ..... 2 RF ..... 7 DK 8
G5. ...Vitamin C?	YES (GO TO SUPPLEMENT FORM) ..... 1 NO ..... 2 RF ..... 7 DK 8
G6. ...Vitamin E?	YES (GO TO SUPPLEMENT FORM) ..... 1 NO ..... 2 RF ..... 7 DK 8
G7. ...Calcium?	YES (GO TO SUPPLEMENT FORM) ..... 1 NO ..... 2 RF ..... 7 DK 8
G8. ...Vitamin D?	YES (GO TO SUPPLEMENT FORM) ..... 1 NO ..... 2 RF ..... 7 DK 8

G9. ...Thiamine or Vitamin B1?	YES (GO TO SUPPLEMENT FORM) ..... 1 NO ..... 2 RF ..... 7 DK 8
G10. ...Riboflavin or Vitamin B2?	YES (GO TO SUPPLEMENT FORM) ..... 1 NO ..... 2 RF ..... 7 DK 8
G11. ...Vitamin B6? (VITAMIN B6 IS ALSO KNOWN AS PYRIDOXINE)	YES (GO TO SUPPLEMENT FORM) ..... 1 NO ..... 2 RF ..... 7 DK 8
G12. ...Vitamin B12? (VITAMIN B12 IS ALSO KNOWN AS CYANOCOBALAMIN)	YES (GO TO SUPPLEMENT FORM) ..... 1 NO ..... 2 RF ..... 7 DK 8
G13. ...Folic Acid?	YES (GO TO SUPPLEMENT FORM) ..... 1 NO ..... 2 RF ..... 7 DK 8
G14. ...Niacin?	YES (GO TO SUPPLEMENT FORM) ..... 1 NO ..... 2 RF ..... 7 DK 8
G15. ...Selenium?	YES (GO TO SUPPLEMENT FORM) ..... 1 NO ..... 2 RF ..... 7 DK 8
G16. ...Iron?	YES (GO TO SUPPLEMENT FORM) ..... 1 NO ..... 2 RF ..... 7 DK 8
G17. ...Zinc?	YES (GO TO SUPPLEMENT FORM) ..... 1 NO ..... 2 RF ..... 7 DK 8
G18. ...any other nutritional supplement or herbal preparation?	YES (GO TO SUPPLEMENT FORM) ..... 1 NO ..... 2 RF ..... 7 DK 8

H. MEDICATIONS

I'd like to ask you about some health conditions and your use of medications – including ones that were prescribed for you, ones you bought at the store without a prescription or ones given to you by some one else. I'd like to know about anything you took during the period three months before you became pregnant (with CHILD'S NAME) and during your entire pregnancy. This would be the period from (DATE) to (DATE) or the entire time period shown on your pregnancy calendar.

If you have any prescription or non-prescription medications you took during that time period available in the house now, please refer to the information on their bottles for these questions.

H1.	During the pregnancy, did you have morning sickness, nausea, or vomiting?	YES .....1 NO (GO TO H2) .....2 RF (GO TO H2) .....7 DK (GO TO H2) 8

H1a. During which months of your pregnancy did you have nausea or vomiting?	MONTH	YES	NO	PREG. ENDED	DK
	1	...1	...2	...3	...8
	2	...1	...2	...3	...8
	3	...1	...2	...3	...8
	4	...1	...2	...3	...8
	5	...1	...2	...3	...8
	6	...1	...2	...3	...8
	7	...1	...2	...3	...8
	8	...1	...2	...3	...8
	9	...1	...2	...3	...8

H1b.	Did you take any medication for nausea or vomiting?	YES (GO TO SUPPLEMENT FORM) .....1 NO .....2 RF .....7 DK 8
H2.	Did you take medicine for sugar diabetes?	YES (GO TO SUPPLEMENT FORM) .....1 NO .....2 RF .....7 DK 8
H2a.	Did you develop diabetes during your pregnancy?	YES .....1 NO (GO TO H3) .....2 RF (GO TO H3) .....7 DK (GO TO H3) 8

H2b.	During which month of your pregnancy were you diagnosed with diabetes?	MONTH ☐☐
H3.	Did you take medication for high blood pressure or hypertension?	YES (GO TO SUPPLEMENT FORM) ..... 1 NO ..... 2 RF ..... 7 DK 8
H4.	Did you take medication to help you lose weight?	YES (GO TO SUPPLEMENT FORM) ..... 1 NO ..... 2 RF ..... 7 DK 8
H5.	...to help you sleep?	YES (GO TO SUPPLEMENT FORM) ..... 1 NO ..... 2 RF ..... 7 DK 8
H6.	...to help you stay awake?	YES (GO TO SUPPLEMENT FORM) ..... 1 NO ..... 2 RF ..... 7 DK 8
H7.	...for anxiety, depression or your mood?	YES (GO TO SUPPLEMENT FORM) ..... 1 NO ..... 2 RF ..... 7 DK 8
H8.	...for epilepsy, convulsions, or seizures?	YES (GO TO SUPPLEMENT FORM) ..... 1 NO ..... 2 RF ..... 7 DK 8
H9.	...for allergies, asthma, or hay fever?	YES (GO TO SUPPLEMENT FORM) ..... 1 NO ..... 2 RF ..... 7 DK 8
H10.	...for acne or other skin problems?	YES (GO TO SUPPLEMENT FORM) ..... 1 NO ..... 2 RF ..... 7 DK 8
H11.	...for headache, joint pain, arthritis or other pain? This includes aspirin, Tylenol, Advil, ibuprofen, etc.	YES (GO TO SUPPLEMENT FORM) ..... 1 NO ..... 2 RF ..... 7 DK 8

H12.	...for urinary tract infection or any other infection, inflammation, fever? This includes antibiotics, aspirin, Tylenol, Advil, ibuprofen, etc.	YES (GO TO SUPPLEMENT FORM) ..... 1 NO ..... 2 RF ..... 7 DK 8
H13.	...for a thyroid condition?	YES (GO TO SUPPLEMENT FORM) ..... 1 NO ..... 2 RF ..... 7 DK 8
H14.	...for upset stomach, gas or an ulcer? (NOT INCLUDING WHAT THEY TOOK FOR MORNING SICKNESS H1b)	YES (GO TO SUPPLEMENT FORM) ..... 1 NO ..... 2 RF ..... 7 DK 8
H15.	...for constipation?	YES (GO TO SUPPLEMENT FORM) ..... 1 NO ..... 2 RF ..... 7 DK 8
H16.	...for hormonal or metabolic problems or anything you saw an endocrinologist for? (OTHER THAN DIABETES)	YES (GO TO SUPPLEMENT FORM) ..... 1 NO ..... 2 RF ..... 7 DK 8
H17.	...to help you conceive a child, such as Clomid or other fertility drugs?	YES (GO TO SUPPLEMENT FORM) ..... 1 NO ..... 2 RF ..... 7 DK 8
H18.	Did you take any other medications, including herbal preparations, during the 3 months before you became pregnant and during your pregnancy that you have not already told me about?	YES (GO TO SUPPLEMENT FORM) ..... 1 NO ..... 2 RF ..... 7 DK 8

J. CIGARETTE SMOKING

Now I'd like to ask you some questions about some personal habits including cigarette smoking and drinking alcohol.

J1.	Have you ever smoked cigarettes regularly? By this I mean smoking more than 100 cigarettes at any time in your life.	YES ..... 1 NO (GO TO J10) 2
J2.	How old were you when you first started smoking cigarettes regularly?	YEARS OF AGE ☐☐
J3.	Did you smoke any cigarettes during the three-month period before you became pregnant (with CHILD'S NAME)?	YES ..... 1 NO (GO TO J4) ..... 2 RF (GO TO J4) ..... 7 DK (GO TO J4) 8
J3a.	During the three months before your pregnancy, about how many cigarettes did you smoke a day?	<1 PER DAY ..... 1 1 PER DAY ..... 2 2-4 PER DAY ..... 3 ½ PACK (5-14) ..... 4 1 PACK (15-24) ..... 5 1 ½ PACK (25-34) ..... 6 2 PACKS (35-44) ..... 7 > 2 PACKS ..... 8 RF ..... 97 DK 98
J4.	Did you smoke any cigarettes during the first three months of your pregnancy (with CHILD'S NAME)?	YES ..... 1 NO (GO TO J5) ..... 2 RF (GO TO J5) ..... 7 DK (GO TO J5) 8
J4a.	During your first three months of your pregnancy, about how many cigarettes did you smoke a day?	<1 PER DAY ..... 1 1 PER DAY ..... 2 2-4 PER DAY ..... 3 ½ PACK (5-14) ..... 4 1 PACK (15-24) ..... 5 1 ½ PACK (25-34) ..... 6 2 PACKS (35-44) ..... 7 > 2 PACKS ..... 8 RF ..... 97 DK 98

J5.	Did you smoke any cigarettes during the fourth, fifth and sixth months of your pregnancy (with CHILD'S NAME)? This is the second trimester.	YES ..... 1 NO (GO TO J6) ..... 2 RF (GO TO J6) ..... 7 DK (GO TO J6) 8
J5a.	During your fourth, fifth, and sixth month of pregnancy about how many cigarettes did you smoke a day?	<1 PER DAY ..... 1 1 PER DAY ..... 2 2-4 PER DAY ..... 3 ½ PACK (5-14) ..... 4 1 PACK (15-24) ..... 5 1 ½ PACK (25-34) ..... 6 2 PACKS (35-44) ..... 7 > 2 PACKS ..... 8 RF ..... 97 DK 98
J6.	Did you smoke any cigarettes during the seventh, eighth, and ninth months of your pregnancy (with CHILD'S NAME)? This is the third trimester.	YES ..... 1 NO (GO TO J7) ..... 2 RF (GO TO J7) ..... 7 DK (GO TO J7) 8
J6a.	During your seventh, eighth, and ninth month of pregnancy about how many cigarettes did you smoke a day?	<1 PER DAY ..... 1 1 PER DAY ..... 2 2-4 PER DAY ..... 3 ½ PACK (5-14) ..... 4 1 PACK (15-24) ..... 5 1 ½ PACK (25-34) ..... 6 2 PACKS (35-44) ..... 7 > 2 PACKS ..... 8 RF ..... 97 DK 98
J7.	Do you smoke cigarettes now?	YES ..... 1 NO (GO TO J9) 2
J8.	On the average, how many cigarettes do you now smoke per day?	PER DAY ☐☐ GO TO J10
J9.	How long ago did you stop smoking?	YEARS ☐☐ MO ☐☐
J10.	During the three months before or during your entire pregnancy did anyone (besides you) regularly smoke cigarettes in your home, workplace or any other place near you?	YES ..... 1 NO (GO TO SECTION K) ..... 2 DK (GO TO SECTION K) 8

J11a.	During the three months before you became pregnant did someone smoke in your home, workplace or any other place near you?	YES.....1 NO.....2 RF.....7 DK.....8
J11b.	During the first three months of your pregnancy did someone smoke in your home, workplace or any other place near you?	YES.....1 NO.....2 RF.....7 DK.....8
J11c.	During the fourth, fifth, and sixth month of your pregnancy did someone smoke in your home, workplace or any other place near you?	YES.....1 NO.....2 RF.....7 DK.....8
J11d.	During the seventh, eighth, and ninth month of your pregnancy did someone smoke in your home, workplace or any other place near you?	YES.....1 NO.....2 RF.....7 DK.....8

K. ALCOHOL CONSUMPTION

Now I'm going to ask you some questions about drinking alcoholic beverages which include liquor, beer, or wine.

K1.	Have you ever drank alcohol regularly, this includes beer, wine, wine coolers, mixed drinks, cocktails, or shots of hard liquor, at any time in your life?	YES ..... 1 NO (GO TO SECTION L) ..... 2 RF (GO TO SECTION L) ..... 7 DK (GO TO SECTION L) 8		
K2.	When you drank alcohol, what type(s) of alcohol did you usually drink? Did you drink....	YES	NO	DK
K2a.	Beer.....	1	2	8
K2b.	White wine.....	1	2	8
K2c.	Red wine.....	1	2	8
K2d.	Mixed drink.....	1	2	8
K2e.	Shot liquor.....	1	2	8
K2f.	Other liquor..... SPECIFY: _____ ☐☐	1	2	8
K3.	During the <u>three-month period before</u> you became pregnant (with CHILD'S NAME), did you drink any alcohol? This includes the same types of alcohol that I mentioned before.	YES ..... 1 NO (GO TO K4) ..... 2 RF (GO TO K4) ..... 7 DK (GO TO K4) 8		
K3a.	During this three-month period before you became pregnant, how often did you usually drink alcohol? Was it...	Every day or most days ..... 1 3-4 times per week ..... 2 1-2 times per week ..... 3 1-3 times per month ..... 4 Less than once per month ..... 5 RF ..... 7 DK 8		

K3b.	On the days you drank alcohol during the three-month period before you became pregnant, how many drinks did you usually have? Was it…	8 or more per day ..... 1 5-7 per day ..... 2 3-4 per day ..... 3 1-2 per day ..... 4 Less than 1 per day ..... 5 RF ..... 7 DK 8
K3c.	During the three-month period before you became pregnant, what was the greatest number of drinks that you had in one 24-hour period? Was it…	More than 24 ..... 1 12-23 ..... 2 6-11 ..... 3 3-5 ..... 4 1-2 ..... 5 RF ..... 7 DK 8
Now, I'd like to ask you about the time during your pregnancy. This includes any time between the estimated date you became pregnant (with CHILD'S NAME), shown in a red box on your pregnancy calendar, and the end of your pregnancy.		
K4.	During your <u>entire pregnancy</u> did you drink any alcohol?	YES ..... 1 NO (GO TO SECTION L) ..... 2 RF (GO TO SECTION L) ..... 7 DK (GO TO SECTION L) 8
K5.	During the <u>first three months</u> of your pregnancy, did you drink any alcohol?	YES ..... 1 NO (GO TO K6) ..... 2 RF (GO TO K6) ..... 7 DK (GO TO K6) 8
K5a.	During the <u>first three months</u> of your pregnancy, how often did you usually drink alcohol? Was it…	Every day or most days ..... 1 3-4 times per week ..... 2 1-2 times per week ..... 3 1-3 times per month ..... 4 Less than once per month ..... 5 RF ..... 7 DK 8
K5b.	On the days you drank alcohol during the <u>first three months</u> of your pregnancy, how many drinks did you usually have? Was it…	8 or more per day ..... 1 5-7 per day ..... 2 3-4 per day ..... 3 1-2 per day ..... 4 Less than 1 per day ..... 5 RF ..... 7 DK 8

K5c.	During the first three months of your pregnancy, what was the greatest number of drinks that you had in one 24-hour period? Was it…	More than 24 ..... 1 12-23 ..... 2 6-11 ..... 3 3-5 ..... 4 1-2 ..... 5 RF ..... 7 DK 8
K6.	During the fourth, fifth, and sixth months of your pregnancy, did you drink any alcohol? This is the second trimester.	YES ..... 1 NO (GO TO K7) ..... 2 RF (GO TO K7) ..... 7 DK (GO TO K7) 8
K6a.	During the fourth, fifth, and sixth months of your pregnancy, how often did you usually drink alcohol? Was it…	Every day or most days ..... 1 3-4 times per week ..... 2 1-2 times per week ..... 3 1-3 times per month ..... 4 Less than once per month ..... 5 RF ..... 7 DK 8
K6b.	On the days you drank alcohol during the fourth, fifth, and sixth months of your pregnancy, how many drinks did you usually have? Was it…	8 or more per day ..... 1 5-7 per day ..... 2 3-4 per day ..... 3 1-2 per day ..... 4 Less than 1 per day ..... 5 RF ..... 7 DK 8
K6c.	During the fourth, fifth, and sixth months of your pregnancy, what was the greatest number of drinks that you had in one 24-hour period? Was it…	More than 24 ..... 1 12-23 ..... 2 6-11 ..... 3 3-5 ..... 4 1-2 ..... 5 RF ..... 7 DK 8
K7.	During the seventh, eighth, and ninth months of your pregnancy, did you drink any alcohol? This is the third trimester.	YES ..... 1 NO (GO TO SECTION L) ..... 2 RF (GO TO SECTION L) ..... 7 DK (GO TO SECTION L) 8

K7a.	During the seventh, eighth, and ninth months of your pregnancy, how often did you usually drink alcohol? Was it…	Every day or most days ..... 1 3-4 times per week ..... 2 1-2 times per week ..... 3 1-3 times per month ..... 4 Less than once per month ..... 5 RF ..... 7 DK 8
K7b.	On the days you drank alcohol during the seventh, eighth, and ninth months your pregnancy, how many drinks did you usually have? Was it…	8 or more per day ..... 1 5-7 per day ..... 2 3-4 per day ..... 3 1-2 per day ..... 4 Less than 1 per day ..... 5 RF ..... 7 DK 8
K7c.	During the seventh, eighth, and ninth months of your pregnancy, what was the greatest number of drinks that you had in one 24-hour period? Was it…	More than 24 ..... 1 12-23 ..... 2 6-11 ..... 3 3-5 ..... 4 1-2 ..... 5 RF ..... 7 DK 8

L. OCCUPATIONAL HISTORY

Now I'd like to ask you about any jobs you may have had during the one-year period before (CHILD'S NAME was born/ your pregnancy ended), that includes the three months before you became pregnant thru the time you delivered. Please include any part-time or full-time jobs you had at home, on a farm or outside your home. If you had more than one job or changed jobs but were with the same employer, I'll ask about each one separately.

L1.	Did you have any part-time or full-time jobs, either inside or outside of your home, during the year before (CHILD'S NAME was born/ your pregnancy ended)?	YES ..... 1 NO (GO TO SECTION M) 2
	I'll begin with the job you had earliest in this period and end with the job you had just before (CHILD'S NAME was born/ your pregnancy ended).	
L2a.	What was the name of your employer (EXACT NAME) or were you self-employed?	_____
L2b.	What kind of business or industry was this (for example, manufacturing, retail, education, restaurant, work at home or on a farm)?	_____ ☐☐☐
L2c.	What kind of work did you do or what was your job title? (for example, homemaker, farmer, engineer, office assistant, waitress, manager, teacher?)	_____ ☐☐☐
L2d.	What were your most frequent activities or duties (for example, typing, keeping account books, helping customers, supervising employees, caring for children, teaching)?	1. _____ ☐☐☐ 2. _____ ☐☐☐ 3. _____ ☐☐☐
L2e.	During the year before (CHILD'S NAME was born/ your pregnancy ended), approximately how many hours per week did you work at this job?	HOURS PER WEEK ☐☐
L2f.	When did you begin working at this job?	MONTH ☐☐ YEAR ☐☐☐☐

L2g.	When did you stop working at this job? IF STILL WORKING AT THIS JOB, ENTER CURRENT DATE.	MONTH ☐☐ YEAR ☐☐☐☐
L3.	Did you have any other full-time or part-time jobs either inside or outside of your home during the year before (CHILD'S NAME was born/ your pregnancy ended)?	YES.....1 NO (GO TO SECTION M).....2
L3a.	What was the name of your employer (EXACT NAME) or were you self-employed?	_____
L3b.	What kind of business or industry was this (for example, manufacturing, retail, education, restaurant, work at home or on a farm)?	_____ ☐☐☐
L3c.	What kind of work did you do or what was your job title? (for example, homemaker, farmer, engineer, office assistant, waitress, manager, teacher?)	_____ ☐☐☐
L3d.	What were your most frequent activities or duties (for example, typing, keeping account books, helping customers, supervising employees, caring for children, teaching)?	1. _____ ☐☐☐ 2. _____ ☐☐☐ 3. _____ ☐☐☐
L3e.	During the year before (CHILD'S NAME was born/ your pregnancy ended), approximately how many hours per week did you work at this job?	HOURS PER WEEK ☐☐
L3f.	When did you begin working at this job?	MONTH ☐☐ YEAR ☐☐☐☐
L3g.	When did you stop working at this job? IF STILL WORKING AT THIS JOB, ENTER CURRENT DATE.	MONTH ☐☐ YEAR ☐☐☐☐

L4.	Did you have any other full-time or part-time jobs either inside or outside of your home during the year before (CHILD'S NAME was born/ your pregnancy ended)?	YES.....1 NO (GO TO SECTION M).....2
L4a.	What was the name of your employer (EXACT NAME) or were you self-employed?	_____
L4b.	What kind of business or industry was this (for example, manufacturing, retail, education, restaurant, work at home or on a farm)?	_____ ☐☐☐
L4c.	What kind of work did you do or what was your job title? (for example, homemaker, farmer, engineer, office assistant, waitress, manager, teacher?)	_____ ☐☐☐
L4d.	What were your most frequent activities or duties (for example, typing, keeping account books, helping customers, supervising employees, caring for children, teaching)?	1. _____ ☐☐☐ 2. _____ ☐☐☐ 3. _____ ☐☐☐
L4e.	During the year before (CHILD'S NAME was born/ your pregnancy ended), approximately how many hours per week did you work at this job?	HOURS PER WEEK ☐☐
L4f.	When did you begin working at this job?	MONTH ☐☐ YEAR ☐☐☐☐
L4g.	When did you stop working at this job? IF STILL WORKING AT THIS JOB, ENTER CURRENT DATE.	MONTH ☐☐ YEAR ☐☐☐☐

M. INCOME

Now I'd like to ask you about your income. Please be assured that, like everything else in this interview, all of this information will be kept strictly confidential.

M1.	In the year before you became pregnant (with CHILD'S NAME), what was your total household income? This would have been from (DATE 1 year before conception) to (DATE of conception) Was it less than ten thousand dollars, more than fifty thousand dollars or somewhere in between?	Less than $10,000 (GO TO M3) ..... 1 More than $50,000 (GO TO M3) ..... 2 In between ..... 3 RF (GO TO M3) ..... 7 DK (GO TO M3) 8
M2.	Would you say it was....? (IF RESPONSE IS $20,000, ROUND UP TO THE HIGH RANGE, $20-30,000; DO THE SAME FOR $30 –$40,000)	$10-20,000 ..... 1 $20-30,000 ..... 2 $30-40,000 or ..... 3 $40-50,000 ..... 4 RF ..... 7 DK 8
M3.	How many people were supported by this income, including both adults and children?	# OF PEOPLE ☐☐ DK = 98

N. CLOSING AND FUTURE CONTACT INFORMATION

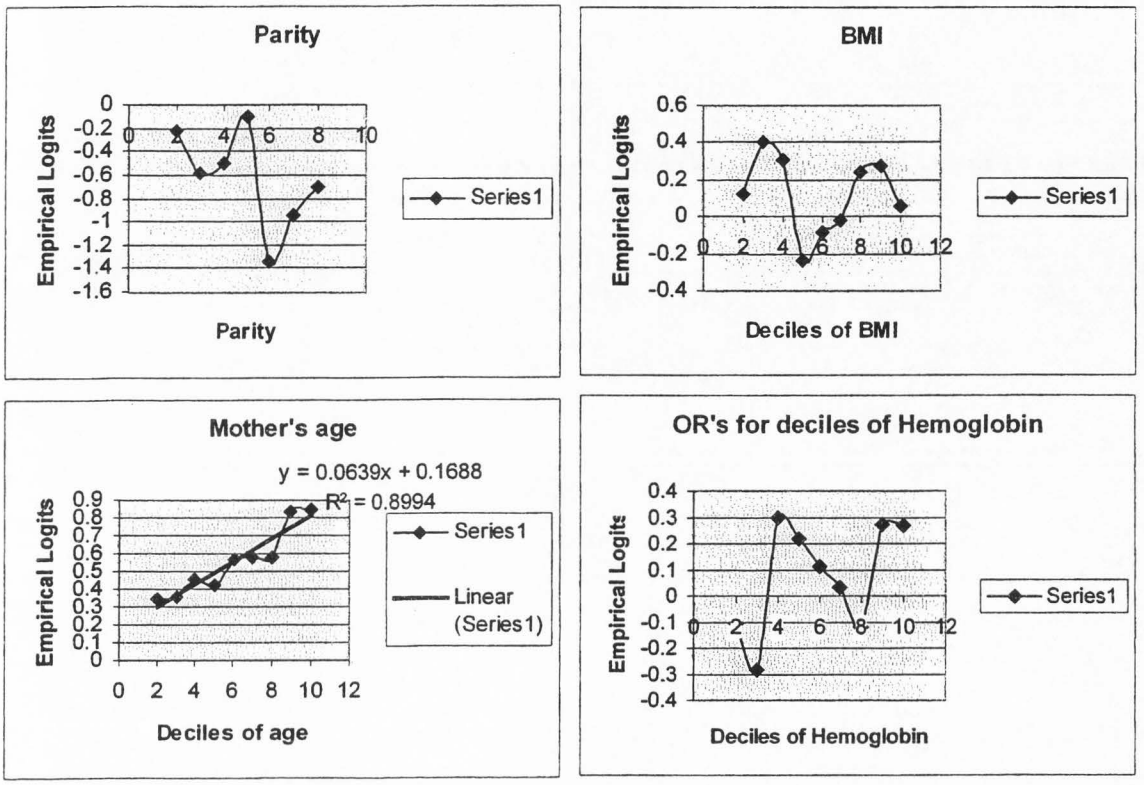
That completes the interview. Thanks very much for doing this with me today. Your contribution to this study will help us greatly in our efforts to better understand the causes and prevention of cleft lip and cleft palate.

N1.	In case we need to get in touch with you in the future, would you be willing to give me the name and address of someone who does not live with you who should always know where you will be? This information will be kept separate from your questionnaire. It will be locked except when needed by the research team.	YES ..... 1 RF (GO TO N2) ..... 7 DK (GO TO N2) 8
N1a.	PRINT LEGIBLY FIRST NAME: LAST NAME: RELATIONSHIP TO RESPONDENT: STREET ADDRESS: CITY, STATE: ZIP CODE: (AREA CODE) TELEPHONE NUMBER:	N1b. PRINT LEGIBLY FIRST NAME: LAST NAME: RELATIONSHIP TO RESPONDENT: STREET ADDRESS: CITY, STATE: ZIP CODE: (AREA CODE) TELEPHONE NUMBER:

N2.	COMPLETE FORM CALLED "ARRANGEMENTS FOR BLOOD AND DNA SAMPLE COLLECTION".	INFORMATION OBTAINED ..... 1 UNABLE TO OBTAIN INFORMATION (EXPLAIN) .. 2 EXPLAIN: _____ _____ _____
N3.	TIME INTERVIEW WAS COMPLETED	☐☐ : ☐☐ AM PM

P. ADDITIONAL INTERVIEWER OBSERVATIONS		
P1.	INTERVIEWER ASSESSMENT OF QUALITY OF INTERVIEW	GOOD ..... 1 FAIR (EXPLAIN IN P2) ..... 2 UNSATISFACTORY (EXPLAIN IN P2) 3
P2.	WAS THERE ANYTHING UNUSUAL ABOUT THIS INTERVIEW THAT YOU WOULD LIKE TO DESCRIBE?	_____ _____ _____ _____ _____ _____

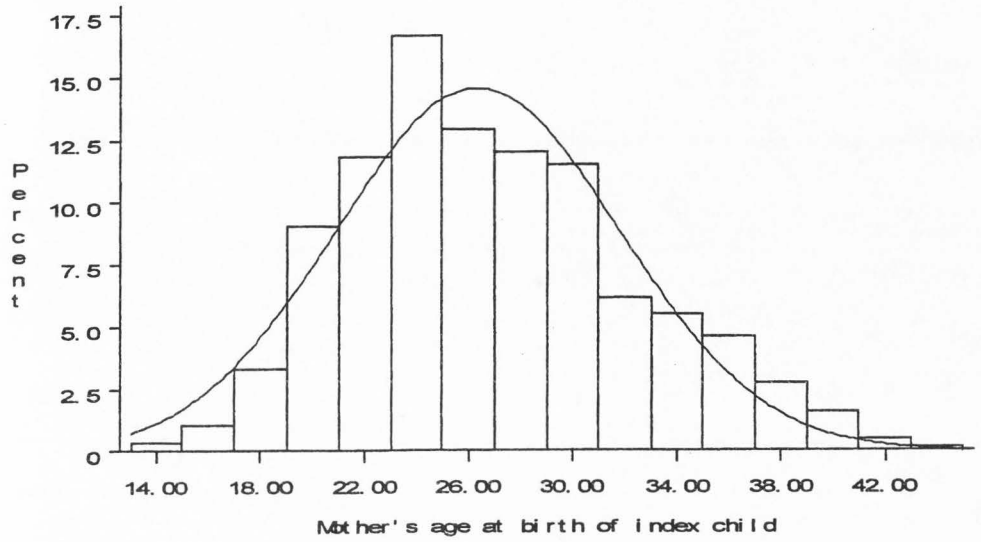
APPENDIX B: Statistical Notes

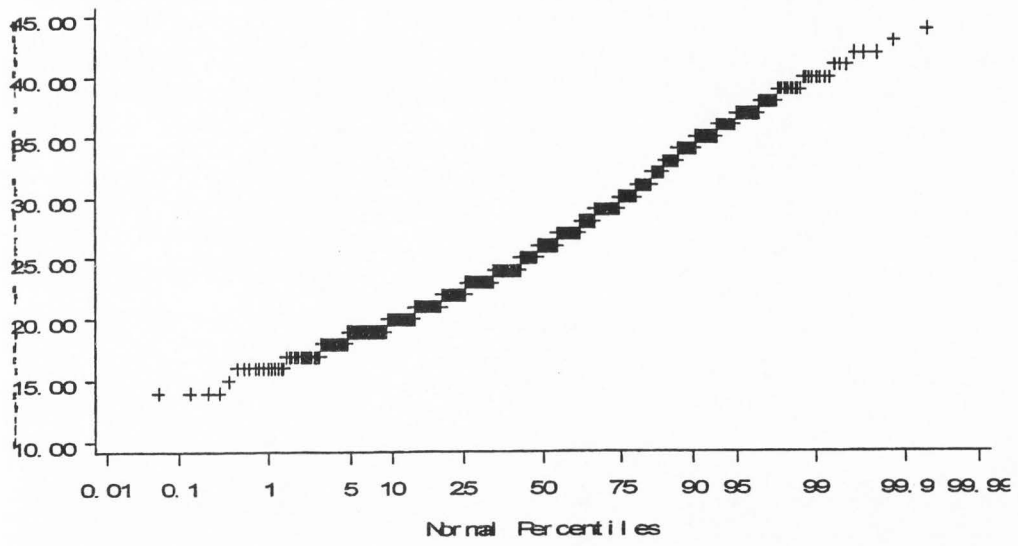


Figures 1-4: Graphs showing the non-linear relationships between the variables parity, hemoglobin and BMI and risk of clefts and the linear relationship between clefting and mother's age.

Table 1: Table showing the chi squared p-values for categorical variables- investigating the relationships between possible model covariates in order to avoid multi-colinearity problems during modeling.

	Edu	Ethnic	Income	Alcohol	Religion	Marital	Folate	Gender
Education								
Ethnicity	.0001							
Income	.0001	.0001						
Alcohol	.0001	.0615	.0172					
Religion	.0001	.0001	.0001	.0001				
Marital	.0001	.0001	.0001	.0001	.0001			
Folate	.1897	.6389	.9325	.9421	.0433	.2746		
Gender	.5011	.3621	.6882	.7383	.1581	.7164	.8137	
Parity	.0656	.8822	.0001	.0111	.2760	.0035	.0562	.0015





Figures 5-6: Histogram and normal quantile plot showing the distribution of mother's age.

Note: Transformations did not appear to normalize the distribution of mother's age as evidenced by histograms, normal quantile plots and formal tests for normality, which were all highly significant even after transformation.

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A15_Book_ISBN:
A16_Journal_Month: July
A17_Journal_Year: 2003
A18_Journal_Volume: 227
A19_Journal_Issue_Number: 3
A20_Copy_Pages: 389
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A23_Your_Title: Smoking and Oral Clefts in Utah
A24_Publication_Date: April 10th 2006
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A14_Book_Author:
A15_Book_ISBN:
A16_Journal_Month: January
A17_Journal_Year: 1998
A18_Journal_Volume: 75
A19_Journal_Issue_Number: 2
A20_Copy_Pages: 134
A21_Maximum_Copies: 6
A22_Your_Publisher: Utah State University
A23_Your_Title: Smoking and Oral clefts in Utah
A24_Publication_Date: April 10th, 2006
A25_Format: print
A31_Print_Run_Size:
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24/03/06

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Dear Ms Moss

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