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GENETIC EPIDEMIOLOGY OF CLEFT LIP AND PALATE

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INTRODUCTION
Clefts of the lip and palate are birth defects with multifactorial etiology, involving important surgical, speech, social, behavioral, and developmental implications. Approximately 70% of clefts are nonsyndromic—they do not involve other types of abnormalities such as shorter limbs, shorter digits (toes or fingers), wider set eyes, and many other symptoms. A number of environmental factors also play a role in craniofacial deformities. These factors include nutritional deficiencies such as insufficient folic acid consumption or absorption, smoking, and alcohol consumption, factors relevant to socioeconomic status.

The purpose of this review is to present information concerning cleft lip and palate that has been observed and confirmed in past studies, apply this information to reviews of recent epidemiological and molecular genetics studies, evaluate factors surrounding these complex diseases, and create suggestions or questions applicable to future research. The papers reviewed include the examination of candidate genes, environmental factors, or a combination of both. Differences in disease distribution in populations will also be discussed.

BACKGROUND OF CLEFT LIP AND PALATE
Phenotypic Characteristics (Please see Appendix A)

Cleft lip and cleft palate result in two distinct phenotypes that differ in genetic complexity. Cleft lip is a separation of the two sides of the lip. This can include the bone of the upper jaw. Cleft palate involves an opening in the roof of the mouth, so that two sides of the palate do not join together during development. Cleft lips and palates can be unilateral or bilateral.

A cleft lip or palate can result during fetal development between the sixth and eleventh week of pregnancy. Parts of the lip and/or palate fail to come together properly. Some clefts occur more often in specific families. Other clefts are linked to certain syndromes (Syndromic Clefting).

Treatment for cleft lip/palate include primary repair (within the first ten weeks), palatal repair (9-12 months), secondary repair (4-6 years), alveolar cleft repair (8-10 years), and final repair (if needed, 14-16 years), which increase in complexity of surgical repair, respectively. All of these methods of repair involve reconstructive surgery. The type of cleft determines the type of and degree of surgery necessary. The common notation for cleft lip with or without cleft palate is CL/P, and cleft palate only is CP, for cleft lip only is CL.
Disease Distribution

In the United States, birth defects are the leading cause of infant mortality accounting for approximately 20 percent of infant deaths, seventy percent of which result from undetermined causes (Lidral, 2000). The Texas Pediatric Surgical Associates report an incidence of 1 per 700 newborns. The CDC reports a total of 3,259 babies born with Cleft lip/palate in the United States in 2000, and a total of 3,123 in 1999 (3). The prevalence of CL/P varies between ethnic groups whereas CP does not. CP is found more predominantly in males whereas CL/P affects more females. CL/P varies between ethnic groups whereas CP does not (Lidral, 2000). Lidral et al. Estimate that orofacial clefts comprise only half of craniofacial anomalies, occurring in 1-2 per 1,000 live births among Caucasians, the group with the highest prevalence of clefting. As of 2000, 20 to 50 percent of orofacial clefts were associated with over 300 syndromes (syndromic clefting). Many genes associated with CL/P and CP have been identified because of the ability to identify a disease gene (with Mendelian segregation) using linkage analysis. Because cleft lip and cleft palate are two types of craniofacial deformities not associated with orofacial syndromes, they have been distinguished as nonsyndromic clefting. This makes linkage analyses more difficult and fewer genes have been associated with the two disorders.

Approximately 1 in 1500 live births in Caucasians results in CP, whereas the incidence of CL/P is slightly higher, occurring in 1-2 per 1000 live births in Caucasians. The highest incidence of CL/P has been found in populations of Asian descent, followed by populations of Caucasian descent, and is least found in populations of African descent (Lidral, 2000). CP, however, does not vary significantly between racial backgrounds, with the exception of an increase in the Maori and Finnish populations (Lidral, 2000). Sex ratios vary for CP and CL/P, indicating that more females than males are affected with CP and more males than females are affected with CL/P.

The majority of genetic research for orofacial clefting has been spent on CL/P, but no recognizable mode of inheritance for CL/P has yet been identified. Complex inheritance patterns have been identified, however, in families with a history of clefting. There is also an increased risk for children of individuals with CL/P and an increased risk in monozygotic, but not dizygotic, twins.

Factors of Inheritance

The mode of inheritance patterns for CL/P has been described as being autosomal dominant, autosomal recessive, or a major gene-environment interaction. Between 2 and 20 genes (most likely between 3 and 6 genes) interact multiplicatively with each other and are involved in the etiology of CL/P (1). It has also been inferred that one of these loci may represent a major gene with a relative risk for first-degree relatives of up to 12 people. Candidate gene studies composed of small collections of families or patient populations are conducted in order to investigate these “major genes.”

The mode of inheritance for CP is also complex, with a correlation of CP patients to a positive family history for clefting.

Disease genes have been identified in studies using Linkage Mapping, a technique that searches for the co-segregation of alleles acting as genetic markers for a disease phenotype. If a marker and a disease mutation are relatively close to each other on the same
chromosome, they most likely involve linkage (1), and thus are more likely to be transmitted together in incidences of crossing-over events during meiosis where sister chromosomes are paired. Recombination frequency can also be calculated to find the genetic distance, i.e. markers in closer proximity to each other are more likely to be crossed over together (cotransmitted or linked).

Linkage has demonstrated a great deal of power in the identification of genetic disorders, when large families or several small families with mutations in the same gene are obtained for research. This linkage indicates that the disease is homogeneous (1). In heterogeneous diseases, involving more than one gene in order to cause the disease, linkage may not be detectable if several smaller families are used. Thus, CL/P and CP have been complicated to analyze genetically due to the heterogeneity of the diseases and the inability to adequately define the genetic model for either phenotype. Additionally, finding enough data to support large pedigrees with a history of nonsyndromic clefting is challenging.

A study done in a Finland populations by de la Chapelle in 1993 found that the Founder Effect, observed in genetically isolated populations results in a higher degree of homogeneity, allowing for certain deleterious conditions such as genetic diseases to proliferate within the population (1, de la Chapelle, 1993). Diastrophic dysplasia, a recessive form of Syndromic Cleft Palate, is one of such diseases found in the Finnish population studied (1). This finding provides insight into migration patterns of populations throughout history that resulted in more isolated populations that contain deleterious alleles for mutations such as those associated with CL/P and CP. This may be significant to consider on an epidemiological and evolutionary scale when analyzing these disorders.

Three specific genes under analysis in Lidral/Murray labs at the University of Iowa Human Genetics lab have been identified to have a role in the etiology of CL/P and CP are TGFA, TGFB3, and MSX1. Linkage disequilibrium has been observed between TGFB3 and CL/P in two Caucasian populations (1). In addition, a possible gene-environment interaction between TGFA and smoking with CP was identified in a Maryland population in 1995. In a Californian population, smoking demonstrated a significant risk factor for both CL/P and CP in a 1996 study. More recent studies, including one by Paul Romitti in 1998, UI College of Public Health, have found a gene-environment interaction between TGFB3, MSX1, and both smoking and alcohol in an Iowa population (7,8).

Additional Risk Factors

Risk factors for orofacial clefting and other craniofacial anomalies include smoking, stress, alcohol consumption, diet, exercise, and medications, especially medications that alter natural steroid levels in the body. One study that provided evidence for such a steroidal relationship was conducted by Dr. Michael Melnick, Dr. Tina Jaskoll, et al., at the University of Southern California (5). This study found that not genes alone, but a genetic “circuit” is responsible for palate formation. The “surge” that causes the genetic circuit to break is due to a high influx of steroid hormones. An investigation involving female mice was performed showing that female mice given steroid hormones during pregnancy had more offspring characterized by cleft palate (5,6).
Other studies have shown a significant increase in the risk for nonsyndromic orofacial clefting (in the form of cleft palate or cleft lip, or both) associated with mothers who smoke while pregnant. One study conducted at the Center of Disease Control and Prevention found that the combination of smoking in the presence of the null allele of GSTT1 in the mother resulted in an increased risk for clefting in the child three fold, compared to the presence of the wild type allele and no smoking. When both the mother and child had the null allele, in combination with smoking, the risk of clefting was even higher. The presence of the null allele or smoking alone was not associated with an increased risk for clefting, with possibly one exception. A similar study was also conducted in a Dutch population involving the CYP1A1 gene (9).

Recent investigations also carried out by the CDC found linkage between the drug, Acutane, a prescription acne treatment, and birth defects, if the drug was taken during pregnancy (4). The label for the drug now contains warnings for women expecting children or women of reproductive age (Boston University Acutane Survey, 2000). The specific birth defects involved were not determined.

**Treatment**

The most common form of treatment for children with CL/P and CP is surgery. Bone grafting is one type of surgery that involves grafting bone of the dental ridge of the upper jaw (maxilla). The most common way for this reconstruction is to move small pieces of bone from the hip, head, ribs, or leg and move it to an area of the cleft near the teeth. This type of surgery allows for 1) support for unerupted teeth and teeth next to the cleft, 2) support for the lip and nose which improves symmetry, 3) the formation of a continuous upper gum (alveolar) ridge, which creates a more natural appearance and stability to the ridge, and 4) an improvement in the stability of the front part of the roof of the mouth (premaxilla), when bilateral cleft is present. Typically, this procedure is most successful in patients younger than 10 years. After this type of surgery, one or more of three forms of oral support are needed: 1) movement of adjacent teeth into the bone graft; 2) prosthetic replacement (dental bridge); or 3) dental metallic bone implants.

One of the most widespread discrepancies in treatment of Cleft lip and palate is the inability to financially support surgery or the lack of access to resources and staff trained for such surgical procedures. In developing countries where the only hospital with the capacity to perform such surgical techniques may be one that is in the capital city, the cost of such may be even higher as a result. Thus the child desiring surgery will be responsible for transportation costs as well as enormous costs of surgery.

**Dietary Effects**

Folic acid supplements have also been used in some investigations for the prevention of birth defects, including orofacial clefting. Folic acid, also known as folate, is a B-vitamin that can be found in some enriched foods and vitamin pills. If women have enough of it in their bodies before pregnancy, this vitamin can decrease the risk for neural tube defects (NTDs), which cause defects in a baby's brain (anencephaly) or spine (spina bifida) (3,8). The recommended dose of folic acid is 400 micrograms per day, which is not difficult for many women in developed nations, but may be much more dif-
difficult for pregnant women in developing nations.

A study was done in a Chinese population in 1998 in order to determine the effectiveness of folic acid in reducing rates of neural tube defects (NTDs) in two areas of China. The study showed that in northern China, which has a high incidence of NTDs, women who took 400 micrograms (0.4mg) of folic acid daily at least 80% of the time before and in the early stages of pregnancy reduced their risk of having an NTD-affected pregnancy by 85%. Among participating women in southern China, where the incidence was lower (similar to the U.S. NTD rate), the reduction in risk was 40% (3).

SIGNIFICANCE OF THE DISEASE

The Impact of Craniofacial-Oral-Dental Conditions on the Quality of Life

Diseases and disorders such as cleft lip and palate that present characteristic differences in the affected individuals can disturb a person’s well-being and self-esteem. This includes functional, psychological, social, and economic consequences. In children with cleft lip or cleft palate, they not only experience problems with breathing, eating, and speaking, but may also have problems adjusting socially, which can affect their education, occupation, and behavior. Oral facial pain may also affect some individuals because of the richness of nerve endings sensitive to stimuli in the craniofacial region.

Economic Effects

Any long-term illness creates a multitude of economic hardships, even with the assistance of insurance, friends, and relatives. The common trend is that those with more money receive better medical care. This is significantly apparent in comparisons between medical care in developed nations such as the U.S. and Western Europe and developing countries. Differences in risk of birth defects including orofacial clefting is also of more significant to U.S. populations who are poor, racial/ethnic minorities, refugees-immigrants, and people lacking dental or medical insurance.

Estimates for the lifetime costs of the multiple surgeries and other medical, dental, and rehabilitation therapies for treating cleft lip or cleft palate at a minimum of $100,000, which poses a significant problem in most countries, especially developing nations. The lack of treatment can result in significant chronic oral-facial pain, and the risk of illegal drug use can become a factor.

REVIEW OF LITERATURE

A population-based study by Tolarova and Cervenka compared cases of orofacial clefts including cleft lip (CL), cleft palate (CP), or cleft lip and palate (CL/P), considered being “typical clefts,” to cases of “atypical” clefts (median, transversal, or oblique facial clefts), in relation to the conditions in which they occur. A sample of 4,433 cases was ascertained from 2,509,881 California births. The cases were classified into eight categories: isolated cleft anomalies, sequences of the primary defect, chromosomal aberrations, monogenic syndromes, results of known teratogens, associations, multiple congenital anomaly (MCA) of unknown etiology, and conjoined twins. The birth prevalence of isolated CL+-P was 0.77 per 1,000 births (CL 0.29/1,000, CL/P 0.48/1,000) and of isolated CP, 0.31 per 1,000 births. Non-Hispanic whites had the greatest prevalence.
of isolated clefts; Asians slightly lower prevalence, and blacks the lowest. Asians had the lowest prevalence of Robin sequence and Non-Hispanic Whites the highest, twice that of Hispanics. Hispanics, followed by Asians, had the highest prevalence of CL+:P with MCA; non-Hispanic Whites had the lowest. Asians had the lowest prevalence of CP; in Whites and Hispanics it was close to twice as high. Blacks had the highest CL:CL/P ratio, followed by non-Hispanic Whites and Asians; Hispanics had the lowest. Isolated anomalies constituted 61.67% of clefts. There were 3.9% sequences, 8.79% chromosomal aberrations, 6.02% monogenic syndromes, 0.2% known teratogens, 0.79% associations, 18.55% MCA of unknown etiology, and 0.1% in conjoined twins, for the total sample.

As a whole, this study represents an evaluation of each child on a case-by-case level, and provides a framework for genetic counseling and other studies focused on causes and prevention of orofacial anomalies. This publication will serve as an excellent reference for researchers of cleft lip and palate because it organizes extensive previous research publications into a more compact and understandable format. It also serves as a method for picking up trends in orofacial clefting occurrence in different populations, which can be based upon genetic and environmental contexts. In addition to these I would also note the application of such data to social, cultural, and historical aspects of each population as well.

Confounding variables in this study included (but were not limited to) environmental factors that contribute to orofacial clefting and other birth defects. Because of environmental influence, such as nutrition (or malnutrition in some populations in developing countries). Because of this variable, the authors developed a subgroup referred to as Orofacial Clefts in Known Environmental Syndromes. This included individuals in which the orofacial cleft was part of the spectrum of environmental embryopathy or fetopathy caused by a known teratogen such as fetal alcohol syndrome or Dilantin syndrome.

Selection biases in this paper may have occurred. This was possible because the researchers selected the literature to include in the study, and which papers not to include. The authors did not discuss a bias within each of the papers selected. Furthermore, if researchers wanted to exclude papers that would have distorted their classifications towards desirable results, they could have done so. If similar biases existed for each individual paper, it is possible that such biases could have accumulated, skewing results of Toralova et al. Simple classification of typical and atypical clefts provided little to no biases because of strict medical characteristics for each type.

James investigated the relationship between intrauterine hormone profiles and cleft lip and palate. There is now substantial evidence for intrauterine hormone profiles that determine the sex of the offspring. This study claims that it is possible that these hormone levels are suspect for causing clefts. This argument would be further instated by the fact that clefts are sexually dimorphic, thus hormones that determine gender during fetal development may contribute to differences in sex distribution of clefting. An example of this has been applied to studies of polydactyly, which occurs more frequently in males, and probands with polydactyly reportedly have a highly significant excess of unaffected brothers. It is possible that the cause of polydactyly is high levels of maternal testosterone (which would also be expected to produce male offspring) (James, 1999).
James also noted the higher prevalence of CL/P in females and higher prevalence of CP in males, as being a related entity. He described a possible relationship of CL/P and CP to maternal hormone levels as follows: if $T, E, G,$ and $P$ are standardized values of a woman’s testosterone, estrogen, gonadotropin, and progesterone, respectively, then the value of $F$ can be calculated as follows:

$$F = \frac{(T+E)}{(G+P)}$$

According to this hypothesis, a low intrauterine value of $F$ would be associated with CP (more speculative) and a high value of $F$ with CL/P.

The author also noted that steroid differences may exist between population groups and contribute to differences in CL/P and CP rates for each group.

The retrospective case-control study was conducted by searching through the literature, locating nine papers giving sex ratios of sibs of probands with CL/P and CP. Sibs of probands with CL/P have a significantly higher sex ratio than the sibs of probands with CP. In general, mothers of CL/P offspring have a tendency to produce boys whereas mothers of CP offspring have a tendency to produce girls.

This paper appeared to be very subjectively written, somewhat opinionated. The author did not make many references to other researchers. This could have resulted in a significant amount of bias. The sole author frequently makes note of “his hypothesis,” and uses first person in various parts of the publication. This does not discredit any of the information he provides, but it creates a less scientific, less professional atmosphere of the paper. The author also makes note of the “flexibility of his hypothesis,” concerning maternal intrauterine hormone levels. He notes that not only do the four previously mentioned hormones play a role and at different levels in determining both sex and risk of orofacial clefting, but that other hormone levels such as growth hormone and thyroid hormone may play a role as well. The author does not go on to elaborate how they affect clefting—i.e. if certain studies have found such results, or if cleft lip/palate are more affected by certain hormones than others, etc. As a reader, this makes the paper even more vague than it already is and provides less concrete of a conclusion for the investigation. The lack of clarity in the conclusion makes me wonder why the paper was even published yet, without further scientific investigation.

Overall, this publication may serve as a stepping-stone or reference of interest for future researchers. It may be hard to understand for some, and make perfect sense for others. The paper also brings up some interesting concerns of epidemiological investigations that are often more subjective than laboratory-based studies. Despite the “subjective feeling” that this paper portrayed, it raised important questions concerning causative factors associated with clefting. Further investigation is needed concerning Dr. James’ hypotheses, involving both himself and other researchers. This would ensure the replicability of his methods and results.

In a study by Huie, Kasper, Arm, Greenberg, and Hirschhorn, the relationship of Glycogen Storage Disorder type II (GSDII) and clefting is examined. GSDII results from a genetic deficiency of lysosomal acid _-glucosidase (acid maltase), an autosomal recessive disorder in which intralysosomal accumulation of glycogen primarily affects function of skeletal and cardiac muscle. In previous studies, 3 out of 100 cases of cleft lip were found to have GSDII and 2 of 35 referral cleft patients at the New York University
Medical School in New York, New York, were found to have a co-occurrence of GSDII. This co-occurrence is greater than the estimated frequency of nonsyndromic cleft lip with or without cleft palate of 1 in 700 to 1,000 (referring to the general population, non-GSDII individuals). Previous studies have shown a minor cleft lip/palate locus on chromosome 17q, which is close to the locus for GSDII. This investigation involved a case study involving a molecular genetic analysis of the two referral patients (Patient I and Patient II) with GSDII and cleft lip.

Patient I (of Dutch descent) was homozygous and the parents heterozygous for an intragenic deletion of exon 18, which is common in Dutch patients. Patient II was heterozygous for the mutation (delta 525T), which is also a common mutation in Dutch patients, and a novel nonsense mutation Gln58Stop in exon 2, the first coding exon. The mother of the patient was heterozygous for delta 525T and the father for the Gln58Stop. These findings may represent a coincidence and the contiguous genetic mutations may not interact. Or these co-occurrences of GSDII and cleft lip/cleft palate could represent a modifying action of alpha-glucosidase deficiency on unlinked or linked genes that result in increased susceptibility for cleft lip. Essentially, this paper proposes the possibility of a contiguous gene syndrome rather than a pleiotropic effect of GSDII or a coincidental occurrence with clefting.

This study was thoroughly discussed and well presented. The scientists objectively stated their findings in a clear and concise manner. Confounding variables are extensive, when generally discussing cleft lip and palate, due to the numerous genetic and environmental contributions to the disease. There were few confounding variables for this particular study. One reason is because of the small sample size (two cases), which provided thorough molecular, historical, social, and medical evaluation of each patient. Thus, in contrast to some studies where a larger sample size is desirable, this study illustrated the benefits of a small sample size. The downfall to this, however, is the decreased ability to apply these cases to a larger population. As a whole, this study serves as a tool to pioneer new study approaches, which may use larger sample sizes. The small sample size may also be due to the fact that a co-occurrence of GSDII and clefting is rare, making recruitment difficult. If there were significant co-factors of either GSDII or clefting, the investigators would have noted such occurrences in their discussion of the findings.

Further investigation of this co-occurrence is required before any associations are confirmed. This would include the use of the same DNA fingerprinting techniques (PCR amplification) in the same or different laboratories, involving more patients both from Dutch and non-Dutch backgrounds.

A study by Bauer, Romitti, and Reynolds (1999) aimed to address the reporting aspects of occupational health risks specific to women. This has significant value due to the increasing number of women entering the workforce in recent years. Women who work in areas where there are potential occupational reproductive hazards are important for consideration with regards to the prevalence of orofacial clefting and other birth defects. The approach to the study of occupational hazards can vary based on who is reporting the incidences of exposure: mothers/workers, or hygienists/supervisors.

This investigation involved a population-based study of orofacial clefts involving 87 cases and 102 controls, all of which were mothers. The purpose of the study was to
assess the effects of women in the work force exposed to occupational reproductive haz­ards, while pregnant. Four occupational agents were assessed: video display terminals, paints, solvents, and agricultural chemicals. The exposure was determined by yes/no cri­teria assigned to the selected classes of agents for a one-year period prior to the child’s delivery. The exposure status for each class agent was assigned by industrial hygienists reviewing the occupational history for each mother of the study. The industrial hygienist-assessed exposure was used as the “gold standard,” and sensitivity and specificity of maternal reports were calculated for change agreement between the two exposure assessment methods.

Other risks involved included health factors that affect the working class more so than other classes in society. These factors may influence both reporting trends and health aspects affecting fetal development. Some of these risk factors include diet, smoking, alcohol, exercise, and stress. These factors were not extensively discussed in the paper, which may provide some additional insight into the reporting trends observed in such work environments. In addition, education programs about chemical and occupa­tional safety may vary in difference places of employment—existing some and not exist­ing in others—, which can influence reporting, or periconceptual occupational exposure. This could be either a less educated staff that are less likely to take note of such expo­sures (no education program places of employment) or perhaps a place of employment without and educational program about hazards and occupational risks may be less likely to take preventative measures of such risks and thus increase the number of staff exposed to such risks. Essentially, the awareness and education of the women in the study may vary both within and between work places.

Biases of the women making the reports may exist due to opinions of the govern­ing bodies of the place of employment. This could include workers who exaggerated or underestimated exposures because of an influence by their supervisors or governing bod­ies (This is my suggestion and is not based on information provided in the paper). Authors of the study noted differences in biases depending on the type of exposure assessed. For example, higher estimates of exposure were found for video display termi­nals, which may reflect a greater public attention focused on this exposure in recent years or the mother’s ability to more easily identify the exposure. Lower estimates found for remaining classes of agents may be due to less awareness of such exposures by mothers or that these classes of agents involve transient or one time exposure events that are not everyday occurrences. The authors also noted that the results of the study were applicable to only Caucasian mothers of children with orofacial clefts, and only four types of occupational hazardous exposures.

The results showed that sensitivity estimates for cases were the highest for video display terminals (77%) and lowest for agricultural chemicals (14%). Estimates for con­trols were 74% and 14%, respectively. The specificity estimates tended to be high for both groups. These results suggest that screening questions alone may not be the preferred method of obtaining occupational exposure histories, seen when the time period of interest is fairly short and recent.

The comparison of maternal assessed and industrial assessed exposures varied in a 1988 study by Eskenazi and Pearson, under similar circumstances of this study. However,
the exposures of the study of Eskenazi and Pearson involved a variety of other occupational toxic exposures, and the "gold standard" was established differently. Thus, comparisons were applicable but not substantial.

The results of screening questions alone were found not to be the preferred method of obtaining occupational exposure histories, even when the time period of interest is short and recent in occurrence. Unlike previous studies that evaluated lifetime exposures, this study found that underreporting of occupational exposures existed even for recent, one-year period of interest. Based on these results, it was recommended that future studies focus more attention on quantitative methodologies to assess maternal periconceptual exposure to occupational agents in population-based studies.

Overall, the study was well presented but a larger sample size, considering the large number of women in the workforce and the large number of possible places of employment would have been of value. Recall bias played a large role in decreasing the significance of the results, but the authors did an excellent job writing their discussion section, including comments on these aspects of the investigation. It would be interesting to change methods of reporting of occupational hazards in some of the places of employment of the study, and repeat the study again to determine if such changes make a difference in the results obtained.

A study by Faron, Drouin, Pedneault, Poulin, Laframboise, Garrido-Russo, and Fraser (2001) involved a case-report of a woman with malabsorption and nutritional deficiencies in addition to obesity who recurrently gave birth to children with cleft lip. The study marks factors of environmental and nutritional deficiencies that may play a role in congenital anomalies. Previous research has concentrated on vitamins and essential nutrients that are key to early development, and in a deficiency of such, can result in congenital anomalies. This case is an example of a condition that inhibits absorption of vitamins and essential nutrients, essential becoming responsible for the deficiencies of such nutrients observed. This study is of importance because it allows doctors and health care professionals to be aware of malabsorption deficiencies or related syndromes occurring in women and take these conditions into consideration when assessing the patient and factor in these aspects when advising women trying to get pregnant or who are already expecting mothers.

This study reported a woman, nonsmoker, 31 years of age, French-Canadian, gravida 3, para 1, with renal insufficiency, morbid obesity, and a malabsorption syndrome. She had interstitial nephritis attributed to chronic pyelonephritis in childhood. When she was age 11, she was diagnosed with fibilateral femora necrosis, attributable to an unrecognized congenital subluxation of both hips associated with obesity. At age 21, she was treated with Sopinaro's intestinal bypass (biliopancreatic bypass with partial gastric resection and selective vagotomy). She developed malabsorption syndrome symptoms two years later. Surgeries to reverse these symptoms were unsuccessful. When she became pregnant, medical difficulties escalated, including severe renal tubular acidosis, hypernatremia, hypercholesteremia, hypocalcemia, (with frequent secondary tetanic episodes), anemia, hypoproteinemia, hyperparathyroidism, and multivitamin deficiency. She was age 26 at this time and the pregnancy ended in spontaneous abortion. At age 29 she had a spontaneous rupture of membranes, developed chorioamnionitis and five weeks
later gave birth to a stillborn male infant at 24 weeks of gestation. The infant had a left unilateral cleft lip-palate anomaly, karyotype 46 XY. The woman had been administered calcium and multivitamin supplementation during pregnancy. Following delivery, both serum beta-carotene and vitamin A were very low levels.

At the start of her subsequent pregnancy in 1994 the woman had a serum creatine level of 235 _mol/L (normal range is 44-97) and urea level of 13mmol/L (normal range is 2.5-6.4). She took prescribed vitamins during the first three trimesters of pregnancy and reduced daily doses following this tie. Her medical condition was stable and she delivered a healthy male. His only physical abnormality was a cleft unilateral cleft lip-palate. No other major anomalies were found. The father of the child had no abnormality and no report of microforms of cleft lip in his relatives. The mother also did not have cleft lip in her family.

This study is an important part of the literature related to clefting because it provides evidence of orofacial clefting that resulted primarily from vitamin and nutrients deficiency. Since the mother and father both had no known hereditary factors of cleft lip or cleft palate in their pedigrees, it was suggested that the malabsorption deficiency alone was what caused cleft lip in the male infants. If this is true and confirmed in further case-reports or case studies, it can show that although genetic factors play a large role in cleft lip and palate, they may not always be required in order for the disease to occur. This would apply especially to underdeveloped nations with a higher number of people suffering from malnutrition, but also to individuals who have a malabsorption deficiency. The authors did not discuss whether they perceived the malabsorption deficiency as a genetically inherited trait. Since the woman noted that she had relatives who also suffered from obesity, it can be suggested that obesity or perhaps the malabsorption deficiency that can lead to obesity is genetically related. It would also be useful to determine where the gene(s) for malabsorption deficiency are located, i.e. whether they are located close to or on the same chromosome as genes found to contribute to clefting.

Beaty, Wange, Hetmanski, et al. (2001) conducted a case-control study of newborns in Maryland between 1992 and 1998. A total of 171 nonsyndromic oral clefts and 182 unaffected controls were part of the study to identify both genetic and environmental risk factors. Isolated nonsyndromic oral clefts (CL, CP, and CL/P) were recruited and exposure plus family history data were collected. DNA was collected from all cases and their parents, plus controls.

In dramatic contrast to the findings in the study by Faron et al., this study found no significant association between environmental risk factors and CL, CP, or CL/P. The only significant risk factors were genetic, as indicated by differences in allele frequencies between CP and controls for Msh homeobox homolog 1 gene (MSX1), transforming growth factor _, (TGF_), transforming growth factor _3, (TGF_3), and BCL3. Only MSX1 showed significant differences in allele frequencies between CP cases and controls. MSX1 also displayed evidence of linkage disequilibrium with a susceptibility gene controlling risk for CP.

The environmental factors included in the study were: maternal smoking, vitamin use, urinary tract infection, or recreational drug use in either univariate analysis or after adjusting for maternal age and education. More control mothers were found to report
alcohol use during the critical time period of pregnancy (one month before conception through the first trimester) compared to case mothers. Siblings of cases were found to have a ten-fold increased risk compared to siblings of controls. The four candidate genes studied were Msh homeobox homolog 1 gene (MSX1), transforming growth factor beta (TGF beta), transforming growth factor beta 3 (TGF beta 3), and BCL3. MSX1 was the only candidate that displayed allele frequencies discrepancies between CP cases and controls. MSX1 was also displayed an association with linkage disequilibrium with a susceptibility gene controlling CP.

These findings showed a strong genetic component of oral clefts. The ratio of risk in first-degree relatives of cases compared to the general population was 30. Models of inheritance may vary, and include single gene models with reduced penetrance, multifactorial models, or a combination of the two. Multilocus or oligogenic models have also been suggested. None of the above models, however, has yet served to sufficiently characterize the discrepancies of oral clefts observed between cases and controls both in this study and in previous studies, specifically considering familial aggregation of oral clefts. The researchers concluded that most of the environmental factors analyzed in the study did not contribute to a risk for isolated, nonsyndromic oral clefts, although alcohol consumption was seemingly protective (a higher amount of alcohol was consumed during the first trimester in controls than in cases).

In order to ascertain the status of the cases, all cases underwent a complete physical examination by a clinical geneticist, and syndromic cases or those with another major congenital anomaly (nonisolated clefts) were excluded from the study. The controls were obtained by recruiting unaffected infants in newborn nursery at the Johns Hopkins Hospital, and at a large pediatric practice in suburban Baltimore County. A total of 182 controls were obtained.

Control of confounding variables included differences in age, education levels, and race. Case parents showed a lower level of education than controls, which was statistically significant. More case parents fell into the high school or less group (38 percent of fathers and 29 percent of mothers of CL/P cases; 48% of fathers and 36% of mothers of CP cases; compared to 11% of fathers and 10% of mothers of controls), for Caucasians. Thus, maternal education and age were included as covariates in logistic regression models for environmental exposures. CL/P and CP were also considered as separate groups in order to minimize heterogeneity.

Since clefting occurs early in development, during the first trimester (0-3 months), the environmental effects were applied to this time period only. Smoking during this time was not shown to be statistically significant. This could have been due to the small number of heavy smokers included in the study. Passive smoking, including second hand smoke exposure via a family member or coworker was also shown to not be statistically significant, including maternal age and education adjustments. Maternal alcohol use during pregnancy decreased in the same manner as maternal smoking, beginning at the point when the mother was aware of the pregnancy. This showed that in both cases and controls, awareness of the negative consequences of smoking and drinking during pregnancy was noted and behavioral changes were made accordingly. Vitamin use in mothers, including varying amounts taken during pregnancy, were examined and showed that the proportion of women who regularly used vitamins prior to pregnancy was very low (17%
CL/P, 18% CP case mothers compared to 36% control mothers). These percentages sharply increased during the first trimester for both cases and control mothers. By the third trimester, more than 90% of both cases and controls mothers took supplementary vitamins. Despite differences between cases and control mothers in vitamin supplementation prior to and during the first trimester of pregnancy, following adjustment for age and education, the results were not significant.

Medications were also taken into consideration for adjustment and showed no significant difference between cases and controls. Considering recreational drug use, two mothers reported use of cocaine, marijuana, or heroin during the critical time period. Ironically, however, none of these effects contributed to case status.

Familial aggregation was conducted to track oral cleft among first-degree relatives (parents and sibs), for both cases and controls. Other aspects of the analysis included Medical history, multivariate analysis, familial aggregation of oral clefts, allelic effects of gene environment interaction, analysis of case-parent trios.

The only significant bias in this study may have resulted from an under-representation of racial groups apart from Caucasians. Researchers noted they had attempted to recruit other racial groups but were less successful and the number of cases found in such groups was also lower. Because of this, other cofactors may have been omitted from the study that could have contributed to environmental factors. This includes smoking, alcohol consumption, the type of alcohol consumed, etc. Considering alcohol type, researchers did not specify whether the alcohol consumed was wine, beer, or hard alcohol. They also did not specify the amount of alcohol consumed (i.e. if the mother was once a heavy drinker or is usually limited to one or two servings per day). This may alter results because of some studies that show benefits of a glass of wine consumed per day. It is possible that this level of alcohol does not impair development, but in fact aides it because of the impacts it has on the mother's health. I could not find much in the literature relating to extensive research related to alcohol type consumed and differences in health that result, but it is not so obtuse to completely disregard as a consideration at this point.

Overall, the study was extremely well done, very thorough, and quite informative. The epidemiologists and geneticists involved were familiar with the literature of oral clefting studies research, and multiple variables involved in the epidemiology of oral clefting.

Vitamin supplementation may not prove to be of use to an otherwise healthy, well fed population. However, research of vitamin supplementation as a variable of cleft lip and cleft palate can be taken into consideration without including an analysis of the daily diet of the mother, prior to and during the first trimester of pregnancy. Thus eliminating vitamin supplementation from the analysis, to save time, work and resources, or to include an analysis of the daily diet as well.

DISCUSSION

These six studies were chosen for review because of their comparability as well as contrasting results. The last two studies in particular highlight contrasting viewpoints regarding the significance of environmental cofactors such as diet. The studies included factors related to nutrition, genetics, occupational hazards exposure, or a co-occurrence of a disease resulting in symptoms that affect the efficiency of vitamin and nutrient absorption,
despite a balanced diet. Only one study was review that specifically focused on a candidate gene study, in order to reduce redundancy. As a consequence, the review of studies only brushed the surface of current and recent research. Instead, this paper was a comprehensive outline, encompassing the multifactorial characteristic of such complicated diseases, cleft lip and cleft palate. The extensiveness of the types of research that can be done on these disorders alone, which are nonsyndromic, also highlights the need for research not only on these disorders but particularly on more complex disorders that may involve even more complex contributing factors.

Just as laboratory methods differ in regard to sensitivity and specificity regarding the presence or absence of an antibody in a serum sample, so can environmental discrepancies exist regarding categorization of behavioral patterns of drug and alcohol consumption, exercise, diet, and general health of the mother that can contribute to the development of a fetus. Establishing a difference between light smoking and heavy smoking is one example of a level of sensitivity and specificity that must be determined by the researchers. If the sensitivity was too high (i.e. only heavy smoking was considered as a risk factor but less so light smoking), then the negative affects of smoking (even light smoking) in some patients could be missed.

Despite similar methods of categorization, differences in the recruitment may exist and the samples and controls may be representative only for a specific population. In a population where environmental contributions are more influential, results may be shown to be statistically significant. For example, for Study VI, if the same study was conducted in a Philippine population as well as the Maryland population, vitamin supplementation may result to be statistically significant. This could be attributable to a generally higher prevalence of malnutrition in Philippine populations, and perhaps a more dramatic response to vitamin supplementation than that observed in the Maryland population. Studies completely and currently underway at Lidral/Murray laboratories address this problem by conducting the same tests on Philippine as well as Iowa samples. This eliminates biases specific to a racial group or geographic location as well as biases in the laboratory methods performed by the investigators. Unfortunately I was not able to discuss my own research experience in the lab, concerning the RYK gene, a candidate gene for clefting. However, perhaps discussion on this will be of use for the next paper related to this topic.

Despite the findings of the study by Beaty et al., environmental factors are of value for study, and further research is necessary. The degree to which such factors contribute to clefting may depend on the degree of exposure. Since smoking has been determined to be a causative factor of birth defects in studies beginning in the 1970s, one would expect maternal smoking during the perinatal time period to increase the risk of clefting. Studies of heavy smokers and the incidence of cleft lip and palate resulting in no association would be required in order to support the hypothesis that environmental factors are perhaps overestimated, as implied by the Beaty et al. study. The same analysis applies to alcohol, second hand smoke, vitamin supplementation, exposure to occupational hazards, and other types of exposures suspect of increasing the risk for cleft lip and cleft palate.

In the Faron et al. study, malabsorption of nutrients alone sufficed to induce cleft lip, since pedigree analysis showed no familial background of clefting for either the father or mother (thus a less likely possibility that the clefting was genetically caused). Studies
involving both environmental and genetic factors show that both factors may contribute to clefting.

The complexity of the causative factors associated with cleft lip and palate are implied not only in these studies but also in most studies in the literature. This is due to the obvious complexity of the disease itself. Similar to other diseases that involve both genetic and environmental factors, such as cardiovascular disease (CVD) and cancer, continuing research is imperative in order to gain a better understanding of these diseases. Comprehension of all of the contributing factors and the most dominant contributing factors will allow for more proper treatment, earlier diagnosis, and perhaps involve gene therapy implications, which require bioethical considerations.

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APPENDIX A:
Page 2 of Texas Pediatric Surgical Associates Webpage with Descriptions and examples of Cleft lip and Cleft palate.