

4

Role of environment in CFA

4.1 Socioeconomic status and orofacial clefts

The investigation of the relationship between socioeconomic status and the prevalence of various health outcomes has provided important clues as to etiology. For example, the observation of an increasing risk of neural tube defects with decreasing socioeconomic status was one of the clues to a dietary hypothesis for these defects (Elwood and Colquhoun, 1992).

Little attempt has been made to investigate whether the risk of orofacial clefts varies by socioeconomic status. Womersley and Stone (1987) examined the prevalence at birth of orofacial clefts within Greater Glasgow (Scotland) during the period 1974-1985, according to housing and employment characteristics recorded in the 1981 census. The highest rates were observed in areas with high proportions of local authority housing with young families, high unemployment and a preponderance of unskilled workers, whereas the lowest rates were found in affluent areas with high proportions of professional and non-manual workers in large owner-occupied or high-quality housing. Most of this pattern was accounted for by CP, with less variation in CL/P.

A variety of different indicators of socioeconomic status have been developed (Liberatos et al., 1988). In an international context, it seems appropriate to use one that is specific to the local area, and one that can be compared between countries, e.g. years of schooling. As socioeconomic status can be difficult to determine at the level of the individual, especially for women, there has been increasing interest in developing, and using, area-based measures of material deprivation as a proxy for socioeconomic status (Townsend, 1987; Carstairs and Morris, 1990).

4.1.1 Orofacial clefting, socioeconomic status, nutrition and dietary supplements

Socioeconomic status may have a number of associated variables contributing to the explanation, such as nutrition, smoking, alcohol, illnesses and infections. These factors tend to have been studied retrospectively in some parts of the world and such studies are now being carried out prospectively in Denmark and Norway with regard to reproductive outcome. Other aspects of nutrition not well studied are the effects of obesity/starvation and it may be useful in future studies to record height and weight to get a measure of body mass index in relation to orofacial clefts.

4.1.2 Conclusions

The evidence for prevalence of OFC being greater in the lower socioeconomic classes remains equivocal, the less well-developed countries having a greater proportion of the population in the lower socioeconomic classes.

The overall conclusion is that socioeconomic status and OFC are not well studied. One of the barriers to investigation of the role of socioeconomic status in orofacial clefting is that common criteria for the description of low socioeconomic status do not exist and, in those studies where socioeconomic status or social class have been examined, different criteria have been used, thus making valid inter-centre comparisons impossible.

4.2 Nutrition and orofacial clefts: general issues

There is considerable interest in the effects of maternal nutrition, during the peri-conceptual period, on the occurrence of several types of congenital anomalies. This interest has been stimulated by the finding in a randomized controlled trial that maternal peri-conceptual folic acid supplementation reduces the recurrence risk of neural tube defects (MRC Vitamin Study Research Group, 1991). The role of maternal peri-conceptual vitamin status is now being debated in relation to:

- orofacial clefts (Tolarova and Harris, 1995; Shaw et al., 1995a; Czeizel, 1996; Hayes et al., 1996);
- limb defects (Shaw et al., 1995b);
- conotruncal heart defects (Shaw et al., 1995b; Botto et al., 1996; Scanlon et al., 1998);
- and urinary tract malformations (Li et al., 1995; Czeizel, 1996).

4.2.1 *Variation in diet*

BOX G

Worldwide variation in diet

Dietary patterns vary greatly between different parts of the world. In rural areas of developing countries, diets may depend solely on what a family or local community produces. As the use of cash is extended, a greater variety of foods becomes available in local markets or shops. In economically developed societies and in urban areas in developing countries, diets are influenced not only by food supplies grown and processed locally but also by those available nationally and internationally (World Cancer Research Fund, 1997).

The diets typically consumed in rural parts of Africa, Asia, Latin America and Oceania often rely on one or two staple cereal foods. In China, India and other low-income countries of Asia, cereals tend to be dominant. Rice dominates in Asia, wheat in North Africa, maize in Latin America, and maize and starchy roots in sub-Saharan Africa (World Cancer Research Fund, 1997).

As countries develop economically, consumption of the dominant staple cereal foods declines. There is a fall in the overall consumption of foods of plant origin and replacement with increasing amounts of foods of animal origin, notably meat, meat products and dairy products. Sugar consumption also tends to increase. Compared with the diets of less developed societies, such diets are lower in fibre and other bioactive compounds found in foods of plant origin. An ever-increasing proportion of food in industrialized societies is processed (World Cancer Research Fund, 1997).

Within some of the most economically-developed countries, this process has slowed and, for some population subgroups, has reversed. For example, in some northern European countries and within North America, there is a trend towards increasing consumption of vegetables and fruits, and decreasing consumption of red meat, fat, full-fat milk, other dairy products and sugar in the form of sucrose (World Cancer Research Fund, 1997).

4.2.2 *Diet in pregnancy*

During the 40 weeks of pregnancy, an average 12.5-15.0 kilograms are gained (Lederman, 1991). This may be lower in populations with chronic food shortage, or when weight-gain limitation is recommended, as was the case in the United States in the 1960s. In view of the weight gain during pregnancy, an increased food intake would be expected. There have been few studies of intake changes during pregnancy in the same women. The available studies suggest some increased intake in mid-gestation (Lederman, 1991; Brown and Kahn, 1997) but the relationship of this to

Assessment of dietary intake is problematic

intake prior to pregnancy, or around the time of conception, is unclear. In a study of about 550 women in Minnesota (United States) recruited prior to pregnancy and followed at monthly intervals until 6-8 weeks postpartum, the peak increase in total energy intake, and peak decrease in energy expenditure, occurred within the first nine weeks of pregnancy (Brown and Kahn, 1997). Postpartum energy intake declined and energy expenditure increased.

About 50% of pregnant women experience nausea or vomiting during early pregnancy (Kullander and Kallen, 1976; Klebanoff et al., 1985). It appears that women experiencing nausea and vomiting tend to cut down or stop their consumption of alcohol, coffee, tea and other potentially harmful beverages, and also stop smoking (Hook, 1976; Golding, 1986), but the effects on maternal diet appear to have been little documented. It has been suggested that elevated estrogen levels early in pregnancy are the main cause of vomiting, but the evidence is inconclusive (Zhang and Cai, 1991).

4.2.3 Biochemical markers and gene/nutrient interaction

Assessment of dietary intake is problematic. The most established method in nutritional epidemiological investigation of chronic diseases is the food frequency questionnaire (FFQ) in which the primary aim is to obtain a relative ranking of subjects in terms of their reported intake, rather than to determine their absolute intake. Misclassification is recognized as a major problem.

In addition to food frequency data, it is also useful therefore to have biochemical markers of nutrition but, because metabolism is under genetic control, these measures are not the same but complementary. One promising area for future research in the influence of socioeconomic status and nutrition in OFC is the examination of genetic polymorphisms which effect nutrient metabolism, e.g. MTHFR and folate receptors, with study designs aimed to examine gene/environment interaction. While these hypotheses are generated on the basis of biological plausibility, there might well be gene/environment interactions with no apparent biological plausibility, such as reports of interaction between TGF α (transforming growth factor) and multivitamins, and TGF α and smoking. In developing countries there is a need to design FFQs and collect data on nutrition in close consultation with the local indigenous people. There may be a tendency for FFQs to exclude important groups of food that are being consumed. It is also important to realize that people eat foods and not nutrients – which makes it challenging to identify the effects of specific nutrients.

4.2.4 Conclusions

In planning or appraising a study of nutritional epidemiology, in addition to the usual considerations of bias, confounding and chance, important criteria are:

- (1) use of a validated dietary instrument that estimates total energy intake;
- (2) appropriate adjustment for total energy intake in statistical analysis;
- (3) whether any biological markers used are appropriate for the hypotheses under test, and the possible effect of their use on participation rates.

The importance of multi-centre collaborative efforts in looking at diet and nutrition is the broad range of exposure that will reduce the impact of misclassification. However, it is recognized that this is also likely to introduce more heterogeneity.

4.3 Folic acid: nutritional biochemistry and orofacial clefts

4.3.1 Folic acid in reproduction

The terms “folic acid” and “folate” both refer to the same vitamin, whereby folate is the polyglutamate natural form and folic acid is the monoglutamate synthetic form. Adequate maternal folate status is crucial to all stages of pregnancy from conception to delivery. Folate nutrition seems to have a dual role in determining pregnancy outcome. One of these is the long-established role in fetal maturation that may place a requirement for supplementation to prevent maternal anaemia in late pregnancy (Scott and Weir, 1998). The other is the newly-perceived role in the prevention of congenital defects during early embryonic development.

4.3.2 Maternal folic acid deficiency

Peri-conceptual folic acid supplementation can prevent the majority of neural tube defects (NTDs) (MRC Vitamin study, 1991; Czeizel and Dudas, 1992). The mechanism does not seem to be a correction of maternal clinical folate deficiency (Kirke et al., 1993). Nevertheless, there is a strong inverse relationship between a mother’s early-pregnancy red cell folate concentration and her risk of having an NTD-affected birth (Daly et al., 1995). This, along with other genetic and environmental evidence, indicates that a complex interaction of folate-related nutritional and genetic influences underlie the etiology of NTDs. The evidence of folate

Early trials
using vitamin
supplementation to
reduce recurrence
of orofacial clefting
were inconclusive

involvement with other congenital defects is not as strong, but is nevertheless encouraging (Finnell et al., 1998). Early trials using vitamin supplementation to reduce recurrence of orofacial clefting were inconclusive. Many of these studies were small, non-randomized and the treatment preparation was a multivitamin containing folic acid. Other evidence suggesting a link between folate and orofacial clefts included positive associations between clefts and (a) maternal use of anticonvulsants and other known folate antagonists, or (b) maternal cigarette and alcohol abuse (both of which interfere with folate status). In addition, some animal studies showed that feeding folate-deficient diets or administration of antifolate drugs to pregnant rats could induce craniofacial abnormalities in rat embryos. It has been suggested that maternal folic acid supplementation plays a role in the prevention of non-syndromic orofacial clefts, i.e., cleft lip with or without cleft palate (CL/P). Using a case-control design, Wong et al. (1999) investigated vitamin-dependent homocysteine metabolism in 35 mothers with non-syndromic orofacial cleft offspring and 56 control mothers with non-malformed offspring. A standardized oral methionine-loading test was performed, in which fasting and afterload plasma total homocysteine, serum and red-cell folate, serum vitamin B12 and whole-blood vitamin B6 levels were determined. The test showed that both fasting ($p < 0.01$), as well as afterload ($p < 0.05$) homocysteine concentrations, were significantly higher in cases compared to controls.

Hyperhomocysteinemia, defined by a fasting and/or afterload homocysteine concentration above the 97.5th percentile, was present in 15.6% of the cases and in 3.6% of controls (odds ratio (OR) 5:3, confidence interval (CI) 1.1 to 24.2). The median concentrations of serum ($p < 0.01$) and red-cell ($p < 0.05$) folate were significantly higher, and vitamin B6 concentrations appeared to be significantly lower ($p < 0.05$) in cases compared with controls. No significant difference was observed between groups for vitamin B12. These preliminary data offer evidence that maternal hyperhomocysteinemia may be a risk factor for having non-syndromic orofacial cleft offspring. In a more recent study among Irish orofacial cleft cases an increased prevalence of a genetic variant of a folate-related enzyme, previously shown to cause increased risk of NTDs, was found (Mills, 1999; Shields et al., 1999). Homozygosity for this common polymorphism occurs in between 5 to 25% of populations worldwide. The variant phenotype expresses reduced enzyme activity and adversely affects folate status (Molloy et al., 1997). This study recognizes the possibility of population differences in genetic susceptibility, and the need for research on gene/environment interaction.

4.3.3 Folic acid metabolism

It would clearly be unethical at this point to conduct a randomized placebo-controlled trial of folic acid and clefts, given the proven benefit of folic acid in preventing NTDs. Thus the identification of a role for folate or indeed other nutrients will have to be pursued by other means. In other words, it will be necessary to study genetic, nutritional or environmental markers of risk. A randomized controlled trial of different doses would be theoretically possible; there are questions with regard to ethics in study design which are discussed in more detail in Section 7.4 below. From a mechanistic point of view there are good reasons why aberrations in folate metabolism might cause congenital abnormalities. Within the cell, the overall function of the folate co-factors is to accept 1-carbon units from several sources and donate them to other molecules in a variety of enzyme reactions. These 1-carbon units are required for the production of purines and pyrimidines for DNA synthesis and to maintain a supply of methyl groups for the methylation of DNA, proteins, neurotransmitters, etc. (Scott and Weir, 1998). Early embryonic development requires extensive DNA synthesis. An adequate capacity to methylate DNA is crucial in the control of gene expression and thus would be an essential component of cell differentiation and development. Thus, genetic variations in folate-related enzymes, altered nutrition or environmental factors influencing folate status could all be considered to be potential risk factors for congenital malformations and candidates for research into the underlying causes of craniofacial anomalies.

4.3.4 Etiologic heterogeneity in OFC

There are, however, several difficulties associated with this approach. The first of these is etiologic heterogeneity of orofacial clefts, apart from the 20% or so that are syndromic due to specific mutations. It is quite possible that a specific fraction of orofacial clefts are related to folic acid or other multivitamins, but these are submerged under a sea of non folate-related defects. Some of these etiologies may be responsive to folate or other nutrients, others may not, making it difficult to find positive effects. Secondly, any potential genetic or biochemical markers of moderate risk may be difficult to detect unless the majority of syndromic cases can be ascertained and excluded from study sets. Thirdly, it will be important to have the capability of monitoring the nutritional or biochemical biomarkers that may be affected by new polymorphisms which are discovered in candidate genes. This means that a system involving collection of blood and perhaps immortalized cells should be set in place for future analyses. However, the logistics of such an undertaking would need to be carefully considered so that the task is comprehensive enough

to be effective without breaking the back of an entire research endeavour. Finally, while conclusive evidence exists for a specific protective role of folate in prevention of NTDs, this is not the case for orofacial clefts. The present indications of nutrient protection are derived from multivitamin preparations and not just folate.

4.3.5 Research strategy to deal with data gaps

In terms of approaches one could take to improve our level of evidence, there are many problems in carrying out good controlled studies to look at the role of folate and one of the biggest obstacles to progress is the heterogeneity of the study population. To minimize the problem in identifying folate-related defects, it will be essential to carefully categorize samples by type of defect, to identify (and exclude) syndromic cases where possible, and to control methodologic and demographic parameters which might confound biochemical and genetic analyses. In terms of identifying factors that influence folate status, genetic influences might play a major role. This was highlighted in a study of mono- and di-zygotic twins (Mitchell, 1997) that suggested that as much as 46% of the variance in red-cell folate concentrations might be attributable to additive genetic effects.

4.3.6 Uses and limitations of FFQ data as an alternative to blood samples

Misclassification is undoubtedly a problem with FFQs but surprisingly few biomarkers give a clear picture of nutritional intake. The inter-correlation between nutrients is also a problem for either FFQs or biochemical measurement. In the case of folate, at least, FFQs alone are very flawed, particularly when carrying out retrospective studies – most studies find that food folate intake does not have a high correlation with red-cell folate levels (correlation approximately 0.4). The chance of finding a folate-related effect on data derived from FFQs alone would have to be very small; nevertheless, the precise and detailed information requested by these questionnaires may possibly give one a false sense of security in the data. There are also practical difficulties with food tables in field conditions – particularly in assessing poorly nourished people in developing countries.

The alternative or complement to questionnaires for nutrient measurement is blood sampling and carrying out case-control studies on nutrient levels (or bio-markers such as homocysteine), provided disease status does not affect nutrient levels. Having overcome the logistics of sampling, there is an important issue in deciding from whom to collect blood (case, mother, father, or controls) and when the most appropriate

Most studies find that food folate intake does not have a high correlation with red-cell folate levels

time to take a blood sample would be. While this was not resolved it was, however, recognized that the major problem of measurement bias in biochemical analyses and inter-laboratory differences in methodology to measure blood levels of folates could be overcome by centralizing and standardizing sample analyses in a reputable laboratory, using another laboratory to ensure quality control.

4.4 Other specific nutrients and orofacial clefts¹

4.4.1 Vitamin B-6

Vitamin B-6 has been shown to protect against teratogen-induced clefts in many animal studies. Vitamin B-6 is the generic term for 3-hydroxy-2-methylpyridine derivatives that have the biological activity of pyridoxine. This vitamin plays many vital roles in amino acid metabolism, including transamination and decarboxylation reactions, and is the coenzyme in the degradation of homocysteine; there are thus many potential pathways in which vitamin B-6 protects against orofacial clefts. Vitamin B-6 deficiency alone was demonstrated to cause cleft palate and other birth defects in mice (Davis et al., 1970). Miller (1972) demonstrated that dietary vitamin B-6 also prevented the induction of clefts by vitamin A excess, cyclophosphamide, and beta-aminopropionitrile; hence the role of vitamin B-6 in cleft prevention may be complex and involve several different mechanisms.

Despite the extensive investigation of the role of vitamin B-6 in animal models of clefting since the 1950s, there is little information on the relevance of vitamin B-6 to clefts in humans. Use of anti-nausea medications has been associated with a reduced risk of congenital heart defects in the Atlanta Birth Defects Case-Control Study (Erickson, 1991), and vitamin B-6 may have a role in this pathway (*see also Section 7.3.2*).

4.4.2 Vitamin A

In experimental animals, vitamin A has been described as a “universal teratogen” (Schardein, 1993). The possible teratogenicity of dietary and supplementary vitamin A intake in the peri-conceptual period or early pregnancy in humans is controversial (Rothman et al., 1995; IARC Working Group, 1998; Miller, 1998). The debate has focused in particular on anomalies of structures derived from cranial neural crest cells, of which orofacial clefts are the most common type. There are considerable differences in the minimum teratogenic dose between species (IARC Working Group, 1998). The identification of genetic polymorphism at retinoic acid effector loci (RARA, AA7, MSX1) in

¹ See also Section 7.3.

humans raises the question as to whether there are inter-individual variations in susceptibility to the possible teratogenic effects of high intakes of vitamin A (*see also Section 7.3.4*).

4.4.3 Zinc

Studies associating maternal zinc nutriture to the risk of orofacial clefts in humans are extremely limited. Only one study has been conducted to evaluate the association by independently analysing the risk of orofacial clefts from other malformations. In addition, there have been a few investigations involving a limited number of cases of orofacial clefts, where no meaningful statistical analysis was possible (Flynn et al., 1981; Soltan and Jenkins, 1982; Stoll et al., 1999).

4.5 Lifestyle, occupational and other environmental factors in orofacial clefting

4.5.1 Cigarette smoking

Maternal cigarette smoking in pregnancy²

BOX H

Maternal cigarette smoking during pregnancy has long been associated with a moderate increase in the risk of orofacial clefts (Andrews and McGarry, 1972; Kelsey et al., 1978; Khoury et al., 1987; Shaw et al., 1996; Kallen, 1997; Werler et al., 1990; Ericson et al., 1979; van den Eeden et al., 1990), although some studies have not confirmed such an association (Evans et al., 1979; Shiono et al., 1986; Malloy et al., 1989; Hwang et al., 1995). A recent meta-analysis of published literature (Wyszynski et al., 1997) produced a summary:

- OR of 1:29 (95% CI 1.18 to 1.42) for CL/P associated with maternal smoking during pregnancy; and
- OR 1:32 (CI 1.10 to 1.62) for CP.

As in many epidemiological studies on birth defects showing weak effects, several potential methodological problems can obscure a true causal association (Khoury et al., 1992). For instance, several studies have not considered the following separately: CL/P and CP; isolated and multiple forms (Khoury et al., 1989). In most studies, there is no evidence of a linear dose-response relationship between cigarette consumption and risk of orofacial clefts. However, if such an association were confirmed, cigarette smoking might account for as much as 20% of orofacial clefts in the general population (Khoury et al., 1989). Parallel investigation of genetic susceptibility and of gene/environment interaction in relation to smoking would also be of interest.

² See also Section 7.2.1.

4.5.2 Alcohol drinking³

Heavy alcohol drinking during pregnancy is known to alter embryonic development, and cleft palate has been described as an associated defect in 10% of severe cases of fetal alcohol syndrome (Lemoine, 1992). An increased risk of CL/P specifically was found in association with a heavy intake of five drinks or more per day (OR=3.0; 95% CI 1.1 to 8.5), a category which concerned only 0.5% of control mothers (Werler et al., 1991). In a recent study in the United States (Iowa), maternal consumption of more than 10 drinks per month was associated with increased risks for isolated CL/P (OR=4.0; 95% CI 1.1 to 15.1) and isolated CP (OR=1.8; 95% CI 0.3 to 12.1), statistically significant only for CL/P (Munger et al., 1996). Paternal drinking was not associated with orofacial clefts (Savitz et al., 1991). One problem in the quantitative interpretation of the few studies on maternal alcohol consumption and orofacial clefts is the wide range of consumption across studies, in which similar effects can be found for a consumption of 5 drinks per day in 1 study and 10 drinks per month in another. In a systematic review presented at the WHO consensus meeting in Utah (May 2001), Little noted that the interpretation of the relationship between alcohol and orofacial clefts may be complicated by publication bias. In a number of studies of smoking, alcohol has been considered as a potential confounder, but no primary results relating to alcohol have been presented.

4.5.3 Other environmental risk factors

There is an association between orofacial clefts and epilepsy, but some controversy about whether it is the disease or the treatment with anti-epileptic drugs (AEDs) such as phenytoin or phenobarbital that is important. It has been estimated that the risk of CL/P among a new-born of a treated epileptic mother may be as high as 1%, i.e. about 10 times the population average (Dravet et al., 1992; Johnston and Bronsky, 1995). In general, as far as it is possible to separate effects of disease and therapy, risks associated with treatment with AED (especially polytherapy) are higher than those associated with disease alone (not treated) (Abrishamchian et al., 1994; Wyszynski, 1996). Among all AEDs, phenytoin has been more specifically associated with the risk of orofacial clefts (Johnston and Bronsky, 1995; Dravet et al., 1992), and the folic acid antagonistic effect is a possible mechanism (*see below*). To help resolve this, examination of familial aggregation and the rate of clefts in the offspring of men with epilepsy can be undertaken.

³ See also Section 7.2.2.

4.5.4 *Other illnesses and medications*

A number of other environmental factors may influence the occurrence of orofacial clefts:

- **Viruses:** acute viral infections and cold have both been reported as having associations with clefts (e.g. Czeizel and Hirschberg, 1997), and there may be confounding by hyperthermia.
- **Folic acid antagonists:** possibly a factor in CLP but not CP (Hernandez-Diaz et al., 2000).
- **Benzodiazepines:** some studies show an increase in risk – retrospectively, but not prospectively.
- **Corticosteroids:** some studies show an association, but the difference between topically- and systemically-applied corticosteroids requires further investigation.
- **Retinoids and tretinoin:** known teratogens in animal experiments, but there is little evidence of their association with orofacial clefts.

4.5.5 *Occupational exposures*

Pesticides/herbicides, water contaminants and occupational exposures have been examined in relation to OFC. Registry data (Ericson et al., 1979; Hemminki et al., 1981) and large-scale studies (McDonald et al., 1988) have suggested associations between orofacial clefts and maternal occupation (health workers, the repair-services industry, industrial trade or agriculture). Subsequent studies among health workers have not confirmed an increased risk (Matte et al., 1993). Maternal occupational exposure to solvents has been related to orofacial clefts in the early study by Holmberg et al. (1982), and subsequent studies in France (Cordier et al., 1992; Laumon et al., 1996) and Europe (Cordier et al., 1997). Teratogenesis with trichloroethylene and tetrachloroethylene in water has been suggested and associations with farming work have indicated a possible role of pesticides, confirmed in some published studies (Gordon, 1981; Thomas et al., 1992; Nurminen et al., 1995) but not in others (Shaw et al., 1999). It is important to specify the study period as this may affect the type and intensity of exposure, and the measures in place to protect against potential adverse effects of exposure (e.g. regulations about use of respirators, etc.).

Occupations of the father in the printing industry, as a painter (Erickson et al., 1979), motor vehicle operator (Olshan et al., 1991), fireman or farmer (Schnitzer et al., 1995) have been associated with an increased risk of orofacial clefts.

4.6 Conclusions

- Main gaps in knowledge are in the examination of co-teratogens and gene/environment interaction – for example: with alcohol in fetal alcohol syndrome (FAS) are there co-teratogens such as folate deficiency, and is there a threshold beneath which alcohol is safe? and with alcohol drinking, is there an indication of a dose response in terms of risk, with greater than 500 ml per day showing a significant association?
- Smoking, alcohol, epilepsy, certain medications and environmental factors may explain a small but appreciable portion of birth defects.
- It is important to be able to differentiate the exposure and the genetic predisposition so that those at risk can be identified and selectively counselled.
- General advice regarding alcohol and smoking in relation to disease tends to be ineffective in achieving significant changes in behaviour. Novel strategies surrounding birth defects may achieve better results. However, one major issue in the reporting of associations with exposures is the distinct possibility of publication bias in the literature.

5

Role of genes in CFA

The genetics of craniofacial anomalies and of cleft lip and palate, in particular, as the single most important sentinel defect of this group is highly complex. As is evident from this summary report and others that accompany it, etiologies are many-fold and complex and include single-gene causes, chromosomal disorders, polygenic interactions, environmental risks, gene/environment risks, and even the likely role of chance. Studies in this area began formally in the 1930s and 1940s with the work of Paul Fogh-Andersen and subsequently continued in the 1950s with Clark Fraser (1968). In the ensuing years much has been learned about the genetics of craniofacial anomalies and the recent advances in the progress of the Human Genome Project with the availability of almost complete human and mouse sequence provide unique and special opportunities to further these studies in powerful ways.

At the same time that the genetics is advancing, it is also clear that many questions remain, including even basic questions of phenotype definition and strategies for gene identification. Equally importantly, these studies need to be carried out in conjunction with other investigators whose primary interests and abilities lie in the areas of epidemiology, environment, nutrition, and clinical trials and prevention. The success of folic acid interventions in preventing neural tube defects provides a benchmark against which other preventive strategies for birth defects can be measured and the hope is that improvements in surgical techniques, speech pathology, dental care, nursing, psychological and paediatric care, and the many other fields involved with children with CFA will occur in concert with studies of etiology and prevention. By working together we can all provide a better future for children born with CFA, in the hope that prevention of these defects occurring in children will also be soon on the horizon.

5.1 Embryogenesis

By working
together we can
all provide a better
future for children
born with CFA

Development of craniofacial structures represents the complex interactions of many genes and environmental triggers. Studies of monozygotic twins whose facial appearances are almost completely overlapping in recognizable phenotypic features tell us that the role of genetics is almost 100% determinant in providing the outline of normal facial structures. Similarly, studies of monozygotic twins show a much higher concordance rate for non-syndromic forms of cleft lip and palate than would be found in dizygotic twins or siblings, again supporting the strong role of genetics in the etiology of defects of development. Nonetheless, concordance is only between 40% and 60% for clefting in monozygotic twins, which strongly supports the observation that the role of *in utero* environment or possibly some element of stochastic variation is also critical in determining which child might be born with which particular form of craniofacial disorder. Independent of non-syndromic forms of cleft lip and palate are many other defects of craniofacial regions, including other forms of clefts, craniosynostosis, and ocular and ear anomalies that have equally wide and disparate causes. During the course of the meeting, the etiology and pathogenesis of both orofacial clefting and craniosynostosis were reviewed in detail by Dr Michael Cohen (Cohen, 1995). In addition, the entire topic of craniofacial development has recently been extensively reviewed and reported upon by Geoffrey Sperber (2001), and this text as well as other recent publications on embryogenesis can serve as valuable resources for individuals with an interest in craniofacial disorders. Recent references include extensive lists of genes that have already been shown to play an important role in facial development; these genes can, in many cases, be divided into the roles that they play in a variety of morphogenetic pathways. These can include genes identified as growth factors, cytokines, self-signalling molecules, structural proteins (such as collagens or extra cellular matrix proteins), and other forms of morphogens or signalling molecules. Table 6 below lists a few of the genes that, from available genetic evidence, play a role in facial development; this list is in no way comprehensive and is, in fact, changing almost daily.

Table 6: Identified genes/clefts

Genes	Syndromes
CDKN1C	Beckwith-Wiedemann
COL11A1	Marshall
COL11A2	Stickler/Nance-Insley
COL2A1	Stickler/Kniest
CREBBP	Rubinstein-Taybi
DHCR7	Smith-Lemli-Opitz
DTDST	Diastrophic dysplasia
FGD1	Aarskog
FGFR2	Apert
FKHL15	Hypothyroidism
GLI3	Grieg/Pallister-Hall
GPC3	Simpson-Golabi-Behmel
KAL1	Kallman
L1CAM	MASA
LMX1B	Nail-patella
MID1	Opitz
MITF	Waardenburg 2A
PAX3	Waardenburg
PEX1,2,5,6,12	Zellweger
PTCH	Basal cell nevus
SHH	Holoprosencephaly
SIX3	Holoprosencephaly
SOX9	Campomelic dysplasia
TREACLE	Treacher Collins
TWIST	Saethre-Chotzen

Source: Murray JC, 2002

The availability of web sites provides opportunities to update the ongoing lists of candidates, as do the databases of clinical disorders involving craniofacial structure; these databases now identify many hundreds of such disorders. Valuable web sites for discussions of clinical aspects of human craniofacial disorders are listed below.

Table 7: Web sites

OMIM http://www3.ncbi.nlm.nih.gov/Omim/searchomim.html A listing of Mendelian disorders and genes; comprehensive for humans and extensively referenced with descriptive and historical data.
Human dysmorphology database http://www.hgmp.mrc.ac.uk/DHMHD/dysmorph.html A searchable database that provides both human and mouse homologies and also allows identification of disorders based on clinical, phenotypic and laboratory features.
Gene Tests/ Gene Clinics http://www.geneclinics.org/ Gene Tests and Gene Clinics are complementary databases. Gene Clinics provides descriptions of many genetic disorders, with an emphasis on management and diagnosis. Gene Tests provides a listing of both clinical and research laboratories currently carrying out molecular studies on a wide range of human disorders, including those involving craniofacial structures.

5.2 Clinical definition of craniofacial anomalies

This topic included discussions by Drs Michael Cohen, Marilyn Jones and Howard Saal of how craniofacial disorders can be defined from a broad perspective, with focused discussions on what would constitute the difference between non-syndromic and syndromic forms of cleft lip and palate and cleft palate only (Jones, 1988).

From the perspective of syndromic identification, many syndromes are now undergoing a revolution in their description as molecular abnormalities of individual genes are defined and redefined. This has been particularly evident in the description of the craniosynostosis syndromes as a variety of fibroblast growth-factor receptor genes, as well as at least one homeobox gene, have been demonstrated as having mutations that are etiologic for those disorders previously described as phenotypes. The situation has become immediately complex with different genes demonstrating mutations with apparently similar phenotypes, such as Pfeiffer syndrome associations with both FGFR1 and FGFR2 mutations, as well as the same gene having mutations that would have been separated on the basis of phenotypic appearances, such as Crouzon's and Pfeiffer's and mutations in FGFR2. Extensive discussions regarding the role that molecular definitions should play in conjunction with clinical delineation took place.

From the perspective of non-syndromic forms of clefting, the discussion was equally wide-ranging. Historically, based on animal as well as human segregation analysis and recurrence risk studies, cleft lip with or without cleft palate has been separated from cleft palate only. It is now evident

The description of what constitutes non-syndromic forms of clefting ... has yet to be fully resolved

that there can be at least occasional overlap between these phenotypes, as has been demonstrated for MSX1 mutations in at least one large family that includes individuals with isolated cleft palate, as well as cleft lip and palate (van den Boogaard, 2000). It has also been recognized for the last few decades in the case of the autosomal-dominant van der Woude's syndrome. Thus, the historic separation of these two categories on embryologic and genetic grounds – while still a valuable tool – is not 100% representative of observational data.

The description of what constitutes non-syndromic forms of clefting was also extensive and has yet to be fully resolved. This discussion is important in that studies undertaking genetic mapping of cleft lip and palate have increased power when phenotypes can be accurately and reproducibly identified. Thus, the ability to generate sub-phenotypes based upon what might have previously been thought of as normal variation is especially critical. In addition, associated major and minor anomalies can have an important impact on whether cases are included or not included in a study and, until molecular definitions begin to separate what should or should not be included in a particular definition, the discussion and criteria need to be established on the basis of clinical and embryologic grounds. Some definitions of non-syndromic clefting disorders would exclude any child with any other major organ system malformation, as well as a number of minor malformations, while other systems might allow the inclusion of a single major, or one or two minor, malformations. Recent developments in ultrasound also afford the opportunity to look for sub-clinical manifestations of clefts, such as deficiencies of the orbicularis oris muscle; these can also be very valuable tools for generating such sub-phenotypes.

In conclusion, and discussed further in Section 6 below, it is clear that these issues need to be formally addressed in any study that is carried out, and that investigators engaged in collaborative studies need to have consensus views for case inclusion and exclusion. Until the molecular phenotypes begin to help sort this out, both narrowly as well as broadly defined phenotypes may be used in genetic mapping studies; the availability of powerful computer analytic programmes also affords the opportunity to carry out multiple sets of analyses on subsets of clinically-defined cases, all drawn from a common larger data set. Table 8, below, shows some disorders where affected individuals might present as a “non-syndromic” cleft.

Table 8: Single-gene disorders that can mimic non-syndromic clefting

Phenotype	Single-gene disorder
Cleft lip and/or palate	CPX
Cleft lip and/or palate	EEC
Cleft lip and/or palate	CLPED1
Cleft lip and/or palate	VDWS

Source: Murray JC, 2002

5.3 Mouse models

The utility of the mouse for comparative studies of human genetic disorders has been widely acknowledged since the early 1900s. This work has become even more valuable as the ability to generate gene-specific knockouts or over-expression transgenics has become available. Coupled to the utility of the mouse as an experimental organism in which embryo manipulation can be carried out, is the very powerful genetics available through this system in which many generations of controlled breeding can be performed in a relatively short period of time. Finally, since the mouse is a mammal, many of its embryologic and developmental processes are closely related to those of the human. In the area of craniofacial development, studies of the mouse have been especially productive. A large number of knockout and transgenic animals that have been generated demonstrate disruptions of craniofacial structures and have provided opportunities to investigate genes identified in development. Evaluation of genes whose expression pattern also supports a role for development of craniofacial structures has also been critical. Particularly relevant models in the mouse come from knockouts of *MSX1* (Satokata and Maas, 1994), *TGF β 3* (Proetzel et al., 1995) and *SKI* (Colmenares et al., 2002). Spontaneously arising mutations, particularly ones in which the defects are focused on a specific craniofacial structure, such as the cleft models *CLF1* and *2* studied by Diana Juriloff (2001), have also been particularly relevant. And finally, the work of investigators, such as Robert Erickson and Scott Diehl (1997), in carrying out genome-wide strategies to look at gene/environment interactions and the role of teratogens in mouse models of clefting has also been very fruitful in providing localizations to regions that have high homology to human chromosomes as a way to better understand these forms of interactions. The availability of large amounts of mouse DNA sequence and very detailed mouse genetic maps and reagents for carrying out mapping also make the mouse an especially productive engine for the study of craniofacial anomalies. During the course of the meeting, details as well as new data were presented by Drs Diehl, Erickson and Juriloff and

provided opportunities for investigators working in human genetic systems to interact directly.

5.4 Genotyping

Advances as well as current strategies revolving around the issue of genotyping were discussed, particularly as they relate to humans. Genotyping includes the genetic analysis of variation and, for purposes of studies of cleft lip and palate, can be applied to genome-wide searches for gene or locus identification or to association studies using candidate-gene analysis. In addition, discussion about the use of chromosomal anomalies in gene finding was also provided. Besides the methodologies involved in the genotyping *per se*, discussions over strategies and particular analytic approaches were also carried out.

5.4.1 Strategic approach

BOX I

Strategic approaches

The cleft lip and palate genetics literature is a fusion of studies that have made use of candidate-gene and association analysis with a more limited number of studies that have used a linkage or genome-wide approach. A recent review summarized the “state-of-the-field” in 2002 (Murray, 2002) with loci on chromosomes 1, 2, 4, 6 and 14 holding particular interest. The difficulties in studying a complex disease, such as cleft lip and palate, include identification of a sufficient number of families in order to have the ability to effectively carry out a genome-wide approach. Thus, many early studies as well as current studies have made use of candidate-gene approaches to look at cases and have compared allelic frequencies with a control population. Very recently the ability to carry out direct candidate-gene sequencing has also been incorporated into some studies. While no single approach is likely to provide all the answers, there have been some preliminary successes with each of the above-mentioned approaches. In addition to the strategic approach selected, individual methodologies are also rapidly changing – as is common in molecular biology – and ongoing evaluation of the specific methodologic approaches will also be key for projects selected within individual laboratories. Finally, the ability to coordinate either analytic techniques or specific methodology and marker selection were important issues also discussed.

5.4.2 Analysis

Analytic approaches can use, for genome-wide searches, either parametric or non-parametric analyses. High-density genetic maps (Broman et al., 1998) and public resources for genotyping such as the NIH-sponsored Centre for Inherited Disease Research (CIDR) provide opportunities for even modestly-funded investigators to undertake such searches. Parametric analysis is the standard linkage approach in which the mechanism of inheritance pattern needs to be specified and can greatly benefit when a single large family is available. A recent report by van den Boogaard (2000) illustrates the utility of this approach when a single large family segregating for cleft lip and palate was identified, shown to be linked to the MSX1 locus on chromosome 4, and a point mutation resulting in a stop codon within this gene eventually identified. This family is especially remarkable in that many of the individuals have a phenotype that, if viewed in isolation, would be readily characterized as non-syndromic cleft lip and palate and raises the possibility of this disorder, at least in some cases, being caused by mutations in MSX1. The difficulty of this approach is that large families, such as the one described by van den Boogaard, are rare and may not provide insights into the most important or frequent genes involved in non-syndromic forms of clefting. Pools of such families can also be used in standard linkage analysis, and this approach has been used for many other complex disorders.

Within ... the
parametric and
non-parametric
categories
there are many
competing
analytic strategies

A compliment to the parametric approach is the non-parametric approach or the affected-pedigree member technique. This approach is best exemplified by sib-pair analysis in which pairs of affected siblings are identified and evidence for statistical aberrations in the proportion of alleles shared either by identity or descent established through genotyping. This approach, though powerful in that genetic mechanisms do not have to be specified, is unable to provide the more defined locus identification that will come about through linkage approaches. Most investigators would now choose to assemble a collection of families in which either analytic strategy, in general, could be applied and then carry out complimentary analysis using a multiplicity of approaches. Even within each of the broad categories – parametric and non-parametric – there are many competing analytic strategies that are discussed in more detail in other publications. Furthermore, the addition of analysis of variance approaches in which the severity of the phenotype can be taken into account, as well as the addition of environmental variables as an analytic variable, are also important considerations in current study designs (Almasy and Blangero, 1998). Although only one large genome-wide search has been carried out (Prescott et al., 1999), there are now under way genome-wide approaches from other laboratories; it is likely that over the next few years additional evidence from these searches will be provided.

Several candidate loci searches using 10 to 40 families have already been reported (Carinci et al., 2000; Marazita et al., 2002). Table 9 below summarizes some linkage work done in humans.

Table 9: Human loci/genes for suggested linkage

Position	Disorder*	Method**	Cloned	[Candidate]
1p36	NS	L/CH	–	[SKI]
1q32	VDWS	L/CH	IRF6	–
2p13	NS	L/LD	TGF α	–
3q27	EEC3	L/KO	P63	–
4p16	NS	LD/CH/KO	MSX1	–
4q31	NS	L/LD	–	–
6p23	NS	L/CH	–	[AP2, EDN1]
11q23	ED4	L	–	–
14q24	NS	LD/KO	TGF β 3	–
9q13	NS	L/LD	–	–
Xq21	CPX	L/CH	TBX22	–

Source: Murray JC, 2002

* Disorders:	NS:	non syndromic
	VDWS:	Van der Woude syndrome
	EEC3:	ectodermal dysplasia/ectrodactyly and clefting syndrome 3
	ED4:	ectodermal dysplasia and clefting syndrome 4
	CPX:	X-linked cleft palate and ankyloglossia
** Methods:	L:	linkage
	CH:	chromosomal rearrangement
	LD:	linkage disequilibrium
	KO:	mouse knockout

The complements to family-based approaches are those that use case and control populations. These studies are best carried out when a candidate gene or locus is available as they depend on the phenomenon of linkage disequilibrium, active over only very short physical distances of DNA. This limits the study to a handful of 10 to perhaps 100 loci, given current fiscal realities and available markers. The selection of candidate genes can often take place using the descriptions provided through developmental biology or mouse models, and frequently utilizes genes shown to be expressed in the developing palate or genes whose disruption in a knockout mouse, for example, would result in a cleft lip or palate phenotype. Judicious selection of candidate genes can be an effective tool in identifying a genetic component of a common disorder. These studies in cleft lip and palate were initiated in 1989 with the study of Ardinger et al. in which evidence for the role of TGF α was provided and a case-control approach was

followed, using non-syndromic cleft lip and palate as the cases and convenience controls, selected from the same geographic area as the cases were collected. Since this publication, the literature has expanded greatly with a number of additional studies, including those using more powerful analytic techniques, that have provided both positive and negative results. A summary of these studies, given in Table 10 below, would seem to support some evidence that both TGF β 3 and MSX1 are genes involved in clefting.

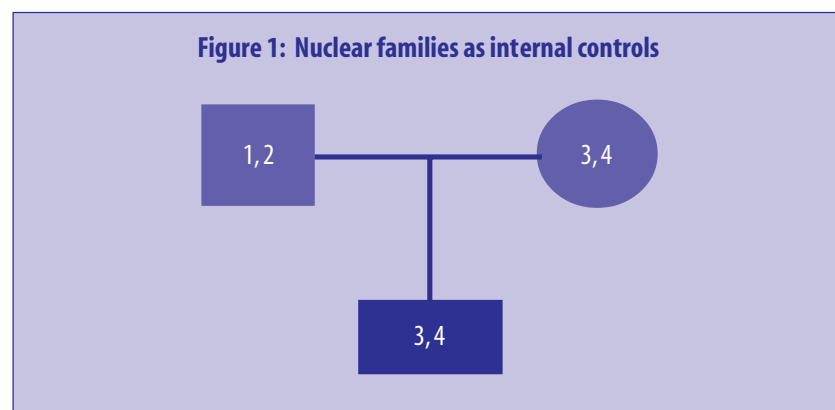
Table 10: Candidate-gene studies for CL/P

Gene	Analysis	Result	Author
TGF α	meta	OR=1:43 (1.12 to 1.80)	Mitchell
MSX1	case-control	p<0.005	Lidral
MSX1	AFBAC*	p<0.04	Lidral
MSX1	TDT	p<0.001	Vieira
TGF β 3	TDT	p<0.008	Lidral
TGF β 3	TDT	p<0.01	Maestri
TGF β 3	TDT	p<0.02	Vieira

* Affected family member-based controls.

Source: Murray JC, 2002

The addition of newer analytic strategies, such as the transmission disequilibrium test (TDT) (Spielman and Ewens, 1996) and likelihood ratio test (LRT) (Umbach and Weinberg, 2000) tests in which transmission distortion or family-based allelic controls to prevent the confounding of ethnic matching, provides for even more powerful platforms for the collection of information. In addition, it is now possible and feasible to collect hundreds of families with a focus on nuclear triads, consisting of an affected child with the mother and father (as shown below in Figure 1 below), in which substantial power for detecting even small gene effects is available.



As the selection of candidate-gene panels also becomes more robust, these approaches are likely to be successful.

5.4.3 Sample collection

Sample collection issues are of paramount importance and were discussed widely. While it is easy to collect samples in the form of buccal or cheek swabs, for example, the DNA available from these is limited and, at the present time, is unlikely to comprise enough for a genome-wide search. Whole blood samples are more robust, both in terms of the quality and quantity of DNA available, and are usually sufficient to apply to genome-wide searches in which approximately 20 micrograms of high quality DNA would be required. Whole blood, however, can present challenges in collection and, in the case of small infants, may be limited by available quantities. Additional advantages of whole blood include the possibility of saving plasma or serum for analysis of other analytes, such as micronutrients or storing cells for subsequent RNA or protein studies. Other tissues, including cord blood, placenta and materials obtained from the site of surgery, also provide opportunities for other forms of analysis. Materials obtained at the site of surgery, for example, might be useful for looking at abnormalities of gene expression found in affected tissues. While there is no single sample collection strategy that can solve all the financial and technical problems, the issues raised by these were important considerations for the group as a whole and, from ongoing studies, it is clear that a variety of study designs have been selected as most appropriate for particular projects. For example, the large collaborative study under way, sponsored by the US Centres for Disease Control, has chosen buccal swabs as these can be obtained via the mail from individuals who self-collect on themselves and their children. The advantage is that this is a very cost-effective approach; it allows for the collection of thousands of samples yearly on a limited budget and also provides limited amounts of DNA for analysis. Other studies have collected blood-spot samples and these may prove to be especially effective when studying newborn populations. These samples are useful in that they can be stored indefinitely and inexpensively but are compromised by the limited quality and amount of their DNA; there may be challenges to comprehensive analysis of DNA from such samples where only certain genotyping approaches may work.

5.4.4 Collaborative strategies

See Box J, facing page.

Collaborative strategies

A variety of efforts, already under way, foster collaborative interactions in the study of cleft lip and palate. A few of these are described below.

1. Estudio Colaborativo Latino Americano Malformaciones Congenita (ECLAMC)

ECLAMC is a collaboration, established in the mid-1960s in South America, in which up to 100 participating hospitals have one or more volunteer paediatricians who collect demographic and clinical data on a wide spectrum of structural birth defects, including cleft lip and palate. The data is entered in a common format and returned to a central repository for storage and evaluation. While this collaboration makes use of volunteer physicians, it has proven to be highly effective and currently collects data on approximately 200 000 cases per year. Numerous studies have been published by this group, including some relevant to cleft lip and palate suggesting, for example, that altitude or ethnicity may be important roles in determining risks for clefts. Recently the group has also incorporated blood sampling from children and parents into their strategy, and it is likely that this will provide extremely powerful data for analyses, given the large number of samples available.

2. US Centers for Disease Control and Prevention (CDC)

For the last five years, the US Centers for Disease Control and Prevention has sponsored an eight-location collaboration to collect data from 2400 cases and 800 controls per year, with a collection of 30 structural birth defects, including cleft lip and palate. In addition, biological sample collection in the form of cheek swabs, collected from infants and their parents, has also been incorporated to complement an extensive interview of the mother in which data regarding pregnancy risks, such as drug exposures, outcomes, nutritional factors and family history, are all incorporated. Data, as well as biological samples, are stored in a central repository and made available to collaborating investigators for addressing specific hypotheses. Because there are such detailed characterizations of environmental exposures along with the collection of DNA samples, this project has enormous power to study gene/environment interactions across a broad geographic range in the United States.

3. European Collaboration on Craniofacial Anomalies (EUROCRAN)

A multi-centre collaboration funded by the European Union (Contract Number: QLGI-CT-2000-01019) was established, combining existing networks that have already been established by EUROCLEFT and the European Science Foundation (ESF) (<http://www.esf.org>). A pan-European, multi-centre, multidisciplinary effort has evolved. The innovation arises from the involvement of international experts at the cutting edge of research in their respective fields, and the application of advances in basic sciences and molecular biology to clinical research is seen as the way forward. A number of ground-breaking work packages have been undertaken, collectively aimed at improving knowledge on the etiology and pathogenesis of craniofacial abnormalities, introducing precise diagnostics/risk assessment, developing therapeutics and producing the best (evidence-based) treatment protocols. These research efforts are being extended to Eastern Europe, and the ultimate objective is to pursue their implementation further afield (*see Annex 1*).

4. European Registry for Congenital Anomalies and Twins (EUROCAT)

EUROCAT is a European network of registries for the epidemiologic surveillance of congenital anomalies. EUROCAT began in 1979 and currently surveys more than 900 000 births per year. Through its work on harmonization of methodology, particularly for ascertainment, EUROCAT has become an established reference centre for population-based information on congenital anomaly prevalence and time trends. The EUROCAT collaborative framework seeks to exploit the power of transnational collaboration in data collection and exchange of expertise to address issues of concern on birth-defects prevention and service delivery (<http://www.lshtm.ac.uk/php/eu/eurocat>).

5.5 Recent developments

While the field of craniofacial anomalies and genetic studies is rapidly moving, a few comments about recent developments are useful. Genes continue to be cloned for a variety of syndromic forms of cleft lip and palate and, very recently, the first craniofacial anomaly identified through linkage – X-linked cleft palate/ankyloglossia syndrome – has had its gene (TBX22) identified (Braybrook et al., 2001). In this case, a transcription factor, TBX22, has been shown to be at fault, and this further opens the door for additional investigations of other transcription factors or their pathway members in non-syndromic forms of clefting. In a complimentary report, the Spritz group (Sozen et al., 2001) has provided evidence that heterozygotes for the PVRL1 gene, which had previously been shown to have etiologic mutations in the Margarita Island ectodermal dysplasia clefting syndrome (Suzuki et al., 2000), had heterozygotes that have an increased frequency of non-syndromic clefting in populations studied in Venezuela. This raises the possibility that heterozygotes for syndromic forms of clefting might occasionally be at increased risk for non-syndromic forms and that, potentially, gene/environment interactions might further complicate this story. This is an important and exciting finding that opens the door to many additional forms of investigation. Candidate-gene studies have continued to be expanded and Terri Beaty's group (2002) has also recently reported additional evidence for the role of the MSX1 homeobox gene in cleft lip and palate. The gene for the van der Woude and Popliteal pterygium syndromes, interferon regulatory factor 6 (IRF6), has also been reported (Kondo et al., submitted). Finally, new efforts at genome-wide approaches are under way and are likely to contribute new information in the near future.

6

Gene/environment interactions

6.1 Introduction

The role of genes, genetic susceptibility and gene/environment interactions (GEI) in the etiology of orofacial clefts remains largely unknown. However, with the availability of the human genome sequence, researchers have increasing opportunities to study the role of genes and gene/environment interactions in human health and disease (Schutte and Murray, 1999). Discussions, led by Lorenzo Botto, sought to examine these opportunities and the major accompanying challenges in three main areas:

- **The first area relates to data:** to identify and, if possible, rank the major data gaps separating our current knowledge from that needed for clinical and public health action.
- **The second area relates to methods:** how to conduct, analyse and present studies of multiple genetic and environmental factors in ways that efficiently fill the data gaps.
- **The third area relates to people and institutions:** how to learn more and more quickly, using the unique opportunities inherent in international collaboration.

Gaps and challenges in the study of GEI in orofacial clefting

Data challenges

- Representative populations
- Focus on common exposures and gene variants

Methodology challenges

- Improved assessment of environmental exposures
- Careful design, complete presentation
- Systematic assessment of risks and impact

Collaboration challenges

- Use, share, pool data
- Sample size – more people, more countries
- Standardized methodologies

6.2 Data challenges

6.2.1 Representative populations

Because the ultimate goal is population-based action (prevention, intervention), scientists need data that is representative of populations. For example, the frequency of gene variants and exposures should come from population-based surveys, the risk estimates from population-based case-control studies, and so on. Such requirements for population-based studies can be a major constraint to study design and conduct; ultimately, however, there is no known alternative for gathering population-based data. Some measures of risk (e.g., the effect of genes alone, departure from multiplicative interaction) could be provided by family studies or case-only studies that are not population based. Such studies can be very useful. However, the full spectrum of gene effects and gene/environment interactions and estimates of attributable fraction require, for identification or confirmation, population-based studies such as population-based case-control studies, as discussed below.

6.2.2 Focus on common exposures and gene variants

There are many genes and exposures that one could study. Indeed only a handful of gene variants and exposures have been studied in relation to orofacial clefting, leaving options virtually limitless. From the preventive perspective that underlies this discussion, it is natural to suggest an initial focus on factors that might contribute to the greatest fraction of cases in the population, i.e., factors with the highest attributable fraction. The latter is a function of the factor's relative risk and its frequency in the population. Because the relative risk is difficult to gauge in advance, frequency of exposures might be a reasonable factor to consider in ranking the potential interest of exposures. This concept is put into numbers in Table 11 (below) which summarizes the population-attributable fraction of a hypothetical exposure, given a range of associated relative risks and exposure frequencies.

Table 11: Population-attributable fraction in relation to frequency of exposure and relative risk

Frequency of exposure	Relative risk (RR)						
	1.2	1.5	2	3	5	10	20
0.0001	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.001	0.00	0.00	0.00	0.00	0.00	0.01	0.02
0.01	0.00	0.00	0.01	0.02	0.04	0.08	0.16
Fever	0.05	0.01	0.02	0.05	0.09	0.17	0.31
Obesity	0.1	0.02	0.05	0.09	0.17	0.29	0.47
	0.3	0.06	0.13	0.23	0.38	0.55	0.73
Smoking	0.5	0.09	0.20	0.33	0.50	0.67	0.82
	0.7	0.12	0.26	0.41	0.58	0.74	0.86
No supplement	0.9	0.15	0.31	0.47	0.64	0.78	0.89

Source: Dr Lorenzo Botto (unpublished data)

Studying small relative risks is, however, challenging as it requires large sample sizes and careful assessment of bias and confounding. Multi-centre and international collaboration with common protocols might be a useful strategy to overcome some of these difficulties. Finding GEI that involve common exposures might also be useful in confirming the role of such exposures in the etiology of orofacial clefting, particularly when the exposure alone is associated with low increased risk (e.g. smoking) that might be due entirely to unrecognized bias or confounding.

Finally, because of the potential impact of these common factors, negative studies become very important. Their replication and publication should therefore be encouraged.

6.3 Methodology challenges

The problems in gene/environment interaction research reside mainly with the *a priori* specification of the interaction model and with the statistical power required. It is also felt that there are difficulties in measurement of the environmental exposure.

It should also be noted, however, that genotype may effect the level of a biomarker and this is particularly important when examining nutrient status.

6.3.1 *Improved assessment of environmental exposures*

... look not only
at genes alone, or
at environmental
factors alone,
but also at their
interaction

The problems in gene/environment interaction are mainly with the environmental aspect. With genes it is possible to carry out more analyses in shorter time periods with good reliability, but better assessment methods are urgently needed for assessment of environmental factors, as well as issues such as measuring versus reporting – the former being more objective while the latter is easier and less expensive.

Environmental exposures are now usually based on maternal reports, often taken months or years after the relevant exposure period. Objective biomarkers of exposure and effect are, for the most part, lacking. Biologic samples for measurement of environmental exposures (urine, hair, serum, whole blood) are difficult to obtain – more so than DNA sources – as are environmental samples (air, water, soil). The precision and validity of GEI studies is a function of the validity and precision of both the genetic and the environmental component, making improvements of environmental measurements a priority in GEI studies.

6.3.2 *Careful design, complete presentation*

Currently, several approaches are being used. Some classic published studies of GEI in OFC were conducted using the population-based case-control design (Denmark and the United States (Iowa and California)). In recognition of the genetic predisposition and GEI, a study design in the United Kingdom adopted a strategy using both case triads and control triads (ITSMAGIC Consortium) and a large ongoing study in the United States is based on a similar design. Some ongoing studies from Europe and the United States are based on case-triad designs. At least one large ongoing study in the United States is based on a mixed case-control design, using both case triads and control triads. These designs were carefully chosen as being the best for the objectives of the studies, given practical constraints; the hope is that the cumulative knowledge so obtained can be integrated to completely characterize, in the sense discussed above, the population-based indices of GEI in orofacial clefting.

It is important to look not only at genes alone, or at environmental factors alone, but also at their interaction. A simple and effective way of looking at gene/environment interaction is exemplified by the 2 x 4 table approach using a case-control model. This approach allows for the study of the effects of each factor or gene alone, joint effects, and the assessment of interaction in terms of departure from any specified model, be it additive or multiplicative (or other).

6.3.3 Systematic assessment of risks and impact

In addition to the summary measure of interaction (be it additive or multiplicative), it is useful to derive and present the component factors, i.e., the effect of the genotype alone, the exposure alone, and the joint effect of both genotype and exposure. For each of these factors, it is useful to present three numbers: the frequency among controls, the relative-risk estimate, and the attributable fraction. These numbers (the frequency, risk and impact for the three components of interaction and the summary measure) neatly summarize many important aspects of a GEI.

6.4 Collaboration challenges

6.4.1 Use, share, pool data

Like most research, results from studies of OFC carried out independently are often difficult to compare because the studies are relatively small and often use different classifications of exposures and outcomes. Indeed, one of the most common sentences in published reports may be variations of “comparison with other studies is difficult because of methodologic differences”. Such comparisons, however, might still be possible if one reverts to the original, individual-level data. Thus collaborative, primary-pooled analyses might be an efficient strategy to maximize the information yield of already-conducted studies. In addition, international collaboration might benefit from the sharing of unpublished data from studies that may have been published in part, perhaps using a common repository of unpublished tables. Pooling data from such tables might be appropriate in some cases, provided there is an awareness of differences in data-collection methodology, biases and confounders, and that any subsequent evaluation or analysis recognizes these factors.

6.4.2 *Sample size*

BOX L

More people, more countries

Sample size is a fundamental issue in GEI studies. In the case of orofacial clefting studies, the challenge of sample size is evident in the published literature where the expected number of cases in the relevant exposure category is usually very small, often less than 10 and sometimes less than 3. Carefully conducted multi-centre and international collaboration might provide a useful strategy to study larger numbers of people, provided there is adequate control of confounding and elimination of biases.

Most data on GEI in orofacial clefting derives from studies of small, wealthy populations (e.g., Denmark and the United States (Iowa, California)). Whilst this is to some extent unavoidable, it underscores the need for similar data in populations that are geographically and ethnically diverse. Orofacial clefting occurs more frequently and causes more morbidity and mortality in the less wealthy countries (Schutte and Murray, 1999; Rosano et al., 2000). Finding GEI that are relevant to these populations (and simple, inexpensive, low-tech prevention strategies) would satisfy elementary requirements for social justice.

Also, broadening the range of exposure probably makes misclassification have a smaller impact than improving the precision of exposure assessment would.

6.4.3 *Standardized methodology*

In disorders that are thought to have a polygenic multi-factorial etiology, as is the case for non-syndromic orofacial clefting, there is a compelling need for researchers to be able to compare their data on putative environmental and genetic factors. The fundamental principle on which multi-centre collaborative research works is that there is a consistency in the methodology of data collection, thus enabling combined analysis.

A multidisciplinary multi-centre European initiative, supported by the European Science Foundation (ESF) has, as one of its main objectives, sought to define in a number of key areas the important data and accompanying methodology of this data collection. The common factor which brought this body of expertise together was a research interest in orofacial clefts and, because of the polygenic multi-factorial etiology and evidence of heterogeneity, this group sought to develop consistent protocols across populations with variable genetic backgrounds, lifestyles, diets and environmental exposures. The parallel development of global networks in CFA research, through funding from the European Union, the NIH and WHO, will enable researchers throughout the world to benefit from these “common core protocols”.

While these have been developed in the context of orofacial clefting, they may provide useful information in the wider context of reproductive outcome – in particular, for other birth defects also suspected of having a polygenic multi-factorial etiology.

6.5 Conclusions

The study of GEI in orofacial clefting has achieved some remarkable successes, and developments in genetic technology promise that such successes are only the beginning (Schutte and Murray, 1999). The eight challenges presented here might stimulate discussions that could lead to useful collaboration. The task ahead is still enormous. There are thousands of gene – gene/environment interactions possible and 99.96% of genes in the population remain untested. In those that are tested, genotype frequencies vary in different populations. Shared priorities, clear planning and international collaboration are likely to be key factors in progressing from basic science to population-based opportunities for primary prevention worldwide.

7

Prevention of CFA

7.1 Meeting objectives

BOX M

Objectives of the WHO meeting on the prevention of CFA

- Identify environmental and behavioural factors with established associations with orofacial clefts and other craniofacial anomalies (CFA) and recommend global public health initiatives for the prevention of CFA caused by these factors.
- Review evidence regarding the role of specific maternal, nutritional factors in the etiology of orofacial clefts and other CFA.
- Reach a consensus regarding the role and importance of nutritional supplementation trials in evaluating the causal role for specific nutrients in the etiology of orofacial clefts and other CFA.
- Discuss aspects of the design of orofacial cleft and CFA-prevention trials and their ethical, legal, social and financial implications.
- Make recommendations regarding the resources needed to implement international collaborative studies of CFA prevention with common core protocols.

7.2 Environmental and behavioural factors and orofacial clefts

Craniofacial anomalies are among the most common birth defects and, of these, orofacial clefts are the most frequent. As described in the *World Atlas of Birth Defects* (World Health Organization, 1998) and in Section 2.1 of this report, there is a great deal of variation in the occurrence of orofacial clefts in different populations throughout the world. It is likely that this is due to both environmental and genetic factors. Poverty has been previously associated with an increased risk of neural tube defects and, more recently, with the occurrence of orofacial clefts (discussed in Section 4.1), providing evidence that environmental factors play an important role in both type of birth defects. Data from Brazil, China and the United States (Utah) presented at the WHO/Utah meeting support the view that the pattern of occurrence of neural tube defects is different from that of orofacial clefts across geographic areas and time periods, indicating that the environmental factors that cause these defects are not the same. The specific components of the environment of the poor, relating to orofacial clefts, are unclear but could include exposure to tobacco smoke, alcohol, occupational or residential exposures to teratogens, and poor nutritional status.

7.2.1 Tobacco and orofacial clefts

Maternal cigarette smoking is perhaps the best studied environmental risk factor for orofacial clefts. As summarized above in Section 4.5.1, maternal tobacco use during pregnancy has been consistently associated with a modest elevation in risk of orofacial clefts. Given the frequency of the habit among women in the United States, smoking may account for as much as 20% of orofacial clefts in the country's population. The risk of orofacial clefts attributable to smoking may be underestimated because exposure of pregnant women to passive smoking in the home and workplace has not usually been taken into account.

Over one billion people worldwide smoke and nearly three-quarters of these live in developing countries, often with relatively low levels of public and political support for effective tobacco control measures. (Aghi et al., 2002). Numerous reports have documented that smoking prevalence rates among women aged 15-25 years have steadily increased globally over the past decade (Windsor, 2002). It was estimated that in 1995, 12-14 million women worldwide smoked during their pregnancy and, when passive smoking was accounted for, 50 million pregnant women, out of a total of 130 million, were exposed to tobacco smoke during their pregnancy (Windsor, 2002). The second wave of the epidemic of tobacco-related

The association between maternal smoking and orofacial clefts may not be widely appreciated by international health organizations

diseases is resulting from women being actively targeted by tobacco companies and taking up smoking in increasing numbers (Kaufman and Nichter, 2002). The traditional habit of chewing tobacco among women in many populations may also represent an under-studied source of tobacco exposure during pregnancy.

The association between maternal smoking and orofacial clefts may not be widely appreciated by international health organizations. The US Surgeon-General's *Report on Women and Smoking* notes that, while the overall risk of birth defects does not appear to be related to maternal smoking, certain specific birth defects have been including orofacial clefts, limb reduction defects, and urogenital defects (Office of the US Surgeon General, 2001). Orofacial clefts were not mentioned however in the most recent WHO report, *Women and the Tobacco Epidemic: Challenges for the 21st Century* (Samet and Yoon, 2002). The tobacco-related health effects of stillbirth, prematurity and intrauterine growth retardation are much more common and better studied than orofacial clefts, yet the topic of orofacial clefts may have powerful and persuasive effects if incorporated into public health campaigns on the consequences of maternal smoking. The images of faces of disfigured children have been used to establish some of the world's largest medical charity organizations that are devoted to providing free orofacial cleft surgeries in under-served populations. Similar images might prove effective in public health campaigns to protect pregnant women from tobacco smoke and other environmental teratogens.

7.2.2 Maternal alcohol use and craniofacial anomalies

Maternal alcohol use during pregnancy is a well-known cause of the fetal alcohol syndrome. Delegates at the WHO/Utah meeting reported that the occurrence of the fetal alcohol syndrome ranges between 1 per 1000 births in western industrialized countries, 8-10 per 1000 in selected Native (North) American populations, and 108 per 1000 in selected South African populations. The populations at high risk for the fetal alcohol syndrome are almost always impoverished, have easy access to alcohol and, in many cases, have experienced rapid deterioration of their traditional culture and subsistence patterns. The fetal alcohol syndrome represents an extreme example of the effects of maternal alcohol consumption during pregnancy and the pattern of alcohol consumption usually involved – binge drinking – is also extreme. While the characteristic and severe features of the fetal alcohol syndrome are mainly neurologic, resulting in diminished cognitive and behavioural functions, animal and human studies have shown that midline craniofacial anomalies, including orofacial clefts may also occur (Kotch and Sulik, 1992; Johnson et al., 1996).

There is enough evidence of a firm causal relationship between maternal alcohol consumption and CFA

Women are more commonly exposed to lower levels of alcohol intake during pregnancy than occurs during the binge drinking associated with fetal alcohol syndrome. Alcohol drinking takes place in a variety of social contexts that may include the modifying or confounding effects of diet, smoking and drug use; it is thus understandable why the association between maternal alcohol use and risk of isolated birth defects is not entirely consistent. Maternal alcohol use during pregnancy has been associated with an increased risk of isolated orofacial clefts in some, but not all, studies, as discussed in Section 4.5.2. An examination of the social and dietary context in which alcohol consumption takes place may help to clarify its relationship to orofacial clefts and other CFA. For example, the risk from alcohol consumed while drinking beer at a bar with non-nutritious snacks and exposure to active or passive smoking is not likely to be equivalent to that when the same amount of alcohol is consumed by drinking wine with a nutritious meal. Despite some remaining uncertainties about the relationship between patterns of alcohol consumption and the risk of isolated orofacial clefts, enough evidence exists of a firm causal relationship between maternal alcohol consumption and craniofacial anomalies and other adverse reproductive health effects to warrant strong, worldwide, public health measures to discourage maternal alcohol consumption near the time of conception and during pregnancy.

7.2.3 Other maternal exposures related to craniofacial anomalies

Maternal exposures to possible teratogenic medications and chemicals in the workplace and residence were reviewed above in Section 4.5. These teratogens may be critically important to women exposed to them but do not seem as widespread as nutritional deficiencies and tobacco and alcohol exposures; they do not, thus, seem to be ideal choices for broad, population-based, intervention studies. Birth-defect prevention efforts related to medications might ideally be focused on clinical approaches, and occupational exposures to teratogens might best be studied further, with prevention efforts targeted at specific occupational groups.

7.3 Maternal nutrition and orofacial clefts

Adequate nutrition of the mother at the time of conception and in the first trimester of pregnancy appears to be important for the normal development of the lip, palate and other craniofacial structures of the fetus. Much experimental evidence for this view has accumulated from studies of laboratory animals in which specific nutritional deficiencies were induced either by dietary manipulation or by the administration of specific nutrient antagonists. Observational studies of human populations are highly supportive of an important role for maternal nutrition in

normal craniofacial development but, with this approach, it has been difficult to identify the specific nutrients involved because of the high intercorrelation of the many nutrients in multivitamin preparations, fortified foods and healthy dietary patterns. A comprehensive review of laboratory animal and human epidemiologic studies of maternal nutrition and orofacial clefts is available (Munger, 2002). Taken together, the evidence from laboratory animal experiments and human observational studies point to folic acid and vitamin B-6 as leading candidate nutrients that may be useful in the prevention of orofacial clefts, and a lesser body of evidence implicates riboflavin (vitamin B-2) and vitamin A.

7.3.1 Folic acid

The role of maternal dietary folate intake in orofacial clefts has been difficult to determine in human case-control studies

Animal models for the study of folate deficiency as a cause of fetal death, orofacial clefts and other birth defects were first established in the 1940s by Nelson, using a combination of dietary folate deficiency and folate antagonists (Nelson and Evans, 1947; 1949; Nelson et al., 1950). Folate antagonists were eventually found to cause craniofacial and other birth defects in mice, rats and chickens, and folate supplementation was found to prevent orofacial clefts in a breeding line of dogs with a genetic predisposition to orofacial clefts (Elwood and Colquhoun, 1997). Medications that disrupt folate metabolism have been shown in human case-control studies to be associated with an increased risk of birth defects, including orofacial clefts (Hernandez-Diaz et al., 2000). The role of maternal dietary folate intake in orofacial clefts has been difficult to determine in human case-control studies because folates from food sources have a wide range of bioavailability and folic acid supplements are usually taken with other vitamins, minerals and trace elements that may also have protective effects against orofacial clefts. Studies of genetic variation of folate-dependent enzymes may yield clues about the role of folate in orofacial clefts, but to date genetic studies have not altered the current state of equipoise: the MTHFR C677T thermolabile genotype was found to be associated with an increased risk of orofacial clefts in Ireland (Mills et al., 1999) but not in the United States (California) – (Shaw et al., 1998; 1999).

7.3.2 Vitamin B-6

Vitamin B-6 (pyridoxine and closely related compounds) is known to protect against orofacial clefts induced in laboratory animals by teratogens including corticosteroids (Fraser and Fainstat, 1951; Kalter, 1957; Peer et al., 1958; Bonner and Slavkin, 1975; Melnick et al., 1981), vitamin A excess (Yamaguchi, 1968), cyclophosphamide (Dostal and Schubert, 1990), and beta-aminopropionitrile (Jacobsson and Granstrom, 1997). Deoxypyridine, a vitamin B-6 antagonist, was shown to induce orofacial

clefts (Miller, 1972) and vitamin B-6 deficiency alone was sufficient to cause cleft palate and other birth defects in mice (Davis et al., 1970). Less information is available from human studies on the possible role of vitamin B-6 in orofacial clefts (*see Section 4.4.1*).

In a case-control study in the Netherlands, mild maternal homocysteinemia was associated with an elevated risk of nonsyndromic orofacial clefts (Wong et al., 1999). Biochemical studies revealed that case-mothers had lower levels of whole blood vitamin B-6 (measured as pyridoxal-5'-phosphate) compared to controls; no differences were found in levels of serum vitamin B-12 and case-mothers had higher levels of serum and red-cell folate compared to controls. Thus, in the Netherlands poorer vitamin B-6 status was associated with a higher risk of orofacial clefts and one possible mechanism may have been elevated homocysteine levels in mothers with poorer vitamin B-6 status.

The worldwide occurrence of vitamin B-6 deficiency is not well described although it is known to be a regional problem in poorer populations of Asia where highly polished rice is the dietary staple and few other dietary sources of vitamin B-6 are available (Bamji et al., 1979). These populations also appear to have elevated rates of orofacial clefts. Vitamin B-6 deficiency is also induced by use of certain medications, including isoniazid for the treatment of tuberculosis, and oral contraceptives (Sauberlich et al., 1972).

7.3.3 Riboflavin (vitamin B-2)

Riboflavin (vitamin B-2) deficiency was found by Warkany in the 1940s to cause skeletal malformations and orofacial clefts in laboratory rats (Warkany and Nelson, 1940). In further studies of the timing of deficiencies during gestation, Warkany found that riboflavin supplementation before Day 13 prevented the malformations but later supplementation did not, thus establishing the principle of a critical period in embryonic development for the susceptibility to nutritionally-induced birth defects (Warkany, 1954). Further studies by others confirmed that riboflavin deficiency caused birth defects in rats (Noback and Kupperman, 1944; Giroud and Boisselot, 1947; Leimbach, 1949; Piccioni and Bologna, 1949; Giroud and Boisselot, 1951), mice (Kalter and Warkany, 1957), and fowl (Lepkovsky et al., 1938; Romanoff and Bauernfeind, 1942).

Despite the findings that riboflavin deficiency caused orofacial clefts and other birth defects in laboratory animals, it does not seem to have been the subject of research in studies of human orofacial clefts. This is an important gap in current knowledge because riboflavin deficiency is one of the most common vitamin deficiencies worldwide (Sauberlich, 1984); it commonly co-occurs with vitamin B-6 deficiency (Bamji et al., 1979) and is closely interrelated with vitamin B-6 metabolism (Sauberlich, 1999).

7.3.4 Vitamin A

Both excessively high and low levels of vitamin A intake during pregnancy have been associated with an increased risk of orofacial clefts and other craniofacial anomalies. Hale was the first to report that maternal vitamin A deficiency caused eye defects, orofacial clefts and other birth defects in experiments with pigs (Hale, 1933; 1935). Human vitamin A deficiency is widespread, especially in developing countries around the world (West et al., 1999). Birth defects related to vitamin A deficiency may be unnoticed in impoverished populations because of the larger burden of other health problems. In a case-control study in Japan maternal consumption of vegetables rich in the plant form of vitamin A, β -carotene, was associated with a reduced risk of CL/P (Natsume et al., 1999).

Most subsequent research on vitamin A-related compounds and craniofacial anomalies in laboratory animals has involved excess exposure to retinoic acid and other retinoids (Kochhar et al., 1984; Abbott and Pratt, 1988; Abbott and Birnbaum, 1990; Whitby et al., 1994; Soprano and Soprano, 1995; Ross, 1999). Human clinical studies have revealed that fetal exposure to retinoid compounds may result in severe craniofacial anomalies (Lammer et al., 1985) and dietary exposures to high levels of vitamin A may also be important. In a prospective study of more than 22 000 births to women in the United States, craniofacial anomalies and other malformations were more common in women who consumed more than 10 000 IU of vitamin A in the peri-conceptional period (Rothman et al., 1995).

7.4 Nutritional supplementation

BOX N

Trials of maternal nutritional supplementation and orofacial clefts

Several attempts have been made to conduct human trials to evaluate maternal vitamin supplementation during pregnancy as a means of preventing orofacial clefts; these were first motivated by the seemingly promising results of experiments in laboratory animals. The first published reports appeared in 1958 and described attempts in the United States to give mothers supplementary multivitamins but the studies were very small; few methods and no statistical analyses were reported (Conway, 1958; Douglas 1958; Briggs, 1976). Other attempts at vitamin supplementation trials for the prevention of orofacial clefts were attempted in Europe (von Krebig and Stoeckenius, 1978; Schubert et al., 1990) and these authors made claims for the effectiveness of the treatments, yet each of these studies also had insufficient data to allow an evaluation of the results.

7.4.1 The Czech orofacial-cleft prevention trial

Tolarova et al. began a trial of vitamin supplementation for the prevention of orofacial clefts in high-risk Czech women in 1976 (Tolarova, 1982). High-risk mothers were defined as those who had given birth to a child with a cleft or who had a cleft themselves. Participating mothers were advised to take a multivitamin preparation daily, during the period three months before conception until the end of the first trimester. The daily multivitamin dose included:

vitamin A (6000 IU),
vitamins B-1 (3 mg), B-2 (3 mg), B-6 (3 mg),
vitamin C (150 mg),
vitamin D (300 IU),
vitamin E (6 mg),
nicotinamide (30 mg),
calcium pantothenate (3 mg), and
folic acid (10 mg).

The exclusion of non-compliant participants in a clinical trial may seriously bias the results

The “treated” mothers were those who accepted supplements and the “controls” were those who refused or failed to comply. Results reported in 1982 revealed that 1 of 85 “supplemented” pregnancies and 10 of 212 “unsupplemented” pregnancies were affected with orofacial clefts (Tolarova, 1982). Later updates (Tolarova, 1987; Tolarova and Harris, 1995) revealed that 3 of 211 “supplemented” pregnancies and 77 of 1824 “un-supplemented” pregnancies were affected with orofacial clefts (Fisher exact p-value, one-sided test, $p = 0.03$; two-sided test, $p = 0.058$). Important limitations of the Czech study include lack of random assignment of mothers to the treatment and control groups and exclusion of non-compliant participants from the analyses. The mothers in the supplement-treated group received additional interventions that the control group did not receive, including advice to conceive in the late spring and summer months because of the greater availability of fresh fruit and green vegetables and a lesser risk of respiratory tract infections. The exclusion of non-compliant participants in a clinical trial may seriously bias the results, even if the trial begins with random assignment; this is the basis for “intention-to-treat” analyses in the design of modern clinical trials (Meinert, 1986). Because of these design limitations and the lack of statistical significance, the results of the Czech trial are not interpretable.

7.4.2 The Hungarian birth-defects prevention trial

The Hungarian Family Planning Program (HFPP) was the setting used by Czeizel and colleagues for a clinical trial to test the efficacy of periconceptional multivitamin supplementation in the primary prevention of birth defects (Czeizel and Dudas, 1992; Czeizel, 1993a, b; Czeizel et al.,

1994; Czeizel and Hirschberg, 1997; Czeizel, 1998; Czeizel et al., 1999). Participating women were given genetic counselling, and health advice regarding nutrition, smoking and alcohol use. The inclusion of health education on known reproductive hazards for all participants in the trial is laudable and is an early example of the provision of minimum local standards of care in a trial, an ethical issue that has emerged in more recent discussions. Participating women were randomly assigned to receive either a multivitamin or a trace-element tablet daily for the period one month before conception, until the third month of gestation. The trial was double-blind. The multivitamin contained:

vitamins A (6000 IU until 1989 and 4000 IU thereafter),
 B-1 (1.6 mg), B-2 (1.8 mg), B-6 (2.6 mg), B-12 (4 ug),
 C (100 mg), D (500 IU), E (15 mg);
 folic acid (15 mg);
 nicotinamide (19 mg);
 calcium pantothenate (10 mg);
 biotin (0.2 mg);
 four minerals, including calcium (125 mg), phosphorus (125 mg),
 magnesium (100 mg) and iron (60 mg); and
 three trace elements, including copper (1 mg), manganese (1 mg)
 and zinc (7.5 mg).

A trial of primary prevention must have a larger sample size than a recurrence-prevention trial to demonstrate a given treatment effect

The trace-element control group took a tablet with the same amounts of copper, manganese and zinc, with the addition of vitamin C (7.3 mg) and lactose (736 mg). Based on an “intention-to-treat” analysis, there was a significant reduction in NTDs (0 in 2471 vitamin-supplemented pregnancies versus 6 in 2391 trace-element-only treated pregnancies; $p = 0.02$), but no significant difference between the treatment groups was observed in the occurrence of a small number of orofacial clefts (4 among the vitamin-supplemented group and 5 in the trace-element-only supplemented pregnancies; $p = 0.57$) (Czeizel, 1998; Czeizel et al., 1999). Thus, the Hungarian trial showed a significant protective effect of multivitamins in reducing the primary occurrence of NTDs, but the trial was too small to determine whether or not multivitamin use prevented orofacial clefts. The Hungarian trial underscores the point that a trial of primary prevention must have a larger sample size than a recurrence-prevention trial to demonstrate a given treatment effect. Another difficulty in interpreting the lack of a treatment effect for orofacial clefts in the Hungarian trial is that the control group received trace elements, including copper and zinc, that may have lowered the risk of orofacial clefts, thus possibly obscuring a treatment effect in the multivitamin group.

7.4.3 *Prevention trials*

BOX 0

Future directions for orofacial-cleft prevention trials

The trials of maternal nutritional supplementation for the prevention of orofacial clefts conducted to date have been uninformative because of inadequate sample sizes and methodologic flaws. Further understanding of maternal nutrition and orofacial clefts will require that specific nutritional hypotheses and state-of-the-art trial design be applied in appropriate high-risk populations. Investigators interested in birth defects prevention would benefit from collaboration with others involved in prevention trials in different areas of reproductive health. Professor Keith West spoke at the WHO/Utah meeting about his experience in conducting large-scale nutritional intervention studies related to maternal and child health in Bangladesh, Indonesia, Nepal, the Philippines and Thailand. His most recent trial assessed the effect of vitamin A supplementation in reducing mortality related to pregnancy in women of reproductive age in a rural and undernourished population in Nepal. Nearly 45 000 women participated in the double-blind, cluster-randomized, placebo-controlled trial and over 22 000 pregnancies were followed. The results of the trial showed that supplementation to women of reproductive age with either preformed vitamin A or beta carotene in recommended dietary amounts significantly lowered mortality related to pregnancy (West et al., 1999). The Nepalese trial and others like it have studied reproductive outcomes such as maternal and infant death, prematurity and low birth weight – factors that are far more common than birth defects, in general, or orofacial clefts in particular.

One of the most difficult challenges in future orofacial-cleft prevention trials will be in recruiting many thousands of high-risk women in their reproductive years. These efforts will lead investigators to high-risk populations in culturally and economically diverse settings. This important research must be done according to current ethical standards – and this is not a straightforward issue because ethical standards continue to evolve and no single set of ethical standards is applicable in every setting around the globe. The lively discussion at the WHO/Utah meeting on appropriate ethical standards for prevention trials for human orofacial clefts reflected the larger sphere of international debate on ethical standards for human experimental trials.

7.5 Ethical issues

BOX P

Ethical issues related to studies of maternal nutrition and birth defects

Professor Richard Smithells, one of the founders of studies of the role of folic acid in human neural tube defects, gave a personal account at the WHO/Utah meeting of the early stages and evolution of his involvement in this area of research. Smithells faced many dilemmas because his personal convictions and dedication to patients collided at times with the mandates of ethical review boards, the opinion of colleagues, and the popular press. At an earlier stage he was not allowed to proceed with a correct randomized trial of folic acid for the prevention of neural tube defects. Later, however, when he was personally convinced that folic acid could prevent neural tube defects and had hence lost his state of equipoise, ethical review boards and health officials in the United Kingdom had become convinced that the time for a randomized, controlled clinical trial had arrived. Professor Smithells believed ethics were very personal and individual; relative rather than absolute. This view was echoed later by many of the meeting delegates.

Professor Smithells recognized the need for “someone else” to conduct the definitive trial of folic acid supplementation for the prevention of recurring neural tube defects and stepped aside. He listed several lessons he learned from this experience:

- (1) What you judge to be ethical or unethical depends on what you believe – ethics are perhaps relative rather than absolute.
- (2) If a thing is worth doing, it is worth doing properly – and that means getting it right the first time around if you can. If a randomized trial is possible – and it isn’t always – it is to be preferred.
- (3) The more circumstantial evidence there is that something works, especially from non-randomized or uncontrolled studies, the more difficult it is to launch a randomized study later. If you spend too long snapping at the heels of a problem, you may lose the opportunity to “go for the jugular and sort it out in one”.

7.5.1 Ethical guidelines for research involving human subjects in orofacial-cleft prevention trials

Professor Robert J. Levine reviewed recent developments and current controversies in the international guidelines involving human subjects in research, with a focus on the recent revisions of the *Declaration of Helsinki* by the World Medical Association (WMA, 2000) and the 2001 draft revisions of the *International Ethical Guidelines for Biomedical Research Involving Human Subjects* by the Council for International Organizations of Medical Sciences (CIOMS, 2001). Professor Levine pointed out that problems inherent in the *Declaration of Helsinki* include an artificial distinction between therapeutic and non-therapeutic research and outdated views of contemporary ethical thinking, particularly in the area of placebo controls. This situation has led to widespread debate and has prompted the WMA and CIOMS to revise their recommendations (current drafts are available on the web sites of these groups). The discussions of placebo and control groups at the Utah/WHO meeting paralleled the broader international debates, with many divergent views being expressed on the basic definitions of placebo and control groups and their proper use.

A complete discussion of ethical issues related to biomedical research in general and to prevention trials in particular was beyond the scope of the WHO/Utah meeting and these topics are covered in detail in the references cited above. There was, however, detailed discussion on several ethical aspects of orofacial-cleft prevention trials, relating to the development of nutritional intervention trials for the prevention of orofacial clefts in industrialized and technologically developing countries and resource-poor populations. The following summary of ethical issues is a result of the presentations made by Professors Levine and Smithells, and discussions with the meeting delegates.

7.5.2 *Equipoise*

The balance of equipoise is usually tipped by the accumulation of results from many separate studies

Equipoise

BOX Q

A fundamental requirement for the justification of a clinical or community-based intervention trial is a recognized state of uncertainty or unresolved dispute among expert clinicians and researchers regarding which therapeutic or preventive measures are superior. The term *equipoise* is often used to describe the state of equilibrium between view points. The requirement for equipoise before embarking on a trial should be most stringently applied when the treatments or interventions being tested are for lethal or disabling medical conditions (World Medical Association, 2000; Council for International Organizations of Medical Sciences, 2001).

Chalmers described the ideal conditions for an ethical clinical trial as a test of the perfect null hypothesis in which individual physicians have no idea as to whether a treatment is better than a placebo or if two alternative treatments differ in effectiveness (Chalmers, 1978; 1979). Freedman derided this view, labelling it *theoretical equipoise*, and proposed as a replacement the term *clinical equipoise* to describe the situation where both risks and benefits were considered as critical parts of the justification for a clinical trial (Freedman, 1987). Freedman allowed that individual clinicians may differ in their judgements about alternative treatments yet ideally join together in a trial to resolve the dispute; the situation described earlier by Professor Smithells regarding neural tube defects and folic acid supplementation is an example of this situation. The common purpose is to develop compelling evidence that one treatment is better than another (or better than placebo) so that other physicians and scientists who have not participated in the trial will be convinced of the results and change their pattern of practice. Unambiguous results are also needed to convince elected officials of the need to change public health policies through acts of legislation.

The information needed to establish a state of equipoise includes data from animal experiments, observations from human case-control and cohort studies, and evaluation of previous trials, if they exist. Professor Meinert pointed out that, in most of the important controversies in medicine and public health, there has been no single, definitive trial and the balance of equipoise is usually tipped by the accumulation of results from many separate studies.

7.5.3 Appropriate study design and ethics

The Helsinki and CIOMS guidelines begin from the position that all studies involving human subjects must conform to generally accepted scientific principles, be based on a thorough knowledge of scientific literature, and employ the latest advances in study design and practice. Ethical review cannot be separated from review of study design and scientific methods. Research that is unsound or deficient because of lack of statistical power to detect treatment effects will not only result in a waste of the participants' time and the resources of sponsoring agencies but will also expose the participants to risk, even if slight, without the prospect of benefits. Further discussion of trial design, important in advancing knowledge of the prevention of orofacial clefts, appears in Section 7.6 below.

Ethical review
cannot be
separated
from review
of study design
and scientific
methods

7.5.4 Local health priorities and applications of findings

Sponsoring agencies and investigators should make every reasonable effort to ensure that a prevention trial is responsive to the health needs and priorities of the participating local populations and that the intervention can and will be made available to the local populations within a reasonable period of time. These considerations become especially important in populations or communities with limited resources. According to the CIOMS guidelines it is not sufficient to justify a prevention trial because of a high prevalence of the health condition of interest; it is also necessary that the intervention being studied, if found to be beneficial, could reasonably be introduced into the local population at the conclusion of the study. If the intervention being evaluated, such as nutritional supplementation, is too expensive or impractical to distribute in the population participating in the trial, and if the knowledge gained about the intervention is used to benefit other populations that have the resources to employ the intervention, then the study is exploitative and therefore unethical (CIOMS, 2001). Detailed baseline studies are needed to describe local health priorities, common maternal and child health problems, the birth prevalence of orofacial clefts and other important birth defects; dietary patterns and biochemical studies are needed as a baseline measure of maternal nutritional status. In most populations half – or more – pregnancies are not precisely planned, therefore nutritional interventions should have the potential to be introduced via dietary improvements and food fortification in the population at large to improve intake of vitamins in the peri-conceptional period. In most populations the more clinical approach of providing nutritional supplements in pill form will not reach a significant number of women in the peri-conceptional period; some notable exceptions however have included China and Hungary, and other areas where family planning and prenatal health care receive strong cultural and governmental support.

7.5.5 Selection of research subjects

The benefits and burdens of intervention trials and other research should be equitably distributed both within and between populations. According to CIOMS Guideline 12 “no group or class of persons should be required to bear more than its fair share of the burdens of participation in research; similarly, no group should be deprived of its fair share of the benefits of research” (CIOMS, 2001). In some areas it is possible that certain groups have been overused as study subjects where research institutions have had access to local patient populations. This is a particular concern when it is easy to recruit impoverished persons as research subjects because they are willing, due to their desperate condition, to participate – in exchange for a trivial (from the viewpoint of the sponsor) payment. This is a larger concern for pharmaceutical trials conducted among the poor – especially when the results are used to benefit wealthier populations, than for investigations of the specific conditions of the poor, as in studies of malnutrition and nutritional deficiencies in populations with a high risk of orofacial clefts.

7.5.6 Placebos and other control treatments

According to Article 29 of the *Declaration of Helsinki* (WMA, 2000):

“the benefits, risks, burdens, and effectiveness of a new method should be tested against those of the best current prophylactic, diagnostic, and therapeutic methods. This does not exclude the use of placebo, or no treatment, in studies where no proven prophylactic, diagnostic or therapeutic method exists.”

Professor Levine pointed out that a major weakness of the Helsinki guidelines is that trials appear to be ruled out in resource-poor countries if the standard of “best current method” is mandated as the control treatment yet is not locally available due to scarcity, high cost, or both (Levine 1999; 2000). According to Levine, this weakness in the Helsinki guidelines is the root of the most bitter controversy in research ethics over the past 30 years, precipitated by the trial of a short duration AZT regimen in the prevention of perinatal transmission of HIV-infected pregnant women. The medication that was the “best available method” at that time in industrialized countries cost 80 times the annual per capita health expenditure in sub-Saharan countries; and this cost did not take into account the advanced medical resources required to administer the medication. As early as 1993 this dilemma led to the recognition that an absolute standard of “best available treatment” could not be applied worldwide and that special arrangements had to be made for trials in low-resource countries. The CIOMS guidelines (CIOMS, 2001) now recognize that there are circumstances in which use of a control treatment other than the “best current method” is justified if:

There is currently no nutritional intervention for women that is known to prevent orofacial clefts in their offspring

- (1) the scientific and ethical review committees in both the country of the sponsoring institution and the host country determine that use of the “best current method” as a control would be likely to invalidate the results of the research or make results inapplicable in the host country;
- (2) plans to make the therapeutic product reasonably available in the host country or community are securely established; and
- (3) a process of planning and negotiation, including justification of a study in regard to local health-care needs, has taken place with the health authorities in the host country before the research begins.

The three most important micronutrient deficiencies worldwide – iron, vitamin A and iodine – are causes of maternal and child illness and death, overwhelmingly greater in number than those affected by birth defects. Iron, vitamin A, and iodine are inexpensive in industrialized countries, yet scarce and difficult to distribute in resource-poor countries, underscoring the point that nutritional interventions face ethical dilemmas similar to those raised in the case of the AZT trials for the prevention of perinatal HIV transmission in Africa.

Folic acid supplementation for women in all populations appears to be the “best current method” of peri-conceptional care for the prevention of neural tube defects in industrialized countries but appears difficult to implement in many low-resource countries with health agendas crowded with a growing number of recommended health-related interventions.

There is currently no nutritional intervention for women that is known to prevent orofacial clefts in their offspring. At first glance this seems to be the ideal state of clinical equipoise, making the test of a nutritional intervention versus placebo timely. The issue becomes complicated quickly when folic acid supplementation, known to reduce the risk of neural tube defects in several populations, is proposed as a preventive intervention to reduce the occurrence of orofacial clefts. Many of the delegates at the WHO/Utah meeting felt that any study that did not provide 400 micrograms of folic acid per day to all mothers was unethical because folic acid would be “withheld” from mothers and they would be at higher risk of having a child with a neural tube defect. Some delegates extended the view that folic acid supplementation was mandatory for women participating in birth defect studies of any design, including observational cohort studies. Others felt that public health action to provide folic acid to women of reproductive age (and many other nutrients important to reproductive health) was well under way through public health campaigns to increase dietary intake of folates and folic acid-containing vitamins in the peri-conceptional period and through food fortification (in Chile, the United States and a growing number of other countries). Thus placebo-controlled

studies of higher levels of folic acid supplementation, as an “add-on” study to the increasing baseline intake of folic acid, was viewed by other delegates as ethical.

The use of placebos is currently being debated by the WMA and CIOMS and the delegates at the WHO/Utah meeting were not successful in reaching a consensus either – indeed the basic definition of a placebo was not even widely agreed upon. Some investigators have added to their “placebo” other vitamins, minerals, trace elements, vaccinations, or treatments for parasites – each thought to be unrelated to the condition under study – as a way to provide some inducement for participation, even though the real benefits may have been difficult or impossible to quantify. This kind of comparison becomes difficult to interpret if later evidence arises that one of the additives to the “placebo” group indeed alters the risk of the outcome under study; if this is the case then the “placebo” is really an active control treatment. In a nutritional supplementation trial a strict placebo would include no active compounds and would be identical in appearance to the hypothesized active treatment, in most cases a pill or an injection. Anything else that is compared to a hypothesized active treatment should be referred to as an active control treatment (Meinert, 1996). In the Hungarian birth defects prevention trial the group actively treated with multivitamins was compared to a “trace element control” group that received a tablet with the same amounts of copper, manganese and zinc as the “active treatment” group received, but with the addition of vitamin C and lactose. The Hungarian study thus did not employ a true placebo-control group and concerns have been raised that, since zinc nutriture might be related to the risk of birth defects and zinc was provided to both groups, the occurrence of NTDs (and perhaps orofacial clefts and other birth defects) may have been reduced in both groups, obscuring the treatment effect of the other nutrients. The trial of the Medical Research Council (MRC) trial to prevent NTDs employed a control group that received tablets with iron and calcium (without the main “active” treatments compared, folic acid alone or folic acid plus multivitamins) rather than a true placebo control group. This was recently criticized by Turner (Turner et al., 2001) who speculated that exposure to high levels of iron and calcium (among control mothers who took more than one pill per day) may have interfered with zinc nutriture and raised the risk of NTDs. Turner’s re-interpretation of the MRC results has been disputed by Moore (Moore, 2001). An important lesson from this experience is that investigators should rigorously define their control groups or risk endless re-interpretations of their study findings.

The use of control groups in nutritional intervention trials is thus complex and there is no global consensus on the precise guidelines for their use

The use of control groups in nutritional intervention trials is thus complex and there is no global consensus on the precise guidelines for their use. Investigators designing trials should follow the general principles regarding control treatments outlined in the *Declaration of Helsinki* and clarified by the CIOMS guidelines, but should decide on the appropriateness of control groups in consultation with the institutional review boards representing the sponsoring institutions and local populations participating in the study.

7.5.7 *Standard of care*

Highest attainable and sustainable standard of care

BOX R

In response to the deep divisions over the ethics of HIV-prevention trials among pregnant women in resource-poor countries and other similar dilemmas, a new standard of care for therapeutic methods in clinical trials has emerged in recent revisions of the Helsinki and CIOMS ethical guidelines: the “highest attainable and sustainable therapeutic method” (Lurie et al., 1994; Aaby et al., 1997; Levine, 1999; 2000; WMA, 2000; CIOMS, 2001). Professor Levine has recently published a detailed analysis of these developments (Levine, 1999; 2000) and discussed this at the WHO/Utah meeting.

“Highest attainable” therapy means that under the conditions of a clinical or community-based intervention trial, the level of therapy in the given location should be “the best one can do.” The level of care available in a resource-poor population should define the minimum ethically-acceptable standard. “Sustainable” means the level of care, medical treatment, or nutritional supplementation that can be expected to be maintained by the local population after completion of the trial. These new standards are closely linked to the principles of addressing local health priorities in a research programme and ensure the application of the findings of the trial in the local population. The introduction of interventions of therapies that are not locally available and sustainable may undermine local health services and priorities. According to Levine, the main benefit of adhering to the standard of available and sustainable therapies “tends to facilitate the efforts of resource-poor countries to develop needed therapies and preventions that are within their financial reach. Until the imbalances in the distribution of wealth among nations of the world are corrected, this appears to be the best we can do” (Levine, 2000).

7.6 The design of orofacial-cleft prevention trials

Timing is the essence of an intervention trial because the state of equipoise may be a narrow window of opportunity. A feeling of urgency however should not lead investigators to start assigning treatments to participants until the infrastructure is in place and the study protocol is developed, data forms are established and tested, field staff are hired and trained for participant recruitment, data intake and analyses, and a mechanism has been established to independently monitor the trial (Meinert, 1986). No single trial is likely to be definitive and trials are needed in diverse populations in both industrialized and technologically developing countries.

7.6.1 Selection of the study population

Trials in high-risk populations are more likely to detect a treatment effect than trials in low-risk populations, and at lower cost and with greater speed. A recurrence-prevention trial of orofacial clefts in a high-risk population will still require that several thousand births are evaluated; a primary prevention trial would require tens of thousands of births. For planning a trial, baseline studies of cleft occurrence and recurrence are needed, as well as a good sense of whether the local population is willing to participate in a trial.

7.6.2 Specification of the test treatment or treatments

The choice of a specific nutrient intervention or interventions should be based on prior laboratory animal studies, observational studies of human populations, and detailed studies of biochemical indicators of nutritional status in the population of interest. The investigators must consider whether the goal of the study is to investigate dose levels of nutrients to correct inadequate dietary intake or higher pharmacological doses that might be necessary to overcome acquired or genetically-based metabolic problems. Well-targeted nutritional hypotheses will have greater public health benefits than the broad approach of multivitamin supplementation because knowledge of the specific nutrients involved could lead to food-based interventions that would ultimately reach a far greater number of women of reproductive age than programmes to encourage the use of supplements in the peri-conceptual period would. Factorial and dose-response study designs are highly efficient ways to answer several complex questions about multiple treatments and doses in a single trial.

7.6.3 *Specification of the placebo or other control treatment*

Many investigators may be tempted to avoid the difficult issues regarding the use of placebo controls or active treatment controls discussed above by attempting to make comparisons between participants receiving the test treatment and so-called “historical controls” (untreated persons from an earlier time period in the same geographic area) or “geographic controls” (untreated persons from a different geographic area in the same time period). Use of historical or geographic controls almost always leads to unclear findings and confusion, thus should be avoided. The use of placebos and active treatment controls was discussed in detail previously.

7.6.4 *Outcome measure for evaluating the study treatment*

Orofacial clefts appear to be the only group of CFA to be common enough at present for a trial. Since cleft lip with or without cleft palate seems to be etiologically distinct from cleft palate alone, a trial should have its primary focus on one group or the other. The issue of detecting and evaluating early pregnancy losses should be carefully considered.

7.6.5 *Bias-free method for assigning patients to the study treatments*

Test and control treatments should be randomly assigned to participants. In the assignment of treatments, “haphazard” does not equal “random” thus formal mechanisms should be in place and monitored to assure true random assignment of treatments.

7.6.6 *Double masking of treatment status*

The treatment status should be concealed from participants and investigators to avoid bias in the attention given to each participant. Because curiosity seems to be a universal human trait, even the most dedicated co-investigators and field staff may be tempted to decipher the treatment allocations, thus much attention should be given to this issue.

7.6.7 *Monitoring*

An independent data, safety, and monitoring committee (DSMC) should be established to regularly review progress of a trial. This committee should have access to all information gathered in the trial, including the treatment allocations of participants. Side-effects and compliance of participants should be closely monitored by the trial field staff and study investigators and reported to the committee.

7.6.8 Analysis by assigned treatment

Investigators should analyse and report results according to the original treatment assignment of participants. This is the only analytical approach that is compatible with the randomized design and it avoids treatment-related selection bias in the composition of the treatment groups. Analysis by assigned treatment provides a conservative and realistic measure of the treatment effect that remains after losses due to participant or health-care provider rejection of the treatments.

7.7 Conclusions

7.7.1 Environmental and behavioural factors related to CFA

Craniofacial anomalies are among the most common birth defects and, of these, orofacial clefts are the most common. Most discussions in the WHO/Utah meeting focused on orofacial clefts but the points raised may be relevant for many other craniofacial anomalies. Orofacial clefts appear to have substantial environmental causes, thus the potential for primary prevention seems considerable. The pattern of occurrence of orofacial clefts is different from that for neural tube defects therefore their causes may also be different.

7.7.2 Tobacco

Maternal tobacco use has been consistently associated with risk of orofacial clefts. This association is modest, yet the attributable risk may be of public health importance because many women are exposed to passive smoking and tobacco use is rapidly increasing among women, especially in technologically developing countries. National health agencies and voluntary organizations may be unaware of the association between maternal tobacco use and orofacial clefts.

7.7.3 Alcohol

Maternal alcohol use has been associated with risk of orofacial clefts in some – but not all – studies. The type and context of alcohol consumption differs considerably across populations and more consistent methods are needed for the assessment of maternal alcohol intake.

7.7.4 Maternal nutrition and orofacial clefts

There is considerable circumstantial evidence that maternal nutritional factors may be related to the occurrence of orofacial clefts, the most common of CFA. The most promising candidate nutrients include folic acid and vitamin B-6 (pyridoxine) and a lesser body of evidence suggests roles for riboflavin (vitamin B-2) and vitamin A.

7.7.5 The need for nutritional supplementation trials

The current state of equipoise regarding maternal nutrition and orofacial clefts makes intervention trials of specific nutrients an urgent priority. Further understanding of the role of maternal nutrition in CFA will require well designed and expertly conducted trials. No single trial is likely to be definitive and trials are needed in diverse populations in industrialized and technologically-developing countries and resource-poor populations.

7.7.6 Ethics and design of orofacial-cleft prevention trials

Poorly conceived and conducted trials are unethical because they waste limited resources and further delay the discovery of effective interventions. Intervention trials should employ strict random assignment of participants to treatment groups, include either a placebo or other appropriate control group, include an adequate sample size, be double-masked, monitored by an independent data and safety committee, employ intention-to-treat analyses, and use appropriate procedures to obtain informed consent from each participant. Comparison of an active treatment group to “controls” from a different time period or geographic location is unlikely to yield an interpretable result. Trials in high-risk populations are not only more likely to detect a treatment effect than trials in low-risk populations, but also at lower cost and with greater speed. The choice of nutrient interventions should be based on prior detailed studies of biochemical indicators of nutritional status in the population of interest.

7.7.7 International cooperation

Role for WHO, governmental agencies and non-governmental organizations

BOX 5

An orofacial-cleft recurrence-prevention trial is far more feasible than a trial of prevention of primary occurrence, but will still require many thousands of high-risk mothers. Orofacial cleft surveillance systems and registries need to be further developed and linked to provide the critical infrastructure for orofacial-cleft prevention trials. A current and urgent need is linkage of existing birth defects registries, harmonization of methods of data collection and data management, and the development of these activities in technologically-developing countries and resource-poor populations. Public health action is needed on other fronts as research on the causes of CFA continues. The association between maternal smoking and alcohol use during pregnancy and the risk of orofacial clefts is strong enough to warrant inclusion of this information in public campaigns to reduce exposure to these teratogens in women of reproductive age.