Pacific Craniofacial Team and Cleft Prevention Program

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ABSTRACT

There is no doubt modern genetics have greatly influenced our professional and personal lives during the last decade. Uncovering genetic causes of many medical and dental pathologies is helping to narrow the diagnosis and select a treatment plan that would provide the best outcome. Importantly, having an understanding of multifactorial etiology helps direct our attention toward prevention.

We now understand much better our own health problems. In some cases, we can modify our lifestyle and diet in order to prevent "environmental factors" from triggering the mutated genes inherited from our parents. Good examples are diabetes and cardiovascular diseases. If we realize we might have inherited genes for cardiovascular problems from several ancestors who had heart attacks, we already know that these genes will make us only "susceptible" for disease. Those who exercise, watch one's weight, diet, and carefully monitor one's lifestyle will very likely — though possessing "susceptibility genes" — stay healthier and, maybe, will never experience any cardiovascular problems.

In principle, the same applies for craniofacial anomalies, especially for nonsyndromic cleft lip and palate. One needs to understand genetic and environmental causes of nonsyndromic orofacial clefts in order to prevent them.

With all this in mind, the Pacific Craniofacial Team and Cleft Prevention Program have been established at the Department of Orthodontics, University of the Pacific Arthur A. Dugoni School of Dentistry in San Francisco. A partnership with Rotaplast International, Inc., has

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made it possible for the faculty, orthodontic residents, and students to participate in 27 multidisciplinary cleft medical missions in underdeveloped and developing countries by donating professional and educational services, and, last but not least, by collecting valuable data and specimens to further research.

A significant number of research studies, including 15 master of science theses, have been accomplished in UOP's Craniofacial Genetics Laboratory, with contributions by faculty, undergraduate and graduate students. It has been leading to a better understanding of etiology of nonsyndromic orofacial clefts. It has been learned that genetic factors and environmental factors are ethnicity-specific and, in many places throughout the world, location-specific. Thus, a specific protocol for cleft prevention has to be worked out based on genetic and nutritional studies of each specific population group in order to be effective. This is our ultimate goal.

While the 19th century has been called the "century of biochemistry" and the 20th century referred to as the "century of physics," the 21st century may be nicknamed the "century of molecular biology." The influence of molecular biology has been strongly felt in all fields of medicine and dentistry. More and more details are being uncovered about the functioning of the human body, and about the roles of a genetic background and its interactions with the environment.

There is no doubt that human genetics and medical genetics are important parts of today's modern health care in its all three main branches: diagnostics, treatment, and prevention. Among present health problems worldwide, the treatment and prevention of congenital anomalies is an area of very serious concern. The combined efforts of scientists and health care providers sharing information and skills, and collaborating on research projects at domestic and international levels are absolutely crucial for the improvement of care of the patients affected with congenital anomalies.

The Division of Craniofacial Genetics at the Department of Orthodontics at Pacific Arthur A. Dugoni School of Dentistry is a leader in primary prevention of one of the most common and most serious congenital anomalies: cleft lip and palate.

During the last six years since the craniofacial genetic research (including a busy molecular genetic laboratory) was established at the University of the Pacific Arthur A. Dugoni School of

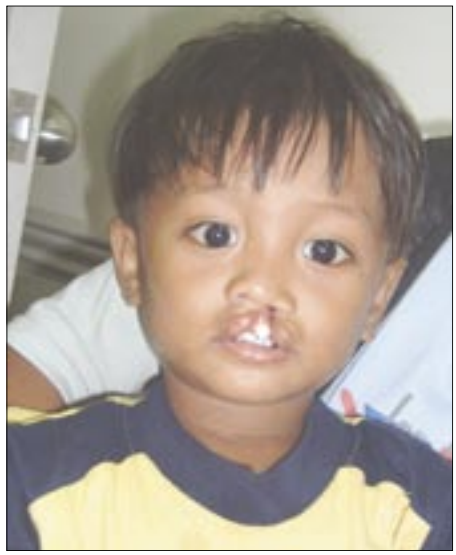

Figure 1a. A Filipino boy affected with unilateral cleft lip and palate on the left side, before surgery.

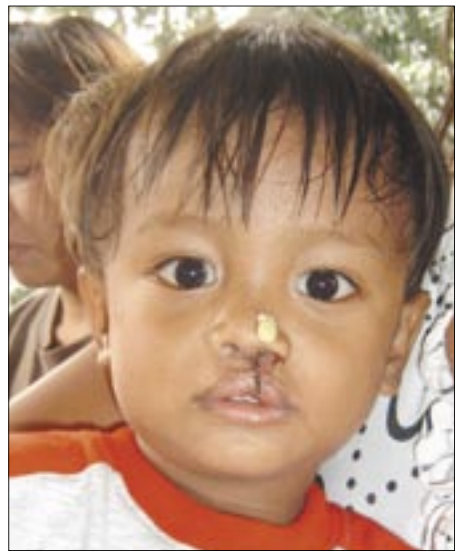

Figure 1b. Same child two days after surgery.

Figure 2. Drs. Donald Poulton and William Olin examining cleft patients in Caracas, Venezuela. Dr. Olin is a professor at the University of Iowa in Iowa City, Iowa.

Figure 3. Dental/genetic team in Venezuela (Drs. Cooper Owens, Poulton, Marie Tolarová, Charles Brodsky, and Javier Mir).

Dentistry, efforts have been strongly focused on finding causes of orofacial clefts and on development of the best clinical protocol for their prevention.

A partnership with the Rotaplast International, Inc., the nongovernmental organization that provides free reconstructive surgeries to underprivileged children affected with cleft lip and palate worldwide (**Figures 1a and b**), allowed UOP's faculty, residents, and students not only to deliver professional services and acquire new experience, but also to bring back valuable data and specimens for genetic research (**Figures 2-4**). A participation of dental professionals (dentists and orthodontists) in the Rotaplast cleft medical missions is now a firm part of the graduate program. It has brought another dimension to education and training of the school's graduates. With no exception, the work of residents and their contributions to the success of each mission is highly prized. They are bringing back from those two weeks — working many times more than 12 hours a day — not only what they learned professionally, but also warm feelings in their hearts remembering their patients whom they helped to start a better future. During the last five years, the department professionally participated (in dental and genetic fields) in 40 Rotaplast cleft medical missions (**Figures 5-8**).

Since 2000, 15 residents of the school's graduate program in orthodontics either have accomplished or have been working toward their master of science in dentistry theses in the craniofacial genetics field. Many more students got their first research experience and excitement in the craniofacial genetics group. At present, the school has 23 DDS or IDS students. Residents and students presented their research results not only on Pacific Research Days, but also at California Dental Association, American Association for Dental Research, International Association for Dental Research, and American Association of Orthodontics meetings. In addition, there have been 12 visiting scholars who not only learned a great deal of population genetic and molecular genetic techniques, but also significantly supported research of residents and students, and thus contributed to many research projects (**Figure 9**).

What We Know About Orofacial Clefts

Orofacial clefts include a cleft lip, either unilateral or bilateral, that can occur either alone, or together with a cleft palate, and a cleft palate alone. Orofacial clefts develop during the embryonic period due to a failure of embryonic facial processes to fuse in a specific timeframe, specifically, cleft lip between six and nine weeks of pregnancy, and cleft palate between nine and 12 weeks of pregnancy.

Cleft lip and palate anomalies are the most common and most serious congenital anomalies of the orofacial region and the second most common congenital anomalies in general. Their birth prevalence in California is 1.77 per 1,000 births, one in every 566 newborns.[1,2] A baby with a cleft is born every two min-

utes somewhere in the world, accounting for 660 babies born with a cleft every day. This adds up to 230,000 children born with clefts every year worldwide. With a projected population growth that is estimated at 1.8 million per year, the number of new cases will be increased by 3,200 babies with cleft every year. More details are given Section 1.

Clefts have a significant genetic component[2,3] (Section 2). Therefore, individuals affected with cleft and also their nonaffected relatives are at a statistically significant higher risk to have a child with a cleft compared to the general population. Each individual affected with a nonsyndromic cleft lip and palate has during his/her lifetime from eight to 12 relatives whose risk for having a child affected with orofacial cleft is from 10 to 40 times higher than a risk in the general population. The highest risk of recurrence, on average 4 percent (40 times higher than in the general population), is for the first-degree relatives, i.e., for siblings of an individual with a cleft and for their children. In other words, at least four out of 100 parents who have had one child affected with a cleft, or who themselves were born with a cleft, will have a baby with a cleft.

Environmental factors also play a significant role in etiology of orofacial clefts. Important toxic factors and nutritional deficiencies interacting with the genetic background for clefts are dealt with in Section 3.

A treatment of children affected with orofacial clefts is challenging, lengthy, and requires a multidisciplinary team approach. An estimated average lifetime medical cost for a treatment of one individual affected with cleft lip with or without cleft palate, CL±P, in the United States is about $101,000.[4] This includes an immediate cost of $30,000/case in the first year of life. Based on an estimate of 7,500 newborns with orofacial clefts/year in the United States, in this year alone, the lifetime medical cost for babies born with orofacial cleft in the United States will total $750 million or more. A prevention of this anomaly can save not only suffering, but also millions of dollars.

Figure 4. Drs. Cory Costanzo and Thomas Ellerhorst preparing specimens for DNA analysis in Guatemala.

The authors were among the first to explore an inverse relation between folic acid intake and the risk of recurrence for CL±P.[5,6] Families with a high risk of recurrence are not only the first on the list of those who need prevention, but they are also the best target population for a prevention effort. They represent a preselected population with respect to phenotype homogeneity and, therefore, they have the highest probability of a positive preventive effect, as well as of the highest return of monetary investment. More about prevention will be covered in Section 4.

Section 1. Epidemiology

The authors conducted extensive genetic and epidemiological studies of orofacial clefts in two large population samples: the Czech population and the California population.[1,3,7] The authors were also specifically interested in the prevalence of clefts in Hispanic populations and evaluated population-based samples of Hispanics from California.[8]

To determine the proportion and birth prevalence of "typical" orofacial clefts (cleft lip, cleft palate and cleft lip and palate) and "atypical" clefts (median, transversal, or oblique facial clefts) and conditions for their occurrence, the authors analyzed a population-based sample of 4,433 cases ascertained from 2,509,881 California births.[1] The birth prevalence of isolated CL±P was 0.77 per 1,000 births and of isolated cleft palates, 0.31 per 1,000 births. Non-Hispanic whites had the greatest prevalence of isolated clefts; Asians a slightly lower prevalence; and blacks the lowest. Asians had the lowest prevalence of cleft palates; in whites and Hispanics, it was almost twice as high.

Section 2. Molecular Genetics

Over the past decade, there has been

Figure 5. Drs. Jamson Wu and Christopher Anderson with their patient (wearing premaxilla cup) and her mother in Guatemala City.

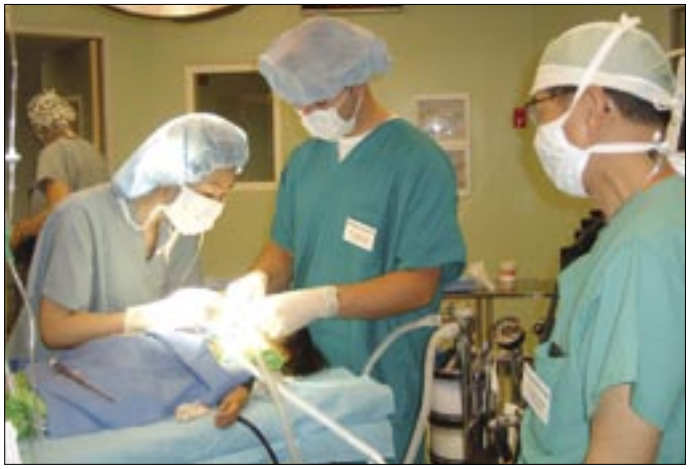

Figure 6. Drs. HeeSoo Oh and Costanzo in operating room in Guatemala.

a considerable interest in identifying genes that contribute to the etiology of orofacial clefting. Recent advances in modern molecular biology, new methods of gene therapy, and the availability of complete genome sequences led to understanding of the roles of particular genes associated with embryonic development of the orofacial complex.

The first candidate gene that showed an association with nonsyndromic cleft lip and palate was transforming growth factor alpha, TGFA in a Caucasian population.[9] Transforming growth factor beta 3 gene TGFB3 and MSX1 were found to be a strong candidate genes involved in orofacial clefts and dental anomalies.[10,11] Oh and Porter suggested that allele 4 (9 CA-repeats) occurs significantly more often in cleft population compared to controls.

In 1994, the methylenetetrahydrofolate reductase MTHFR gene was cloned and since then, 17 mutations have been described, including clinically most significant C->T substitution at nucleotide 677.[12,13] This common mutation has been identified as the first molecular risk factor for neural tube defects and for cleft lip and palate.[14,15] In the authors' Mendoza study, a significant association was found with mutated allele and CL±P, strongest in cases of bilateral clefts.[16] At present, MTHFR deficiency is considered to be the most frequent hereditary defect of folate metabolism.[17]

Studies from the authors' Craniofacial Genetics Laboratory have been focused on mutations of various candidate genes and their roles in etiology of nonsyndromic cleft lip and palate in different populations.[18] Costanzo suggested a strong association of reduced folate carrier gene (RFC1) with nonsyndromic cleft lip and palate in Guatemala.[18] In collaboration with the University of Colorado, the authors demonstrated a highly significant association between poliovirus receptor-like gene (PVRL1) and NCLP in northern Venezuela.[19]

Based on the authors' results, it seems very likely that a different spectrum of genetic factors constituting a genetic susceptibility to nonsyndromic cleft lip and palate exists in different populations. The authors' studies strongly suggest that a spectrum of genes participating in the etiology of orofacial clefts, as well as spectrum of environmental factors triggering a genetic susceptibility created by a combination of these genes, is "location specific," i.e., varies in different countries and different locations.[20]

Recently, Zucchero reported that variants of interferon regulatory factor 6, IRF6, gene might be responsible for 12 percent of nonsyndromic cleft lip and palate.[21]

In summary, based on recent studies, approximately 15 percent to 20 percent of nonsyndromic cleft lip and palate are determined by combinations of MSX1, RFC1, IRF6 and TGFB3 gene polymorphisms.

Section 3. Gene-environment Interactions in Etiology of Orofacial Clefts

The factors contributing to etiology of orofacial clefts include folic acid intake and mutations related to folate metabolism, poor maternal nutrition,

smoking, alcohol and drug consumption, and a presence of other altered genes (so-called candidate genes) known to be associated with orofacial clefts. Studies looking at the role of smoking with TGFA and MSX1 suggested that mutations in these genes might be susceptible to detrimental effects of maternal smoking.[22]

The authors' pilot study of gene-environment interactions in the etiology of cleft lip and palate anomalies was conducted in Mendoza, Argentina, in collaboration with University of Nijmegen, the Netherlands.[15,16]

Altogether, 140 families of individuals affected with orofacial cleft and 110 control families were analyzed. Both cases and controls came from a middle or low social class. Data on socioeconomic status, diet composition, other lifestyle information, blood levels of folic acid and vitamins were compared between cases and controls and their mothers. In general, the diet of families of cleft patients was poorer than that of the controls. The results of the red blood cell and plasma analysis showed significantly lower levels of folate in Argentineans compared to a Dutch control sample. Evaluation of MTHFR 677CT polymorphism in case and control groups revealed a significantly higher frequency of mutations in cleft populations, indicating that problems behind compromised folate metabolism can occur on a genetic level. It was concluded that exogenous factors, including lifestyle characteristics, together with nutrition, may play an important role in the etiology of the orofacial clefts in Argentina, however, even in the presence of normal amounts of dietary folate, the fetus of a mother carrying this mutation, or fetuses that are carrying it themselves would be at much greater risk of developing a cleft.[16] Later, a detailed nutritional study of the Mendoza cleft population revealed a low daily intake of folate and high intake of Vitamin A in the diets of mothers of cleft children.[23]

The authors' studies on periconceptional supplementation of the mothers' diet with folic acid showed a 65 percent to 82 percent decrease in recurrences and a 27 percent to 50 percent decrease in occurrences.[5,6,24] These results strongly suggest the major role that vitamins and folic acid play in the etiology of orofacial clefts.

Even when the 677 CT mutations in MTHFR seem to increase the susceptibility for clefting, the authors hypothesize, that this circumstance may be overcome by supplementation with folic acid. Thus, the nutrition seems to play an important role in triggering the genetic susceptibility for orofacial clefts and probably for other dysraphic congenital anomalies as well.

Section 4. Prevention of Orofacial Clefts

There is no doubt orofacial clefts are going to be the next congenital anomaly (following neural tube defects), for which a primary prevention — most likely involving folic acid supplementation — will become a part of health recommendations and policies. There is clear evidence for a role of folic acid in the prevention of neural tube defects.[25-27]

The size of the preventive effect was found to be directly proportional to a given dose of folic acid.[28] Moreover, there are numerous articles pointing to a preventive effect of folic acid in other dysraphic congenital birth defects.[29] A high number of scientific communications have presented suggestions or evidence for a preventive effect of folic acid on orofacial cleft anomalies.[5,6,24,28,30]

Figure 7. Dr. Ellerhorst with his patient in Guatemala.

In a nonrandomized interventional study, the authors found a dramatic reduction of cleft recurrences after periconceptional supplementation by multivitamins and high dose of folic acid. The first results were published in *Lancet* in 1982, and the complete final evaluation followed later.[5,6] The authors prospectively evaluated 221 pregnancies in women at risk for a child with CL±P. The 10-step protocol included multivitamin supplementation and folic acid (10 mg/day), beginning at least two months before a planned conception and continuing for at least three months thereafter. A comparison group was comprised of 1,901 women at risk for a child with CL±P who received no supplementation, and gave birth within the same period as the study group. In the supplemented group a 65.4 percent decrease of recur-

Figure 8. Drs. Ellerhorst, Costanzo and Oh with their cleft patients.

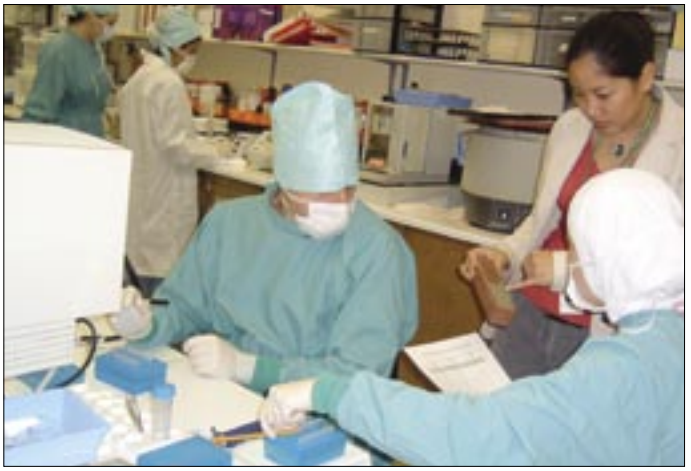

Figure 9. Craniofacial Genetics Laboratory at Pacific Arthur A. Dugoni School of Dentistry. The team is working on DNA isolation and analysis from different saliva specimens. (From left to right: Drs. Aurora Patino; Laura Reid and Gabriela Pitigoi-Aron, Department of Restorative Dentistry; Drs. Midori Obara (orthodontics 2007), and Alia Al-Jabeiti, (orthodontics 2008).

Table 1

Prevention of CL±P by Periconceptional Vitamin Supplementation (Particularly With a High Folic Acid)

Proband	Nonsupplemented (without/with cleft)	Supplemented (without/with cleft)	Efficacy expected occurrence	Decreased by (%)
CL±P (1)	1,824/77	211/3	8.67	65.4
Male with CL±P (2)	1,149/42	129/1	4.58	78.2
Female with CL±P (3)	675/35	82/2	4.14	51.7
Unilateral CL±P (4)	1,511/55	163/1	5.76	82.6
Bilateral CL±P (5)	313/22	48/2	3.29	39.2

[1]Fisher's exact test was used for all results. (1) P=0.030579; (2) P=0.063169; (3) P=0.227924; (4) P=0.02433612; (5) P=0.3734264.

rence of a cleft was observed (**Table 1**). Subset analysis by a patient's sex and severity of cleft showed the highest supplementation efficacy in individuals with unilateral clefts (82.6 percent decrease). No efficacy was observed for female individuals with bilateral CL±P. Generally, the efficacy was greater for subgroups with unilateral than with bilateral clefts and for male individuals.

Similarly, a large population-based case control study in California demonstrated that periconceptional use of multivitamins, which usually contain 0.4 mg or more of folic acid, reduced the risk for CL±P by approximately 27 percent to 50 percent. This was based on data derived from a population-based case-control study of fetuses and live-born infants with orofacial anomalies (731 mothers with an infant with a cleft and 734 mothers with unaffected baby).[24]

However, the most interesting results that actually strongly support the authors' justification for using a high dose of folic acid in the prevention of nonsyndromic cleft lip and palate are those of Czeizel and his colleagues. The first of his study of periconceptional supplementation with a multivitamin

containing a low "physiologic" dose of folic acid (0.8 mg) did not show any preventive effect.[28] However, a following study indicated a reduction of nonsyndromic cleft lip and palate after the use of high doses of folic acid (3-9 mg) in the early postconceptional period, pointing out "a dose-dependent effect" of folic acid in the prevention of orofacial clefts.[28]

During the last several years, an optimal design for an orofacial cleft prevention trial has been extensively discussed.[31-34] The authors are aware there are several key questions that need to be addressed in future scientific studies in order to clarify the highly probable association between cleft lip and palate anomalies and a lack of vitamin intake.[31] A proposal for a multicenter randomized double-blind trial of primary prevention of clefts has been developed by the authors' group and only a lack of funding is holding them back from carrying out the study that would lead to an efficient cleft prevention protocol.

Conclusion

Regardless of excellent surgical results and an advanced multidisciplinary treatment approach, the birth of a child with cleft lip and palate is a serious event, which should not happen without strong effort to prevent it, especially if we have tools in our hands that can lead to a birth of a healthy child. ■■■■

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