Addressing the global challenges of craniofacial anomalies

Report of a WHO meeting on International Collaborative Research on Craniofacial Anomalies
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World Health Organization

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<td>AIDS</td>
<td>autoimmune deficiency syndrome</td>
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<tr>
<td>Bmp</td>
<td>bone morphogenetic protein</td>
</tr>
<tr>
<td>BCL1</td>
<td>B-cell lymphoma 1</td>
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<tr>
<td>BHMT</td>
<td>betaine–homocysteine methyltransferase</td>
</tr>
<tr>
<td>CBS</td>
<td>cystathionine betasynthase</td>
</tr>
<tr>
<td>Clfl</td>
<td>murine cleft lip mutant gene 1</td>
</tr>
<tr>
<td>Clf2</td>
<td>murine cleft lip mutant gene 2</td>
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<tr>
<td>CLPTM1</td>
<td>cleft lip and palate transmembrane protein 1</td>
</tr>
<tr>
<td>CYP1A1</td>
<td>cytochrome oxidase detoxification enzyme</td>
</tr>
<tr>
<td>DNA</td>
<td>deoxyribonucleic acid</td>
</tr>
<tr>
<td>ECLAMC</td>
<td>Estudio Colaborativo Latino Americano de Malformaciones Congenita</td>
</tr>
<tr>
<td>EUROCAT</td>
<td>European Registry of Congenital Anomalies and Twins</td>
</tr>
<tr>
<td>EUROCRAN</td>
<td>European Collaboration in Craniofacial Anomalies</td>
</tr>
<tr>
<td>EUROHAZCON</td>
<td>European Study on Hazardous Waste Landfill Sites</td>
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<tr>
<td>FGFR1</td>
<td>fibroblast growth factor receptor 1</td>
</tr>
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<td>folate receptor alpha</td>
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<tr>
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<tr>
<td>IRF</td>
<td>interferon regulatory factor</td>
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<tr>
<td>MADRE</td>
<td>MATernal DRug Exposure</td>
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<tr>
<td>MSX1</td>
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<td>methylene tetrahydrofolate reductase</td>
</tr>
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<td>NAT1</td>
<td>N-acetyl transferase 1</td>
</tr>
<tr>
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<tr>
<td>PTCH</td>
<td>patched homologue (<em>Drosophila</em>)</td>
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<td>polio virus receptor</td>
</tr>
<tr>
<td>PVRL1</td>
<td>polio virus receptor-related protein 1 precursor</td>
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<tr>
<td>PVRL2</td>
<td>polio virus receptor-related protein 2 precursor</td>
</tr>
<tr>
<td>RFC</td>
<td>reduced folate carrier</td>
</tr>
<tr>
<td>RNA</td>
<td>ribonucleic acid</td>
</tr>
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<td>Shh</td>
<td>sonic hedgehog</td>
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<td>SIMWALK2</td>
<td>statistical genetics computer application for haplotype, linkage and identity by descent</td>
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<tr>
<td>TGFα</td>
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<td>TGFβ3</td>
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<tr>
<td>TP63</td>
<td>tumour protein 63</td>
</tr>
<tr>
<td>TRANSMIT</td>
<td>transmission disequilibrium test software</td>
</tr>
<tr>
<td>V274I</td>
<td>variant 2741 of the <em>IRF6</em> gene</td>
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Executive summary

**International Database on Craniofacial Anomalies**

In September 2002, the National Institute of Dental and Craniofacial Research (NIDCR) in the United States of America and the programme on Human Genetics of the Department of Chronic Diseases and Health Promotion at WHO initiated the International Database on Craniofacial Anomalies and assigned its coordination to the International Centre on Birth Defects.

The main aims of the International Database on Craniofacial Anomalies are:

- to encourage existing databases to share their data in order to create a specific, worldwide database;
- to encourage the establishment of new databases to contribute to the worldwide database;
- to present the collected data in an accessible way and to make available specific data to stimulate research on primary and tertiary prevention and on better treatment of craniofacial anomalies; and
- to stimulate scientific and lay organizations to collect and share relevant data and information on persons affected by craniofacial anomalies.

**The International Perinatal Databases of Typical Orofacial Clefts:** The International Database on Craniofacial Anomalies is setting up the International Perinatal Databases of Typical Orofacial Clefts on the basis of international collaboration with existing resources. Data on typical oral clefts are more reliable then those on other craniofacial anomalies, and the coding systems are well developed. The definition of other craniofacial anomalies, their detection perinatally and their diagnosis and coding are more problematic.

The diagnoses included are: isolated oral cleft, with the appropriate code; multiple congenital anomalies, with a code identifying the number of major unrelated defects present; or syndrome, with the Online Mendelian Inheritance in Man or expanded International Classification of Diseases Revision 10 (ICD-10) code. Other information collected includes the distribution of rates by clinical presentation of the child (isolated or multiple malformations, syndrome), maternal age, sex, defect phenotype (e.g. bilateral or unilateral cleft lip), twin status, pregnancy outcome (e.g. termination of pregnancy), birth weight and length of gestation.

**Website:** On the basis of the recommendations of a meeting in 2001 on a global registry and database on craniofacial anomalies, held in Bauru, Brazil (WHO, 2003), WHO developed a website for the craniofacial anomalies project ([www.who.int/genomics/anomalies](http://www.who.int/genomics/anomalies)), which is designed to give visibility to the aims and objectives of the project; to create online access to the International Database on Craniofacial Anomalies; and to set up an online directory of resources.

This website has been useful not only for communication but also for forming networks and links among persons interested in research on craniofacial anomalies. A regular influx of feedback and queries from members of the public, the scientific community and others on the content of the website is sought, as well as more information from communities.

**The future:** The Perinatal Databases of Typical Orofacial Clefts plans to increase the number of participating registries and to collect data on other craniofacial defects. A few programmes
will be selected in which the identified problems have been solved. In parallel, structural changes will be encouraged in existing registries.

*Treatment in developing and industrialized countries*

*Care for persons with cleft lip and/or palate in developing countries:* Care for persons with cleft lip and/or palate in developing countries encounters certain limitations: overseas missions often focus on service and neglect training and research; resources, such as personnel for research and service, are lacking; and there are insufficient research facilities, data collection, storage and data processing. Problems are also met in maintaining communication and support for a database system.

The health-care system is important in sustaining research and establishing long-term follow-up. A standardized programme for the care of persons with cleft lip and/or palate should be established and then adapted in each country to the prevalent socioeconomic conditions, government policy and available health-care infrastructure. While developed countries have more services and better access to care, they have made many mistakes, from which developing countries can learn and thus avoid repeating them.

Local services must be strengthened by training specialists. For instance, clinicians should be trained in interdisciplinary team management and standardization of protocols, and researchers should be instructed in assessment of long-term overall outcomes and focus on reducing the health-care burden. Both collaborative clinical research and basic research on the etiology of cleft lip and cleft palate in developing countries with large numbers of patients are important to identify the causal factors and increase the possibilities for future prevention.

*Benefits of inter-centre comparisons:* Professionals entrusted with the provision of health care have an obligation to review the success of their practices and, when shortcomings are revealed, to take remedial action. This should be done continuously, in what is sometimes known as clinical audit, which has been defined as “the systematic critical analysis of the quality of care including procedures for diagnosis and treatment, the use of resources and the resulting outcome and quality of life for the patient” (Long, 1996). Clinical audit is often divided into evaluating the way in which care is delivered and its outcomes. Cycles of outcome audit are more easily established when the intervention is common and the consequences are clear-cut and quickly observable. Audit of care for cleft lip and palate presents a considerable challenge because of the lengthy follow-up required, the complexity, subtlety and number of relevant outcomes and, above all, the relatively small number of cases.

Experience in Europe has demonstrated that inter-centre collaboration offers significant advantages, by allowing comparisons of the delivery and outcome of treatment, establishment of future goals and exchange of successful practices. Perhaps the greatest benefit of inter-centre comparisons is the cooperative spirit they foster and the gradual diminution of rivalry. It is no coincidence that most participants in cohort studies also participate in multicentre randomized clinical trials. Working closely together also leads to sharing of past successes and failures and facilitates fruitful collaboration, such as in developing rating scales and formulating new research questions.

WHO advocates collaboration among representatives of all the disciplines involved in the care of persons with cleft lip and/or palate, especially between clinicians and laboratory scientists, to ensure progress.
Genetics

With respect to genetics, the techniques, analytical approaches and populations that will be most useful for gaining a better understanding of the causes of craniofacial abnormalities were discussed, with particular reference to those with strong genetic components. While environment and stochastic events can predispose to craniofacial anomalies, the role of genetics is compelling.

Genes involved in palate development: Both the study of animal models and human studies have been useful in identifying genes that play a direct role in human palate development. Two recent reports are particularly relevant. In the first, mutations were identified in the gene that encodes the transcription factor interferon regulatory factor 6 (IRF6), which results in van der Woude syndrome, an autosomal dominant disorder. This syndrome resembles an isolated cleft but is accompanied in most cases by lip pits, caused by mutations in a single gene, whereas the commoner isolated cleft is a complex trait caused by multiple gene mutations or environmental insults. It was demonstrated recently that a common haplotype associated with IRF6 contains a mutation that results in an attributable risk of approximately 12% for all common forms of cleft lip and palate.

Another gene, FGFR1 was identified in cases of Kallmann syndrome, an autosomal dominant disorder typically characterized by infertility and anosmia. About 5% of patients with Kallmann syndrome, however, have clefts of the lip and/or palate, and, as in van der Woude syndrome, some persons present with clefts as the only component of the phenotype. Studies on other genes that play a role in human palate development (P63, PVRL1, TGFA, and TBX22) were reviewed recently (Murray & Schutte, 2004) or were reported (SATB2) (FitzPatrick, 2003).

A remarkable feature of the genes IRF6, FGFR1 and also MSXI is that mutations in any of them are associated with dental anomalies and ‘mixed clefting’, disorders in which cases of isolated cleft palate and cleft lip (with or without cleft palate) occur in the same pedigree. Clefts of the lip or clefts of the lip and the palate arise in the primary palate, whereas clefts of the palate alone occur in the secondary palate. The existence of mixed clefting disorders indicates that identical mechanisms cause the two forms, which were previously separated on the basis of embryological and genetic evidence. The presence of dental anomalies in some persons with mutations in each of these three genes suggests that the same pathways are common to tooth development.

Signal transduction pathways in palate development: The essential role of certain signalling pathways in palate development has been shown in a number of studies. Muscle-specific homebox (Msx1), bone morphogenetic proteins (Bmp4 /Bmp2) and sonic hedgehog (Shh) are pathways that are essential for palate development in mice, as they drive the epithelium and mesenchyme interactions that support cell proliferation and palate growth. FGFR1, SATB2 and TBX22 are also involved in palate growth in humans and mice, although their exact position in a known pathway remains to be determined. The involvement of these genes and their hypothesized interactions suggest that many elements are involved in palate development. These candidate genes can be investigated by DNA re-sequencing or statistical analyses.

Collection and storage of genetic data. Analysis depends on sample collection. Rapid, cost-efficient collection of small samples, as exemplified by blood spots or cheek swabs, and collections of whole blood or cell lines, which allow for more extensive analysis of protein and RNA, both have strengths and weaknesses. International collaboration is essential, as the causes of clefts are likely to be diverse, although some underlying gene and environmental causes are common to all anomalies. Multicentre collaborations, of which several are under way, provide an opportunity to collect large numbers of samples, providing sufficient power to confirm linkages or associations.
Parallel research efforts and multidisciplinary approach. New findings from studies with animal models and insights gained in developmental biology allow better understanding of the pathways involved in human embryogenesis in general and in palate development in particular. Additional model systems are needed, as are further analyses of human biological and epidemiological data. Such studies will lead to better diagnoses, interventions to improve clinical outcomes and preventive strategies for human birth defects. Interaction among clinicians, epidemiologists, statisticians, molecular biologists and developmental biologists will result in the most rapid progress.

Gene–environment interactions and oral clefts

Environmental factors: Although genes play a substantial role in facial embryogenesis, the environment plays a critical role in modulating genetic effects. At least three major classes of environmental triggers have been studied: teratogens, nutrients and maternal illness. Maternal smoking, for example, has been recognized as an important covariate in clefting. Other teratogens that increase the risk for cleft lip and palate after maternal ingestion include pharmaceuticals such as the anticonvulsant phenytoin, benzodiazepines and pesticides, such as dioxin. Nutrients, such as vitamins and trace elements, and cholesterol metabolism also affect embryonic development. Folate in particular plays an important role in neural tube formation. The recognition that folic acid supplementation can decrease the risk for neural tube defects represents, with treatment of Rh disease and phenylketonuria, one of the great public health successes of the twentieth century. Furthermore, cholesterol is an essential component of Shh signalling.

Maternal illness, infection or hyperpyrexia during critical periods of embryogenesis can also play a role, although the mechanism involved is less clear. Nevertheless, two genes that are essential for palate development, IRF6 and PVRL1, are members of gene families that modulate the immune response to infection. This finding suggests the need for a more critical examination of whether exposure to infectious agents during the first trimester increases the risk for clefting.

Investigations of gene–environment interactions: Investigations of the etiology of oral clefts continue to be confounded by the extreme heterogeneity within groupings. The results of such investigations have been inconclusive partly because of a lack of statistical power to detect or exclude interactions, partly because of differences between studies in whether both the infant and the mother have been genotyped and partly because replication has either not been performed or not been reported. In studies of polymorphisms of xenobiotic metabolism in which only the fetal genotype was investigated, epoxide hydrolase 1 was strongly expressed in fetal liver; however, the other enzymes coded for by these genes might be expressed only after birth. The timing and the tissue in which a gene is expressed might be important in interpreting these findings, but few data are available. Furthermore, gene variants have tended to be considered one at a time, whereas variants of multiple genes might modulate the effects of exposure.

Several study designs have been used or proposed for the investigation of gene–environment interactions. Studies in which cases were compared with unrelated controls are the commonest, but case–parent studies have been used to investigate clefts because of concern about population stratification or participation of controls. Other design options include cohort studies for biobanks, case-only studies and randomized controlled trials. No one design is ideal for each situation, and a range of designs is needed to build up evidence.

Challenges in investigations of gene–environment interactions include exclusion of false-positive results, exposure assessment and specifying a model for the postulated interaction. The sample size requirements for detecting interactions might be too great for single studies;
however, meta-analyses can be limited by differences in exposure definition and assessment and differences in methods of estimating joint effects, pooled analysis of data on individual subjects and inclusion of new multicentre studies. In the first two approaches, reporting bias and identifying studies are major potential problems. The last two approaches are resource intensive, requiring collaborative initiatives and assembling data (and samples) on large numbers of persons in a standardized way.

**Prevention**

A WHO report on prevention of craniofacial anomalies (WHO, 2002) listed maternal cigarette smoking as the best-studied environmental risk factor for oral clefts and noted that consistent associations had been observed in epidemiological studies. Subsequently, more evidence has accumulated that maternal exposure to tobacco smoke increases the risk for oral clefts by an average of 30% and that sizeable proportions of oral clefts in populations can be attributed to this cause. The time has come for concerted public health action to publicize evidence that exposure to tobacco smoke is a cause of oral clefts and to use this finding in campaigns to reduce the rates of smoking among women of reproductive age. As discussed in the previous report and re-emphasized here, the disfigured faces of children affected with craniofacial anomalies might motivate people to support public health activities far more than statistics on widespread causes of morbidity and mortality among children.

While there is less evidence that maternal alcohol drinking is a cause of oral clefts, the causative role of alcohol use during pregnancy in fetal alcohol syndrome is well known. Strong, worldwide public health campaigns are therefore warranted to reduce alcohol drinking during pregnancy.

Several nutrients, including folate, vitamin B₆, zinc and vitamin A, have been associated with risk for oral clefts, and other nutrients and dietary patterns have also been implicated. Case–control studies involving use of biomarkers of nutritional status, genetic markers and histories of illnesses and use of medications during pregnancy appear to be the most useful means for studying the nutritional causes of oral clefts. Such studies are best performed with representative populations from broad geographical areas. Clinical trials will be useful for testing specific nutrients and dietary patterns for the prevention of oral clefts, but more data from observational studies are needed. Both observational and experimental studies must be based on well-functioning birth defects surveillance systems.

A key approach to discovering the role of environmental factors in the etiology of oral clefts and other craniofacial anomalies will be a concerted global effort to form a network of registries and researchers in both industrialized and developing countries. The aims of the existing registries in industrialized countries are to:

- harmonize ways of working and definitions;
- promote use of local and regional services;
- add value by use of registry data, publication and dissemination;
- work with local authorities to increase understanding;
- stimulate contact with registries, not only to obtain data, but to create policies; and
- create a means for evaluating primary prevention policies, such as folic acid supplementation.
Role of the World Health Organization

Over a 5-year period, WHO has facilitated a series of consensus meetings on craniofacial anomalies involving the world’s leading experts in a range of disciplines and with broad international representation. This initiative has resulted in an evidence-based approach to research in surveillance, etiology and treatment, and recommendations have been made.

The humanitarian and scientific objectives in the field of craniofacial anomalies are to improve the quality of care and of treatment strategies side by side with primary prevention. In order to harmonize and globalize research in this field, WHO aims to coordinate population-based work, such as collection of data and biological samples, testing of candidate genes and genetic markers and production of guidance on research protocols and analytical approaches.

Significant progress has already been made in setting up collaborative research and defining protocols and guidelines for future research in genetics, gene–environment interactions, surveillance and surgical and non-surgical care. Developing countries that are participating in international networks are playing an increasingly significant role in these research projects.
1. INTRODUCTION

Craniofacial anomalies affect a significant proportion of society. Cleft lip and/or palate, for example, occurs in approximately 1 per 500–700 births, the ratio varying considerably by geographical area and ethnic grouping. The costs of these anomalies in terms of morbidity, health care, emotional disturbance and social and workplace exclusion are considerable for affected individuals, their families and society. It is estimated that 80% of orofacial clefts are non-syndromic and of multifactorial origin, both genetic and environmental, the latter being especially important from the point of view of prevention. Craniofacial anomalies require surgical, nutritional, dental, speech, medical and behavioural interventions and impose a substantial economic and societal burden (Berk & Marazita, 2002).

Current research on craniofacial anomalies falls into three spheres: etiology, prevention and treatment. Unfortunately, much research is conducted independently, with little evidence of a coherent global strategy. The goal of the initiative outlined in this report is to reduce duplication of effort and achieve broader coverage of important research needs, by bringing researchers into collaborative international partnerships, and to develop a global consensus on the research directions and research protocols in this area.

Research on the genetic basis of craniofacial anomalies has benefited enormously over the past decade from recombinant DNA technology. The genes involved in over 50 craniofacial syndromes have either been mapped to a chromosome or isolated and their structure identified. This achievement relates, however, to only a fraction of the known craniofacial syndromes. The pathogenesis of the commonest forms of these anomalies (non-syndromic) is especially challenging because they appear to arise from complex polygenic interactions with environmental factors. A coordinated international approach to this work will not only provide an effective means of sharing data, samples and resources, but will result in strategic exploitation of geographical and ethnic variations in incidence and pathogenesis.

Research that might lead to the prevention of craniofacial anomalies is based primarily on isolated case–control studies in Asia, Europe, Latin America and North America. These projects were carried out independently, and consistent conclusions have yet to emerge about viable interventions, such as dietary supplementation in the periconceptual period. Once again, international standardization of research protocols, consensus on preventive interventions suitable for clinical trials, and the performance of trials in an international framework will enhance both their validity and consistency.

The treatment of craniofacial anomalies has, so far, escaped the rigours of contemporary health technology assessment, and there is no consensus on optimal management of even the commonest conditions. The availability of homogeneous samples of adequate size for randomized trials and long-term follow-up for each of the many subgroups of craniofacial anomalies represents a formidable challenge, and multisite cooperation is a prerequisite. In the developing world, the costs of rehabilitation and problems of access put treatment beyond the reach of vast numbers of affected individuals. Research is urgently required on systems of delivering care under various geographical and economic circumstances.

The craniofacial anomalies project of the WHO programme for human genetics

In 2000, the National Institute for Dental and Craniofacial Research, United States, and the WHO programme for human genetics initiated a project to form an international network for planning, designing protocols and setting up a database for international collaborative biomedical, epidemiological, clinical and behavioural studies on craniofacial anomalies. The goal of this initiative was to bring together researchers in different countries through collaborative partnerships and to reach a global consensus on the directions of research, with
common protocols. A further objective was to create a directory of research resources and a publicly accessible internet-based research database. Three meetings were held to advance these objectives.

The first meeting, held in Geneva, Switzerland, in November 2000, included concurrent workshops on research on the genetic basis of craniofacial anomalies, gene–environment interactions and treatment. The second meeting, held in Utah, United States, in May 2001, addressed the prevention of craniofacial anomalies; and the third meeting, held in Bauru, Brazil, in December 2001, considered registration of craniofacial birth defects, which thereafter became the fifth theme. Detailed reports of these meetings are available (WHO, 2002, 2003) and can be found on the website www.who.int/genomics.

Since 2001, a collaborative network has been set up. The objective of the final meeting of the project, in December 2004, of which this publication is the report, was to review progress since initiation of the project and to bring all interested parties together to discuss further steps to optimize the surveillance, treatment and research strategies and infrastructure for craniofacial anomalies in various parts of the world.
2. INTERNATIONAL DATABASE AND REGISTRY OF CRANIOFACIAL ANOMALIES

The definitions of the terms ‘database’, ‘register’ and ‘registry’ were taken from the Dictionary of Epidemiology (Last, 1995), as shown in Box 1. More specifically, the term ‘registry’ was used to identify local systems that maintain a register, and the term ‘database’ was used to identify a set of registries or a set of cases collected by registries, commonly at international level.

**Box 1. Definitions of epidemiological terms used**

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<thead>
<tr>
<th>Term</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Database</td>
<td>An organized set of data or collection of files that can be used for a specific purpose</td>
</tr>
<tr>
<td>Register</td>
<td>Actual document or file of data on all cases of a particular disease or other health-relevant condition in a defined population, such that the cases can be related to a population base. With this information, incidence rates can be calculated. If the cases are followed up regularly, information on remission, exacerbation, prevalence and survival can also be obtained.</td>
</tr>
<tr>
<td>Registry</td>
<td>System of continuous registration</td>
</tr>
</tbody>
</table>

From Last (1995)

Registries and databases can be created for a specific health condition or for a category of conditions, for example cancers, birth defects or rare diseases. Examples of registries and databases for birth defects in general and more specifically for craniofacial anomalies are presented in Box 2. A method to improve the classification of craniofacial anomalies was discussed.

**Box 2. Registries and databases referred to**

<table>
<thead>
<tr>
<th>Registries</th>
<th>All birth defects</th>
<th>Craniofacial anomalies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Registries</td>
<td>Philippines Birth Defects Registry</td>
<td>Philippines Oral Cleft Registry</td>
</tr>
<tr>
<td>Databases</td>
<td>National Birth Defects Prevention Network, United States</td>
<td>International Perinatal Databases of Typical Orofacial Clefts</td>
</tr>
<tr>
<td></td>
<td>International Clearing-house for Birth Defects Surveillance and Research</td>
<td>International Database on Craniofacial Anomalies</td>
</tr>
<tr>
<td></td>
<td>European Registry of Congenital Anomalies</td>
<td>The Indian Association of Plastic Surgeons database on oral clefts</td>
</tr>
</tbody>
</table>
2.1 REGISTRIES OF BIRTH DEFECTS

Philippines Birth Defects Registry
The Philippines is an archipelago composed of more than 7000 islands, situated in South-East Asia between the South China Sea and the Philippine Sea. The division of the country into numerous small islands accounts for the diversity of the culture of the Filipino people. It has a population of 80 million, with a population growth rate of approximately 2.0% per year (1.7 million newborns per year) and an infant mortality of 27–28 per 1000 live births.

In 1999, the Institute of Human Genetics of the National Institutes of Health, United States, and the Department of Health started the Philippines Birth Defects Registry. The justification for setting up this Registry was that information on birth defects was limited, the precise prevalence at birth had not yet been established, and birth defects in other parts of the world rank among the top 20 causes of death throughout life and are the third leading cause of death during infancy.

The Registry is hospital-based, with 66 participating hospitals out of a total of 1700 throughout the country. During the first 5.5 years of activity, about 558 000 livebirths were evaluated, representing about 6% of all births in the country, and 8250 birth defects were registered (1.47%).

Birth defects are diagnosed in livebirths and classified and coded according to ICD-10. The entry point to the Registry is the hospital nursery, and birth defects diagnosed after discharge from the nursery are not registered. For each malformed child, a special data collection form is filled out by the physician in charge. This form, reproduced in Appendix 1, is considered to be exemplary and could be used in other developing countries.

Reporting of cases from all 7000 islands of the Philippines archipelago poses a huge challenge. Until methods of communication and data transmission are improved, many cases, particularly those in infants born in communities, will remain unreported. There is also under-recognition of specific congenital syndromes with associated cleft lip and/or palate. At present, the only cases reported to the Registry are isolated oral clefts. Minor types of oral clefts, such as cleft uvula, are also underreported. Surgeons and physicians should be encouraged to provide accurate, specific diagnoses of different types of cleft.

Birth defects surveillance system in China
A register was established in 1992 in collaboration with the Centres for Disease Control and Prevention, Atlanta, Georgia, United States. It covers 33 counties in four provinces of Hebei and Shanxi in the north of the country around Beijing and Jiangsu and Zhejiang in the south around Shanghai. It is based on a population of about 20 million. The annual number of births is about 170 000.

Data are collected from 800 hospitals and clinics in the two areas, which are equipped with computers to enter the data and to transmit them electronically every day to county maternal and child health institutes. The county institutes transfer the data to the National Center for Maternal and Infant Health at Peking University Health Science Centre every month, and real-time monitoring is performed. The target population for birth defects is all births from 20 gestational weeks. Each livebirth or stillbirth is examined, and a report on any suspected malformation is sent to the county maternal and child health institute within 24 h. The county institute sends a staff member to take photographs of infants with suspected malformations and stillbirths within 24 h. All cases of birth defects and stillbirths are reviewed independently
by paediatricians at the National Center for Maternal and Infant Health monthly and at the Centers for Disease Control and Prevention twice a year.

The register is linked to the primary health-care surveillance system, in which women are enrolled from marriage and their health monitored until 6 weeks after the birth of a child, and also to the child health-care surveillance system, which provides information from each childhood examination (four times in the first year after birth, two in the second year and one in each subsequent year up to 7 years).

Since 1997, the registry has been monitoring congenital heart defects among newborns and infants in the southern counties. In 2001, a biobank was set up to collect biological samples for use in nested case–control triad studies. In 2002, a pilot study was initiated to register internal defects.

National Birth Defects Prevention Study, United States

In 1996, the United States Congress directed the Centers for Disease Control and Prevention to establish the Centers for Birth Defects Research and Prevention. This directive was formalized with the passage of the Birth Defects Prevention Act of 1998 (Public Law 105-168), which authorized the Centers for Disease Control and Prevention to collect, analyse and make available data on birth defects; set up regional centres to conduct applied epidemiological research for the prevention of birth defects; and provide the public with information on preventing birth defects. Centres have been established in Arkansas, California, Iowa, Massachusetts, New York, North Carolina, Texas and Utah, as these states already had programmes with nationally recognized expertise in birth defects surveillance and research. The Centers for Disease Control and Prevention coordinate the Centers for Birth Defects Research and Prevention and participate in the National Birth Defects Prevention Study as the ninth study site.

The National Birth Defects Prevention Study is a large, population-based case–control study of major birth defects (Yoon et al., 2001) and is a collaborative effort among nine centres. The purpose of the study is to identify environmental and genetic factors that contribute to the occurrence of birth defects. It focuses on birth defects of unknown etiology and excludes infants with chromosome abnormalities or single-gene disorders. Each centre contributes about 300 case infants and 100 control infants to the study per year.

The study has three main components. First, through existing birth defects surveillance systems, each centre identifies and collects information on case infants who have any of the major birth defects listed in Box 3. Clinical geneticists at each centre review and classify the clinical information for each case (Rasmussen et al., 2003), and the information on those eligible for the study is stored in a central database. Each centre also identifies a random sample of livebirths with no major birth defect from the same population and period as the case infants, to serve as control infants in the study.

Second, the mothers of case and control infants participate in a computer-assisted telephone interview in Spanish or English. Mothers who cannot complete an oral interview in either of these languages are excluded. The interviews include questions about pregnancy and medical history, occupational and environmental exposures, life style, diet and medication use. Mothers are interviewed for the study between 6 and 24 weeks after their estimated date of delivery; those who cannot be reached within this time are excluded from the study. All the interview data are maintained in a centralized database.
### Box 3. Birth defects eligible for inclusion in the National Birth Defects Prevention Study

<table>
<thead>
<tr>
<th>Defects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anencephaly, craniorachischisis</td>
</tr>
<tr>
<td>Spina bifida</td>
</tr>
<tr>
<td>Encephalocele, cranial meningocele, encephalomyelocele</td>
</tr>
<tr>
<td>Holoprosencephaly</td>
</tr>
<tr>
<td>Hydrocephalus</td>
</tr>
<tr>
<td>Dandy–Walker malformation</td>
</tr>
<tr>
<td>Anophthalmia, microphthalmia</td>
</tr>
<tr>
<td>Cataracts, glaucoma and related eye defects</td>
</tr>
<tr>
<td>Anotia, microtia</td>
</tr>
<tr>
<td>Conotruncal heart defects</td>
</tr>
<tr>
<td>Single ventricle</td>
</tr>
<tr>
<td>Septal heart defects (atrial, ventricular and atrioventricular)</td>
</tr>
<tr>
<td>Ebstein malformation</td>
</tr>
<tr>
<td>Obstructive heart defects (right and left ventricular outflow tract defects)</td>
</tr>
<tr>
<td>Anomalous pulmonary venous return</td>
</tr>
<tr>
<td>Heterotaxia</td>
</tr>
<tr>
<td>Choanal atresia</td>
</tr>
<tr>
<td>Cleft lip and/or palate</td>
</tr>
<tr>
<td>Cleft palate</td>
</tr>
<tr>
<td>Oesophageal atresia and/or tracheoesophageal fistula</td>
</tr>
<tr>
<td>Intestinal atresia or stenosis</td>
</tr>
<tr>
<td>Biliary atresia</td>
</tr>
<tr>
<td>Hypospadias, second- or third-degree</td>
</tr>
<tr>
<td>Renal agenesis or hypoplasia</td>
</tr>
<tr>
<td>Exstrophy, bladder</td>
</tr>
<tr>
<td>Exstrophy, cloaca</td>
</tr>
<tr>
<td>Limb deficiency, intercalary</td>
</tr>
<tr>
<td>Limb deficiency, longitudinal</td>
</tr>
<tr>
<td>Limb deficiency, transverse</td>
</tr>
<tr>
<td>Limb deficiency, not elsewhere classified</td>
</tr>
<tr>
<td>Craniosynostosis</td>
</tr>
<tr>
<td>Diaphragmatic hernia</td>
</tr>
<tr>
<td>Sacral agenesis</td>
</tr>
<tr>
<td>Omphalocoele</td>
</tr>
<tr>
<td>Gastrochoschisis</td>
</tr>
<tr>
<td>Amnion rupture sequence</td>
</tr>
</tbody>
</table>
Third, after completing the interview, the mothers receive a kit for collection of buccal cells from the mother, father and infant, to allow identification of genetic factors that might affect the risk for birth defects (Rasmussen et al., 2002). Buccal cells are collected by rubbing a cytobrush across the inside wall of the mouth. A portion of the DNA that is collected from the families is stored in a specimen bank at the Centers for Disease Control and Prevention for long-term research projects on birth defects. A central database that includes records of all samples collected and the results of quality control marker tests is maintained by the Centers.

The information gathered from the interviews and the results of analysis of the DNA samples will be invaluable for studying the associations between genetic susceptibility to environmental exposures for a broad range of carefully classified birth defects. The unprecedented statistical power of this collaborative study will enable scientists to study the epidemiology of some rare birth defects, and the compiled data and banked DNA will facilitate future research as new hypotheses and improved techniques emerge. The collaborative nature of the study increases its statistical power for assessing risk factors and allows consideration of etiologically distinct subtypes. The collaborators are conducting a number of studies of potential risk factors for orofacial clefts, including:

- maternal smoking and environmental tobacco smoke (Honein et al., 2004);
- assisted reproductive technology (Reefhuis et al., 2004);
- maternal intake of multivitamin and mineral supplements and cereals; and
- examination of candidate genes, maternal cigarette smoking and gene–environment interactions.

Numerous additional projects are planned or in progress, and better understanding of the causes of craniofacial anomalies is expected over the next 2–5 years. The numbers of cases in the study make it possible to look at separate subgroups; for example, risk factors for isolated cases (only one major birth defect) can be assessed separately from those for two or more major defects in at least two organ systems, and subtypes of craniofacial anomalies can be studied (e.g. cleft palate with notation of Pierre Robin sequence in the medical record).

The National Birth Defects Prevention Study provides a model for collaborative research on craniofacial anomalies, as all centres follow a standard, mutually agreed protocol and contribute data to centralized databases.

2.2 REGISTRIES OF CRANIOFACIAL ANOMALIES

The Philippines Oral Cleft Registry

The incidence of all forms of oral clefts in the Philippines is estimated to be 1 in 1136 livebirths. This figure includes all types of cleft palate (hard or soft), cleft lip and cleft lip and palate and some cases of cleft uvula.

Recognizing the burden of oral clefts in the Philippines, the Institute of Human Genetics of the National Institutes of Health, United States, invited organizations involved in the care of patients with such anomalies to a series of meetings and consultations, with the objective of forming a network for systematic data collection and collaborative research. Thus, the Philippines Oral Cleft Research Group was organized, comprising the Institute of Human Genetics of the National Institutes of Health, United States, the Philippines Association of Plastic, Reconstructive and Aesthetic Surgeons, the Philippines Society of Otolaryngology–
Head and Neck Surgery, the Operation Smile Philippines Foundation and the Philippines Band of Mercy. The specific objectives of the Philippines Oral Cleft Research Group are:

- to establish the Oral Cleft Registry for patients seen by members of the participating organizations in the Philippines;
- to determine the incidence of oral clefts in the population covered by the network of those organizations;
- to identify possible risk factors for oral clefts;
- to provide statistics to assist in national policy and programme planning;
- to make recommendations for adoption of the registry on a larger scale; and
- to conduct awareness campaigns among the constituents of the above institutions and organizations on the importance of reporting oral clefts.

Through this network, the Philippines Oral Cleft Research Group intends to identify all cases of oral clefts in the country, including patients in the community. Both the Operation Smile Philippines Foundation and the Philippines Band of Mercy organize community surgical missions all over the country. The Philippines Society of Otolaryngology–Head and Neck Surgery and the Philippines Association of Plastic, Reconstructive and Aesthetic Surgeons treat cases in hospital and also provide free service during community missions organized by the Philippines Band of Mercy and Operation Smile. The Institute of Human Genetics of the National Institutes of Health, United States, serves as the base of the registry, where all data are submitted and consolidated. The Institute also provides technical support and equipment for database entry.

The four-page oral cleft registry form includes the following:

- notifier’s data;
- general data;
- patient data, including diagnosis, classification of cleft lip and/or palate (ICD-10 code) and drawings of the lesion or lesions;
- maternal history, including exposure to radiation, drugs or chemicals, metabolic illnesses or infections and intake of vitamins;
- family history of oral cleft; and
- findings of a physical examination.
During a 6-month pilot operation, 1648 data forms were received. Of those encoded, half reported both cleft lip and palate, 18.4 % reported cleft lip only, and 15.6% reported cleft palate only; the rest were unspecified. Table 1 shows the number and percentage of each cleft type. These preliminary data were presented at a meeting of collaborators, where problems of data collection, database entry and information technology support were discussed. The format and content of the registry form are being amended.

**Table 1. Numbers of reported cases of each type of cleft in the Philippines during a 6-month pilot operation, 2000**

<table>
<thead>
<tr>
<th>Organization</th>
<th>Cleft lip and palate</th>
<th>Cleft lip only</th>
<th>Cleft palate only</th>
<th>Unspecified</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Operation Smile</td>
<td>93</td>
<td>29.0</td>
<td>32</td>
<td>10.0</td>
</tr>
<tr>
<td>Philippines Band of Mercy</td>
<td>590</td>
<td>59.4</td>
<td>203</td>
<td>20.4</td>
</tr>
<tr>
<td>Philippines Society of Otolaryngology—Head and Neck Surgery</td>
<td>37</td>
<td>41.6</td>
<td>18</td>
<td>20.2</td>
</tr>
<tr>
<td>Philippines Association of Plastic, Reconstructive and Aesthetic Surgeons</td>
<td>26</td>
<td>66.7</td>
<td>8</td>
<td>20.5</td>
</tr>
<tr>
<td>Unspecified</td>
<td>78</td>
<td>47.6</td>
<td>43</td>
<td>26.2</td>
</tr>
<tr>
<td>Total</td>
<td>824</td>
<td>50.0</td>
<td>304</td>
<td>18.4</td>
</tr>
</tbody>
</table>

In collaboration with Utah State University, United States, training was provided to staff of the Institute of Human Genetics of the National Institutes of Health in mapping data with a geographical information system, which allows identification of patient location and clustering of cases. This information will be used for scientific investigations, resource management and development planning.

The Philippines Oral Cleft Research Group also plans to undertake a study on the correlation between maternal vitamin intake and prevention of oral clefts. A survey is being conducted among mothers to assess their willingness to participate in the study.

**Association of Plastic Surgeons of India study on oral clefts**

To estimate the birth prevalence of orofacial clefts in the Indian population, the Association of Plastic Surgeons of India studied several homogeneous geographical areas in India, each with a population of about 100,000 persons. Ten areas were identified, two in the north, two in the south, two in the west, two in the east and two in the centre of the country. A comprehensive questionnaire was prepared, and social workers or volunteers questioned every household in each area and entered the information into a database.

The first surprise was that the incidence was much lower than was generally believed. In one study in rural Maharashtra, with a tribal population, there were only 10 cases of cleft lip, in persons ranging in age from 2 to 43 years, and none of cleft palate. None of the cases had
been operated. On a subsequent visit to this area, photographs of children with cleft lip and palate were shown to elders and educated persons, who reported that infants with these deformities all died within a few days of birth, as they could not suckle at the breast. Spoon-feeding was unheard of, and there were no visiting doctors or health workers to tell the parents how to feed infants with cleft palates. Enquiries were also made about consanguinous marriages and the occurrence of clefts in the extended family to determine whether cleft lip and palate ‘ran in families’.

In an attempt to gauge the role of environmental factors in the causation of clefts, special emphasis was placed on family income, diet and habits and whether the mother smoked, drank alcohol or took allopathic (e.g. vitamins) or herbal drugs.

The main problem encountered was that the volunteers filled in the questionnaire in their local language, so the data could not be entered into a database at a central location. Ideally, local representatives who speak both the local language and English would feed the data into the database; however, this would be a Herculean task, given that there were about 20 000 forms from each area. Another problem faced by the volunteers was to obtain accurate information about the intake of vitamins and drugs. Most of the population had no idea which vitamins had been prescribed by local health workers. Furthermore, most villagers were unlikely to tell the volunteers about the intake of drugs like hashish and ganja.

The results from five of the ten designated areas are shown in Table 2.

<table>
<thead>
<tr>
<th>Area</th>
<th>Total population</th>
<th>No. of cases in population and rate</th>
<th>Total no. of children</th>
<th>No. of cases among children and rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gujarat</td>
<td>55 986</td>
<td>7</td>
<td>27 722</td>
<td>7</td>
</tr>
<tr>
<td>Assam</td>
<td>~ 57 000</td>
<td>79</td>
<td></td>
<td>1 : 7998</td>
</tr>
<tr>
<td>West Bengal</td>
<td>108 076</td>
<td>76</td>
<td></td>
<td>1 : 721</td>
</tr>
<tr>
<td>Talvada (Mumbai)</td>
<td>86 427</td>
<td>4</td>
<td></td>
<td>1 : 1422</td>
</tr>
<tr>
<td>Coimbatore</td>
<td>103 147</td>
<td></td>
<td>38 317</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1 : 1161</td>
</tr>
</tbody>
</table>

2.3 INTERNATIONAL DATABASES OF BIRTH DEFECTS

European Registry of Congenital Anomalies

The European network of population-based registries for epidemiological surveillance of congenital anomalies was started in 1979 and covers more than 1 million births per year. It is made up of 39 registries in 19 countries, giving a coverage of 25% of the newborn population of Europe. These high-quality, multiple source registries ascertain terminations of pregnancy as well as births. The network provides a standardized database on > 250 000 cases of congenital anomalies among livebirths, stillbirths and terminations of pregnancy.

The European Registry of Congenital Anomalies and Twins (EUROCAT) is a WHO Collaborating Centre for the epidemiological surveillance of congenital anomalies. It is supported by the Public Health Programme of the European Commission Public Health Directorate. EUROCAT manages research by collaboration in working groups and
committees, such as the Classification and Coding Committee and the Working Group on Folic Acid and Neural Tube Defects. The aims of EUROCAT are shown in Box 4.

**Box 4. Objectives of the European Registry of Congenital Anomalies and Twins (EUROCAT)**

- **To provide essential epidemiological information on congenital anomalies in Europe.** The EUROCAT network provides the means for data management, data harmonization and dissemination of collected data. The primary means of dissemination of prevalence data is the website: www.eurocat.ulster.ac.uk/pubdata. Users can specify the region, year and congenital anomaly of interest to them to obtain customized tables of prevalence among livebirths, stillbirths and terminations of pregnancy after prenatal diagnosis. There are links to registry descriptions and other methodological detail.

- **To facilitate early warning of new teratogenic exposures.** The EUROCAT strategy is to act as a rapid communication and reaction centre. Statistical packages have been developed for local surveillance, and new drug coding will allow better analyses of drug–malformation associations. Multiple malformations are monitored constantly.

- **To evaluate the effectiveness of primary prevention.** Folic acid supplementation for reducing the prevalence of congenital anomalies is an example of primary prevention based on linkage of surveillance data, research and attention to the health-care burden (www.eurocat.ulster.ac.uk/pubdata).

- **To assess developments in prenatal screening.** A number of reports (www.eurocat.ulster.ac.uk/pubdata) address the frequency of ultrasound diagnoses of severe structural malformations and the joint effects of increasing the average maternal age and increasing prenatal screening of the prevalence of Downs syndrome among livebirths.

- **To act as an information and resource centre for the population, health professionals and managers about clusters or risk factors of concern.** Both enviromental and genetic factors have been shown to influence the prevalence of craniofacial anomalies. EUROCAT conducts research into accidents and disasters (e.g. Chernobyl), industrial and agricultural sources of pollution and clusters identified by surveillance or communities.

- **To act as a catalyst for setting up and connecting registries that collect comparable, standardized data throughout Europe.** As Europe expands, new countries and regions have become members (e.g. Hungary, Poland).

The strategy adopted by EUROCAT for research into craniofacial anomalies comprises three branches:

- an epidemiological approach to evaluation of the distribution of craniofacial anomalies,
- research into etiological factors, and
- policy practice and prevention.

These three areas are underpinned by the extensive surveillance data collected by EUROCAT and shared through the International Clearing-house for Birth Defects Surveillance and Research for the world register. Details of relevant research are given in Section 4 of this report.
International Clearing-house for Birth Defects Surveillance and Research

Since its inception in 1974, the International Clearing-house for Birth Defects Surveillance and Research, formerly known as the International Clearing-house for Birth Defects Surveillance and Research, or the Clearing-house, has systematically monitored birth defects. Forty registries now collaborate (see Appendix 2) to exchange the results of monitoring for 40 types of birth defect (see Appendix 3) among 3.5 million births annually (International Clearing-house for Birth Defects Monitoring Systems, 2003).

The Clearing-house has a head office and coordinating centre, the International Centre on Birth Defects, set up with financial support from both nongovernmental and governmental organizations (mainly in Italy, Norway and the United States and the European Commission), and has established official relations with several like-minded international bodies, including other birth defect networks and WHO. As a result, the Clearing-house has been able to continue the exchange of data on and monitoring of birth defects, to conduct epidemiological and public health research and to help other countries to set up or improve their surveillance systems. These three areas—surveillance, research and capacity building—have a common goal of reducing disease and promoting healthy outcomes through primary prevention, representing the natural evolution of the original mission of the Clearing-house.

Monitoring: Data received annually are used in public health research, such as assessments of birth defect rates in relation to folic acid use. After updating and further review, these data were used to create a World Atlas of Birth Defects, now in its second edition, giving the prevalence and burden of congenital malformations worldwide (International Clearing-house for Birth Defects Surveillance and Research, 2003). Updated information on these and other resources can be found in the annual reports of the Clearing-house (www.International Centre on Birth Defects.org). In these and other activities, the Clearing-house increasingly collaborates with other organizations, such as EUROCAT and the National Birth Defects Prevention Network in the United States.

Searching for unsuspected teratogens among medications: Maternal use of medications, such as thalidomide and, more recently, retinoic acid, has accounted for epidemics of birth defects. The safety during pregnancy of many medications is not established conclusively. Because medications are used frequently during the first months of pregnancy, often before the pregnancy is recognized, even small teratogenic risks can translate into many affected infants. Registries of congenital malformations can provide data to identify the teratogenic effects of medications. The Clearing-house thus set up the MADRE (MAternal DRug Exposure) project, as a collaborative study among several registries (Robert et al., 1994), which is described in Section 6 of this report, in the context of prevention of birth defects.

Public health genetics research: International collaboration in genetics can help to elucidate the fundamental molecular epidemiology of genes of public health importance, as the basis for studying gene–disease associations (Khoury & Little, 2000). Such collaboration also helps researchers to assess associations across a wider and therefore more informative range of genotype frequencies and environmental exposures than would be feasible in one country. At the same time, collaboration provides access to larger study populations, with a resulting improvement in the study’s statistical power. These aspects are discussed and illustrated by examples in Section 4.

Strategic direction: In 2004, on the occasion of the 30th anniversary of the Clearing-house, it initiated a strategic plan to examine current activities and redirect its efforts. The strategic planning team consisted of a selected group of committed programme directors with diverse opinions. The lessons learnt from past activities—both strengths and weaknesses—were used to plan future directions. The Clearing-house has been an invaluable international forum, in which epidemiological data on structural congenital anomalies have been compiled,
exchanged and published. These activities will continue, with the following recommended changes, subject to member ratification:

1. Surveillance rather than monitoring will be emphasized.
2. Researchers who are not members of the Clearing-house will be invited to collaborate with members in using the diverse population-based data, the core strength of Clearing-house programmes, to test research hypotheses.
3. Surveillance programmes, which include children’s disabilities other than structural congenital anomalies, will be invited to join the Clearing-house.
4. There will be less emphasis on quarterly statistical monitoring and more emphasis on constant communication among Clearing-house members.
5. The name of the organization will be changed to the International Clearing-house for Birth Defects Surveillance and Research to reflect the new strategic direction.

Focus on developing countries and international collaboration: As international priorities change and technical capacity increases, the scope of activities for organizations such as the Clearing-house appear not so much to change but rather to expand. Etiological research, with its new tools, such as molecular genetics, can progress with registry-based international collaboration. Developing countries, where now most births occur, must be supported in dealing with birth defects and genetic diseases. In this time of information explosion and internet-based communication, international networks can help provide the multilingual content and a supra-national conduit for disseminating crucial information on the impact, health outcomes and prevention of birth defects. These possibilities can be achieved only through a conscious investment in international collaboration. Efforts over the past 30 years have produced remarkable results, but with limited funding and much in-kind work by partner programmes. The results bear testimony to the activity of many but hardly constitute a policy for sustainability.

Support has been provided by several institutions, both governmental (e.g. the Italian and Norwegian Governments, the Centers for Disease Control and Prevention and the National Institutes of Health in the United States and the European Commission) and nongovernmental (e.g. the March of Dimes). What is now needed is a concerted, sustained effort by the international community, through national and international agencies, to support international activities and thus realize the possibilities of the surveillance of, research on and primary prevention of birth defects for the world.

2.4 INTERNATIONAL REGISTRIES OF CRANIOFACIAL ANOMALIES

International Database on Craniofacial Anomalies

International databases of persons with congenital malformations are a crucial basis for accumulating and disseminating facts that can lead to improved health and the prevention of unnecessary suffering. Ensuring that the contents of these databases are accessible to researchers worldwide should be a fundamental tenant of any international collaboration on surveillance. On this premise, using craniofacial malformations as a model, the National Institute of Dental and Craniofacial Research in the United States and the WHO programme on Human Genetics in September 2002 promoted the development of the International Database on Craniofacial Anomalies and assigned its coordination to the International Centre on Birth Defects, the head office of the Clearing-house. It was also decided that the International Database would be made available on the web, to ensure dissemination of the database and of the aggregate data gained from its analysis. The WHO Human Genetics
programme was entrusted, with the International Centre on Birth Defects, to ensure that the website was set up and maintained.

The first activity of the International Database was to collect cases from all the existing national and regional databases and to ensure that the data would be available for further research. The main aims of the Database are:

- to encourage existing databases to share data, thus creating a specific worldwide database;
- to encourage the establishment of new databases to contribute to the International Database;
- to present the collected data in a suitable way or to make available more specific data to stimulate research on primary and tertiary prevention and better treatment of craniofacial anomalies; and
- to encourage scientific and lay organizations to collect and share relevant data and information on persons affected by a craniofacial anomaly.

*International Perinatal Databases of Typical Orofacial Clefts*

The International Database on Craniofacial Anomalies is setting up the International Perinatal Databases of Typical Orofacial Clefts (see Appendix 4). The Database is starting with typical orofacial clefts because data on these anomalies are the most reliable in existing resources. Typical oral clefts are the only craniofacial defects that have a simple, shared definition, can be detected perinatally, can be diagnosed simply by observation and are coded in well-defined coding systems. Problems persist in the definition, perinatal detection, diagnosis and coding of all other craniofacial anomalies (Appendix 5).

As of November 2004, 62 registries had joined the International Perinatal Databases of Typical Orofacial Clefts, representing 36 countries on all five continents (Appendix 6). Data are available on over 5 million births, and registries are expected to update their submissions yearly. The definitions used in the International Perinatal Databases are shown in Box 5. For each case with a typical oral cleft, registries send at least the following information to the International Centre on Birth Defects: case identification code, date of birth (at least month and year), sex, birth weight, gestational age, single or twin status, vital status (termination of pregnancy, livebirth, stillbirth), available verbatim description of the oral cleft and of any other associated anomaly (including the name of a syndrome if recognized), and all codes used for describing the oral cleft as well as any other malformations present.

At the International Centre on Birth Defects, cases of oral clefts associated with holoprosencephaly and cases of cleft uvula or submucous cleft palate are excluded. All cases with a description or code of Pierre Robin anomaly are revised, and all cases with micrognathia are evaluated for a diagnosis of Pierre Robin sequence. The clinical geneticist evaluates all cases with two or more codes and with a description of any anomaly associated with the typical oral cleft. All syndromes are evaluated, and more information is requested when the diagnosis is not clear. Each case is coded by two sets of codes: for the diagnosis and for the defects. The diagnosis can be isolated oral cleft with the appropriate code, multiple congenital anomaly with a code identifying the number of major unrelated defects present or syndrome, with the Online Mendelian Inheritance in Man or expanded ICD-10 code identifying the syndrome.
Box 5. Definitions used in the International Perinatal Databases of Typical Orofacial Clefts

<table>
<thead>
<tr>
<th>Definition</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cleft lip (749.1) Q36</td>
<td>A congenital malformation characterized by partial or complete clefting of the upper lip. Exclusions: median cleft lip part of holoprosencephaly sequence; rare and oblique facial clefts</td>
</tr>
<tr>
<td>Cleft lip and palate (749.2) Q37</td>
<td>A congenital malformation characterized by partial or complete clefting of the upper lip with clefting of the alveolar ridge and/or the hard palate. Exclusions: any oral cleft part of the holoprosencephaly sequence; rare and oblique facial clefts</td>
</tr>
<tr>
<td>Cleft palate (749.0) Q35</td>
<td>A visible congenital malformation characterized by a closure defect of the hard palate and/or soft palate behind the foramen incisivum without cleft lip. Exclusions: submucous cleft palate, occult cleft palate, cleft uvula. In some databases, cleft palate includes Pierre Robin sequence.</td>
</tr>
<tr>
<td>Pierre Robin sequence (756.03) Q87.08</td>
<td>A congenital malformation characterized by a closure defect of the palate behind the foramen incisivum without cleft lip, associated with significant micrognathia with or without clinically relevant glossoptosis (reposition of tongue) or respiratory distress</td>
</tr>
<tr>
<td>Isolated case</td>
<td>Any case with only one major defect registered; in this database, only a typical orofacial cleft and its sequence (e.g. nose deformities)</td>
</tr>
<tr>
<td>Associated defects, multiple congenital anomaly or multimalformed infant</td>
<td>Any case with a major defect other than the orofacial cleft and its sequence (e.g. nose deformities)</td>
</tr>
<tr>
<td>Syndrome</td>
<td>Any case with a diagnosis of a syndrome or a recognized pattern of multiple malformations</td>
</tr>
</tbody>
</table>

On the basis of the recommendations of the meeting on the global registry and database on craniofacial anomalies in Bauru, Brazil (WHO, 2003), the WHO Human Genetics programme developed a website for the project (www.who.int/genomics/anomalies), designed to fulfill three objectives: to give visibility to the project’s aims and objectives; to allow online access to the International Database on Craniofacial Anomalies and to create an online directory of resources on craniofacial anomalies. Launched in November 2003, this website contains information on all collaborative research, communicates the aims and achievements of the collaboration and disseminates all documentation and data resulting from their work. All meeting reports can be found on the website, with online access to aggregated data from the International Database on Craniofacial Anomalies, which is provided regularly by the International Centre on Birth Defects to the Human Genetics programme.

The International Database on Craniofacial Anomalies is a centralized, comprehensive source of information on individual cases of craniofacial anomalies gathered regularly from a global network of birth defect registries and clinical databases. It is designed to provide simple access to aggregated data on typical orofacial clefts from countries throughout the world. The data are updated every 3 months. Cases are analysed by the International Centre on Birth Defects in Rome, Italy. Aggregated data are available as summary tables of cumulative data by register and as tables of aggregations by country or region (see Box 6). Clear instructions to help researchers obtain data on individual cases are outlined on the website.
### Box 6. Data provided to each registry and set of registries included in the International Database on Craniofacial Anomalies

1. Number of cases by type and total rates, by register
2. Distribution by type: isolated, associated with other major malformations, syndromes by register
3. Total cases, by type and by set of registries
   - Number of cases by set of registries
   - Rates, with 95% confidence intervals
   - Proportion of terminations of pregnancy
   - Sex ratio (expressed as male : male + female) with 95% confidence intervals
     - Births
     - Terminations of pregnancy
   - Median and interquartile ranges for birth weight (only for live- and stillbirths)
   - Proportions of live- and stillbirths under 2500 g
   - Median and interquartile ranges for gestational age (only for live- and stillbirths)
   - Proportions of live- and stillbirths under 37 weeks of gestation
   - Proportions of live- and stillbirths with intrauterine growth restriction (< 10% of weight for gestational age)
   - Rate of twin deliveries: twin deliveries / twin deliveries + singleton deliveries
   - List of syndromes
4. Isolated cases, by type and set of registries
   - Number of cases by set of registries
   - Rates, with 95% confidence intervals
   - Proportion of terminations of pregnancy
   - Sex ratio (expressed as male : male + female), with 95% confidence intervals
     - Births
     - Terminations of pregnancy
   - Median and interquartile ranges for birth weight (only for live- and stillbirths)
   - Proportions of live- and stillbirths under 2500 g
   - Median and interquartile ranges for gestational age (only for live- and stillbirths)
   - Proportions of live- and stillbirths under 37 weeks of gestation
   - Proportions of live- and stillbirths with intrauterine growth restriction (< 10% of weight for gestational age)
   - Rate of twin deliveries: twin deliveries : twin deliveries + singleton deliveries

All resources recommended by members of the collaboration and team leaders and sent to the Human Genetics programme are added to the directory. This website has been useful not only for communication but also to generate networking among persons interested in research on craniofacial anomalies. Regular feedback and queries are expected from members of the public, the scientific community and others on the content of the website.

As of July 2004, information had been collected on 5432 cases of typical orofacial clefts among 3 529 582 births (Table 3).

The variation in rates internationally is due mainly to differences in the rates of cleft lip and/or cleft palate (Figure 1). Data from the 25 registries in Europe indicate that the rates of cleft lip and/or palate correlate directly with latitude ($r = 0.61$, $p < 0.01$), with higher rates in the north (Figure 2). No such correlation is seen for cleft palate ($r = 0.31$, $p = 0.10$). The higher the rates of cleft lip and/or palate, the more severe are the defects of the primary palate. This finding is consistent with the prediction of a multifactorial model, that the commoner the defect in a population the higher the proportion of severe forms of that defect.
Table 3. Numbers and rates per 10 000 deliveries of typical oral clefts by area covered by the International Database on Craniofacial Anomalies

<table>
<thead>
<tr>
<th>Set of registries</th>
<th>No. of registries</th>
<th>No. of cases</th>
<th>Total no. of births</th>
<th>Rate per 10 000 deliveries*</th>
</tr>
</thead>
<tbody>
<tr>
<td>North Europe</td>
<td>10</td>
<td>874</td>
<td>549 709</td>
<td>15.9</td>
</tr>
<tr>
<td>Central Europe</td>
<td>10</td>
<td>1126</td>
<td>716 106</td>
<td>15.7</td>
</tr>
<tr>
<td>South Europe and Israel</td>
<td>10</td>
<td>325</td>
<td>364 375</td>
<td>8.9</td>
</tr>
<tr>
<td>North America</td>
<td>13</td>
<td>2030</td>
<td>1 369 431</td>
<td>14.8</td>
</tr>
<tr>
<td>South America and Mexico</td>
<td>10</td>
<td>399</td>
<td>229 253</td>
<td>17.4</td>
</tr>
<tr>
<td>Japan</td>
<td>1</td>
<td>644</td>
<td>270 656</td>
<td>23.8</td>
</tr>
<tr>
<td>South Africa</td>
<td>2</td>
<td>34</td>
<td>30 052</td>
<td>11.3</td>
</tr>
<tr>
<td>Total</td>
<td>56</td>
<td>5432</td>
<td>3 529 582</td>
<td>15.4</td>
</tr>
</tbody>
</table>

*Including terminations of pregnancy

Figure 1. Dependence of variation in rates of ‘all oral clefts’ on variations in rates of cleft lip with cleft palate

Rates of all oral clefts, cleft lip with cleft palate, cleft lip and cleft palate by main areas included in the International Database on Craniofacial Anomalies.

The heterogeneity of the rates all oral clefts is due mainly to variations in those of cleft lip with cleft palate. The rates of cleft lip and of cleft palate are more homogeneous, except for a high rate of cleft lip in Japan and low rates of cleft palate in Japan and southern Europe.
Figure 2. Correlation between latitude and rates of isolated cleft lip and/or palate in Europe

Other information currently available from the International Perinatal Databases of Typical Orofacial Clefts includes the distribution of rates by clinical presentation (isolated, multi-malformed, syndrome), maternal age, sex, defect phenotype (e.g. bilateral or unilateral cleft lip), twinning status, pregnancy outcome (e.g. termination of pregnancy), birth weight and length of gestation. More information can be found on the websites www.who.int/genomics/anomalies/idcfa/en/ and www.international_centre_on_birth_defects.org.

The next steps for the International Perinatal Databases of Typical Orofacial Clefts are to enlarge the number of participating registries and to collect data on other craniofacial defects. Unfortunately, the existing data cannot be used to conduct international collaborative studies on risk factors because of:

- lack of ‘normal’ controls in many registries;
- differences in the kinds of risk factors collected;
- lack of standard definitions of exposures (e.g. smoking during the first trimester, before pregnancy, throughout gestation);
- lack of a standard way of collecting information (e.g. smoking given only as yes or no information);
- the heterogeneity of the quality of information (e.g. collected at a special interview with the mother, collected by a routine interview at birth); and
- the wide range of missing data on risk factors among registries.
A possible solution would be to encourage existing registries to emulate the few programmes in which these problems have been solved.

Other long-term aims of the International Database on Craniofacial Anomalies are related to the persons affected by craniofacial defects, to improve answers to their queries and to evaluate them from a database. For this purpose, international collaborative databases should be set up for a single clinical setting (e.g. surgical departments) or for a specific condition. Details of the International Surgical Departments Database of Craniofacial Anomalies are given in Appendix 7. Strategic alliances should be formed between researchers, health-care providers and parent support groups to set up registers of specific conditions, such as the International Database of Moebius Syndrome. Similar databases for rare condition like Apert or Treacher Collins syndromes might be started.

A database cannot be seen simply as a collection of cases but should be used for practice and research. We are preparing examples of on-line meta-analyses, one for systematic collection of data on the prevalence of oral clefts and the other for the evaluation of benzodiazepines as a possible risk factor for oral cleft. These systematic reviews will be available on the website, where authors and researchers can contribute, check and make specific comments.

The general goal of the International Database on Craniofacial Anomalies is to assemble multiple databases, each with its methods and aims, which can collectively help answer questions relevant to people with craniofacial anomalies, their families and their health-care providers.
3. COLLABORATIVE CLINICAL RESEARCH

At the consensus meeting held in Geneva in November 2000, three themes and several priorities for clinical research were established (WHO, 2002).

Evidence-based care

The focus is to replace current uncertainty and confusion about clinical care with sound evidence based on rigorous clinical research. A critical mass of expertise in clinical research and sufficiently large samples of patients are needed for clinical trials with adequate power. Initial efforts should include:

- trials of surgical methods for various orofacial cleft subtypes, not just unilateral clefts;
- trials of surgical methods to correct velopharyngeal insufficiency;
- trials of use of prophylactic ventilation tubes (grommets) for middle-ear disease in patients with cleft palate;
- trials of adjunctive procedures in the care of patients with clefts, especially those that place a burden on the patient, family, or medical services, such as presurgical orthopaedics, primary dentition, orthodontics and maxillary protraction;
- trials of methods for managing perioperative pain, swelling and infection, and nursing;
- trials of methods to optimize feeding before and after surgery;
- trials addressing the special circumstances of care in developing countries in respect of surgical, anaesthetic and nursing care; and
- trials of various types of speech therapy, orthodontic treatment and counselling.

Collaborative groups should be set up, or the networking of existing groups should be improved, to standardize outcome measures, in particular on psychological and quality-of-life measures and economic outcomes. For rare interventions, prospective registries should be established to hasten collaborative monitoring and critical appraisal, equivalent to phase-I trials. Relevant topics would be craniosynostosis surgery, ear reconstruction, distraction osteogenesis for hemifacial macrosomia and other skeletal variations, midface surgery in craniofacial dysostosis and correction of hypertelorism.

Quality improvement

Methods are needed for monitoring and improving the delivery of clinical services. International adoption of guidelines for the provision of clinical services and for the maintenance and analysis of minimum clinical records of care of patients with clefts is proposed. Various independent registries of clinical outcomes exist, and efforts should be made to harmonize their data.

Access and availability

Strategies are needed to maximize access to adequate levels of care by all affected persons, irrespective of nationality. In large parts of the world, routine public health services are unable to afford treatment for craniofacial anomalies. Three general approaches can be identified: high-volume indigenous centres of excellence, contracts between nongovernmental
organizations and local hospitals and volunteer short-term surgical missions. These efforts could be sustained in the long term by:

- a survey of the charitable organizations involved and the scale of their work;
- appraisal of the cost–effectiveness and clinical effectiveness of various models of aid;
- promotion of dialogue between various nongovernmental organizations to reach agreement on codes of practice and on the most appropriate forms of aid for local circumstances, with emphasis on support that favours indigenous long-term solutions;
- initiation of clinical trials on specific surgery in developing countries, one-stage operations, optimal late primary surgery, anaesthesia protocols (e.g. local anaesthetic, inhalation sedation) and antisepsis; and
- establishment of common core protocols for genetic, epidemiological and nutritional studies.

Globalization presents many challenges to equal access to quality treatment for clefts, related to cultural differences, economic, social and environmental factors and the sustainable development of countries. Furthermore, there are limited global health resources for the care of persons with orofacial clefts. The largest numbers of newborns with oral clefts are found in countries with high birth rates, where there are invariably limited resources for addressing the problem (Lee, 2002). Resource-rich countries with high technology and good health-care systems have fewer patients, while developing countries with minimal resources have high loads and fewer systematically arranged facilities and support. One way of balancing the needs of well-developed and developing countries would be to institute an international collaborative programme of research and care, involving exchange of knowledge, innovations, technology and communication.

3.1 REGIONAL PERSPECTIVES

Several regions were appraised with regard to the possibilities for and obstacles to collaborative clinical research.

Africa: Clinical resources for craniofacial anomalies are scarce in sub-Saharan Africa because of prevailing economic problems and the high prevalence of communicable diseases, particularly AIDS. For example, in Namibia, despite a high reported incidence of clefts, there are no surgeons who operate on persons with this anomaly. In South Africa, the wealthiest sub-Saharan country, there are about 12 centres for cleft surgery, but they tend to work independently, with no common protocols for quality improvement. Few formal studies of craniofacial anomalies in sub-Saharan Africa have been undertaken. A regional ‘good practice’ reference archive for this region would be valuable.

A number of centres exist in the cities of North Africa, but, as elsewhere in Africa, a survey has yet to be undertaken to identify sites that could participate in collaborative research.

Australia and New Zealand: There are well-developed services in many cities in Australia and New Zealand, although the case load is quite low in some, limiting the potential for collaborative research. Coordination is nevertheless ensured through the Australia and New Zealand Craniofacial Association, and one centre supports centres in Indonesia and Malaysia.
China: In China, there appears to be a large unmet need for treatment of clefts and other craniofacial anomalies. An existing network of several large surgical centres could, however, act as a research partner. Treatment is not free, and follow-up is difficult, speech therapists being few. Of persons who undergo surgery for clefts, only 30% are operated in the first year of life. Thus, surgical trials are needed to define preferable operative techniques for more mature patients. A survey of clinical services and potential collaborating sites would be valuable, as would formulation of a strategy for quality improvement and a ‘good practice’ archive.

Europe: European clinical services were surveyed in 2001 (Shaw et al., 2001). Most of the problems arise from fragmentation of care in numerous small centres. Nevertheless, adoption of consensus recommendations has resulted in restructuring, at least with regard to services for clefts. Several international research collaborations are under way and, within the European Collaboration in Craniofacial Anomalies (EUROCRAN) programme, initiated in 2001, the European Commission is funding a series of multinational projects to extend the network.

India: The Indian subcontinent has not yet been surveyed regarding services and research capability for craniofacial anomalies or clefts. An overview, which might be reasonably representative of neighbouring countries, indicates that there are high levels of unmet need and that access is problematic, as most of the population live in rural communities. Several hundred surgeons are trained in cleft surgery, and there are several large university hospitals, but no protocols for quality improvement exist. The subcontinent undoubtedly has numerous potential partners for clinical trials, although funding follow-up studies will be a challenge.

Latin America and the Caribbean: No survey has yet been conducted on clinical services and research capability in this area. Mexico has at least one large centre in which clinical trials have been completed (Ysunza et al., 1998; Pamplona, Ysunza & Jimenez-Murat, 2001; Ysunza et al., 2001) and which is recognized as a centre of excellence in the region. Brazil also has a centre of excellence, at Bauru. Elsewhere in Latin America, there is probably much unmet need.

South-East Asia: Singapore has already embarked on a surgical trial, in collaboration with a large centre of excellence in Taipei, China (www.nncf.org; www.cgsc.org.tw), resulting in extensive research capability. In Indonesia, there is much unmet need, but six teams for treating clefts are established, which would be potential sites for research collaboration. Centres in Indonesia and Malaysia are collaborating in epidemiological, nutritional and genetic research with agencies in Australia, Europe, Singapore and elsewhere. The local incidence of craniofacial anomalies, such as frontal encephalocele, is reported to be high, and these might be useful targets for multidisciplinary research.

As in Europe, services for treatment of clefts in Japan are fragmented in small centres; however, the Japanese Cleft Palate Association has begun discussions on inter-centre studies and clinical trials. In the Republic of Korea, several high-volume centres are potential sites for collaborative research, and the Korean Cleft Palate Association has begun discussion on inter-centre studies.
Middle East: Much unmet need has been reported in the Middle East, where there are few established centres for treating craniofacial anomalies. A number of university hospitals in the region would be potential partners in research.

North America: North America also has fragmented services for treatment of clefts and other craniofacial anomalies, and it is therefore difficult to obtain sufficient numbers of patients for clinical trials. The emergence of health management organizations is particularly conducive to the fragmentation of services and dissipation of established teams. Lessons might be drawn from the Childhood Cancer Study Group, which has achieved a high level of coverage in the United States, as a result of which a high proportion of affected children are enrolled in trials (Shochat et al., 2001).

The American Cleft Palate–Craniofacial Association has promoted adequate team care, published several sets of guidelines and initiated the Craniofacial Outcomes Registry.

3.2 EUROCLEFT: AN EXPERIMENT IN INTER-CENTRE COLLABORATION

The Eurocleft initiative consists of three elements: a cohort study, a clinical network and studies of treatment within the EUROCRAAN research programme.

Cohort study

The Eurocleft cohort study was initiated in the late 1980s as an inter-centre comparison of the records of 9-year-old children with complete unilateral cleft lip and palate. Its aim was to overcome, at least partly, some of the limitations and potential biases associated with comparisons of outcomes described in reports from single centres. Full accounts of the methods and findings have been presented elsewhere (Asher-McDade et al., 1992; Mars et al., 1992; Mølsted et al., 1992; Shaw et al., 1992a,b; Mølsted et al., 1993a,b). Five of the original six teams agreed to continue follow-up of the cohort to the age of 17. The stated surgical protocols of the respective centres are shown in Table 4.

Outcomes at age 9: Several differences were found among the centres for children aged 9 years. Most reflected differences between centres D and F and the others. Patients who had been treated at centre D showed flattening of the nose, reduced nasal prominence, a short upper lip and a concave profile, and patients treated at centre F patients also had a retracted upper lip and a relative reduction in upper face height. Patients treated at centre D also had a more retruded skeletal profile than those treated at centre B (Figure 3). Major differences were seen in the dental arch relationship (Figure 4). Whereas only 7% of cases treated at centre E were considered to require osteotomy, almost half (48%) of those treated at centre D did so. Success or failure could not be ascribed to particular aspects of the surgical protocols, but the poor outcomes appeared to be related to decentralized services without consistent protocols.

Follow-up: The aims of the follow-up were to quantify the burden of care imposed by the respective protocols; to see whether the ranking of centres for different outcomes at age 9 was predictive for equivalent outcomes at age 17; to assess the satisfaction of patients and parents with the care received and to explore the relations with outcome and burden (Brattström et al., 2005; Semb et al., 2005a,b; Mølsted et al., 2005). A separate comparison of speech outcomes was carried out among the children when they reached 11–14 years (Grunwell et al., 2000).
<table>
<thead>
<tr>
<th>Age</th>
<th>Centre A</th>
<th>Centre B</th>
<th>Centre D</th>
<th>Centre E</th>
<th>Centre F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth</td>
<td>Presurgical orthopaedics (Hotz)</td>
<td>Presurgical orthopaedics (extra-oral strapping)</td>
<td>Presurgical orthopaedics (T-traction)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 months</td>
<td>Lip closure (Millard, Skoog)</td>
<td>Lip and hard palate closure (Tennison, vomer plasty)</td>
<td>Lip (varied methods and timing)</td>
<td>Lip and hard palate closure (Millard, vomer plasty)</td>
<td></td>
</tr>
<tr>
<td>5 months</td>
<td></td>
<td></td>
<td></td>
<td>Lip closure (modified Skoog, Tennison-Randall) and bone grafting</td>
<td></td>
</tr>
<tr>
<td>9 months</td>
<td>Soft palate closure (von Langenbeck, Perko, Wardill, Kriens)</td>
<td>Hard and soft palate closure (varied methods and timing)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18 months</td>
<td></td>
<td></td>
<td></td>
<td>Soft palate closure (modified von Langenbeck)</td>
<td>Hard and soft palate closure (Veau-Wardill-Kilner pushback)</td>
</tr>
<tr>
<td>24 months</td>
<td>Soft palate closure (Wardill pushback)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8–11 years</td>
<td>Bone grafting and hard palate closure</td>
<td>Bone grafting</td>
<td>Bone grafting</td>
<td>Bone grafting</td>
<td></td>
</tr>
</tbody>
</table>
Figure 3. Superimposition of mean plots for craniofacial skeletal structures at age 9 years on cephalometric measurements, in two centres participating in the Euroclef cohort study
Reproduced with permission, from Bearn (2000)

Figure 4. Goslon scores for individual patients at age 9 years by centre participating in the Euroclef cohort study
A Goslon score of 1 represents excellent maxillary prominence, and a score of 5 represents severe maxillary retrusion. This outcome variable can indicate the need for subsequent maxillary osteotomy: cases with scores below 3.5 at this age are probable candidates for osteotomy in their late teens.

The intensity of treatment provided by the five teams in 1976–79 was remarkably different (Table 5). Most notable was the longer hospital stay associated with presurgical orthopaedics in centres D and F. Patients in centre D also had more orthodontic visits before treatment and review and a larger overall number of operations than the other centres. Discussion with these centres indicated that the large differences in the intensity of treatment were due not to clinical need but rather to differences in beliefs and historical practices that had shaped the clinical protocols of the period.
Table 5. Intensity of treatment provided by five teams participating in the Eurocleft cohort study in 1976–79

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Centre A</th>
<th>Centre B</th>
<th>Centre D</th>
<th>Centre E</th>
<th>Centre F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surgery</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean number of operations</td>
<td>4.8</td>
<td>3.3</td>
<td>6.0</td>
<td>4.4</td>
<td>3.5</td>
</tr>
<tr>
<td>Mean days in hospital</td>
<td>33</td>
<td>31</td>
<td>60</td>
<td>24</td>
<td>26</td>
</tr>
<tr>
<td>Early orthopaedics</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Months of treatment</td>
<td>13</td>
<td>0</td>
<td>15</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>No. of visits</td>
<td>11</td>
<td>0</td>
<td>8</td>
<td>0</td>
<td>17</td>
</tr>
<tr>
<td>Days in hospital</td>
<td>0</td>
<td>0</td>
<td>60</td>
<td>0</td>
<td>146</td>
</tr>
<tr>
<td>Orthodontic treatment</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment length (years)</td>
<td>5.6</td>
<td>3.3</td>
<td>8.5</td>
<td>3.5</td>
<td>4.0</td>
</tr>
<tr>
<td>No. of visits for treatment</td>
<td>52</td>
<td>41</td>
<td>54</td>
<td>33</td>
<td>47</td>
</tr>
<tr>
<td>No. of visits for follow-up</td>
<td>11</td>
<td>23</td>
<td>42</td>
<td>16</td>
<td>25</td>
</tr>
<tr>
<td>Total no. of visits</td>
<td>63</td>
<td>64</td>
<td>94</td>
<td>49</td>
<td>72</td>
</tr>
</tbody>
</table>

The statistical method used to compare the five centres was a general linear mixed model applied to longitudinal data (Diggle, Liang & Zeger, 1994). Variance terms were included in the model to account for between-person variation in the intercept as well as fixed factor for assessment point (9, 12, 17 years) and centre. Full details have been reported elsewhere (Shaw et al., 2005).

As Figure 5 indicates, the scores for dental arch relation tended to improve in centres A, B and E, but not in D and F. There was a consistent relation over time for most cephalometric variables, e.g. soft-tissue profile (Figure 6), and for nasolabial appearance.

![Figure 5. Mean scores for dental arch relations at ages 9, 12 and 17 years in centres participating in the Eurocleft cohort study](image)
Figure 6. Mean soft-tissue profiles (facial convexity angle: soft tissue sub-spinale–nasion–supra-mentale) at ages 9, 12 and 17 years in centres participating in the Euroclef cohort study

Not surprisingly, follow-up of these five cohorts of patients from the age of 9 to the age of 17 confirmed the main finding of the first report: that some centres continued to achieve considerably better outcomes than others, at all ages. Perhaps more surprising is the lack of association between the intensity of treatment and the final outcome (Tables 6–8). Especially ironic is the finding that the two centres with the highest intensity of early treatment (hospitalization in order to perform presurgical orthopaedics) achieved the lowest rankings for eventual outcome (Table 6). Patients in the centre with the least favourable outcomes (centre D) also underwent the longest orthodontic treatment and the largest number of orthodontic visits. This result appears to be due partly to the complexity of centre D’s orthodontic treatment protocols, with almost continuous treatment from the eruption of primary dentition, and partly to the unfavourable dentofacial outcomes of primary surgery. (It is now almost 30 years since treatment was begun for these cohorts of patients, and hospitalization for orthopaedics has long been discontinued.)

Table 6. Association between outcome and frequency of treatment in centres participating in the Euroclef cohort study

<table>
<thead>
<tr>
<th>Objective ranking</th>
<th>Centre</th>
<th>Months of treatment</th>
<th>No. of visits</th>
<th>Days in hospital</th>
</tr>
</thead>
<tbody>
<tr>
<td>Best</td>
<td>E</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>13</td>
<td>11</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>5</td>
<td>17</td>
<td>146</td>
</tr>
<tr>
<td>Worst</td>
<td>D</td>
<td>15</td>
<td>8</td>
<td>60</td>
</tr>
</tbody>
</table>

The lack of association between treatment outcome and intensity (Tables 7 and 8) might be informative for future protocols. It justifies an emphasis on simplicity, economy and minimal
burden for the patient, rather than adherence to demanding protocols with unsubstantiated promise.

Table 7. Lack of association between outcome and intensity of treatment in centres participating in the Euroleft cohort study

<table>
<thead>
<tr>
<th>Objective ranking</th>
<th>Centre</th>
<th>Years of treatment</th>
<th>No. of visits</th>
<th>No. of operations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Treatment</td>
<td>Check-up</td>
<td></td>
</tr>
<tr>
<td>Best</td>
<td>E</td>
<td>3.5</td>
<td>33</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>5.6</td>
<td>52</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>3.3</td>
<td>41</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>4.0</td>
<td>47</td>
<td>25</td>
</tr>
<tr>
<td>Worst</td>
<td>(D)</td>
<td>8.5</td>
<td>54</td>
<td>42</td>
</tr>
</tbody>
</table>

Perhaps the most perplexing finding is the inconsistency between objectively rated outcomes and the satisfaction of patients and parents. In some instances, the highest levels of dissatisfaction with treatment outcome were reported by persons treated at the centres with the best objective ratings (Table 8). The possible reasons for this disparity have been discussed elsewhere (Semb et al., 2005b); they highlight the need for concerted work on understanding and measuring patient satisfaction and the provision of more holistic care.

Table 8. Lack of association between outcome and satisfaction with treatment in centres participating in the Euroleft cohort study

<table>
<thead>
<tr>
<th>Objective ranking</th>
<th>Percentage of respondents dissatisfied with</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nasal appearance</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Best</td>
<td>A 64</td>
</tr>
<tr>
<td></td>
<td>E 32</td>
</tr>
<tr>
<td></td>
<td>B 14</td>
</tr>
<tr>
<td></td>
<td>D 45</td>
</tr>
<tr>
<td>Worst</td>
<td>F 33</td>
</tr>
</tbody>
</table>

Benefits and limitations of inter-centre comparisons: Professionals entrusted with the provision of health care have an obligation to review the success of their practices and, where shortcomings are revealed, to take remedial action. Such efforts should constitute a continuous cycle, sometimes known as ‘clinical audit’, which has been defined as “the systematic critical analysis of the quality of care including procedures for diagnosis and treatment, the use of resources and the resulting outcome and quality of life for the patient” (Long, 1996). Often, clinical audit is divided into evaluating the process of care (the way in
which it is delivered) and the outcomes of care (what is achieved). Cycles of outcome audit are more easily established when the intervention is common and the consequences are clearcut and quickly observable. Audit of the treatment of clefts is therefore a considerable challenge, because of the lengthy follow-up required, the complexity, subtlety and number of relevant outcomes and, above all, the relatively small number of cases.

Inter-centre collaboration still offers significant advantages, by providing insight into the processes and outcomes of treatment of comparable services elsewhere, the establishment of future goals and the exchange of clearly successful practices. The participation of two centres in the United Kingdom in the comparisons of 9-year-olds in this series stimulated national assessment and subsequent reorganization of services for clefts (Bearn et al., 2001).

Perhaps the greatest benefit of inter-centre comparisons is the cooperative spirit that they foster and a gradual diminution of rivalry. It is no coincidence that most of the participants in this cohort study and the related Scandcleft study (Friede et al., 1991; Enemark et al., 1993) are now participating in multicentre randomized trials. Close work also allows sharing of successes and failures and facilitates fruitful collaboration, such as on rating scales and on new research questions.

A fundamental limitation of inter-centre comparisons is the inability to distinguish the effects of individual elements of a centre’s protocol on its outcomes, such as the influence of the personnel who deliver a protocol. In the series described above, the two centres with the poorest outcomes used presurgical orthopaedics (of various kinds) but had disappointing results for other reasons, such as participation of low-volume surgeons in centre D and the use of early bone grafting in centre F. This shows that better results are achieved elsewhere without the additional burden and cost of orthopaedics. In the event, centre D discontinued early orthopaedics, adopted the protocols of centre E and changed the way care was delivered from low- to high-volume specialists; while centre F discontinued both early bone grafting and early orthopaedics.

While a series of inter-centre comparisons with large numbers of cases would eventually allow one or another centre to emerge as the best for particular outcomes, this exercise would be of limited value to the clinical community as a whole. Only protocols can be transferred, not clinicians. Inevitably, the definitions of good and bad protocols or good and bad elements of protocols require the explicit setting of a randomized trial. The main distinction between audit and research is that clinical research (principally clinical trials) determines optimal procedures that can be transferred to an infinite number of centres; clinical audit determines whether they have been transferred successfully.

Despite the advantages of collaboration, it has two important limitations as a routine method of clinical audit. First, multiple between-group comparisons increase the sample of cases required, and, secondly, the logistics and costs may be prohibitive. For most teams, reassurance that they are achieving outcomes coherent with competent practice might be sufficient, and they might not need to know their ranking relative to other centres.

One strategy would be to assemble an archive of relevant clinical records that are considered to represent good practice, perhaps drawn from consecutive cases seen in respected centres. As long as other centres collect equivalent consecutive records, matching of cases on relevant characteristics would allow comparisons to be made. This could be done by the centre in question ‘behind closed doors’ or, if preferred, more transparently. For example, the centre’s cases could be mixed with archived cases and rated independently or rated by a panel consisting of the centre’s own personnel who are unaware of the source of the case. With appropriate techniques, such exercises could be managed via the Internet to maximize use of time, although the advantages should be set against loss of the benefit of face-to-face professional interaction and discussion. (See under EUROCRAN below.)
An alternative or complementary approach is use of a registry. Prospective entry of newborn patients would have the particular advantage of establishing a list of consecutive cases that could be used to affirm that follow-up and exclusion bias are not confounders of later comparisons.

The simplest system is for teams to obtain a minimal set of objective measurements on their cases that are subject to negligible measurement error, e.g. prominence of maxillary incisors (overjet) (Morris, Roberts & Shaw, 1994). These could be compared in tables or graphs summarizing ‘good practice’ outcomes. One way of doing this would be to convert values for the reference data to a normal distribution curve. Individual centres could then plot their mean value for a particular characteristic on the curve to determine the extent of any difference from the norm and its statistical significance (Bearn, 2000; Figure 7).

**Figure 7. A simple two-step system for checking the outcomes for a series of consecutive patients with unilateral cleft lip and palate**

Reproduced from Bearn (2000) with permission

Based on the mean value for overjet on the non-cleft central incisor. No special equipment or statistical skills are required, and any appropriate distribution can be selected as the reference base.
All the above would be facilitated by a broad consensus. At the previous meeting held under the auspices of WHO (2002), there was global consensus on recommendations for record-keeping (www.who.int/genomics/publications/en/index.html). Minimum records were defined as those kept for a range of cleft types and treatment episodes by centres that might wish to participate in future international comparisons. In the meantime, researchers should establish norms for outcomes of a range of clefts and undertake collaborative work to refine methods for comparison. Further work on the long-term reliability of early outcome assessment is also a priority if unsuccessful protocols are to be eliminated more rapidly. Longitudinal archives on clefts from clinics around the world could make a significant contribution to this work, by defining which early measurements are most likely to be predictive over time.

Clinical network

In the 1990s, competitive funding initiatives to encourage multi-state cooperation were announced within the European Union. A successful application by the participants of the cohort study provided an opportunity to broaden its original goals. The project ran for 3 years, between 1996 and 1999, in Member States. Teams from central and eastern European states joined the network for a further year (Shaw et al., 2001). The goal of the project was to improve the effectiveness and efficiency of care for European children with clefts.

Each member of the project steering group had responsibility for linking with professionals in clefts in a group of countries for which they had particular expertise, in terms of language or experience. This strategy facilitated the process of nominations by the registered teams, who were asked to suggest individuals in their countries to act as key delegates and to provide a general link to the network. The criteria for nomination included a requirement that the person be an expert in the field of cleft care, with good knowledge and a general overview of the organization of care for clefts in their country. National representatives were chosen from among persons nominated by their colleagues, ensuring a spread of clinical specialties in order to make the network multidisciplinary.

It was evident from previous investigations that the quality of cleft care would vary enormously among regions owing to organizational and surgical differences. It was considered inappropriate to prescribe specific technical protocols for the timing, sequence and design of surgical procedures, orthodontic treatment, speech therapy and so forth, as many of these choices remain highly controversial, and practices vary greatly across Europe. It was hoped, however, that improved organization could be promoted by making recommendations about policies and practice guidelines. Therefore, the steering group and the national representatives forming the Eurocleft network met in a series of workshops to identify areas of agreement and to draw up minimum recommendations in the form of policy statements (Shaw et al., 2001; www.eurocran.org/content.asp?contentID=776) and practice guidelines (www.eurocran.org/content.asp?contentID=777&sid=142954). It was proposed that improvements in the quality of surgical skills would best be achieved by promotion of clinical audit. If the method for assessing outcomes could be defined and established by consensus, European centres could evaluate their own quality of care, compare it with that achieved by other centres and make improvements. Relevant guidelines were agreed at further workshops and circulated to all registered centres, national health authorities and governments (Shaw et al., 2001; www.eurocran.org/content.asp?contentID=779&sid=143236).

A European register of centres where clefts are treated was assembled, resulting in 201 centres in 30 countries. A survey across the network revealed great diversity in national policies, service organization and clinical practices. The greatest challenge to the delivery of adequate service across much of Europe appeared to be organizational. Compliance with key recommendations, such as creation of fully comprehensive teams with a sufficiently large case load to optimize clinical experience and outcome evaluation (e.g. 40 new cases per year
per surgeon, orthodontist and speech therapist), is being achieved in only a few countries. In the workshops, it was clear that the greatest obstacles to progress were human, not economic.

In some countries, policies for the provision of care of persons with clefts are well established. In Denmark, the provision of primary surgery by one surgical team has been formalized in law since 1937. In Norway, a long-standing arrangement of two national centres was confirmed by national policy stating that no care can be provided elsewhere unless requested by one of those teams. In the Czech Republic, Latvia and Slovakia, well-established regional centres are continuing to provide comprehensive services following recent political and economic changes. Some moves towards privatization of health care are considered to present a risk of possible destabilization of established centres. At the other extreme, the concept of team care is still to be adopted. National representatives from Bulgaria, Greece, Italy, Portugal, Romania, Spain and Ukraine, for instance, reported that much care was still provided by individual clinicians, working in isolation, and not in government-funded centres.

Involvement of a wide variety of surgical specialties was reported. Those mentioned most often were plastic surgery (94 of the 201 centres; 46.7%), maxillofacial surgery (59; 29.4%), paediatric surgery (22; 10.9%) and ear-nose-and-throat surgery (4; 2%). The clear lack of a sound evidence base for selecting treatment protocols was reflected in the remarkable diversity of practices across Europe for the surgical care of just one cleft sub-type, unilateral complete cleft of lip, alveolus and palate. No single European team practised the same protocol as any other (except for those participating in the clinical trial described below). That is to say, 201 teams were practising 194 different protocols for one cleft sub-type. Seventeen possible sequences of operation to close the cleft are practised (Table 9). Although 86 (42.8%) teams closed the lip at the first operation and the hard and soft palate together at the second, almost every other conceivable sequence appeared to be practised somewhere. Thus, the total number of operations performed to complete closure of the cleft varied from one (10; 5%), to two (144; 71.1%), to three (43; 21.9%) and four (4; 2%). About half of the registered teams used presurgical orthopaedics, of which 67 (65%) used it routinely. Passive plates were used by 74 (70%) teams, and some teams also used the plate as a feeding plate.

Of the 28 European countries involved in the network, 18 have some form of organization for parents. Such groups vary in activity, effectiveness and influence and take various forms. Some function as officially established national associations, while others operate on a less formal basis. In Germany, for instance, there are several small regional organizations and one major group which takes part in annual meetings with the professional association. Denmark has a well-established group for parents, which has been functioning for over 25 years. Interestingly, in Bulgaria, the parent group is working to bring together national cleft specialists with a view to instigating an association for professionals.

Achieving optimal standards of care for persons with clefts across Europe remains a challenge. The basic principles of care described in the Eurocleft policy statements and practice guidelines are hardly revolutionary; indeed, they describe what most persons (including those in clinical practice or government) would wish for their own children, regardless of country of birth. The potential strength of a European network is that examples of good practice can be easily shared, challenges of a general nature can be easily recognized and understood, and solutions that have been successful in one country can be applied elsewhere. The need is obvious, as the variation among centres in sequence, technique and timing of cleft repair could hardly be greater.
### Table 9. Sequences of operation used to close a cleft among 201 centres in Europe

<table>
<thead>
<tr>
<th>First operation</th>
<th>Second operation</th>
<th>Third operation</th>
<th>Fourth operation</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lip closure</td>
<td>Hard and soft palate closure</td>
<td></td>
<td></td>
<td>42.8</td>
</tr>
<tr>
<td>Lip closure</td>
<td>Soft palate closure</td>
<td>Hard palate closure</td>
<td></td>
<td>15.3</td>
</tr>
<tr>
<td>Lip and hard palate closure</td>
<td>Soft palate closure</td>
<td></td>
<td></td>
<td>10.4</td>
</tr>
<tr>
<td>Lip and soft palate closure</td>
<td>Hard palate closure</td>
<td></td>
<td></td>
<td>10.0</td>
</tr>
<tr>
<td>Lip, hard and soft palate closure</td>
<td>Soft palate closure</td>
<td>Hard palate closure and alveolar bone grafting</td>
<td></td>
<td>5.0</td>
</tr>
<tr>
<td>Lip closure</td>
<td>Soft palate closure</td>
<td></td>
<td>Hard palate closure and alveolar bone grafting</td>
<td>3.5</td>
</tr>
<tr>
<td>Lip and soft palate closure</td>
<td>Hard palate closure and gingivo-alveoloplasty</td>
<td></td>
<td></td>
<td>2.5</td>
</tr>
<tr>
<td>Lip and alveolar closure</td>
<td>Hard and soft palate closure</td>
<td></td>
<td></td>
<td>2.0</td>
</tr>
<tr>
<td>Soft palate closure</td>
<td>Lip and hard palate closure</td>
<td></td>
<td></td>
<td>2.0</td>
</tr>
<tr>
<td>Lip adhesion</td>
<td>Lip closure</td>
<td>Soft palate closure</td>
<td>Hard palate closure</td>
<td>1.5</td>
</tr>
<tr>
<td>Lip and alveolar closure</td>
<td>Soft palate closure</td>
<td>Hard palate closure</td>
<td></td>
<td>1.0</td>
</tr>
<tr>
<td>Lip adhesion</td>
<td>Lip, hard and soft palate closure</td>
<td></td>
<td></td>
<td>1.0</td>
</tr>
<tr>
<td>Lip adhesion</td>
<td>Lip and hard palate closure</td>
<td>Soft palate closure</td>
<td></td>
<td>1.0</td>
</tr>
<tr>
<td>Hard and soft palate closure</td>
<td>Lip closure</td>
<td></td>
<td>Hard palate closure</td>
<td>0.5</td>
</tr>
<tr>
<td>Hard and soft palate closure and alveolo-plasty</td>
<td>Hard palate closure and alveolar bone grafting</td>
<td></td>
<td></td>
<td>0.5</td>
</tr>
<tr>
<td>Lip and soft palate</td>
<td>Hard palate closure and gingivo-alveoloplasty</td>
<td></td>
<td></td>
<td>0.5</td>
</tr>
<tr>
<td>Lip adhesion</td>
<td>Lip closure</td>
<td>Hard and soft palate closure</td>
<td></td>
<td>0.5</td>
</tr>
<tr>
<td>Lip closure</td>
<td>Soft palate closure</td>
<td>Gingivo-alveoloplasty</td>
<td>Hard palate closure</td>
<td>0.5</td>
</tr>
</tbody>
</table>

Some obstacles, especially those arising from specific local circumstances and personalities, need local solutions. These obstacles include:

- the egotism of individuals unwilling to discontinue the practice of treating a few children each year;
- competition between specialties for pre-eminence in the field, e.g. plastic versus maxillofacial versus paediatric versus ear-nose-and-throat surgery;
- local pride, each hospital, town or region wanting its own small team or local service;
- the necessity for teaching hospitals to cover a spectrum of clinical practice; and
- lack of responsiveness of health authorities at local and national level.

All the above problems have confronted the United Kingdom in the recent past, and a national review was instigated by the Government’s Clinical Standards Advisory Group. The review included a national survey, which revealed that Britain’s fragmented, decentralized services were achieving a low standard of clinical care in most areas. As a result, the Government instructed regions to provide care from a single regional centre, each with a fully comprehensive specialist team, typically with two to three surgeons, each responsible for no fewer than 40 new cases for primary surgery per year. In this instance, Government interest was essential in improving services when voluntary methods failed (Bearn et al., 2001).

Several changes were subsequently made in service organization across Europe, e.g. some restructuring of services, with more high-volume centres, better funding of treatment and attempts to improve record-taking and national registration for inter-centre research (Shaw et al., 2001). In some countries, however, there is still resistance to change, with competition for patients between clinical disciplines and lack of collaboration between teams. Often, at government level, care of persons with cleft lip and palate is not seen as a priority, and, in some countries, difficulties with the insurance system lead to fragmentation of services.

**European Collaboration in Craniofacial Anomalies (EUROCRAN)**

The European Collaboration in Craniofacial Anomalies (EUROCRAN), the final element of the Eurocleft initiative, began in 2000. It was conceived to improve European research on clefts and related craniofacial anomalies. It was designed to take forward several lines of research arising from the Eurocleft cohort study and related work in the Eurocleft clinical network. It involves a new partnership with geneticists and epidemiologists assembled under a European Science Foundation programme. It will gain from the extended capability of the research group and from more possibilities for recruitment to clinical and genetic projects via the Eurocleft clinical network. The project has a website to facilitate dissemination and participation (www.eurocran.org) and consists of seven work packages.

1. **Randomized controlled trials of primary cleft surgery**

A group of multicentre randomized trials of primary surgery for infants with complete unilateral cleft lip and palate is designed to test four variations of surgical techniques in three concurrent trials. Infants have been randomized to a surgical method common to all three trials or to the usual local method. A total of 450 infants is being recruited.

2. **A prospective registry of patients undergoing distraction osteogenesis**

This study has been carried out in two parts: the first is a web-based survey of the practice of distraction osteogenesis in Europe, and the second is a prospective registry of cases of distraction. The aim is to provide professionals with guidelines and recommendations for treatment and future research. To date, 150 patients have been registered. The site can be accessed at www.eurocran.net.

3. **Two cohort studies of surgical outcome in unilateral cleft lip and palate**

A cohort of infants with unilateral cleft lip and palate at five centres is being registered with the study coordinating centre before receiving the surgery usually practised locally. At pre- and postoperative times, specified data and clinical records are obtained. A second cohort of consecutively treated 5–7-year-olds with unilateral cleft lip and palate who have previously received surgery are being recalled for standardized assessment. A comparison of perioperative complications and operating costs will be made for the first cohort and of dentofacial growth and nasolabial appearance for the second.
(4) Setting up a good practice archive

An archive of the clinical records of patients treated consecutively for unilateral cleft lip and palate in three centres in Europe that are representative of good practice has been assembled. The records include dental casts, photographs and cephalograms. This archive is a physical resource, which allows teams to monitor the quality of their care. A number of centres (in Tartu, Estonia; Milan, Italy; and Riga, Latvia) have used the archive to compare clinical outcomes as a series of pilot studies. The archive will also be available as a web-based library of clinical outcomes.

(5) A study of gene–environment interactions

A multinational population-based patient–parents triad study is investigating the role of genetic susceptibility polymorphisms and gene–environment and gene–gene interactions in the etiology of orofacial clefting. The method includes a maternal interview on diet and other exposures around conception and DNA sampling of the mother, father and child for gene variant analysis. Over 1000 triads will be recruited into the study.

(6) A chromosome approach to identifying orofacial clefting genes

Patients with orofacial clefting and with apparently balanced chromosome rearrangements have been identified and their breakpoints and clinical phenotypes catalogued. A bank of immortalized cell lines has been established from patients in whom two or more specific breakpoints have been associated with orofacial clefts. Genes that have been interrupted by two or more breakpoints have been identified and are being fully characterized and screened for mutations and polymorphisms that might be used in the study of gene–environment interactions (FitzPatrick et al., 2003).

(7) Molecular diagnosis of monogenic craniofacial anomalies

Sensitive molecular assays for the mutations involved in a number of craniofacial malformation syndromes have been developed, with Treacher Collins syndrome as a model. The techniques are being disseminated to other laboratories (Kondo et al., 2002).

The global context

International collaboration is essential in order to have having adequate numbers of samples for research on the etiology, treatment and prevention of craniofacial anomalies, and also for assembling a critical mass of clinical researchers and basic scientists in fields such as molecular biology, genetics, biochemistry and epidemiology. Those who have taken part in the European experiment described above recognize the challenges of working internationally, including cultural and language barriers, although the latter has become a relatively minor problem for persons fortunate to have English as their first language, given its widespread use. The practicalities of rapid communication have, of course, also been hugely enhanced by the advent of e-mail and teleconferencing.

The requirement of some institutions that their employees demonstrate their performance in research by being listed as the first author on publications has been a deterrent to collaboration. This can be offset by participation in more substantial, adequately powered research projects than those generally arising in a local context and by the emergence of new conventions of multiple authorship in leading journals.

The funding of international research is a mixed challenge. The multicentre advantages of sample recruitment and mix of skills can result in strong applications, but relatively few national funding agencies are keen to see too much of their budget flow beyond their national borders. Strategies are needed to promote international coordination of funding agencies.
Craniofacial anomalies are a highly diverse group of complex congenital anomalies. Collectively, they affect a significant proportion of global society. As for many other aspects of the human condition, there are many advantages in overcoming the challenges of international collaboration.

3.3 STATUS OF INTER-CENTRE COLLABORATION FOR CLINICAL RESEARCH IN THE UNITED STATES

Although the United States is a leader in many areas of the management of patients with clefts and craniofacial anomalies, has many well-organized teams and the largest professional organization in the world in this field (the American Cleft Palate–Craniofacial Association), the country has been unable to generate any significant momentum in inter-centre, collaborative clinical research. The opportunities for this would seem to be immense. Over 100 teams have annual case loads of craniofacial anomalies of over 50 and therefore can provide sample sizes adequate for outcome audits and clinical trials in a timely, cost-effective manner. In a recent survey by the American Cleft Palate–Craniofacial Association of its members, 266 of 555 respondents (48%) indicated an involvement in research; of the 289 who did not, an additional 99 expressed a desire to become involved. In addition, 200 of the respondents reported that they were either currently involved in collaborative research or expressed an interest or willingness to participate. The numbers are likely to be even greater, as the response represented approximately 20% of the membership of the Association. While the research topics of interest mentioned by the respondents covered nearly all aspects of craniofacial anomalies, a significant portion related to clinical topics. Of the current and ongoing research projects listed, however, relatively few were statistically sound, unbiased, inter-centre assessments and comparisons of clinical outcomes; few, if any, were randomized controlled clinical trials. This would suggest that much of the clinical research under way in the United States generates little useful information for establishing sound evidence-based decision-making in clinical care.

Several attempts have been made at various levels to take advantage of these clinical research opportunities. At least one centre has carried out several clinical outcome assessment comparisons on the basis of the data from the Eurocleft study (Vargas, 2002; Flinn et al., 2006). Nevertheless, unlike the European experience, in which the original study generated a groundswell of support, extension of the clinical research approach throughout European centres and led to the establishment of EUROCRAN, Scandcleft and strong financial support from governmental and nongovernmental sources, the experience in the United States has been the opposite. This failure is a reflection of problems and obstacles, some of which may be unique to the United States. A number of problems were evident in the responses to the survey, including lack of funding, lack of research training and lack of time for primary care providers to engage in research projects. Another critical problem in the United States is the current health-care climate, which tends to favour cost containment and access to care over quality of care. This in turn has created a tendency for decentralization of care provision. While having a large number of centres and persons providing treatment for craniofacial anomalies improves patients’ geographical accessibility to care, it also creates fractionation of the study population, thereby reducing the probability of having access to patient samples of adequate size to allow valid research. The entire landscape is further complicated by non-comparable patient populations, non-comparable treatment records, unquantifiable differences in operator skills, and difficulties in letting go of biases. The recently enacted privacy laws in the United States also add additional steps and complications for the sharing of data in collaborative studies. Although collaborative research can be structured without violating patient privacy laws, the rigours of doing so are sufficient discouragement for many clinicians to participate. Finally, there remains a general lack of agreement between centres on minimal
Addressing the global challenges of craniofacial anomalies

standards for reporting and recording outcomes, as well as cost and ethical concerns over taking records which cannot be clearly identified as essential for diagnosis and treatment.

Potential means for overcoming these obstacles are ‘top-down’ solutions, which have been initiated by a number of groups and organizations, including facilitation of standardization of records of treatment histories and outcome data, centralization of data through a registry, and networking between individuals and centres with collaborative research interests. These initiatives have either failed or met with limited success. Most notably, the Craniofacial Outcomes Registry was an undertaking funded by the National Institute of Dental and Craniofacial Research, with the goals of establishing a craniofacial registry for collection of data on the outcomes of care; stimulating improvements in the outcomes of craniofacial health-care services by disseminating the results of treatment procedures to participants in the Registry programme; and providing information on the results of treatment to focus educational and research efforts on identified problems. After several years of funding and enrollment of over 10 000 patients from over 50 teams, the project was discontinued owing to lack of renewal funding.

The database of the American Cleft Palate–Craniofacial Association provides teams with a simple database that enables them to document patients seen and procedures performed and with a rudimentary system that can be expanded to meet their needs and those of teams collaborating in joint clinical research projects. This database is used by over 50 teams in the United States and includes demographic and treatment information on over 15 000 patients. While potentially useful for identifying patient samples that might be appropriate for trials or outcome studies, the database does not, however, contain outcome measures and would not lend itself readily to collaborative studies.

In summary, although the desire, research ability and patient samples are all readily available in the United States, failure to get centres to agree on something as basic as standardization of recording and reporting outcomes, as well as Government hurdles and a serious lack of funding, have resulted in a huge missed opportunity. The most promising solution may lie in the Eurocleft approach. With a core group of interested, experienced clinicians operating at high-volume centres, who are willing to agree on recording significant outcome measures and research protocols, and with possible guidance from persons involved in the successful Eurocleft, Scandcleft and EUROCRAN programmes, progress would still be possible.

3.4 COLLABORATIVE CLINICAL RESEARCH ON CRANIOFACIAL ANOMALIES IN SOUTH AFRICA

In August 2001, the Minister of Health of South Africa presented policy guidelines for the management and prevention of genetic disorders, birth defects and disabilities, which had been drawn up by a national task team whose activities were financed by WHO. It reviewed available knowledge of the burden of birth defects in South Africa, the resources available to respond to this burden and laid down principles, policy and practice for their management and prevention. Six priorities were designated, including cleft lip and/or palate. The guidelines also included a proposal for a medical genetics education programme for primary health-care practitioners.

South Africa has approximately 1.1 million births annually. Limited information is available on the epidemiology of craniofacial anomalies, but, on the basis of a modelled birth prevalence, it can be estimated that as many as 1570 infants with cleft lip and/or palate and another 500 infants with malformations involving the face, ears and neck are born annually. This does not include the single gene and chromosome disorders with craniofacial abnormalities or cleft lip and/or palate due to teratogens, particularly alcohol. To care for this burden, South Africa has seven academic craniofacial units, which serve the 80% of the
population that uses public health facilities. Numerous facilities are available for the remaining 20% of patients who can afford medical insurance.

One of the aims of the South African research programme will be to develop and expand a community-based birth defect surveillance system and then a birth defects registry, initially for the six conditions of highest priority. These facilities could eventually be used for research on gene–environment interactions.

Research on the association between maternal alcohol intake and cleft palate could be conducted in collaboration with the Foundation for Alcohol-related Research, a nongovernmental organization responsible for most of the research on fetal alcohol syndrome in South Africa. The academic craniofacial units also offer possibilities for research on the care of craniofacial abnormalities in a low-resource setting.

Training of primary health-care practitioners throughout South Africa in the medical genetics education programme and other programmes that are being set up should contribute to research on all birth defects, including craniofacial abnormalities. Early recognition and appropriate referral of patients with birth defects should result from these education programmes, which are in the pilot phase and will be in place in 1–2 years.

Little or no research on the genetic basis of and environmental factors associated with craniofacial anomalies has been carried out in African populations. This and comparative studies of etiological factors in other populations deserve urgent attention.

In the developed world, craniofacial and cleft abnormalities are usually treated in childhood, so it is difficult to establish whether children with these conditions have an inherent growth deficiency or whether the growth deficiency is due to the surgical procedures. For example, trigonocephaly is a craniosynostosis that is seen fairly commonly in childhood but has not been reported in adulthood, implying that these patients either die or the condition corrects itself. In Africa, patients with cleft and craniofacial anomalies sometimes do not seek treatment or seek treatment at a late date. This affords an opportunity to observe the natural history of craniofacial anomalies in the absence of surgery.

The major obstacle to research is the increasing pressure on the country’s health services due to the HIV/AIDS pandemic. Research would, however, be possible in an academic setting, financed and undertaken from outside the public health service but working in collaboration. Such partnerships would be welcomed by the National Department of Health’s Genetic Services Directorate. Africa has many other significant medical conditions that take priority because of their high incidence and impact, including malaria, malnutrition and a high infant mortality rate. Cleft and craniofacial anomalies have to compete with these conditions for research resources.

Technical expertise and equipment are lacking or absent in certain countries and in certain areas. A core group of individuals is required in each unit to coordinate research and record data. Nevertheless, while the delivery of treatment for cleft and craniofacial abnormalities is generally poor in Africa, there are good centres where these conditions are treated. These centres have technical expertise, fairly sophisticated technical equipment and good researchers, infrastructure and facilities. They could be called upon to assist and oversee any collaborative and clinical research that is undertaken. Once detailed projects have been established, the appropriate infrastructure and research capacity will have to be obtained. Resources for complex DNA and gene analysis are not currently available; however, with appropriate governance, it would be possible to collect blood samples and send them to Europe or the United States for analysis in collaborative projects.

1 Foundation for Alcohol-related Research, c/o Division of Human Genetics, National Health Laboratory Service and University of Witwatersrand, PO Box 1038, Johannesburg 2000, South Africa
3.5 DATABASES, RESEARCH AND TREATMENT FOR CRANIOFACIAL ANOMALIES IN CHINA

China could be a major partner in international collaborative research. It has a network of university hospitals and a well-established registry based at the University of Beijing. An indication of this potential is the database set up at the Peking University School of Stomatology 10 years ago, which already contains records of more than 8000 persons with cleft lip and/or palate. Current research topics are factors affecting speech after surgery for cleft palate; how hard palate ossification influences maxillary development and velopharyngeal closure; and mechanisms of compensatory articulation.

The major obstacles to significant international collaboration are lack of universal outcome measures; the language difference with regard to evaluating speech outcome; and lack of financial resources for collaborative research.

3.6 OPPORTUNITIES FOR AND OBSTACLES TO COLLABORATIVE CLINICAL RESEARCH IN JAPAN

Collaborative clinical research in Japan is still at an early stage, with limited participation in inter-centre comparisons and no participation in clinical trials. Nevertheless, two projects were conducted in Japan after the meeting held at WHO in 2000. One was a nationwide survey of the occurrence and primary surgery of cleft lip and palate made by the Investigation Committee of the Japanese Cleft Palate Association (Yamashita et al., 2003, 2004), in which 4349 newborns with cleft lip and palate in 108 institutions were surveyed. Preferences with reagrld to the method and timing of primary surgery, including presurgical orthopaedics, were identified. The study population represented 37% of the expected total number of cases, on the basis of a prevalence of cleft lip and/or palate of 1 per 500 births. Each institution saw an average of eight new patients per year (range, 2–94). For cleft lip and palate, most used one-stage closure of the palate, while about one-fifth used two-stage closure. For cleft palate, the palate was usually closed in a one-stage method. A Hotz plate was used in 52% of cases of cleft lip and palate and in 13% of cases of cleft palate.

The other study was a six-centre comparison of treatment outcome in patients with unilateral cleft lip and palate, similar to the Eurocleft study (Asahito et al., 2004; Morita et al., 2004; Negoro et al., 2004; Susami et al., 2004; Tachimura et al., 2004). The study was performed retrospectively under the leadership of Niigata University with the approval of the Japanese Cleft Palate Association. Craniofacial form, soft-tissue profile, dental arch relations, nasolabial appearance and speech at the ages of 8–10 years were evaluated. The results showed a diversity of treatment outcomes in the six centres, but the sample sizes in each centre were small, and this study is considered to be preliminary. Fortunately, several other centres are willing to participate in this project, and randomized controlled trials on primary surgery are being planned.

As the Japanese Cleft Palate Association is the only academic society that has multidisciplinary members in the field of craniofacial anomalies, it is being proposed as the lead group for collaborative research. Although obstacles similar to those faced in European countries are met, the most significant one in Japan is lack of understanding of the aims of collaborative research. It will take time before clinicians understand the benefits. The large number of hospitals in which craniofacial anomalies are treated may also be a complication.
3.7 COLLABORATIVE PROJECTS ON CRANIOFACIAL ANOMALIES IN BRAZIL

In Brazil, the incidence of cleft lip and palate, the commonest form of craniofacial anomaly, is 1 in 650 livebirths. As the Brazilian population is about 170 million, it is estimated that there are more than 260,000 persons with clefts in the country. Despite the known impact on affected individuals and on society, there are few services that offer multidisciplinary treatment and few research groups studying those anomalies in Brazil.

The Hospital for Rehabilitation of Craniofacial Anomalies of the University of São Paulo, known as Centrinho, provides free multidisciplinary care to persons with cleft lip and palate and related anomalies. It has thus far received more than 41,000 patients from all Brazilian states and even neighbouring countries. More than 3500 of the cases are syndromic. The treatment offered is based on plastic surgery, orthodontics and speech pathology, complemented by other medical specialities, such as paediatrics, ear-nose-and-throat surgery, neurosurgery and clinical genetics, other dental specialties such as oral and maxillofacial surgery, implantodontics and prosthodontics, plus nursing, psychology, nutrition and physical therapy. Centrinho also counts on specialized laboratories for diagnosis and research, such as the Laboratory of Respiratory and Speech Physiology, the Laboratories of Genetics, the Laboratory of Experimental Phonetics and the Centre for Audiologic Research. A team of social workers facilitates patient access to treatment and ensures treatment continuity.

The services offered, the team’s high qualification, with 269 specialized professionals (34 with a doctorate degree) and good infrastructure made Centrinho the national reference centre for the study and treatment of cleft lip and palate, and these factors and the high volume of attendance (about eight new cases and 25 operations per day) make Centrinho a potential key site for larger projects. At present, two international collaboration projects supported by the National Institutes of Health, United States, are being developed: on velopharyngeal function for speech after palatal surgery, in partnership with the University of Florida, and a global network for women’s and children’s health research, in partnership with the Estudio Colaborativo Latino Americano de Malformaciones Congenitas (ECLAMC) and the University of Iowa. A randomized trial of pharyngeal flap and sphincter pharyngoplasty in cleft palate is planned on the basis of previous interchanges with the University of Manchester (United Kingdom) and the University of Oslo (Norway).

Certain difficulties have emerged in Centrinho’s involvement in international collaborative research, some of which can be overcome:

- Any project conducted in the institution must receive prior approval by the internal Committee of Ethics of Research in Human Beings and sometimes by a federal committee.

- Despite the large number of patients, the rigid inclusion criteria limit the sample size of patients.

- Despite the large number of qualified professionals, many do not have enough time or interest in participating in long-term studies.

- Some members of each team have a natural resistance to conducting randomized treatment studies.

- The projects are generally too long, making the return in publications very slow.

- The lack of resources limits development of the institution’s research potential.
3.8 CRANIOFACIAL ANOMALIES IN THAILAND AND NEIGHBOURING COUNTRIES

Thailand and neighbouring countries in Asia have high prevalences of cleft lip and palate. A retrospective study at a Bangkok hospital showed a birth prevalence of 1.62/1000 livebirths (Chuangsuwanich et al., 1998), and it was noted that more than half of the patients in the study came from northeastern Thailand, where the population has a low socioeconomic status, indicating that the incidence in this region might be higher. Ruangsit et al. (1993) collected data prospectively by recording births at three hospitals in Khon Kaen, in northeast Thailand, for 6 months during 1993 and found an incidence of clefts of 2.49/1000 livebirths.

As the etiology of orofacial clefts includes interactions between genetic and environmental factors, both epidemiology and demography are necessary for planning health-care services, including preventive measures. The total population of Thailand and the neighbouring countries, Bangladesh, Bhutan, Brunei Darussalam, Cambodia, Indonesia, the Lao People’s Democratic Republic, Malaysia, Nepal, Pakistan, Philippines, Singapore, Sri Lanka and Viet Nam, is 867 906 842, and the estimated incidence of new cases of cleft at birth is 43 019, on the basis of an average incidence of 2/1000 livebirths. Demographic information and the estimated number of new cases of clefts per year are shown in Table 10.

<table>
<thead>
<tr>
<th>Country</th>
<th>Total population</th>
<th>Per capita gross domestic product (US$)</th>
<th>Population living below poverty line (%)</th>
<th>Births/1000</th>
<th>Total no. of Estimated incidence of clefts</th>
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<tr>
<td>Bangladesh</td>
<td>141 340 476</td>
<td>1 900</td>
<td>36</td>
<td>30.0</td>
<td>4 244 455</td>
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<td></td>
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<td></td>
<td></td>
<td>8 489</td>
<td></td>
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<tr>
<td>Bhutan</td>
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<td>1 300</td>
<td>NA</td>
<td>34.4</td>
<td>75 205</td>
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<td>365 251</td>
<td>18 600</td>
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<td>19.3</td>
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<tr>
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<td>13 363 421</td>
<td>1 900</td>
<td>36</td>
<td>27.1</td>
<td>362 550</td>
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<tr>
<td>Indonesia</td>
<td>238 452 952</td>
<td>3 200</td>
<td>27</td>
<td>21.1</td>
<td>5 033 742</td>
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<td></td>
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<td></td>
<td></td>
<td>10 067</td>
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<tr>
<td>Lao People’s Democratic Republic</td>
<td>4 353 893</td>
<td>1 700</td>
<td>40</td>
<td>36.5</td>
<td>158 787</td>
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<td>Malaysia</td>
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<td>9 000</td>
<td>8</td>
<td>23.4</td>
<td>549 720</td>
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<td>1 099</td>
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<td>Nepal</td>
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<td>1 400</td>
<td>42</td>
<td>32.0</td>
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<td>Pakistan</td>
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<td>35</td>
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<td>9 940</td>
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<tr>
<td>Philippines</td>
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<td>4 600</td>
<td>40</td>
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<tr>
<td>Singapore</td>
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<td>Sri Lanka</td>
<td>19 905 165</td>
<td>3 700</td>
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<tr>
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<td>7 400</td>
<td>10</td>
<td>16.0</td>
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<td>2 081</td>
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<tr>
<td>Viet Nam</td>
<td>82 689 518</td>
<td>2 500</td>
<td>37</td>
<td>19.6</td>
<td>1 619 061</td>
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</tr>
<tr>
<td>Total</td>
<td>867 906 842</td>
<td></td>
<td></td>
<td>43 019</td>
<td></td>
</tr>
</tbody>
</table>

NA, not available

Source: Total population, per capita gross domestic product, population living below poverty line and births/1000 population from [www.cia.gov/cia/publications/factbook](http://www.cia.gov/cia/publications/factbook)
The first Thai International Congress on Interdisciplinary Care for Cleft Lip and Palate was held in Khon Kaen in December 2003, when the Thai Cleft Lip–Palate and Craniofacial Association was established. Over 300 participants from 22 mostly Asian countries attended the congress, and the inaugural meeting of the Asia–Pacific Craniofacial Research Collaboration was held. The objectives of the Collaboration are to optimize cleft care, promote collaborative research in craniofacial anomalies and work with other networks, such as EUROCRAN, and other research groups. A further meeting to establish collaboration with countries in the region was held in Manila, Philippines, in February 2004, where emphasis was placed on setting up a comprehensive database and good collaboration in research.
4. INTERNATIONAL PROGRESS IN RESEARCH ON GENETIC DETERMINANTS OF CRANIOFACIAL ANOMALIES

Clefts can be divided into non-syndromic (isolated) and syndromic forms. Persons with isolated clefts have no other physical or developmental anomalies, and most studies suggest that about 70% of cases of cleft lip and/or palate and 50% of those of cleft palate are non-syndromic (Marazita, 2002). The syndromic cases can be subdivided into more than 400 Mendelian disorders (e.g. the Online Mendelian Inheritance in Man, 2003, lists 404) and cases due to chromosome anomalies or teratogens and uncategoryed syndromes. The complex etiology of clefting affords opportunities to identify gene–gene or gene–environment interactions that can shed light on human embryology and its disturbances (Spritz, 2001). This report summarizes the available genetic data, with an emphasis on the isolated or non-syndromic form of cleft lip and palate.

4.1 DEVELOPMENTS

In humans, an exquisitely choreographed cascade of gene expression, cell migration, cell transformation and apoptosis occurring 14–60 days post-conception creates the tissues of the face from the originating oropharyngeal membrane. Neural crest cell migration drives the swellings that form the frontonasal prominence and the paired maxillary and mandibular prominences, and fusion of the prominences result in normal facial relations. By 48 days, the upper lip is continuous, and by 60 days palatal shelf fusion completes facial embryogenesis (Sperber, 2002). Disruption of any of the tightly regulated processes occurring during this time can predispose to cleft lip and/or palate. Genes known to be involved in these processes include transcription factors, growth factors, cell signalling molecules and extracellular matrix proteins. Embryology suggests that clefts of the primary palate that involve the lip with or without the palate result from disruptive mechanisms that are different from those that cause clefts affecting only the secondary palate (Fraser, 1955). The practice of combining cases of cleft lip and/or palate with those of cleft palate in etiological studies has, however, received support, as both cleft palate and cleft lip and/or palate are found in families that segregate mutations for the MSL1 (van den Boogard, 2000), IRF6 (Kondo et al., 2002) or FGFR1 (Dode et al., 2003) gene.

4.2 EPIDEMIOLOGY

Cleft lip or palate affects about 1/700 births, with wide variation by geographical region (Vanderas, 1987; Mossey & Little, 2002) and socioeconomic status (Murray et al., 1997). In general, Asian and American populations have the highest birth prevalences, often as high as 1/500, while European-derived populations have intermediate rates, at about 1/1000, and African-derived populations have the lowest prevalence, at 1/2500. The frequency of cleft lip and/or palate differs remarkably by sex and side of clefting, with a 2:1 male:female ratio and a similar 2:1 left-side:right-side ratio for unilateral clefts. Fogh-Andersen (1942) in Denmark first proposed a role of genetic factors in clefting, and this was subsequently confirmed by segregation analysis (Marazita, 2002) and twin studies (Mitchell, 2002). The identical facial structures observed in normal monozygotic twins gives credence to a strong genetic component in facial development, but the lack of complete concordance for clefts in monozygotic twins (monozygotic twins have 40% and dizygotic twins 4% concordance) ensures that other factors (environmental or stochastic) play a role as well. Estimates of recurrence risk ratios to siblings give a λ of ~ 40. Schliekelman & Slatkin (2002) used maximum likelihood estimation and cleft prevalence rates to estimate the number of loci contributing to the genetic component of cleft lip and/or palate. They reported two peaks of
almost equal effect, where either three or six loci would explain the observations. The confidence interval for the best estimate extended to 14 loci; therefore, studies of genetic etiology need the power to search for genes of this moderate effect size (5–10% per locus for the major loci).

4.3 GENETIC LINKAGE

Until recently, studies of cleft lip and/or palate were limited by insufficient numbers of families and genotyping resources (Mitchell et al., 2002). Five genome-wide screens have now been published (Prescott et al., 2000; Marazita, 2002; Wyszynski et al., 2003; Zeiger et al., 2003; Marazita & Mooney, 2004; Marazita et al., 2004a). No highly significant loci were identified in these studies, but a meta-analysis performed by Marazita et al. (2004b) pinpointed one highly significant locus on 9q. Candidate genes in cleft lip and/or palate have also been sought in association studies with case–controls or case–parents triads (Murray, 2002; Marazita, 2002). With this approach, transforming growth factor α (TGFα) was suggested as contributing to cleft lip and/or palate (Ardinger et al., 1989). Since that first report, many others have demonstrated or refuted association with genes and loci (Murray, 2002).

Consistently positive findings have been obtained for only two genes; both were initially selected on the basis of their expression phenotype in the mouse. The MxL1 knockout mouse has a cleft palate (Satokata & Maas, 1994), and, in humans, MSX1 was shown to have a stop codon in affected members of a large pedigree with clefting and missing teeth (van den Boogaard, 2000). This finding for a single family was extended, to show that about 2% of over 1000 cases of non-syndromic cleft lip and/or palate have mutations in MSX1 (Jezelewski, 2003; Suzuki et al., 2004). Similarly, the Tgfβ3 knockout mouse has a cleft (Proetz et al., 1995), and consistent support has been found for a role of TGFβ3 in human cleft lip and/or palate (Lidral et al., 1998; Beaty et al., 2001, 2002; Vieira et al., 2003a).

A third gene, IRF6, which is causal in van der Woude syndrome, has been shown to play a strong role in the isolated form of clefting as well (Zuccherio et al., 2004) and is described in more detail below. Candidate genes for cleft lip and/or palate in humans can also be found among the Mendelian forms, in which mutations have a wide enough range of expression that some affected individuals present as phenocopies of isolated cleft lip and/or palate (Murray, 2001). Examples include van der Woude syndrome (IRF6, Kondo et al., 2002), Kallmann syndrome (FGFR1, Dode et al., 2003), ectrodactyly–ectodermal dysplasia or clefting (TP63, Celli et al., 1999), X-linked ankyloglossia or clefting (TBX22, Braybrook et al., 2001; Marçano et al., 2004), Gorlin syndrome (PTCH, Kimonis et al., 1997) and heterozygotes for the Margarita Island clefting syndrome (PVRL1, Sözen et al., 2001). Each of these genes might harbour a mutation that could result in or modify the expression of isolated cleft lip and palate.

4.4 GENOME SEQUENCING AND COMPARATIVE GENOMICS

Identification of the draft sequence of the human genome provides unprecedented opportunities to move quickly from locus identification to finding genes and mutations (Wolfsberg et al., 2002). The complete sequences of most regions of the genome are available, allowing direct searches for candidate genes. The human genome sequence has also been coupled to the genomic sequences of other vertebrates, including the pufferfish (fugu), mouse, rat, dog and chimpanzee (Kirkness et al., 2003), allowing comparisons of the genome around candidate genes (Frazer et al., 2003). While searches for mutations have usually focused on amino acid coding regions, genomic regions outside transcribed DNA, which may be involved in gene regulation, can usefully be examined. Conserved regions can lie at a
Addressing the global challenges of craniofacial anomalies

considerable physical distance (as much as 1 megabase; Lettice et al., 2003) from the ‘body’ of the gene, and comparisons of genomic sequences can allow identification of highly conserved nucleotide regions that are likely to be critical for gene regulation.

Study of the genome sequence has also provided evidence that the underlying causes of human inherited disorders are distinct from single-gene inheritance or point mutations. It has been recognized in the past few years that genomic rearrangements have a causal role in some birth defect syndromes. Genomic rearrangements can arise when interspersed repeat elements, lying in tandem, facilitate submicroscopic deletion (or duplication) events (Stankiewicz et al., 2003). A gene caught in a genomic rearrangement can be identified by demonstration of a change in the dosage of that gene. If screening tools are available to detect genome rearrangements, large numbers of persons with a disorder of interest can be examined for evidence of deletions or duplications. The Dancer mutation, described below, is an example of a cleft arising from displaced genomic material, as are the clefts arising in the 22q– syndrome, one of the prototypes for genomic rearrangements (Carlson et al., 1997; Shaikh, Kurahashi & Emanuel, 2001). Advances in high-resolution single nucleotide polymorphism genotyping (Oliphant et al., 2002), quantitative polymerase chain reaction (Germer, Holland & Higuchi, 2000) and comparative genomic hybridization (Pinkel et al., 1998) make searches for small deletions and duplications feasible on a genome-wide or locus-specific basis.

4.5 ANIMAL MODELS OF GENE EXPRESSION

Animal models and data on expression can be powerful tools for identifying candidate genes for a complex trait like cleft lip and/or palate (Murray & Schutte, 2004). Genome-wide searches and transmission disequilibrium tests can be used to identify regions containing tens or even hundreds of genes that could be causal; therefore, a list of priorities must be established for the order in which genes are to be evaluated for a causal role. Animal models with clefts arising spontaneously or from knockout or experiments with the mutagen N-ethyl-N-nitrosourea can suggest a list of genes for which direct sequencing or fine mapping linkage disequilibrium can be used for confirmation.

While cleft palate is a common phenotype in the mouse, cleft lip is rare. Two spontaneous cleft lip mutants, C/lf1 and C/lf2, were identified in a genome-wide scan for susceptibility loci in the mouse strain A/WySn (Juriloff, 2002). Another mutant with high penetrance for cleft lip is the Dancer mutant, shown to arise from a translocation of the p23 gene sequence into the Tbx10 locus, resulting in ectopic expression of Tbx10 under the influence of the p23 promoter (Bush, Lan & Jiang, 2004).

The strongest candidate genes for cleft are those whose normal expression includes the critical time and tissue for lip and palate development. Data on the expression of individual genes has been available in the scientific literature since the 1980s. More recently, at least two global approaches to expression analysis of genes in craniofacial structures have provided a broader view of gene function. The Craniofacial and Oral Gene Expression Network project (hg.wustl.edu/COGENE/) provides public web access to data on gene expression in 24 craniofacial-specific human tissues isolated from day-26–60 embryos, and these data are already contributing directly to the selection of candidate genes (see below). Further, the expression of genes relative to each other in both time and space can be represented visually by optical projection tomography (Sharpe et al., 2002), which can be used to evaluate the expression of new candidate genes and to demonstrate that the expression of existing genes is consistent with hypotheses about their function.
4.6 CHROMOSOME ANOMALIES

Adventitious chromosome anomalies provide important clues about genes involved in clefting. Chromosome deletions and duplications can result in clefts, and regions strongly associated with cleft lip and/or palate have been identified (Brewer et al., 1998). Mapping of balanced translocation breakpoints in persons with clefts can also be used to find genes transected by or adjacent to the breaks, which are then candidates for cleft lip and/or palate. These genes are especially attractive candidates when the phenotype is confined to cleft lip or palate alone or when the cleft occurs in several members of a family segregating with the translocation. Three relevant genes or gene clusters have been identified, one at 2q32 (FitzPatrick et al., 2003), one on 6p (Donnai et al., 1992) and one at 19q13 (Yoshiura et al., 1998), with balanced translocation and cleft lip and/or palate. The SATB2 gene at 2q32 is transected and encodes a 733-amino acid DNA-binding protein of remarkable conservation between human and mouse (differing by only 3 of 733 amino acids). SATB2 is strongly expressed in both the lip and the palate, making it an excellent candidate gene for isolated cleft lip and/or palate. The translocation breakpoint at 19q13 was identified in the CLPTM1 gene in a family segregating for clefts. It is adjacent to two homologues (PVR and PVRL2) to the PVRL1 gene, mutations in which result in Margarita Island clefting syndrome (Suzuki et al., 2000).

Comparative genomic hybridization is performed with gridded clones, typically bacterial or Pl artificial chromosomes, to examine amplified DNA samples from test cases. With different fluorescent tags, differences in dosage of bacterial or Pl artificial chromosome samples that are deleted or duplicated can be identified. With a dense, overlapping array of such clones, the complete genome can be screened. The limitations of comparative genomic hybridization include obtaining a high enough density of overlapping clones to find a small deleted or duplicated region and the cost per sample analysed, which is prohibitive for use of this technique as a screening tool. A second approach is to use single nucleotide polymorphism genotyping on case–parents triads, where deletions are detected as disturbances of expected Mendelian inheritance. One-fourth of such events are detected in this approach, which has the advantage of cost–effectiveness when the same data are used for genetic investigations of linkage or association. The disadvantage is that 75% of deletions in any single nucleotide polymorphism are missed, and duplications are difficult to find. Quantitative polymerase chain reaction assays are highly effective for a locus-by-locus search. Once a rearranged region has been identified by screening, its physical limits can be characterized, and the genes that are deleted can be determined by examination of the genome sequence. Confirmation of a deletion in a case of isolated cleft lip and/or palate corresponds to recognition of a genetic component of clefting. Deletions found in cases of syndromic cleft lip and/or palate indicate that further investigations should be undertaken of genes in the same region for a role in isolated cleft lip and/or palate, as well. The finding of two micro-deletions associated with van der Woude syndrome that led directly to subsequent gene identification (Sander, Schmelze & Murray, 1994; Schutte & Murray, 1999) proves the principle of this approach.

4.7 STATISTICAL ANALYSES

Advances in statistical methods have been made in parallel to the rapid advance in molecular genetic techniques. No one analytical paradigm is optimal for identifying all genes in involved complex disorders, and a combination of approaches is best for exploiting the samples that can be collected, the inheritance patterns and the linkage and association methods available. The advent of the LOD score method was exceptionally useful for analysing single gene traits, and it can be applied to complex traits as well. Model-free or non-parametric linkage analysis of sibling pairs and other affected family members has also been useful. Although model-free approaches cannot identify locations for linked diseases,
alternative strategies for fine mapping can address this difficulty. Both these approaches can be supplemented with linkage disequilibrium and transmission disequilibrium tests. An increasing selection of methods (e.g. family-based association test, likelihood ratio test) can be used to analyse case–controls (for linkage disequilibrium) and case–parents triads (linkage disequilibrium or transmission disequilibrium tests) and more complex family structures. These techniques have been modified recently to deal with confounders such as population heterogeneity. Collections that include DNA samples from many families of variable structure can be analysed by parametric and non-parametric linkage as well as by linkage disequilibrium approaches (each subdivided into specific methods).

As some genes are best found by linkage while others have undetectable linkage signals because of strong linkage disequilibrium, a range of methods should be used on a genotyped family dataset in order to find genes. One subset of gene mapping approaches is based on the observation that in some disorders (including cleft lip and/or palate) there are substantial differences in birth prevalence according to the ancestral origin of the parents. These frequency differences parallel differences in allele frequencies at many polymorphic sites, also depending on the origin of the population. When high- and low-frequency populations admix, the differences can be used to map genes from far smaller sample sizes and marker densities than would be required for similar approaches in distinct populations. When two genetically distinct populations begin to exchange genes through admixing, a powerful pattern of linkage disequilibrium emerges, being observed at all loci that differ in allele frequency in admixed populations, even at loci on different chromosomes. For example, the African–American population derives 10–20% of its genes from European ancestry (Parra et al., 1998). The frequency of cleft lip and/or palate is also remarkably different in African (1 per 2500) and European (1 per 1000) populations (Mossey & Little, 2002). Similarly, as described below, native American groups have a higher rate of clefts (1/500) than admixed European populations, especially for cleft lip and/or palate. Strong evidence for effects of admixing have been seen in South American populations (Vieira, Orioli & Murray, 2002).

4.8 INTERNATIONAL PROJECTS IN GENETICS

The combination of animal models, gene expression studies, molecular advances and analytical techniques necessary to find the genes for cleft lip and palate is now available and can be applied directly to human material. Improvements in knowledge of the causes of cleft lip and/or palate can facilitate investigations into the developmental biology of craniofacial structures and lead directly to improvements in treatment and prevention.

Case ascertainment and data collection

Two significant international collaborative projects are under way.

In the Philippines, a case ascertainment project is being led by a team of four nurse phlebotomists, who collect family histories and samples in clinics run by Operation Smile. Histories have been obtained from 2400 families per year, and 339 families have been identified with two or more members affected, who are suitable for genome-wide analysis. Data on an additional 979 case–parents triads have been collected, with demographic data, clinical photos and diet histories from each family member. The nurses have reciprocal training in the United States each year. They educate the families about infant feeding, refer cases for surgery and re-contact the families for additional phenotyping.

The Estudio Colaborativo Latino Americano de Malformaciones Congenitas (ECLAMC) is a birth defects registry programme that has been active in South America for the past 35 years under the direction of Dr Eduardo Castilla. It involves about 70 hospitals throughout South
America. Its primary goal is to collect detailed epidemiological, demographic and clinical data on each child born with a birth defect in each hospital. The database currently holds information on over 2 000 000 births, making it one of the largest of its type in the world. Collection of biological material from newborns with congenital defects was tested for feasibility in 1998–99 (Orioli et al., 2001).

**DNA sequencing of candidate genes for cleft lip and/or palate**

Gene finding and mutation screening have led to identification of mutations in *MSXI* and *TGFβ3* (Lidral et al., 1998; Jezewski et al., 2003); ectrodactyly–ectodermal dysplasia syndrome and *P63* (Barrow et al., 2002), van der Woude syndrome and *IRF6* (Kondo et al., 2002) and *TBX22* (Marçano et al., 2004). A comprehensive sequencing study has been carried out on cases of cleft lip and/or palate for *MSXI*, based on linkage and linkage disequilibrium studies, chromosome deletions, a large family with a stop codon mutation segregating with clefting, and the *MsoI* phenotype in the knockout mouse. The entire gene was sequenced (two exons and a single intron), including highly conserved non-coding regions found at 5′ and 3′. *MSXI* mutations were found in 2% of 917 persons of European, Asian or native South American ancestry with cleft lip and/or palate (Jezewski et al., 2003). Similarly, 2% of 175 cases of cleft lip and/or palate in Viet Nam were also found to have *MSXI* mutations, and a particular variant (*P147Q*) was found in nine members of three unrelated families who shared a common haplotype on the affected chromosome, suggesting a founder affect (*p = 0.006*) (Suzuki et al., 2004).

**Candidate gene association (transmission disequilibrium test) studies**

Extensive work has been carried out to identify single nucleotide polymorphism in candidate genes (Ardinger et al., 1989; Shiang et al., 1993; Lidral et al., 1998; Machida et al., 1999; Romitti et al., 1999; Barrow et al., 2002; Jezewski et al., 2003; Jugessur et al., 2003a,b). The candidate genes (some with multiple single nucleotide polymorphisms) investigated in case–parents Filipino triads are *IRF6, TGFβ3, TGFα, MSXI, MTHFR, NAT1, PVRL2, LHX8, PAX9, JAG2* and *RFC1*. The data were analysed within the transmission disequilibrium test framework and specifically with the family-based association test (Horvath, Xu & Laird, 2001). Furthermore, haplotype-based transmission disequilibrium statistics were calculated for the markers in *TGFβ3* with the TRANSMIT program (Clayton, Jones, 1999). A global $\chi^2$ test statistic of 16.611 (5 degrees of freedom; $p = 0.005$) was found for *TGFβ3* in haplotypes with a frequency greater than 5%. In 293 Filipino triads, positive associations were seen with *MSXI* in the subset with cleft lip only, indicating that *MSXI* may modify severity. Thus, of the candidate genes so far investigated in the Filipino families, the strongest associations are with *MSXI* and *TGFβ3* (and *IRF6*, as described below). Similar studies with a collection of mother–infant diads within the ECLAMC group and nuclear triads in Brazil (Vieira et al., 2003a) indicate transmission distortion with the same *MSXI* and *TGFβ3* alleles as seen in the Philippines, confirming the role of both genes in cleft lip and/or palate in independent populations.

Another area of investigation is based on gene identification in balanced translocations. The *SATB2* gene has been cloned in translocation studies (FitzPatrick et al., 2003) and found to be located at 2q32. This gene is a highly conserved (730 of 733 amino acids identical between human and mouse) DNA binding protein that is homologous to a family of nuclear matrix attachment site binding proteins suggested to have a role in transcription regulation. While no mutations were found in the initial study of 70 cases of isolated cleft palate, there was both site- and stage-specific expression of the gene in the lip and palate of developing mice.
van der Woude syndrome and IRF6

van der Woude syndrome is a good model for non-syndromic cleft lip and palate in that it is a highly penetrant autosomal dominant syndrome and is distinguishable from isolated clefting by only one minor feature, pits of the lower lip. This gene was first mapped by linkage to 1q32 (Murray et al., 1990) and then narrowed to a critical 350-kilobase region by microdeletions (Schutte et al., 2000). The best candidate genes in this region were then sequenced in rank order in a set of discordant monozygotic twins, to help eliminate rare single nucleotide polymorphisms as causal variants (Kondo et al., 2002). This strategy proved successful, and a stop codon was found only in the affected twin in the IRF6 gene. IRF6 is a member of the transcription factor family of interferon regulatory factor genes, the other members of which are involved in immune regulation and interferon production after viral infection.

IRF6 is expressed in the palate, limb and genital region, consistent with the pattern of phenotypes seen in van der Woude syndrome and the allelic popliteal pterygium syndrome. A total of 124 independent mutations have now been identified in IRF6 (55% of the 226 unrelated affected genes sequenced in all 10 exons, including splice boundaries) in patients with van der Woude syndrome and popliteal pterygium syndrome. Most of the mutations are either missenses that cluster in the DNA and protein-binding domains or nonsense or frameshift mutations scattered throughout the gene. The strategy for identifying the causes in the 45% of cases of van der Woude syndrome that did not have mutations was, first, to ensure that there was minimal locus heterogeneity and no unlinked families, and, then, to identify all regions of human–mouse homology (> 80% for 50 base pairs or more) in introns or in 200-kilobase 5’ or 3’, and, finally, to do quantitative polymerase chain reaction assays with TaqMan probes.

Table 11. Overtransmission of the V allele at V274I in populations with cleft lip and/or palate

<table>
<thead>
<tr>
<th>Birthplace</th>
<th>No. of families</th>
<th>Rare (I) allele (%)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Philippines</td>
<td>403</td>
<td>22</td>
<td>0.000001</td>
</tr>
<tr>
<td>Viet Nam</td>
<td>175</td>
<td>36</td>
<td>0.001</td>
</tr>
<tr>
<td>Japan</td>
<td>115</td>
<td>36</td>
<td>0.69</td>
</tr>
<tr>
<td>China</td>
<td>97</td>
<td>37</td>
<td>0.17</td>
</tr>
<tr>
<td>Brazil</td>
<td>303</td>
<td>11</td>
<td>0.0002</td>
</tr>
<tr>
<td>ECLAMC</td>
<td>158</td>
<td>16</td>
<td>0.86</td>
</tr>
<tr>
<td>Colombia</td>
<td>107</td>
<td>25</td>
<td>0.30</td>
</tr>
<tr>
<td>United States (Iowa)</td>
<td>246</td>
<td>2</td>
<td>0.29</td>
</tr>
<tr>
<td>Denmark</td>
<td>239</td>
<td>4</td>
<td>0.56</td>
</tr>
<tr>
<td>India</td>
<td>50</td>
<td>7</td>
<td>0.23</td>
</tr>
<tr>
<td>Total</td>
<td>1893</td>
<td></td>
<td>&lt; 10⁻⁹</td>
</tr>
</tbody>
</table>

With this method, an amino acid variant, V274I, the only coding sequence polymorphism, was identified in IRF6. To investigate the role of the V274I variant, 1893 families of European, Asian and South American ancestry with isolated cleft lip and/or palate, including 3763 case–
parents triads, for a total of 8003 persons, were genotyped by a kinetic polymerase chain reaction assay (see Table 12). The data were analysed with the family-based association test, the transmission disequilibrium test and multipoint linkage analysis of IRF6; chromosome 1 markers were also analysed in the extended Philippine kindreds with SIMWALK2 (Sobel et al., 1996). The results with IRF6 alone or with other chromosome 1 markers were negative; however, the results of the transmission disequilibrium tests were remarkably significant for overtransmission of the common allele in most populations (see Table 11).

In summary, IRF6 was a significant modifier of cleft lip and/or palate in all ancestral groups examined. The causes may lie in one or more gene variants, either within or 3′ of the gene. This is the first modifier established for a major birth defect, indicating a 12% attributable risk for isolated clefts due to variants in the IRF6 gene.

**Extended, multiplex pedigree linkage analysis**

The Center for Inherited Disease Research in the United States undertook analysis of the genotypes of a collection of families in China, Colombia, India, the Philippines, Turkey and the United States. Samples were submitted in three batches, with about one-half from previous work in the Philippines. Linkage between cleft lip and/or palate and each of the 392 markers was analysed with parametric and non-parametric methods: SimIBD (Davis et al., 1996), single point linkage (LOD scores), multipoint linkage (LODs and HLODs, SIMWALK2; Sobel et al., 1996), and non-parametric linkage statistic (SIMWALK2). One locus on 9q showed a summed LOD of 6.6, and this locus contains four excellent candidate genes—PTCH, ROR2, TGFβR1 and ZNF189. The results of meta-analysis have been reported elsewhere (Marazita et al., 2004b).

A strong signal was also seen on 8p, and 16 single nucleotide polymorphisms for 13 candidate genes were tested over about a 20-cM interval, one of which is FGFR1. Dode et al. (2003) reported that Kallmann syndrome can be caused by inactivating the FGFR1 gene. Kallmann syndrome is an autosomal dominant disorder in which anosmia and infertility are the primary features but in which cleft lip and/or palate is seen in 5% of cases. FGFR1 hypomorphic alleles in the mouse show cleft palate in 80% of cases (Trokovic et al., 2003). As clefting was an important component of the families with FGFR1 mutation, it was hypothesized that some cases of isolated cleft lip and/or palate might have FGFR1 mutations or be cases of Kallmann syndrome with variable expressivity.

**Signal transduction pathways involving FGFR and other genes in palate development**

Epithelial–mesenchymal interactions are essential during the initial stages of palate development. Rice and coworkers (2004) demonstrated the essential role of the Fgf10/Fgfr2/Shh signalling pathway in murine palate epithelial–mesenchymal interactions and integrated this model into the Msx1 pathway. Zhang et al. (2003) demonstrated that Msx1, bone morphogenetic protein 4 (Bmp4), Shh and Bmp2 constitute a pathway that is essential for palate development in mice. It might well be that FGFR1 (Ornitz & Itoh, 2001), SATB2 (FitzPatrick et al., 2003) and TBX22 (Braybrook et al., 2001) are also involved in palate growth in humans or mice, although their exact placement in a known pathway remains to be determined. The involvement of these genes and their hypothesized interactions suggest a broader view of the major determinants of palate development and indicate that additional candidate genes could be investigated by DNA re-sequencing or statistical analyses.
Admixture and population studies

The effect of admixture on clefting rates was examined in South America, where the population of much of the continent is a tri-racial mix of native South Americans, Europeans and Africans (Vieira, Orioli & Murray, 2002). In view of the high rates of cleft in native Americans and the low rates in Africans, mitochondrial and Y chromosome variants were studied in a case–control population derived from the ECLAMC registry. A much higher ($p < 0.00001$) frequency of the D mitochondrial haplotype specific to native Americans was found in the 217 cases of clefts than in the matched controls (next child born with no birth defect in the same hospital). Similarly, the case group had a much lower frequency of a Y chromosome marker specific to African populations ($p = 0.002$). This work demonstrates substantial effects of admixture between the high-incidence Amerindian groups and low-incidence African groups in this population, supporting use of admixture mapping for clefts.

In the past few years, there have been substantial advances in knowledge of the causes of isolated forms of clefting. At least three genes, $MSX1$, $IRF6$ and $FGFR1$, appear to play significant roles, and additional pathways for investigation have been identified. The role of gene–environment interactions is also becoming clearer (see section 5). Although much work remains to be done, these insights will influence genetic counselling in the future and hold promise for improving treatment and prevention.
5. GENE–ENVIRONMENT INTERACTIONS: INTERNATIONAL COLLABORATION

One of the four themes of a collaborative project between WHO and the National Institutes of Health of the United States on craniofacial anomalies was to further knowledge about the etiology and pathogenesis of craniofacial anomalies, by investigating the roles of genes, environmental factors and their interactions. Progress has been made, but much remains to be done. At the meeting organized by the WHO Human Genetics programme on international collaborative research on craniofacial anomalies, entitled *Global Strategies towards Reducing the Health Care Burden of Craniofacial Anomalies* (WHO, 2002), the following recommendations were made in relation to research on gene–environment interactions:

- to undertake more collaborative studies;
- to identify and involve interesting populations;
- to focus on common exposures and gene variants;
- to standardize methods and introduce common core protocols;
- to use innovative study designs and statistical methods;
- to exploit, share and pool data and biological samples; and
- to better assess environmental exposures.

In December 2004, the progress in relation to these objectives was reviewed, and this report describes developments in all areas, new avenues for research and projections for the future. The inevitable overlaps with ongoing genetics research and prevention are discussed and cross-referenced.

5.1 EXISTING COLLABORATIVE STUDIES

A number of interdisciplinary collaborative initiatives were begun after the meeting in November 2000, to encourage exchanges among experts in craniofacial anomalies. A few specifically addressed gene–environment interactions as part of the overall problem of craniofacial anomalies, mainly in relation to orofacial clefts.

Models for interdisciplinary research on craniofacial anomalies are ECLAMC in Latin America (2500 births per month), the National Birth Defects Prevention Study in the United States (nine centres), EUROCAT in Europe (1 million births per year) and EUROCRAN (gene–environment interactions project).

*Estudio Colaborativo Latino Americano de Malformaciones Congenitas (ECLAMC)*

The Latin American Collaborative Study of Congenital Malformations, ECLAMC, has been studying the etiology of birth defects since 1967 by clinical and epidemiological methods. A large hospital-based system was set up in all 10 South American countries to screen newborns with congenital anomalies. Since 1998, ECLAMC has been building up a cell bank and DNA banks of cases of birth defects, to supplement the clinical and epidemiological screening. Cells and DNA from patients will be available for investigators contributing biological material to the bank as well as for other scientists who propose research projects in collaboration with ECLAMC or offer training to Latin American scientific or technical personnel.
The basic epidemiology of oral clefts in South America shows stable secular trends over 33 years (1967–99) for isolated cleft lip and/or palate and for cleft palate, and significantly rising trends for syndromic cases. For cleft lip and/or palate, significant associations have been found with high altitude (above 2000 m), male sex, twinning, low socioeconomic status, maternal illness, self-medication and parental consanguinity. For cleft palate, significant associations have been observed with female sex, twinning, low socioeconomic class and self-medication.

The DNA bank was started in 1998 for oral clefts and in 2001 was extended to all major malformations as well as to randomly selected samples of non-malformed newborns. By July 2004, the stored material comprised DNA samples from 15 460 healthy newborns and 3747 malformed newborn–mother dyads, consisting of 656 cases of cleft lip and/or palate, 123 cases of cleft palate, 62 cases of microtia and 64 cases of holoprosencephaly.

**National Birth Defects Prevention Study, United States**

The National Birth Defects Prevention Study is described in detail in section 2.

The information gathered from the interviews, combined with the availability of DNA, will be an invaluable resource for the study of genetic susceptibility to environmental exposures for a broad range of carefully classified birth defects. The collaborative nature of the National Birth Defects Prevention Study increases the statistical power for assessing risk factors for craniofacial anomalies, and allows careful consideration of etiologically distinct subtypes. The numbers of cases included in the study make it possible to look at subgroups separately; for example, isolated cases (only one major birth defect) and infants with two or more major defects in at least two organ systems can be assessed. Furthermore, subtypes of craniofacial anomaly (e.g. cleft palate with notation of Pierre Robin sequence in the medical record) and phenotypically similar subsets of cleft lip and palate can be analysed in separate groups.

**European Collaboration on Registration of Congenital Anomalies and Twins (EUROCAT)**

EUROCAT, the European network of population-based registries for the epidemiological surveillance of congenital anomalies, was started in 1979 and surveys more than 1 million births per year in Europe. It is made up of 39 registries in 19 countries, giving a coverage of 25% of the birth population of Europe. The high-quality, multiple-source registries ascertain terminations of pregnancy as well as births. The network provides a standardized database on > 250 000 cases of congenital anomaly among livebirths, stillbirths and terminations of pregnancy. EUROCAT is a WHO Collaborating Centre and is supported by the European Commission Public Health Directorate Public Health Programme. EUROCAT manages research through collaboration in working groups and committees, such as the Classification and Coding Committee and the Working Group on Folic Acid and Neural Tube Defects.

The strategy adopted by EUROCAT for research into craniofacial anomalies includes an epidemiological approach to the evaluation of the differential distribution of such anomalies, research into etiological factors and policy practice and prevention. These three areas are underpinned by the extensive surveillance data collected within EUROCAT and shared through the International Clearing-house for Birth Defects Surveillance and Research for the world register.

EUROCAT has produced a guide to coding syndromes, to be published on the website, which will facilitate research into associated genetic factors. Many genes have been implicated in the etiology of cleft lip, with or without cleft palate (Schutte & Murray, 1999), each possibly contributing to genetic susceptibility in a complex network of gene–gene and gene–environment interactions. Cleft lip, with or without cleft palate, also occurs as part of many
single-gene syndromes, some of which might also play roles in non-syndromic cleft lip with or without cleft palate (Sözen et al., 2001). EUROCAT has embarked on an extensive project to document the prevalence of some important syndromes, including craniofacial syndromes. This information will be available on the website, with links to syndrome definition and diagnostic and genetic detail provided by the Orphanet website (www.orpha.net). This collaboration is part of an information system on rare diseases at the European level.

With representatives of the major organizations contributing to the International Perinatal Databases of Typical Orofacial Clefts (see Section 2), EUROCAT serves on the steering committee and provides data from a number of the registries annually or semi-annually. Some of these data for 1980–2002 are also available on EUROCAT’s website: www.eurocat.ulster.ac.uk/pubdata. Users can specify the region, year and congenital anomaly of interest to obtain customized tables.

Some EUROCAT studies that included orofacial clefts are summarized and discussed in the context of prevention in section 6. They are ‘Congenital malformation and maternal occupational exposure to glycol ethers’ (Cordier et al., 1997), ‘Risk of congenital anomalies near hazardous-waste landfill sites in Europe: the EUROHAZCON study’ (Dolk et al., 1998), ‘Maternal occupational risk factors for oral clefts’ (Lorente et al., 2000a), ‘Tobacco and alcohol use during pregnancy and risk of oral clefts’ (Lorente et al., 2000b) and ‘Socioeconomic inequalities in risk of congenital anomaly’ (Vrijheid et al., 2000).

EUROCAT, with its long-established network and proven ability in conducting scientific research, is the ideal tool for support in collaborative research on the roles of the large number of standardized variables collected in the complex etiology of craniofacial anomalies. The EUROCAT network is also a coordinated system through which biological samples can be collected and clinical studies conducted, providing unique points of reference and expertise in craniofacial anomalies. Use of the EUROCAT network could result in harmonization of the efforts of various organizations (e.g. the International Clearing-house for Birth Defects Surveillance and Research and the National Birth Defects Prevention Network) for international research projects, thus avoiding overlap and ensuring the best use of the resources available for research on craniofacial anomalies.

**International Clearing-house for Birth Defects Surveillance and Research**

The history, aims and activities of the International Clearing-house for Birth Defects Surveillance and Research (or Clearing-house) are described in detail in section 2. Its contribution to international collaboration in birth defects has been considerable, providing a range of opportunities for improving public health research and prevention. For example, such collaboration can furnish the fundamental molecular epidemiology of genes of public health importance, as the basis for the study of gene–disease associations. International collaboration also helps researchers to assess such associations across a wider and therefore more informative range of genotype frequencies and environmental exposures than would be feasible in one country. At the same time, collaboration provides quick access to large study populations, with resulting improvement in the statistical power of studies.

One successful collaboration within the framework of the Clearing-house was supported, in part, by the National Center on Birth Defects and Developmental Disabilities of the Centers for Disease Control and Prevention in the United States and the Italian Ministry for Research. In this collaboration, the 677 C→T allele of MTHFR, a folate-related gene, was evaluated in well-defined populations in 14 areas of Australia, the Americas, China and Europe (Wilcken et al., 2003). As summarized in Box 7, significant geographical and ethnic variations were identified in this genotype, which has been associated with an increased risk for neural tube defects (Botto & Yang, 2000). For example, high frequencies of homozygosity for the variant
allele (20% or more of the population) were found in Mexico, northern China and southern Italy. Ethnic variation was remarkable in a population-based sample in the Atlanta area in the United States, with a high frequency of homozygosity (18%) among Hispanics, an intermediate frequency (11%) among whites and a low frequency (< 3%) among African-Americans (Wilcken et al., 2003).

**Box 7. International collaboration in genetics within the International Clearing-house for Birth Defects Surveillance and Research**

<table>
<thead>
<tr>
<th>Goal</th>
<th>Assess international variation in genotypes of public health importance and their relation to adverse birth outcomes; promote capacity for genome analysis, particularly in the developing world.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Approach</td>
<td>Coordinate registries in setting up and running anonymous systematic surveys of unaffected newborns; coordinate interlaboratory quality control.</td>
</tr>
<tr>
<td>Current data</td>
<td>Over 7000 samples from 16 regions, collected systematically; information on the 677 C→T allele of MTHFR.</td>
</tr>
<tr>
<td>Selected findings</td>
<td>Geographical variation in the 677 C→T allele, with high rates of homozygosity in China (particularly in the north), Mexico and southern Italy; ethnic variation in the United States, with high rates among Hispanics and low rates among people of African origin or ancestry; no variation by sex.</td>
</tr>
<tr>
<td>Evolution</td>
<td>Evaluate expansion of the survey to other genotypes (e.g. other folate genes), other areas (e.g. Africa and Asia) and enroll affected infants for association studies.</td>
</tr>
</tbody>
</table>

From a practical perspective, this collaboration experienced widely differing capacities. Some registries (e.g. China, United States) could have undertaken local studies, from sample collection to genetic testing and report writing, but chose to collaborate and be included in a larger, more powerful, more meaningful study. Other programmes (e.g. in Israel and Mexico) had good capacity but little funding; with some financial support (mainly for reagents), they were able to provide data and the participation of populations of interest. In many other programmes, samples could be collected but not analysed; through this collaboration, they were able to participate in novel genetic studies and generate locally relevant data. Some programmes participated in discussions but not in the study; they thus gained knowledge about issues such as study design, sample collection and storage, quality control and data analysis. This successful collaboration provides the basis for further public health research on other genotypes of public health importance through partnerships between developed and developing countries.

The National Birth Defects Prevention Study, EUROCAT, EUROCRAN and the International Clearing-house for Birth Defects Surveillance and Research are all models for collaborative research on craniofacial anomalies. Centres follow a standard, mutually agreed protocol and contribute data to centralized databases. This approach maximizes the possibility of identifying both genetic and environmental risk factors for defects of unknown etiology, including many craniofacial anomalies, within a few years.

### 5.2 IDENTIFICATION AND INVOLVEMENT OF RELEVANT POPULATIONS

Presentations were made on other populations of interest, many of which still have a significant burden of craniofacial abnormalities. This was sometimes due to a high rate of unmet need owing to problems with surgical care services (e.g. India and Thailand), while in other populations the high birth prevalence might be due to consanguinity (e.g. Jordan),
specific gene-pool effects (parts of northern Europe) or a combination of as yet unknown genetic and environmental influences (e.g. China, Japan and the Philippines).

Research initiatives in Denmark were outlined. (1) In a case–control study, the participants were women who had given birth to a child with an orofacial cleft during 1991–94. Information on maternal exposures during the first trimester was obtained from interviews and birth records. Blood spots on filter paper from newborn screening cards were used to obtain DNA. (2) In a genetic study, the database included information from questionnaires and DNA (obtained by cheek swabs) from children born with orofacial clefts in Denmark in 1981–90 and from their parents and siblings. More than 500 families had been ascertained. More recently, DNA was also obtained from grandparents. (3) Registration of multiple births and linkage with the Danish twin register provided data on the occurrence of orofacial clefts among twins. (4) A population-based prospective study included 100 000 pregnant women and their offspring. The women were typically enrolled during their first trimester in 1996–2001 and participated in two interviews during the pregnancy and two after the birth. DNA was obtained from both mother and child. Information on hospitalization and surgeries was retrieved from several national health registers, and 220 children were found to have been registered with orofacial cleft.

Sample collection in the Philippines, supported by a grant from the National Institutes of Health, United States, with assistance from Operation Smile in case ascertainment, is described in detail in section 4.8.

ECLAMC is a birth defects registry programme that has been active in South America for the past 35 years. As described in section 4, ECLAMC introduced collection of biological material in 1998–99 (Orioli et al., 2001) from families of newborns with orofacial clefts (Vieira & Orioli, 2001; Orioli et al., 2002; Vieira, Orioli & Murray, 2002; Jezewski, 2003; Vieira et al., 2003a,b) and from families with holoprosencephaly (Orioli et al., 2001).

Opportunities for and obstacles to collaborative clinical research in Africa were outlined, and unique patterns and profiles of the birth prevalence of certain craniofacial abnormalities were reported. There are nevertheless limitations to the technical expertise, equipment and core personnel available, and, therefore, while biological samples could be obtained, analysis had to be carried out elsewhere. It was recommended that the first priority for collaborative research should be to define the epidemiology of craniofacial abnormalities, to set up and extend the community-based birth defects surveillance system and to establish a birth defects registry. African countries might then be in a position to collaborate internationally in research on gene–environment interactions. A further obstacle to such research, however, is the increasing pressure on health services of the HIV/AIDS pandemic.

A presentation entitled ‘Available database, research and treatment facilities in relation to craniofacial anomalies in China’ outlined opportunities for further research, including gene–environment interactions, in a well-organized, well-documented system, with willingness to collaborate in multicentre studies.

One of the largest craniofacial treatment centres in the world is in Bauru, Brazil, and a paper entitled ‘Brazil as a partner for the development of collaborative projects on craniofacial anomalies’ was presented. This paper highlighted the collaborative research initiatives that have been developed. Centrinho, Bauru, was recognized as a centre of excellence for multidisciplinary surgical and non-surgical management of orofacial clefts. Laboratory research facilities have been extended, enabling genetic and biochemical expertise to be brought to studies on the etiology of craniofacial abnormalities.

Collaborative research opportunities in South-East Asia were outlined in a paper entitled ‘Overview of cleft lip–palate and craniofacial anomalies in South-East Asia’. This presentation highlighted the high birth prevalence of various craniofacial abnormalities,
including cleft lip and palate. A birth defects surveillance programme (hospital-based) and a good health-care system have been developed over the past 20 years. As the research infrastructure is also evolving, collaborative research will be possible, including involvement in genetic and gene–environment interactions initiatives.

A presentation entitled ‘Opportunities and obstacles to collaborative clinical research in patients with cleft lip/palate in Chile’ indicated that the country is a willing and able partner for future multicentre collaborative research. Congenital malformations are a major cause of infant morbidity and mortality in Chile. Medical genetics is becoming increasingly important in the diagnosis and management of birth defects, and research is possible in epidemiology, clinical genetics, cytogenetics and molecular genetics. Since January 2000, flour has been enriched with folic acid (220 µg/100 g); in combination with a good birth defect surveillance system, this should contribute to investigation of the efficacy of folic acid supplements and food fortification in the prevention of birth defects.

Jordan’s experience with craniofacial abnormalities was presented, highlighting the unique features of the Jordanian population, including arranged, mainly consanguineous, marriages; prohibition of abortion on demand; widespread tobacco use but minimal alcohol use; and a high birth rate of 44 livebirths per 1000 population per year. The prevalence of congenital anomalies is 1 in 50 newborns, half having a chromosome abnormality. The prevalence of genetic disorders was reported to vary by ethnic group and age group, but no figures for craniofacial anomalies were presented. The staple diet is bread made of milled wheat and a considerable amount of folate-containing food items; as a result, the diet is folate sufficient.

The situation in India was outlined in three consecutive presentations. The first, entitled ‘Ascertainment of craniofacial anomalies: experiences in India’, pointed to inaccuracies in the ascertainment of craniofacial abnormalities in that country, as most studies have been hospital-based and do not reflect the true incidence in the population. An attempt to carry out ascertainment in geographically defined areas in an accurate population-based survey, with social workers and volunteers making house-to-house enquiries, was described. This study produced interesting data, indicating that some populations have an extremely low prevalence of cleft lip and no cleft palate; however, it was reported that children were indeed born with such disabilities in this population but that they were unable to feed and died of starvation.

The second presentation, entitled ‘Opportunities and obstacles to collaborative clinical research in India’, outlined the epidemiology but acknowledged that there was inadequate documentation of the rates of birth anomalies and that there was a large unmet need. A number of activities were proposed:

- carry out a hospital-based study to determine the frequency of craniofacial anomalies, the denominator being the number of births in hospital;
- study nutritional factors, including folic acid, to understand the role of nutrition and malnutrition in birth defects;
- analyse genetic information on affected children and parents in order to conduct studies of gene–environment interactions and other issues;
- improve public awareness about craniofacial abnormalities and the fact that they can be treated; and
- begin creating a registry for craniofacial abnormalities throughout India.

In the third presentation on India, an overview was given of the clinical and surgical services available. The backlog of unmet need for surgical repair is being addressed in centres of excellence, such as one in Mangalore. These centres are mainly supported by charities, such as SmileTrain and the International Foundation for Craniofacial Abnormalities. A significant shift in emphasis from treatment of craniofacial abnormalities to complementary research is
emerging, with recognition that data on environmental and genetic factors are crucial. The hope for the future is that India will make a significant contribution to worldwide collaborative etiological research.

The presentations all emphasized a willingness to become involved in research and to contribute to collaborative initiatives. Some mentioned existing or potential problems, but many reported new facilities, expertise and infrastructure that will allow more accurate assessment of birth prevalence and facilitate the involvement of these centres in clinical and laboratory research in the future. As large samples are imperative for progress in the field of gene–environment interactions, this multicentre approach should be made a priority.

5.3 BIOLOGICAL PLAUSIBILITY OF COMMON EXPOSURES AND GENE VARIANTS

Environmental factors and gene–environment interactions

Relatively few investigations have been made of gene–environment interactions in the etiology of craniofacial anomalies, and even fewer in which biologically plausibility is a central component. Such investigations would lead to improved understanding of etiology and pathogenesis and provide a background for decisions on public health strategies. So far, all studies have addressed the etiology of isolated or non-syndromic orofacial clefts (Table 12):

- TGFα and smoking (Hwang et al., 1995; Shaw et al., 1996; Beaty et al., 1997; Christensen et al., 1999; Romitti et al., 1999);
- TGFα and vitamin supplements (Shaw et al., 1998a);
- TGFβ3 and smoking and alcohol (Maestri et al., 1997; Romitti et al., 1999; Mitchell et al., 2001);
- MSX1 and smoking and alcohol (Romitti et al., 1999; Mitchell et al., 2001; Beaty et al., 2002);
- polymorphisms affecting xenobiotic metabolism (CYP1A1, EPHX1, GSTM1, NAT1, NAT2, N-acetyltransferase phenotype) and smoking (Hartsfield et al., 2001; van Rooij et al., 2001, 2002a; Lammer et al., 2004);
- polymorphisms affecting xenobiotic metabolism (GSTM1, GSTT1, NAT2) and occupational exposures (Shaw et al., 2003a);
- polymorphism affecting xenobiotic metabolism (N-acetyltransferase phenotype) and maternal medication use (van Rooij et al., 2002a);
- retinoic acid receptor α polymorphisms and maternal intake of vitamin A (Mitchell et al., 2003); and
- polymorphisms affecting folate metabolism (MTHFR, RFC) and maternal folate intake (Shaw et al., 1998b, 1999; Jugessur et al., 2003b; van Rooij et al., 2003a; Shaw et al., 2003b).

The results of these investigations have been inconclusive, partly because of lack of statistical power to detect or exclude interaction, partly because of differences in who was genotyped (in some only the infant, in some only the mother) and partly because replication has either not been done or not been reported. In the studies of polymorphisms affecting xenobiotic metabolism in which only the infant genotype was examined (Hartsfield et al., 2001; Shaw et al., 2003a; Lammer et al., 2004), epoxide hydrolase 1 was strongly expressed in fetal liver, but the other enzymes coded for by these genes might not be expressed until after birth.
Addressing the global challenges of craniofacial anomalies

(Cresteil, 1998; McCarver & Hines, 2002). Timing and the tissue of gene expression are of potential importance in interpreting these findings, but there appear to be few data on these issues. In addition, gene variants have often been considered one at a time, whereas variants of multiple genes would modulate the effects of an exposure (Fryer & Jones, 1999; Sing, Stengard & Kardia, 2003).

Table 12. Studies on gene–environment interactions with common exposures and gene variants

<table>
<thead>
<tr>
<th>Area, period (reference)</th>
<th>Design</th>
<th>Genotyping of</th>
<th>Cleft lip, with or without cleft palate (N ≤)</th>
<th>Cleft palate (N ≤)</th>
<th>Variants reported</th>
<th>Maternal exposures reported</th>
</tr>
</thead>
<tbody>
<tr>
<td>Denmark, 1991–94</td>
<td>Case–control</td>
<td>Infant</td>
<td>233</td>
<td>83</td>
<td>TGFα</td>
<td>Smoking, alcohol, vitamins, eating liver</td>
</tr>
<tr>
<td>(Christensen et al.,</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>TGFβ3</td>
<td></td>
</tr>
<tr>
<td>1999; Mitchell et al.,</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>MSXI</td>
<td></td>
</tr>
<tr>
<td>2001, 2003)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>RARA</td>
<td></td>
</tr>
<tr>
<td>Netherlands, 1997–2000</td>
<td>Case–control</td>
<td>Infant, mother</td>
<td>146</td>
<td>NS</td>
<td>CYP1A1</td>
<td>Smoking, medications, folic acid, folate</td>
</tr>
<tr>
<td>(van Rooij et al., 2001,</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>GSTT1</td>
<td></td>
</tr>
<tr>
<td>2002a, 2003a)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>NAT2</td>
<td></td>
</tr>
<tr>
<td>Norway, 1996–98</td>
<td>Case–parents</td>
<td>Infant, mother, father</td>
<td>173</td>
<td>88</td>
<td>TGFα</td>
<td>Smoking, alcohol, vitamins, folic acid</td>
</tr>
<tr>
<td>(Jugessur et al., 2003a,b)</td>
<td>triad</td>
<td></td>
<td></td>
<td></td>
<td>MTHFR</td>
<td></td>
</tr>
<tr>
<td>United States, California, 1987–89</td>
<td>Case–control</td>
<td>Infant</td>
<td>447</td>
<td>215</td>
<td>TGFα</td>
<td>Smoking, vitamins, occupational chemical groups</td>
</tr>
<tr>
<td>(Shaw et al., 1996, 1998a,b, 1999; Hartshfield et al., 2001; Shaw et al., 2003a,b; Lammer et al., 2004)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>EPHX1</td>
<td>Smoking, alcohol</td>
</tr>
<tr>
<td>United States, Iowa, 1987–94</td>
<td>Case–control</td>
<td>Infant</td>
<td>154</td>
<td>60</td>
<td>TGFα</td>
<td>Smoking, alcohol</td>
</tr>
<tr>
<td>(Romitti et al., 1999)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>TGFβ3</td>
<td></td>
</tr>
<tr>
<td>United States, Maryland, 1984–92</td>
<td>Case–control</td>
<td>Infant, mother, father</td>
<td>186</td>
<td>83</td>
<td>MSXI</td>
<td>Smoking</td>
</tr>
<tr>
<td>(Hwang et al., 1995; Beaty et al., 1997; Maestri et al., 1997; Beaty et al., 2002)</td>
<td>and triad</td>
<td></td>
<td></td>
<td></td>
<td>TGFα</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>TGFβ3</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>MSXI</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>BCL1</td>
<td></td>
</tr>
</tbody>
</table>
Nutrition, smoking, alcohol and other common environmental exposures have been the focus of much of the work on gene–environment interactions. Nutritional factors continue to be studied (Munger, 2002; Munger et al., 2004), as does low socioeconomic status (Murray et al., 1997). Recognized teratogens that contribute to clefts include rare exposures, such as to phenytoin and thalidomide, and also common environmental exposures, such as maternal alcohol or cigarette use (Hayes, 2002; Little, 2004).

Risks due to environmental exposures can suggest metabolic pathways, the disruption of which might play a role in the development of cleft lip and/or palate. The effects of common variables, such as alcohol, smoking, occupational exposures or nutrition, might be amplified by variation in pharmacogenetics. Most population-specific studies (Table 12) and multicentre collaborative studies (Table 13) of gene–environment interactions have concentrated on common exposures and gene variants. Similar studies of non-syndromic cleft lip and/or palate are in their infancy, but some interesting preliminary data suggest that further studies should be conducted on the role of folate-metabolizing enzymes and genes in detoxification pathways (Zeiger et al., 2002).

### Table 13. Selected multicentre collaborative studies

<table>
<thead>
<tr>
<th>Area, period</th>
<th>Design</th>
<th>Genotyping of</th>
<th>Cleft lip with or without palate ( n )</th>
<th>Cleft palate ( n )</th>
</tr>
</thead>
<tbody>
<tr>
<td>EUROCRAN</td>
<td>Case–parents triad</td>
<td>Infant, mother, father</td>
<td>~900</td>
<td></td>
</tr>
<tr>
<td>United States</td>
<td>Case–control and triad</td>
<td>Infant, mother, father</td>
<td>~1000 samples from ~300</td>
<td></td>
</tr>
</tbody>
</table>

Emerging evidence from studies of genetics, animal models, gene expression and environmental correlates suggests a variety of candidate genes for cleft lip and/or palate. Table 14 gives a current listing of candidate genes grouped by gene class; although it must be recognized that any such list is continually evolving.

### Table 14. Candidate gene classes for cleft lip and/or palate

<table>
<thead>
<tr>
<th>Transcription factor</th>
<th>Growth factor</th>
<th>Cell signalling</th>
<th>Folate path</th>
<th>Detoxication</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSX1</td>
<td>TGF( \alpha )</td>
<td>PVRL1</td>
<td>MTHFR</td>
<td>CYP1A1</td>
</tr>
<tr>
<td>TBX22</td>
<td>TGF( \beta 1 )</td>
<td>PVRL2</td>
<td>RFC1</td>
<td>NAT1</td>
</tr>
<tr>
<td>IRF6</td>
<td>TGF( \beta 2 )</td>
<td>PVR</td>
<td>MTRR</td>
<td>NAT2</td>
</tr>
<tr>
<td>LHX8</td>
<td>TGF( \beta 3 )</td>
<td>PTCH</td>
<td>GCP2</td>
<td>GSTM1</td>
</tr>
<tr>
<td>TBX10</td>
<td>TP63</td>
<td>GABRB3</td>
<td>CBS</td>
<td>GSTT1</td>
</tr>
<tr>
<td>DLX1/2/5/6</td>
<td>SKI1</td>
<td>ARNT2</td>
<td>MTHFD</td>
<td>GSTP1</td>
</tr>
<tr>
<td>SATB2</td>
<td>TGF( \beta R1 )</td>
<td>WNT9A</td>
<td>FOLRA</td>
<td>RARA</td>
</tr>
<tr>
<td>RYK</td>
<td>FGFR1</td>
<td>ROR2</td>
<td>BHMT</td>
<td>EPHX1</td>
</tr>
</tbody>
</table>
Biological plausibility forms the basis of many hypotheses in this field, but chance interactions are detected occasionally, as was the case for smoking and the uncommon TGFαC2 allele (Table 15). In Iowa, United States, smoking and alcohol use were investigated as environmental variables and coupled to candidate genes. Elevated risks for cleft palate (smoking) and cleft lip and/or palate (alcohol) were seen when the environmental variable was on the background of specific allelic variants of TGFβ3 and MSXI (Romitti et al., 1999). Seven genes involved in folate metabolism are being investigated, linking the genetic analysis to vitamin use and nutritional data. The preliminary results show a significant association of the MTHFD gene with cleft lip and/or palate. In a related study, 17 genes involved in smoking detoxification paths were examined for phase I and phase II enzymes. The genotyping was perfomed with new real-time polymerase chain reaction assays (Shi et al., 2003), and analysis was conducted with both logistic regression and log–linear models (Weinberg et al., 1998). Preliminary results showed a strong gene–environment component for smoking and the A313G variant of GSTP1 in case–parent samples. GSTP1 is the sole member of the π family of glutathione S-transferase genes and is the dominant GST gene expressed in the lung. It is thus a prime candidate for a gene that would serve as a covariate with maternal cigarette use. Perhaps more remarkably, GSTP1 is also highly expressed in the developing fetus and lies within 100 kilobases of the TBX10 gene, in which ectopic expression results in cleft lip and/or palate in the Dancer mouse mutant.

Three studies were conducted on genetic and environmental risks for clefts with environmental data from a Danish case–control study conducted in 1991–94 and genotyping performed in Iowa, United States. One study suggested that the previously reported association between TGFα and cleft lip and palate might be attributable to confounding by ethnicity (Christensen et al., 1999). A study of the association between TGFβ3 and both cleft lip and/or palate and cleft palate in Denmark found a statistically significant association ($p = 0.05$) between cleft palate and the CA repeat of TGFβ3 (Mitchell et al., 2001). In a study of the interaction between the retinoic acid receptor α locus and intake of vitamin A, no evidence was found that the risks for cleft lip and/or palate and cleft palate are related to the retinoic acid receptor α variant (Mitchell et al., 2002).

Genetic assays of TGFα, TGFβ3, MSXI, MTHFR and RARA in cleft lip and/or palate were performed on 262 case–parents triads from a population-based case–control study of clefts in Norway. Log–linear models (Weinberg, Wilcox & Lie, 1998) were used to estimate the effects of those variants. Effects of TGFα variants on cleft palate were seen, and there was also evidence of gene–gene interactions between MSXI and TGFα (Jugessur et al., 2003). A significant increase in relative risk (4.48; 95% confidence interval, 1.28–15.7; $p = 0.019$) was found with with the TGFα variant when mothers did not take folic acid supplements, but not when they did (relative risk, 1.42; 95% confidence interval, 0.16–12.7; $p = 0.76$) (Jugessur et al., 2003b).

### 5.4 STANDARDIZATION OF METHODS AND SYSTEMATIC ASSESSMENT OF RISKS AND EFFECTS

To optimize research on craniofacial anomalies, consistent protocols for all populations are necessary. For ongoing research on gene–environment interactions, the study design might be a case–control or case triad. In either case, information is collected on cases and, when necessary, on control subjects. Some guidelines on the recruitment of cases and controls have been drawn up by Dr Terri Beaty, and a group supported by the European Science Foundation has issued detailed guidelines on the core information required in eight research areas.
### Table 15. Studies on maternal TGFα and maternal smoking in association with cleft lip and/or palate

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Type of cleft</th>
<th>Cases / controls</th>
<th>Odds ratio with combination of maternal smoking and genotype</th>
<th>Test for interaction</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Smoking</td>
<td>G+/−</td>
<td>G−/−</td>
</tr>
<tr>
<td>Taq1 C2 allele</td>
<td>CL/P</td>
<td>8/11</td>
<td>Yes/no</td>
<td>1.1</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td>CP</td>
<td>12/11</td>
<td></td>
<td>0.8</td>
<td>1.1</td>
</tr>
<tr>
<td>Uncommon allele</td>
<td>CL/P</td>
<td>6/3</td>
<td>≥ 0</td>
<td>0.8</td>
<td>1.8</td>
</tr>
<tr>
<td></td>
<td>CP</td>
<td>5/3</td>
<td>T1</td>
<td>1.0</td>
<td>1.8</td>
</tr>
<tr>
<td>Taq1 C2 allele</td>
<td>CL/P</td>
<td>3/2</td>
<td>Yes/no</td>
<td>0.7</td>
<td>1.8</td>
</tr>
<tr>
<td></td>
<td>CP</td>
<td>2/2</td>
<td></td>
<td>1.4</td>
<td>1.8</td>
</tr>
<tr>
<td>Taq1 C2 allele</td>
<td>CL/P</td>
<td>19/46</td>
<td>Yes/no</td>
<td>1.2</td>
<td>1.6</td>
</tr>
<tr>
<td></td>
<td>CP</td>
<td>5/46</td>
<td></td>
<td>1.2</td>
<td>1.0</td>
</tr>
<tr>
<td>Taq1 C2 allele</td>
<td>CL/P</td>
<td>0/4</td>
<td>≥ 0</td>
<td>1.3</td>
<td>1.8</td>
</tr>
<tr>
<td></td>
<td>CP</td>
<td>2/4</td>
<td>T1</td>
<td>0.9</td>
<td>3.2</td>
</tr>
</tbody>
</table>

CL/P, cleft lip and/or palate; CP, isolated cleft palate; G−/−, homozygous for common allele; G+/−, heterozygous genotype; G++/+, homozygous for uncommon allele

The guidelines proposed for recruiting case and control families are:

1. Document ascertainment of cases and controls.
   a. Population-based
   b. Clinic-based
   c. Diagnosis (for cases), sex and age
2. Collect family history.
3. Collect information on exposures during pregnancy.
4. Collect DNA from case, parents and siblings (extend to affected family members as needed) and from controls (extend to parents and siblings as needed).

The European Science Foundation Scientific Network developed methods to investigate the interaction between nutritional, environmental and genetic factors in early human development, with a demonstration project on orofacial clefts. Common core protocols for gene–environment interactions would include:

- case ascertainment;
• nutritional factors and food frequency questionnaires;
• life style and environment factors;
• obstetric and medical history, including drugs;
• family history;
• clinical assessment of orofacial clefting;
• genetic protocols and assays;
• laboratory biochemical assays; and
• ethics guidelines.

Summaries of each set of guidelines are given below, and comprehensive details can be found on a pdf file under ‘common core protocols’ on the EUROCRAN website www.eurocran.org.

European Science Foundation common core protocols project: minimum datasets

Nutritional factors and food frequency questionnaires. Nutrition remains one of the most useful aspects of research into orofacial clefting. Core nutritional data and a food frequency questionnaire are useful to enable assessment of total energy intake. The reported nutrient intake must be population-specific and must be validated by comparison with a relative ranking obtained by another method, such as diet diary or weight record. Vitamin supplements and food fortification should be included. Food frequency questionnaires are used only for stratification and not as a quantitative tool.

Life-style and environmental factors. It is usual to collect data on exposures such as smoking and alcohol use during the first trimester of pregnancy. It might be questioned whether data on occupational exposure and use of recreational drugs is also essential. If socioeconomic status is to be examined, the most consistent measures must be selected, which might include education, housing, income, occupation or a combination.

Obstetric and medical history. The minimum data should include illnesses and medications used during the first trimester, obstetric history, date of conception, birth control methods used, the timing of awareness of pregnancy and history of morning sickness. The medical history should include common illnesses such as colds and flu as well as medical conditions that might be associated with birth defects. Drug therapy should be related to the medical conditions. Questions on drug therapy should be tailored to the hypothesis, such as use of anticonvulsants in epilepsy and radiotherapy, folate and anti-folate drugs, such as methotrexate, and antimalarials. Previous obstetrical history should be recorded in terms of number of siblings, previous stillbirths and other related congenital abnormalities. Other aspects of the medical history specifically related to the hypothesis should be recorded, e.g. use of vitamin A.

Clinical assessment of orofacial clefting. Ascertainment of congenital anomalies and a precise diagnosis should be based on multiple sources of information. Ascertainment should include: definition of fetal death, definition of induced abortion, coding of congenital anomalies, minor anomalies and a precise diagnosis. The information could include multiple anomalies and syndromes, late diagnosed cases, prenatal diagnosis, ultrasound or maternal serum screening. The subsets of cleft lip and palate are cleft lip, unilateral cleft lip and palate, bilateral cleft lip and palate and right and left. Isolated cleft palate subsets can be classified by, e.g. 22q11 deletions.
Biochemical assays. The two main issues are the methods of sample collection and storage and biochemical analysis. Both can depend on the purpose for which samples are being collected. The core dataset in cases of orofacial clefting should include a full blood count, red cell folate, plasma folate, plasma vitamin B₁₂, plasma homocysteine, plasma vitamin B₆, methylmalonic acid, genetic analysis, vitamin A and other nutrients and immortalized cell lines obtained from lymphocytes.

Genetic protocols and assays. Molecular genetic factors in orofacial clefting include DNA and polymorphisms, adjacent to or within the candidate genes, for identification of etiological genetic loci. Nuclear triads have several advantages, as they can be used in transmission disequilibrium tests, for identifying the effects of the parent of origin, chromosome deletions and uni-parental disomy. The candidate genetic loci fall into four overlapping categories: genes expressed during palatogenesis with temporal and spatial specificity to clefting; chromosome deletions, duplications or translocations that cause orofacial clefts; genes or loci identified in animal models; and genes that possess or control specific biological activities that might explain orofacial clefting.

Samples. The samples collected can include saliva, buccal cells, dried blood spots (Guthrie cards) or blood samples.

Family history. The family history can be subdivided into minimal information (compulsory), complete family history and blood samples from relatives as well as the nuclear triad. The minimal family history should include the immediate family as first-degree relatives, i.e. grandfather and grandmother on both sides, all fathers, siblings and paternal first cousins and all mothers of siblings and maternal first cousins. All malformations in the family should be recorded. The questionnaire should be designed so that minimal information will be collected, but the data should not be computerized, so as to maintain confidentiality.

In order to obtain a family history, the desirable option is an interviewer trained in family investigation using a detailed familial questionnaire. Blood samples from relatives allow genetic analysis, and other siblings should be included whether they are affected or not. Affected relatives other than a parent or sibling should be included, with blood samples from any relative in this branch of the family.

Bioethical issues. Minimum data should be available on informed consent, confidentiality and the principles espoused in the Declaration of Helsinki.

These common core protocols can be accessed on the EUROCRAN website: http://www.eurocran.org/documents/ComCoreProtMaster5-050901.pdf.

5.5 INNOVATIVE STUDY DESIGNS AND STATISTICAL METHODS

Orofacial clefts, which include cleft lip, cleft lip and/or palate and cleft palate, have a complex and heterogeneous etiology, because both genes and environmental risk factors are known to be involved and because any one type of cleft can have multiple causes or reflect the combined action of more than one gene or a gene and environmental risk factors. This high level of etiological complexity must be considered in designing studies of orofacial clefts.

No single genetic model of inheritance has been consistently supported by family studies, and animal models suggest that two or more genes are important in the etiology of orofacial clefts. In addition, among the several recognized environmental risk factors for orofacial clefts are common exposures such as maternal smoking, alcohol consumption and vitamin supplementation. Thus, consideration of multiple genes and environmental exposures and their possible interactions should become routine in designing studies of orofacial clefts.
Studies involving many populations are needed to amass large numbers of subjects in a short time. Thus, the effect of genetic diversity among populations should also be considered in both the analytical phase and the interpretation of results. There are two general strategies for identifying genes that control risk for a complex disease such as orofacial clefts: linkage and association. The two differ in both the underlying scientific questions and the type of data required. Both methods can effectively be conducted with data from many populations, but, again, the effects of genetic and environmental differences among populations should be considered.

**Linkage studies**

Linkage studies usually derive information on families with two or more affected members from multiple sources, favouring large extended families spanning many generations with as many affected members as possible. In conventional tests for linkage, there is relatively little danger of spurious findings (e.g. false-positive results) because the statistical test is narrowly focused on estimating genetic recombination within defined mating types. The extreme ascertainment process involved in collecting information on such heavily affected families often guarantees that the results reflect the effects of a gene rather than some environmental risk factor. One or only a few large affected families can be used to identify genes that cause orofacial clefts, especially if the causal mutation leads to a high probability of being affected (van den Boogaard et al., 2000; Schutte et al., 2000). Linkage analysis can also be done on pairs of affected relatives (typically affected sibling pairs), allowing estimation of shared marker alleles that are identical by descent. Information on smaller affected families can thus be obtained in a more robust, non-parametric approach.

A key strength of linkage studies is their insensitivity to ascertainment bias. In analysing samples from heavily affected families, it would seem impossible to avoid some degree of linkage or locus heterogeneity; however, even samples from a single source routinely provide evidence that orofacial clefts in some families are linked to a given gene or chromosome region, while others are not. This suggests a high degree of heterogeneity and a number of different causal genes. Studying heavily affected families in different populations should add power for detecting linkage, and only minor modifications to the analysis are required to estimate marker allele frequencies in different populations. Marazita et al. (2004b) summarized the results of seven genome-wide studies, which indicated that at least 16 different chromosome regions may contain susceptibility genes for orofacial clefts. Families that are useful for linkage studies are not, however, representative of all cases, as only 12–15% of cases have any family history of orofacial clefts. It is therefore difficult to generalize the findings from linkage studies to the general population of cleft cases.

**Association studies**

Association studies are used to test the more general null hypothesis that frequencies of genetic markers are independent of whether a person has a cleft or not, usually by comparing unrelated cases and controls. Population-based case–control studies allow the drawing of general inferences about what determines risk (genes, environmental risk factors or a combination) as well as estimation of the attributable fraction (the proportion of cases attributable to a particular causal factor). The observed statistical associations between genetic markers and orofacial clefts are, however, notoriously difficult to replicate across populations and may or may not point to a truly causal gene. It is important to understand that a statistically significant association between a marker and a disease can represent: (1) a direct effect of the marker on risk; (2) an indirect effect of the marker on risk due to tight linkage between the marker locus and an unobserved causal gene (called "linkage disequilibrium");
(3) a simple statistical error; or (4) a spurious association due to confounding or population stratification (see below). While linkage analysis is relatively immune to ascertainment bias, researchers using case–control designs must be careful to document their ascertainment sources (clinic, hospital or registry) and to consider differences among populations.

In the traditional case–control study, gene frequencies in groups of unrelated cases and unaffected controls are compared, either as alleles, genotypes or haplotypes (combinations of alleles at two or more genetic markers). Ideally, the cases should represent all infants born with an orofacial cleft, and controls should represent all unaffected infants. Thus, in clinic-based studies, efforts should be made to document the survival of infants undergoing surgical treatment. The controls can be either healthy infants or at least infants without an orofacial cleft (controls with birth defects unrelated to clefting are often used).

One weakness of the usual case–control design is that false-positive results can be obtained due to confounding if cases and controls are drawn from unrecognized sub-populations that differ in both their risk of disease and their marker allele frequencies. The actual importance of confounding remains a matter of debate, but many reported associations between orofacial clefts and markers in candidate genes have been difficult to replicate across populations. Meta-analysis or analysis of pooled data can sometimes overcome this problem, but not always (Mitchell, 1997). The lack of reproducibility of results might reflect lack of statistical power due to small sample sizes in individual studies, various sources of bias in sampling cases or controls, or true heterogeneity among populations. A chief advantage of case–control designs is that they permit formal testing for interaction between genetic markers and environmental risk factors, which may be critical in the etiology of orofacial clefts. Tests for interaction generally require larger samples sizes, however, and are also difficult to replicate across studies (Zeiger, Beaty & Liang, 2005). Furthermore, to the extent that controls are representative of the populations in question, genetic diversity across populations in multicentre and international studies can easily be analysed by looking at the markers in the control group. This will highlight any confounding and avoid obtaining spurious results, albeit at the expense of increased stratification of the data.

**Case triad studies**

Families ascertained from a case can be used in a modification of the usual case–control design to contrast genes that occur in the affected child with those that do not. Family-based tests of association circumvent the problem of confounding by comparing observed marker frequencies to that expected under strict Mendelian transmission of markers, independent of phenotype. The simplest family unit is a case and the two parents, known as a case–parents triad. This study design is particularly suited to studies of birth defects because the parents are generally readily available and it is easy to obtain DNA from all three family members. Rejecting the null hypothesis of complete independence between markers and affected status in a case–parents triad design tests the existence of both linkage and linkage disequilibrium between the marker and an unobserved causal gene. Case–family designs can also be used for markers such as alleles, genotypes and haplotypes. Haplotype analysis has greater statistical power to identify causal genes and has been used in studies of orofacial clefts (Fallin et al., 2003). Case–parents triad designs can be extended to test for gene–environment or even gene–gene interactions (Beaty et al., 2002; Jugessur et al., 2003b). Case–family designs cannot, however, be used to test for effects of environmental risk factors alone, because “controls” are created from the genotypes of the parents and cannot vary in exposure. Thus, case–family study designs are not as flexible as case–control designs.
Practical applications

Both linkage and association studies can be effectively used in multicentre, international studies of orofacial clefts, but each design brings its own limitations and strengths. Linkage studies of heavily affected families are well suited for identifying genes that control risk, but they do not address the role of environmental risk factors and offer limited opportunity to test for interactions. Furthermore, because the families suitable for linkage studies are only a subset of all cleft families, it is difficult to generalize and build inferences about the role of any one gene on risk at the population level. Population-based case–control designs offer the most flexible approach for testing genetic and environmental risk factors, but they require careful definition of the ascertainment scheme, and international studies require extensive checking for differences among populations that could result in confounding, leading inevitably to more stratification in the analysis. Nevertheless, the design itself provides an opportunity to use the controls to measure genetic heterogeneity among populations and adjust the analysis accordingly.

Because case–family studies circumvent the possibility of confounding due to population stratification, they may be preferred for international studies, where there are recognized differences in marker allele frequencies and birth prevalence rates of orofacial clefts. Genetic differences among populations can always be a source of critical heterogeneity in this study design, but it will be less of a problem than in the usual case–control study. Human populations always show a substantial degree of genetic diversity, and this is reflected in both allele and haplotype frequencies, which are critical to identifying causal genes in tests of association. Therefore, a careful evaluation of genetic diversity should be part of any international study. Case–family designs offer the opportunity to analyse genetic diversity among the parents of patients (most of whom will be unaffected). The parents of affected children might not be a representative sample of each population, but they are a reasonable starting point.

Populations can differ in the degree of departure from Hardy-Weinberg equilibrium, i.e. in the strength of linkage disequilibrium among markers separated by the same physical distance. This can be easily documented by examining patterns of linkage disequilibrium in the parents of affected children, even if no controls are available. While the overall patterns of linkage disequilibrium might be roughly similar across human populations, older human populations are likely to be closer to full Hardy-Weinberg equilibrium than younger populations. It should be recognized that recent admixture between genetically distinct populations can also create a high level of disequilibrium, distinct from that due to linkage. Even when there is a high level of genetic diversity among populations, genetic data from international studies can be used to search systematically for genes that control the risk for orofacial clefts.

Concept of nutrigenomics and its application to birth defects

Investigations of gene–disease associations can help to identify the potential effects of a nutrient on a disease or to corroborate indications of nutrient–disease associations. For example, the MTHFR C677T variant affects the metabolism of folate and, in turn, causes elevated homocysteine levels. The relation between this MTHFR polymorphism and coronary heart disease has been investigated in a number of studies; in a recent meta-analysis, persons who were TT homozygotes were found to have a 16% higher risk of coronary heart disease than persons homozygous for the common variant (Klerk et al., 2002). This finding corroborates evidence of a relation between higher homocysteine levels and ischaemic heart disease.

This approach exploits the principle of ‘Mendelian randomization’ (Davey Smith & Ebrahim, 2003), the random passage of genes from parents to offspring during meiosis. On the basis
that certain genotypes can be viewed as proxies for certain exposures, assessment of gene–
disease associations can be used as an indirect method for exploring the causal nature of
environmental exposures. Because of Mendelian randomization, an association between a
disease and a genotype is unlikely to be due to confounding, provided that the study is
designed according to the principles of population-based studies (Clayton & McKeigue,
2001), so that the unconfounded effect of prolonged exposure to a nutrient (or other
environmental agent) the effect of which depends on genotype can be inferred.

The caveats to many observational studies include small sample size, linkage disequilibrium,
population stratification, and gene–gene and gene–environment interactions that might mask
simple gene association effects (Little et al., 2002, 2003). The usefulness of this approach is
also limited by incomplete understanding of gene function and biological pathways important
in the pathogenesis of common diseases.

5.6 EXPLOITATION, SHARING AND POOLING OF DATA AND BIOLOGICAL
SAMPLES

The meeting acknowledged that, while some national and international collaborative projects
on craniofacial anomalies have been initiated, they remain fairly limited and there is scope to
expand them to intercontinental projects. A network of research on gene–disease associations
and related gene–gene and gene–environment interactions in craniofacial anomalies was
proposed by Professor Julian Little in the context of gene–environment interactions. It would
be important to extend such research to associations between craniofacial anomalies and
genetic susceptibility factors. Formation of a network to facilitate meta- and pooled analyses
of associations between genes and craniofacial anomalies and related gene–environment and
genome–gene interactions was therefore proposed.

Rationale

A workshop was organized by the Human Genome Epidemiology Network in November 2004
to discuss methodological issues in the systematic review and meta-analysis of studies of
genome–disease associations and related interactions. A combination of almost unrestricted
genotyping potential, exploratory statistical analyses in studies with limited sample sizes, and
selective reporting is increasing the frequency of spurious claims that cannot be replicated,
when replication is attempted. Null findings are often not published or publication is delayed.
Although it might be argued that evidence in favour of ‘true’ associations will accumulate and
eventually be distinguished from false-positive results, this has not always occurred with
respect to other types of observational evidence, notably on diet and chronic disease. Such
inefficiency can lead to substantial misuse of resources.

The workshop participants recognized that, while study registration is useful for achieving
transparency and avoiding biases in clinical trials, this approach might not be feasible for
studies of gene–disease associations and related interactions because of the diversity of
settings in which such studies can be carried out and the rapidity of their conduct, which is
facilitated by the availability of specimen collections. Therefore, creation of inclusive
registries or networks of investigators and data and sample collections in various fields was
proposed. The WHO International Collaborative Research on Craniofacial Anomalies project,
would be the basis for such a network, and the network on craniofacial anomalies could
exemplify the proposed approach.
Strategy

As a first step, a list would be drawn up of teams working on gene–disease associations and related interactions in craniofacial anomalies, on the basis of the WHO database and literature searches. This network project would allow participation of teams with established data and sample collections, teams with data and sample collections in progress and teams planning to establish data and sample collections.

Formation of the network and registration of research will facilitate the conduct of rigorous systematic reviews, meta-analyses and, for certain topics, pooled analyses of individual data, possibly with additional genotyping.

Challenges in conducting international studies

In some countries (e.g. China and India), once data have been collected, additional governmental approval is required to allow the sharing of certain types of materials (e.g. DNA) among research partners. The need for such approval should be identified before research grants are submitted, as, if certain material cannot leave the country, the protocol might have to be revised to allow a national collaborator to process or analyse samples. Once the analysis protocol has been completed, the results can be presented according to pre-existing guidelines for international collaborations.

Clearly, there are many logistic challenges to be met in order to conduct a multinational genetic study. Given the large sample sizes that are necessary for investigating complex traits such as orofacial clefting, however, international collaborations will continue to be essential. Guarding the confidentiality of human data, providing for informed consent and implementing peer-reviewed study protocols can be lengthy, but the importance of these aspects should outweigh any inconvenience.

5.7 IMPROVED ASSESSMENT OF ENVIRONMENTAL EXPOSURES

Environmental factors are thought to make a significant contribution to the etiology of orofacial clefts, but investigation of their contribution is particularly complex. Nevertheless, improving the quality of exposure assessment can improve the power of a study more than increasing the number of participants.

Environmental exposure can be separated into three categories: nutritional and dietary; life style and occupation; and drugs and medicines. Over the years, there has been significant interest in nutritional factors, such as folic acid, vitamins A, B₆ and B₁₂; medical factors, such as illness and medication; occupational factors, such as glycol ether and solvents; and lifestyle factors, such as alcohol use and smoking. Although many of these exposures are difficult to quantify precisely, assessment of their safety or teratogenic potential is of the utmost importance in studying the etiology of craniofacial abnormalities, particularly orofacial clefts, many of which are known to have an environmental component.

Nutritional and dietary factors

Improving assessment of nutritional factors is addressed in detail in the descriptions of the common core protocols above. Further information is available on www.eurocran.org. Several measures were taken to assess maternal folate status in a population-based case–control study in the United Kingdom, which has not yet been published. The measurements included
dietary and supplemental folic acid intake, analysed by means of a validated food frequency questionnaire; biochemical markers in blood, e.g. red blood cell folate, serum folate, serum homocysteine and vitamin B<sub>12</sub>; and genetic markers, such as MTHFR and FOLR1.

**Life-style and occupational factors**

Biological markers are available for some exposures, such as cotinine or arsenic in urine, elements such as lead and zinc in serum, and nutritional markers in red blood cells or serum. The cost is, however, often prohibitive, and relating measurements taken after birth to events in early pregnancy can be problematic. With regard to teratogenic effects, it is usually assumed that there is a threshold of exposure above which the natural regulatory and repair mechanisms during fetal development are overcome and that lower levels of exposure will not result in a malformation.

Bias can be introduced into studies limited to liveborn cases and controls, as differences in exposure might be related to the probability of prenatal diagnosis and termination (such as social status or maternal age) rather than causal risk factors. Similarly, if cases are limited to survivors of the neonatal period, risk factors for severity, multiple congenital anomalies or survival might be identified, rather than causal factors for the congenital anomaly itself. This also applies to survival during pregnancy, as previously mentioned.

**Drugs and medicines**

About 20 drugs or groups of drugs are known to cause birth defects in humans (Webster & Freeman, 2003). For a drug to cause birth defects, it must be taken at a critical stage of pregnancy at a dose high enough to cause a threshold of exposure for an appropriate duration. More than 90% of pregnant women exposed to most known human teratogens during the first trimester have normal offspring. Epidemiological studies of pregnancy outcomes after exposure to specific drugs often provide superficially reassuring results, but the power of most to detect adverse outcomes is severely limited. Better knowledge of the parameters that determine teratogenicity is needed. The International Clearing-house for Birth Defects Surveillance and Research is well placed to carry out population risk assessment. Examples of drugs for which such systems have led to the identification of associations include valproate and spina bifida and corticosteroids and orofacial clefts.

**Recent studies on environmental exposures**

Recent EUROCAT studies on environmental exposures in relation to the etiology of orofacial clefts are summarized and discussed in the context of prevention in section 6. All publications from EUROCAT since 1979 are available on their website: http://www.eurocat.ulster.ac.uk.

A number of recent studies of gene–environment interactions in orofacial clefts have addressed the assessment of nutrition and lifestyle. The results were as follows:

**Nutrition**

- A detrimental effect of low periconceptional folate intake on the risk of giving birth to an infant with a cleft lip, with or without cleft palate, was more pronounced in mothers with the MTHFR 677TT or MTHFR 1298CC genotype (van Rooij et al., 2003b).
- Periconceptional maternal folic acid supplement use was found to reduce the risk for cleft lip and palate. An additional effect of food folate was shown (van Rooij et al., 2002b).
• Multivitamins conferred protection, reducing the risk for orofacial clefts (Shaw et al., 2004).

• Periconceptional intake of thiamine, niacin and pyridoxine appeared to contribute to the prevention of orofacial clefts (Krapels et al., 2003).

• A low vitamin B12 concentration in mothers increased the risk for orofacial clefts in their offspring (van Rooij et al., 2004a).

• A low maternal vitamin B6 status was consistently associated with an increased risk for cleft lip and/or palate. No consistent association was found with folate status (Munger et al., 2004).

**Maternal smoking**

• An odds ratio of about 2.0 was found for both cleft palate and cleft lip and/or palate, with a dose–response relation, and a possible effect of passive smoking (Little, Cardy & Munger, 2004a).

• Infants with NAT1 polymorphisms are at increased risk if the mother smokes (Lammer et al., 2004).

• Mothers who smoked and carried the GSTT1-null genotype were at greater risk of having a child with orofacial clefting than nonsmokers with the wild-type genotype (odds ratio, 3.2; 95% confidence interval, 0.9–11.6) (van Rooij et al., 2001).
6. INTERDISCIPLINARY RESEARCH ON PREVENTION OF CRANIOFACIAL ANOMALIES

The first WHO meeting on the prevention of craniofacial anomalies was held in May 2001 in Park City, Utah, United States, as a part of the project on international collaborative research on craniofacial anomalies (WHO, 2002). This meeting and the subsequent report focused mainly on orofacial clefts, the commonest craniofacial anomalies, and concluded that there were substantial environmental causes. It also noted that the pattern of occurrence of orofacial clefts was different from that of neural tube defects, and that the major causes of these two groups of common birth defects might therefore differ. These distinctions are important, because they indicate that the known, dramatic effect of folic acid supplementation on reducing the risk for neural tube defects might not be as powerful, or even exist, for orofacial clefts. Likewise, maternal smoking has been consistently associated with an increased risk for orofacial clefts, but not with neural tube defects, yet the association has gone generally unnoticed by advocates of maternal and child health.

Further progress in discovering environmental causes of orofacial clefts and other craniofacial anomalies, with possibilities for intervention and prevention, will require broad global strategies that include interdisciplinary research. Application of research findings on the causes and prevention of craniofacial anomalies will also require a concerted global effort, which should include linkage with other maternal and child health issues that vie for the attention of health policy planners and funding agencies at international and national levels. For craniofacial anomalies to receive greater attention, the broader agenda of birth defects research as a public health issue must first gain more prominence in international discussions of maternal and child health. A key approach might be to emphasize the common environmental factors that are causes of both birth defects and other illnesses and death among children worldwide.

The Bellagio Child Survival Study Group estimated in 2003 that 10 million children die each year from largely preventable diseases, almost all in poor countries, and that over half of the deaths can be attributed to poor nutrition.(Black, Morris, Bryce, 2003; Bryce et al., 2003; Claeson et al., 2003; Jones et al., 2003; Victora et al., 2003). The only preventive interventions and treatments listed by the study group that are directly related to nutrition, however, are breastfeeding and vitamin A and zinc supplementation. Remarkably, the broader issues of improving diets and increasing intakes of a broad range of nutrients have received scant attention. The Child Survival Study Group also failed to mention the role of maternal smoking and alcohol use during pregnancy as important causes of poor child survival. Thus, an opportunity exists for those involved in studies of orofacial clefts, other craniofacial anomalies and other birth defects to focus attention on the importance of these exposures to maternal and child health.

6.1 USE OF REGISTRIES AND HIGH-RISK POPULATIONS IN THE STUDY OF CRANIOFACIAL ANOMALIES IN INDUSTRIALIZED AND DEVELOPING COUNTRIES

The global effort to understand and prevent craniofacial anomalies should involve countries at all levels of development. The rates of occurrence of craniofacial anomalies and other birth defects in the few developing countries in which they have been assessed often appear to be higher than in most industrialized countries. The challenges to and opportunities for monitoring and studying craniofacial anomalies in developing countries were discussed at the meeting. Establishment of an international network of craniofacial anomalies registries and researchers will enhance understanding of the role of suspected risk factors, including
maternal smoking and alcohol use, dietary patterns and nutritional status, poverty and occupational and medical exposures in their causation.

Section 2 describes the role of the major global registries in collecting data from surveillance of birth defects and potential environmental teratogens. The main registries are the International Clearing-house for Birth Defects Surveillance and Research, the EUROCAT network, the National Birth Defects Prevention Study in the United States and the Latin American Collaborative Study of Congenital Malformations (ECLAMC). As international priorities change and technical capacity increases, the scope of activities of organizations such as the Clearing-house do not so much change but rather expand. Etiological research, with its new tools (e.g. molecular genetics), can progress further through registry-based international collaboration. Developing countries, where now most births occur, must be supported in their efforts to combat birth defects and genetic diseases. In these times of information explosion and internet-based communication, international networks can help provide the multilingual content and a supranational conduit for disseminating crucial information on the impact, health effects and prevention of birth defects.

6.2 MATERNAL NUTRITION AND OROFACIAL CLEFTS

Evidence has accumulated that maternal nutrition is related to the risk for orofacial clefts and that many nutrients might be involved in complex interactions with one another, with other environmental factors and with genetic polymorphisms. Attention continues to be focused on folate because of the strong effect of folic acid supplementation in reducing the risk for neural tube defects and the reasonable hypothesis that folic acid might also be related to orofacial clefts, although the association might be complex.

Exposure to high concentrations of homocysteine has been shown to alter the migration of neural crest cells and cause malformations in experimental avian models (Rosenquist, Ratashak & Selhub, 1996). In the Netherlands, vitamin B6 has been implicated in orofacial clefts, perhaps through its role in homocysteine metabolism (Wong et al., 1999). This study shows the usefulness of investigating biochemical markers of maternal nutritional status in case–control studies of clefts. Case mothers had higher mean homocysteine levels than controls in both fasting (12 vs. 9 µmol/l; p < 0.01) and methionine afterload (35 vs. 31 µmol/l; p < 0.05) samples. The mild hyperhomocysteinaemia of case mothers was thus not due to low folate values but might have been related to a poorer vitamin B6 status than controls. In a subsequent case–control study in the Netherlands, infants with orofacial clefts had lower mean serum folate and higher erythrocyte folate values than control infants, but the differences were not statistically significant. No differences were found between case and control mothers in serum or erythrocyte folate in this study (van Rooij, 2003; van Rooij et al., 2003b). In a further case–control study in the Netherlands, periconceptional folic acid supplementation was associated with a reduced risk for cleft lip and/or palate, with an additional, independent protective effect of food sources of folate (van Rooij et al., 2004b).

The nutrients, deficiencies in which might be associated with risk for orofacial clefts, include folate, vitamin B6, vitamin A and zinc; a lesser body of evidence implicates riboflavin (vitamin B2), vitamin B12, ascorbic acid, β-carotene, α-tocopherol, pantothenic acid, biotin, iron and magnesium (Munger, 2002). If even a few of these nutrients are important, many interactions between nutrients and dietary patterns might be related to the risk for clefts, and these might be detectable with current methods of dietary assessment. The general dietary patterns that would result in high intake of these nutrients would be those that are diverse and contain generous amounts of whole grains, fruits and vegetables, moderate amounts of meat, eggs and dairy products, and minimal amounts of highly processed cereal grains and sugars.
Two case–control studies conducted in the Philippines, in which red cell folate was found to be positively associated with the risk for orofacial clefts at one site and negatively associated at another, were discussed. Increased levels of vitamin B₆ in mothers’ blood samples were associated with a reduced risk for orofacial clefts in their offspring in both studies, and a regression analysis of data from both studies showed that the association was significantly modified by vitamin B₆ status (Munger et al., 2004). Further analyses by the group in the Netherlands indicated that B vitamins apart from folate, including thiamine, niacin and vitamin B₆ (pyridoxine), might reduce the risk for orofacial clefts. Krapels et al. (2004) considerably broadened their analyses of maternal nutritional factors and found evidence that energy-adjusted intakes of vegetable protein, fibre, β-carotene, ascorbic acid, α-tocopherol, iron and magnesium were lower in the mothers of infants with orofacial clefts than in controls, and concluded that a maternal diet with increased intake of fruits and vegetables could reduce the risk for orofacial clefts.

The data from the studies in the Philippines were also analysed with respect to plasma zinc concentrations in the mothers and the risk for non-syndromic orofacial clefts in their offspring. Zinc is important in maternal and child health, but intake is often inadequate in developing countries. Hurley and Swenerton (1966) first described high rates of malformations in the fetuses of rat dams given a diet low in zinc during gestation, with cleft palate in 34% of the fetuses. The association between prenatal zinc status and the risk for orofacial cleft in animals has since been well established; however, information with regard to craniofacial anomalies is scarce.

A case–control study was conducted with 74 case mothers recruited at a free surgical mission for oral clefts and 283 control mothers of unaffected children in Cebu province in central Philippines in early 2003. The mean plasma zinc concentration of the mothers of infants with cleft lip and/or palate (9.6 μmol/l) was significantly lower than that of control mothers (10.1 μmol/l; p < 0.05). Low plasma zinc concentrations (< 11.0 μmol/l) were found in 87.7% of mothers of infants with cleft lip and/or palate, 94% of mothers of infants with cleft palate and 72.1% of controls (p < 0.05). The odds ratio for cleft lip and/or palate and cleft palate combined, after adjustment for potential confounding factors, decreased with increasing quartiles of plasma zinc concentration, as follows: 1.0 (lowest quartile, reference), 0.83 (95% confidence interval, 0.37–1.89), 0.70 (0.31–1.58) and 0.26 (0.10–0.70) (p for trend = 0.008). Thus, low plasma zinc concentrations were common among Filipino women of reproductive age, and higher plasma zinc concentrations were associated with a lower risk for orofacial clefts in their children. The results indicate that maternal zinc nutrition should be improved in both developing and developed countries as a public health measure for preventing orofacial clefts.

Case–control studies of dietary patterns, nutrient intake, biomarkers and nutrition-related genes continue to be a cornerstone of research on the causes of craniofacial anomalies. A central assumption of the studies of nutrient biomarkers is that blood levels measured after pregnancy are a useful indicator of biomarker status at the time of conception and in the earliest weeks of pregnancy. Measurements of biochemical markers of maternal nutritional status even years after the affected pregnancy could be useful in case–control studies of human birth defects. The assays could provide measures of the collective effects of variations in dietary and supplement intake of nutrients and variations in nutrient absorption and use that result from interactions with other nutrients, environmental factors and genes. Several studies provide evidence that this approach is valid.
**Intervention trials on nutrition**

At the WHO meeting on prevention of craniofacial anomalies, there was much discussion of the optimal design and ethical aspects of clinical trials for evaluating interventions to prevent orofacial clefts and other craniofacial anomalies (WHO, 2002). Very large sample sizes are required to yield adequate statistical power to detect differences, because the prevalence of orofacial clefts at birth is only about 2 per 1000 births in ‘high-risk’ populations. Trials based on recurrence rates among mothers with previously affected pregnancies (perhaps 10 times higher than the rate of primary occurrence in the population) would still require studying many thousands of women throughout their pregnancies, with an adequate infrastructure to find and recruit the high-risk women individually. Trials are more difficult to mount as more and more circumstantial evidence accumulates about a proposed intervention. The knowledge that deficiencies in many nutrients might be involved in orofacial clefts, the fact that there might be complex interactions between some of the candidate nutrients, and the enormous sample size and cost required for a trial mean that, before definitive trials are undertaken, more refined hypotheses, more detailed preliminary data, broad international cooperation between study sites with adequate birth defects surveillance, and a large amount of funding will be needed. One such trial, described below, has been initiated in Brazil, but cleverer observational studies of orofacial clefts might be required before large-scale experimental trials are initiated.

**Food fortification**: Country-wide folic acid fortification of food products is under way in Chile, the United States and other countries, where monitoring of birth defects is also being carried out, so that trends that coincide with the fortification programmes can be detected. The results obtained with this approach are, however, difficult to interpret because other secular trends might confound the analyses.

The ECLAMC collaboration is being used to study the effects of the intervention with folic acid in Chile. The primary hypothesis is that the prevalences of both neural tube defects and orofacial clefts will be decreased. Since January 2000, on the order of the Ministry of Health, wheat flour used for baking bread is being fortified with 220 µg of folic acid for every 100 g of flour. As there are 83 g of flour in 100 g of bread and as the daily consumption of wheat bread in Chile is at least 200 g, this results in provision of 364 µg of folic acid per day, which is close to the recommended 400 µg/day for the prevention of neural tube defects. Similar legislation was implemented in Argentina in November 2003 and in Brazil in August 2004.

ECLAMC monitors the frequency of neural tube defects, orofacial clefts and other congenital anomalies in these three countries, involving more than 300 paediatricians, obstetricians and medical geneticists working in 86 hospitals. The unique features of the ECLAMC network, which provide its strength, are its multinational nature and its voluntary, cooperative organization; these advantages also create challenges that are not faced by other programmes.

The 4 021 193 births that occurred between 1967 and 1999 in 206 ECLAMC hospitals were subdivided into the 434 624 births in 1969–99 in 18 Chilean hospitals and the 3 586 569 births in those years in 189 hospitals in the other South American countries. The two sub-samples are comparable because all hospitals follow the same operational definitions given in the Manual Operacional del ECLAMC (1995). All live- and stillbirths weighing 500 g or more were included, except in 1967–78, when only livebirths were registered.

The preliminary results revealed that, during the period of observation (2002–04), there was a significant decline in the risk for neural tube defects after folic acid fortification (ratio of observed:expected, 0.55; 95% confidence interval, 0.38–0.78) but no significant reduction in the occurrence of orofacial clefts (ratio of observed:expected, 0.90; 95% confidence interval, 0.70–1.14).
Interdisciplinary experimental studies of maternal nutrition and orofacial clefts in developing countries

A new perspective was given at the meeting on use of birth defects surveillance systems in trials of maternal micronutrients in developing countries. The incidence, prevalence and epidemiology of birth defects remain poorly estimated and understood in most rural regions of the developing world, although evidence suggests that the rates of mild-to-severe anomalies are comparable to or exceed the rates in industrialized countries. Constraints to improving estimates of the burden of birth defects include inadequate health service delivery, particularly with respect to obstetric and newborn care, when assessment is most critical, inadequate clinical skills, lack of standardized reporting systems and modern informatics capability and sparse resources to build and maintain surveillance systems.

Over the past decade, two ‘community laboratories’ have been developed in rural South Asia, where birth defect surveillance has been initiated and maintained for at least 3 years: one comprises a population of ~ 185 000 people living in ~ 450 km² in the south–central plains of Nepal, and the other is a population of nearly 600 000 people living in an area of over 600 km² in rural north-western Bangladesh. The two sites have the requirements for conducting large, community-based intervention trials with micronutrients among children and women of reproductive age. Setting up reporting systems on pregnancies, pregnancy and health outcomes and vital status and organizing the means for reaching mothers, infants and children in each area routinely also made it possible to set up and use birth defect surveillance systems. Each trial was based on initial studies to evaluate the effects of maternal pre- and periconceptional and ante- and postnatal supplementation with weekly low doses of vitamin A or β-carotene in reducing maternal mortality and morbidity, infant mortality and other reproductive and infant health outcomes.

Nepal Nutrition Intervention Project. Sarlahi, the Nepal Nutrition Intervention Project, a collaborative research activity of the Center for Human Nutrition at Johns Hopkins University, Baltimore, United States, and the National Society for the Prevention of Blindness and Eye Diseases in Kathmandu, Nepal, conducted its first nutrition study in Sarlahi between 1989 and 1991. In this randomized trial, the effect of periodic, high-potency vitamin A supplementation in reducing mortality (West et al., 1991; West, 2004) and in improving growth (West et al., 1995) was tested in 30 000 infants and pre-school-aged children. The trial involved training a basic cadre of field workers, developing community rapport and trust, mapping 270 local administrative wards and listing ~ 35 000 houses to permit longitudinal enrollment, dosing and follow-up of children. Mortality among children over 6 months of age was reduced by 30%, but there was no effect on survival during the first 6 months. Concurrently, maternal night blindness was reported in the study area (Katz et al., 1995), and the condition was found to be associated with both vitamin A deficiency and increased morbidity from infections (Christian et al., 1998).

A second cluster-randomized, double-masked, controlled trial was conducted in 1994–97 to assess the effect of giving women of reproductive age a weekly supplement, equivalent to a recommended dietary allowance, of vitamin A, either pre-formed or as provitamin A β-carotene. About 44 000 women were enrolled and dosed weekly before, during and after pregnancy, the outcome of which was evaluated with respect to effects on mortality (West et al., 1999) and morbidity from all pregnancy-related causes (Christian et al., 2000) and fetal loss and infant health and survival (Katz et al., 2000), all in comparison with the group that received a placebo. A home follow-up visit was conducted about 3 months after the birth to evaluate vital status, health, dietary intake and exposure of the infants and mothers to risk factors. Maternal mortality was reduced by 40%, but there was no overall effect on fetal or infant mortality.
As the trial involved supplementing women periconceptionally, the protocol was reviewed and approved by the Teratology Society (JW Hanson, personal communication), in addition to institutional review boards in Nepal and the United States. To assess the safety measures, a birth defect surveillance system was established, and serum retinoic acid metabolites were measured in a sub-sample of pregnant mothers in the three randomized groups. This provided an opportunity to set up a lay field worker-based, clinician-supported birth defect surveillance system in a densely populated, poor, underserved rural population, where > 95% of all births occur at home. Local field interviewers (male) conduct a 3-month post-partum assessment, during which the infant is examined visually according to standard procedures. A clinician (a senior health assistant or a physician) follows up any reports of abnormality. If the infant has died by the time of interview, the interviewer conducts a ‘verbal autopsy’ at the home of the parents to ascertain the condition of the infant and events during the weeks before death, including gross birth defects.

The birth defect surveillance system operating in north-western Bangladesh has been used in a double-masked, placebo-controlled, cluster-randomized trial of maternal supplementation with vitamin A or β-carotene to confirm and extend the findings from Nepal. The project is named ‘JiVitA Bangladesh’, ‘jibhitau’ meaning ‘alive’ in Bengali. The system is modelled on the Nepali system but has been enhanced. Liveborn infants are registered, and, at 3 months of age, undergo a systematic anatomical review by lay field interviewers (female). As in Nepal, the field interviewers are trained to note on a standardized form any perceived abnormality, with a brief description of the condition placed alongside the anatomical variable on the data collection form. By design, the system is designed to err on the side of false-positive misclassification in order to maintain high sensitivity. The week after data entry, a list of infants is generated and conveyed to a team of two research physicians, who conduct a second screening visit to the household, examine the listed infants, and note, diagnose when possible, sketch and digitally photograph all abnormalities. If an infant has died before the verification visit, as in Nepal, a verbal autopsy interview is carried out with the parents, which includes questions in a standardized format about any gross external birth defects.

In the data entry centre, a database and linked digital photo library is generated for each infant or child examined by a team physician. As of early November 2004, 23 295 infants had been visited at a median age of 104 days (interquartile range, 87–172) and evaluated by field interviewers, who reported ‘abnormalities’ in 4336 infants (18.6%), indicating substantial false-positive misclassification with respect to actual birth defects. As of the same date, 877 candidate children had been visited at home by research physicians, of whom 160 (18.2%) were verified as having one or more abnormalities consistent with a diagnosis of birth defect. The JiVitA database will permit further validation of each birth defect by expert reviewers in a secure web-based database that will include access to closed-ended data, textual clinical descriptions, physicians’ sketches of abnormalities and multiple digital photographs. The system should advance efforts to estimate incidence and prevalence and should, with its extensive database on maternal, pregnancy-specific and infant risk factors and exposures, advance the epidemiology of the occurrence and severity of birth defects in rural South Asia.

Brazil–United States oral cleft prevention programme: The aim of this collaborative project is to determine whether folic acid supplementation prevents the recurrence of non-syndromic cleft lip and palate. In view of the ambiguity about the efficacy of such supplementation on the occurrence of this disorder and the lack of evidence for a preventive effect of 400 μg or higher doses of folic acid on its recurrence, the project was initiated in January 2004 in the hospital for the rehabilitation of craniofacial anomalies in Bauru, Brazil, to assess the effects of folic acid on the recurrence of cleft lip and palate in a high-risk group of women.

The study includes women of child-bearing age who either have cleft lip and palate or are the mothers of patients with cleft lip and palate. The aim is to recruit about 2000 women and to
randomize them in a double-blind fashion to 0.4 or 4.0 mg of folic acid supplementation during preconception to the first trimester of pregnancy. Follow-up visits are conducted every 2 months to monitor pregnancy occurrence and measure vitamin B₁₂ and blood folate levels. The pregnancy outcomes (livebirths, miscarriages and stillbirths) are evaluated for cleft lip and palate.

By January 2005, about 400 women were enrolled and five infants had been born to participants. The study is being extended to other sites in Brazil, including craniofacial clinics in Porto Alegre, Salvador, Joinville and Recife. A clinic-based recruitment and follow-up model is being tested to lower the participation burden of subjects. The study demonstrates the possibility of carrying out intervention trials for cleft lip recurrence in high-risk populations where large numbers of cases provide power to detect significant differences. Future studies could address other micronutrients and incorporate genetic risk factors into the analysis.

6.3 MATERNAL SMOKING AND OROFACIAL CLEFTS

The global picture of tobacco use and risk for orofacial clefts and details of studies of tobacco use and orofacial clefts in Scotland was presented. The World Health Report 1999 (www.who.int/pub/en/) noted that about one-third of the world’s population aged 15 years or older smoke tobacco, including some 12% of women. The proportion of women who smoke in developed countries is estimated to be 24%, while the proportion in developing countries is about 7%. Although tobacco consumption by women might be declining in some countries, there are signs overall that the proportion of women who smoke is increasing, particularly in developing countries, where women are specifically targeted by tobacco companies and where it is estimated that tobacco consumption is rising by about 3.4% per year. Globally, about 12 million women smoke during pregnancy each year.

An association between maternal cigarette smoking and orofacial clefts in their offspring has been observed in a number of studies. In meta-analyses, marked variations in relative risks have been observed (Wyszynski, Duffy & Beattie, 1997; Källén, 2001; Little, Cardy & Munger, 2004a); however, in larger studies, the relative risks for cleft lip and/or palate have been in the range of 1.2–1.7, those for cleft palate alone being slightly lower. In the most recent and largest meta-analysis of maternal tobacco use and risk for orofacial clefts (Little et al., 2004b), consistent, moderate, statistically significant associations were found between maternal smoking and cleft lip and/or palate (relative risk, 1.34; 95% confidence interval, 1.25–1.44) and cleft palate (relative risk, 1.22; 95% confidence interval, 1.10–1.35). There was evidence of a modest dose–response relation for cleft lip and/or palate. While these relative risks are not high, the proportion of orofacial clefts attributable to smoking might be substantial, as many women smoke during pregnancy. The association between maternal tobacco smoking and orofacial clefts is strong enough to justify its mention in anti-smoking campaigns.

EUROCAT studies provided supporting evidence that maternal tobacco use is associated with the risk for orofacial clefts (Lorente et al., 2000b). The data were derived from a European multicentre case–control study of 161 infants with orofacial clefts and 1134 control infants. Multivariate analyses showed an increased risk for cleft lip and/or palate associated with smoking (odds ratio, 1.79; 95% confidence interval, 1.07–3.04) and an increased risk for cleft palate associated with alcohol consumption (odds ratio, 2.28; 95% confidence interval, 1.02–5.09). The former risk increased with the number of cigarettes smoked.

In Scotland, statistics from the Information and Statistics Division in 2000 revealed that 28% of women smoked at the start of their pregnancy. In 1996 in Scotland, maternity in-patient discharge records showed that 9.5% of women in deprivation category 1 (least deprived) and
49.6% in category 7 (most deprived) smoked during pregnancy. A positive association between orofacial clefts and deprivation (assessed on the basis of the census characteristics of the area of residence) was observed in Scotland for the period 1989–98, and it is possible that tobacco smoking is responsible. The tobacco control policies that have been formulated indicate that, as yet, the association between orofacial clefts and smoking is not widely recognized. Although cleft lip and palate was listed among the specific birth defects that have been related to maternal smoking in the 2001 report of the United States Surgeon General on men and smoking (http://profiles.nlm.nih.gov/NN/), it was not mentioned in the most recent report of WHO on women and the tobacco epidemic (WHO, 2005).

A number of public health issues are relevant to the debate about legislation on tobacco smoking in general and exposure to passive or environmental tobacco smoke. As described above, in a study in the United Kingdom, a positive association was found between maternal smoking during the first trimester of pregnancy and both cleft lip and/or palate and cleft palate, with a dose–response relation for both types of cleft (Little et al., 2004b). This adds to the considerable body of evidence from other parts of the world. Assuming causality, the population attributable fraction of clefts attributable to maternal smoking is of the order of 22%.

The public health messages surrounding smoking and its association with cardiovascular disease and cancers are a blunt tool in terms of encouraging behavioural change, particularly among the younger generation. It is recognized that the developing fetus will potentially have health problems if the mother is exposed to cigarette smoke (Box 8); however, despite the evidence linking smoking to the distressing structural abnormality of cleft lip and/or palate, no mention is made of the consistent association with smoking. The public health message with regard to smoking and birth defects should be expanded and aimed at the younger population of reproductive age if it is to succeed in contributing to primary prevention of birth defects.

**Box 8. Adverse effects of maternal smoking on reproductive health**

- spontaneous abortion
- premature birth
- low birth weight
- stillbirth
- fetal hypoxia
- structural abnormalities in the fetal brain
- cognitive behavioural abnormalities in the newborn
- sudden infant death syndrome after birth
- attention deficit disorder
- impaired physical growth
- impaired academic attainment

From National Health System Scotland (2003)

In light of the increased targeting of women by tobacco companies, both in developed and developing countries, an association between orofacial clefts and smoking has important implications globally. We suggest that orofacial clefts be incorporated into public health campaigns on the consequences of maternal smoking. It is noteworthy that the images of faces of children with cleft lip have been used to promote some of the world’s largest medical charity organizations. This powerful image might help counterbalance the active targeting of
women by tobacco companies. The association between low socioeconomic status and the prevalence of smoking is illustrated in Table 16. An association between low socioeconomic status and orofacial clefts has been documented by Carmichael et al. (2003) and Clark et al. (2003).

Table 16. Adult smoking prevalence in countries with populations living on less than US $1 a day and extent of childhood malnutrition, as indicators of low socioeconomic status

<table>
<thead>
<tr>
<th>Country</th>
<th>Living on &lt; US$ 1/day</th>
<th>Childhood malnutrition (%)</th>
<th>Smoking prevalence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Men</td>
</tr>
<tr>
<td>Yemen</td>
<td>15.7</td>
<td>46</td>
<td>77.0</td>
</tr>
<tr>
<td>Indonesia</td>
<td>7.2</td>
<td>25</td>
<td>69.0</td>
</tr>
<tr>
<td>Kenya</td>
<td>23.0</td>
<td>22</td>
<td>66.8</td>
</tr>
<tr>
<td>Kyrgyzstan</td>
<td>2.0</td>
<td>11</td>
<td>60.0</td>
</tr>
<tr>
<td>Uganda</td>
<td>82.2</td>
<td>23</td>
<td>52.0</td>
</tr>
<tr>
<td>Nicaragua</td>
<td>82.3</td>
<td>12</td>
<td>51.0</td>
</tr>
<tr>
<td>Viet Nam</td>
<td>17.7</td>
<td>34</td>
<td>50.7</td>
</tr>
<tr>
<td>Bangladesh</td>
<td>36.0</td>
<td>48</td>
<td>48.3</td>
</tr>
<tr>
<td>Zimbabwe</td>
<td>36.0</td>
<td>13</td>
<td>46.0</td>
</tr>
<tr>
<td>Zambia</td>
<td>63.7</td>
<td>24</td>
<td>40.0</td>
</tr>
<tr>
<td>Nepal</td>
<td>37.7</td>
<td>48</td>
<td>39.5</td>
</tr>
<tr>
<td>Pakistan</td>
<td>13.4</td>
<td>38</td>
<td>36.0</td>
</tr>
<tr>
<td>India</td>
<td>34.7</td>
<td>53</td>
<td>29.4</td>
</tr>
<tr>
<td>Malawi</td>
<td>41.7</td>
<td>25</td>
<td>20.0</td>
</tr>
<tr>
<td>Nigeria</td>
<td>70.2</td>
<td>31</td>
<td>15.4</td>
</tr>
<tr>
<td>Ghana</td>
<td>44.8</td>
<td>25</td>
<td>10.8</td>
</tr>
<tr>
<td>Rwanda</td>
<td>35.7</td>
<td>24</td>
<td>7.0</td>
</tr>
</tbody>
</table>

*a Only countries for which data on smoking were available are included.


c From *Tobacco control country profiles* (WHO, 2003)

China has 20% of the world’s population and produces and consumes about 30% of the world’s cigarettes (Peto, Chen & Boreham, 1999), making it the world’s largest national market for cigarettes. Although China has attained ‘lower middle income’ status, 16% (more than 200 million Chinese) live on less than US$ 1 a day. Currently, in China, over 300 million men and 20 million women smoke (Yang et al., 1999).

Future trends in smoking in impoverished and lower-income countries are reflected in the smoking behaviour of young people. Within a project of the Global Youth Tobacco Survey Collaborating Group (2004) and an international surveillance system under the auspices of WHO and the United States Centers for Disease Control and Prevention, schoolchildren aged 13–15 in many countries have been surveyed (Warren et al., 2000). The smoking rates among
the young are highest in western Europe, where one-third of boys and nearly one-third of girls smoke.

6.4 MEDICATIONS AND OCCUPATIONAL EXPOSURES

MADRE (MAternal DRug Exposure) study

The MADRE (MAternal DRug Exposure) study conducted by Clearing-house investigators was set up to study the role of medications and occupational exposures in the causation of orofacial clefts. In section 2, mention was made of the role of registries of congenital malformations in providing crucial data for identifying teratogenic effects of medications. The main features of the registries in involved in the collaborative MADRE project are summarized in Box 9.

Box 9. International screening for drug teratogenicity: the MADRE project

<table>
<thead>
<tr>
<th>Goal</th>
<th>Screen for potential teratogenicity of medications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Approach</td>
<td>Study of cases of birth defects after reported exposure from maternal medication during first trimester of pregnancy. Analysis by a modified case–affected control approach, stratified by registry</td>
</tr>
<tr>
<td>Current data</td>
<td>Over 15 000 cases of birth defects from 12 registries</td>
</tr>
<tr>
<td>Selected findings (anti-seizure medication)</td>
<td>Confirmed classical associations: phenobarbital with orofacial clefts and heart defects; valproic acid with spina bifida, hypospadias and heart defects; and carbamazepine with heart defects. Tested associations suggested in the literature: evaluated and confirmed the reported association between valproic acid and craniosynostosis</td>
</tr>
<tr>
<td>Evolution</td>
<td>Refine classification according to pathogenetic mechanisms; assess specific risks for multiple congenital anomalies; focus on selected major defects (heart defect subtypes, gastroschisis); increase registry participation</td>
</tr>
</tbody>
</table>

In MADRE, medications are cross-tabulated against malformations, and a two-by-two table is constructed for each combination of medication and malformation. An infant is defined as a case if he or she has the malformation in question and as a control infant otherwise. An infant is defined as exposed if the mother used the drug in question during the first trimester of pregnancy and as unexposed otherwise. If the exposures and malformations are unrelated, the drug types and malformation types should be randomly distributed, and deviations from this distribution should show up as increased odds ratios. Such deviations can be used to screen for associations that deserve further study.

The main strength of MADRE is that it provides an adjunctive tool to screen for teratogens, as it is based on available data at low cost. Because it is a screening tool, associations thus identified should be further assessed in other data sets. The international setting and the large sample available from collaborating registries also lead to greater statistical power and provide, to some extent, internal indicators of consistency. For example, an association is increasingly unlikely to be due to chance if it occurs simultaneously in different registries. Finally, because all cases are affected, differential recall of medication use is a lesser concern than in a case–unaffected control design.

The MADRE project now comprises over 15 000 cases. The analyses have revealed several associations, both known (e.g. valproic acid and spina bifida) and new. The new associations are considered hypotheses to be tested on further samples, as was the case for the association between corticosteroids and orofacial clefts. The first suggestion of such an association was
reported in 1994 (Robert et al., 1994), and three subsequent epidemiological studies confirmed a weak association (Carmichael & Shaw, 1999; Park-Wyllie et al., 2000; Edwards et al., 2003). The association was confirmed in 2003 with MADRE data (Pradat et al., 2003). The MADRE database is also used to test associations as they are reported in the literature. For example, the suggested association between valproic acid and craniosynostosis (mainly trigonocephaly) (Lajeunie et al., 2001) was confirmed, while the reported association between trimethoprim and malformations such as neural tube and cardiac defects (Hernández-Díaz, Werler & Mitchell, 2004) could not be replicated.

**EUROCAT in the prevention of birth defects**

Among the objectives of EUROCAT, listed in section 2, and perhaps the most important in the context of prevention is “to provide a ready collaborative network and infrastructure for research related to the causes and prevention of congenital anomalies and the treatment and care of affected children”. In the 10-year period 1994–2004, EUROCAT published 122 scientific publications in a range of journals, as well as reports on current activities, local registry publications, annual reports and special reports. The EUROCAT special report on environmental risk factors, available on the website (http://www.eurocat.ulster.ac.uk), is a review of the literature that will be expanded and updated periodically.

The role of the EUROCAT network in investigating the role of maternal use of medications and occupational exposures in the causation of orofacial clefts was discussed. Glycol ethers are found in a wide range of domestic and industrial products and are used in women’s work environments. On the basis of concern about their potential reproductive toxicity, the risk for congenital malformations related to exposure to glycol ether during pregnancy was evaluated as part of a multicentre case–control study in six regions of Europe (Cordier et al., 1997). The overall odds ratio for congenital malformation associated with exposure to glycol ether was 1.44 (95% confidence interval, 1.10–1.90) after adjustment for several potential confounders. The association with exposure to glycol ethers was particularly strong for three subgroups of malformations: neural tube defects (odds ratio, 1.94; 95% confidence interval, 1.16–3.24), multiple anomalies (2.00; 1.24–3.23) and cleft lip (2.03; 1.11–3.73). In the last subgroup, the risk, especially for an isolated defect, tended to increase with level of exposure.

Waste disposal sites, a potential hazard to health, were studied in a multicentre case–control study of the risk for congenital anomalies associated with residence near hazardous waste landfill sites in Europe (Dolk et al., 1998) A significantly raised odds ratio for residence within 3 km of a landfill site was found for neural-tube defects (odds ratio, 1.86; 95% confidence interval, 1.24–2.79), malformations of the cardiac septa (1.49; 1.09–2.04) and anomalies of the great arteries and veins (1.81; 1.02–3.20). Odds ratios of borderline significance were found for tracheo-oesophageal anomalies (2.25; 0.96–5.26), hypospadias (1.96; 0.98–3.92) and gastrochisis (3.19; 0.95–10.8).

Maternal occupational risk factors for orofacial clefts were investigated in a EUROCAT study by Lorente et al. (2000a). After adjustment for confounding factors, cleft palate was significantly associated with maternal occupation in services such as hairdressing (odds ratio, 5.1; 95% confidence interval, 1.0–26.0) and housekeeping (2.8; 1.1–7.2). The analysis suggested associations between exposure to aliphatic aldehydes (2.1; 0.8–5.9) and glycol ethers (1.7; 0.9–3.3) and cleft lip and/or palate, and between exposure to lead compounds (4.0; 1.3–12.2), biocides (2.5; 1.0–6.0), antineoplastic drugs (5.0; 0.8–34.0), trichloroethylene (6.7; 0.9–49.7) and aliphatic acids (6.0; 1.5–22.8) for cleft palate only. In view of the limited numbers of subjects, these results must be interpreted with caution; however, they indicate the tive need for more research on chemicals of being reprotoxins.
7. CONCLUSIONS AND RECOMMENDATIONS

7.1 SURVEILLANCE AND REGISTRATION OF BIRTH DEFECTS

Surveillance and registration of birth defects underpins all aspects of research into craniofacial anomalies in both developing and industrialized countries. WHO should help to sustain surveillance in those countries where it is already established and to expand the registries programme to parts of the world where no such facilities exist.

Research efforts to parallel this activity should seek to compare the prevalence of craniofacial conditions in different populations; establish and investigate unusual trends; generate research hypotheses to stimulate future research; seek to improve the quality of care; and identify environmental risk factors and genetic predisposition with a view to prevention.

In their presentations, EUROCAT and the International Clearing-house on Birth Defects Monitoring Systems described their views of the future in the context of craniofacial anomalies.

EUROCAT

The recommendations of EUROCAT noted that, in Europe and other parts of the world, there are well-established networks of registries to monitor, register and study craniofacial anomalies. These registers are an important means for reducing the health-care burden represented by these anomalies and for studying etiological factors. WHO could help in:

- harmonizing working methods and definitions;
- promoting the use of existing local and regional services;
- promoting the use of registry data, publication and dissemination;
- working with local authorities to increase understanding;
- stimulating contact with registries, not only to obtain data, but to create policies; and
- creating mechanisms through which primary prevention policies (such as folic acid supplementation) can be evaluated from prevalence data.

International Clearing-house for Birth Defects Surveillance and Research

The Clearing-house has provided an invaluable international forum for the compilation, exchange and publishing of epidemiological data on structural congenital anomalies. These activities will continue, with the following changes recommended by the strategic planning team, to be ratified by members:

- Surveillance rather than monitoring will be emphasized.
- Non-Clearing-house researchers will be invited to collaborate with Clearing-house members to use the diverse population-based data to test important research hypotheses.
- Surveillance programmes, including those on children’s disabilities other than structural congenital anomalies, will be invited to use the Clearing-house.
- There will be less emphasis on quarterly statistical monitoring and more emphasis on constant communication among Clearing-house members.
• The name of the organization will be changed to the International Clearing-house for Birth Defects Surveillance and Research to reflect the strategic direction.

**International Database of Craniofacial Anomalies**

In September 2002, the Human Genetics programme at WHO promoted the International Database on Craniofacial Anomalies and assigned its coordination to the International Centre on Birth Defects, the head office of the Clearing-house. The main aims of the Database are:

• to stimulate existing databases to share their data, creating a specific, worldwide database;

• to encourage the establishment of new databases to contribute to the worldwide International Database;

• to present the collected data in a suitable way or to make available more specific data to stimulate research addressing primary and tertiary prevention as well as better treatment of craniofacial anomalies; and

• to stimulate scientific and lay organizations to collect and share relevant data and information on persons affected by a craniofacial anomaly.

### 7.2 TREATMENT OF CRANIOFACIAL ANOMALIES

At the previous meeting held under the auspices of WHO (2002), a global consensus was reached on recommendations for record-keeping (www.who.int/ncd/hgn/publications.htm). These define minimum record-keeping for a range of cleft types and treatment episodes for centres that might wish to participate in future international comparisons. In the meantime, researchers on clefts should establish norms for outcomes of all types of care and undertake collaborative work to refine methods for comparison. Further work on the long-term reliability of early outcome assessment is also a high priority if unsuccessful protocols are to be eliminated more rapidly. Study of longitudinal archives from cleft clinics around the world could make a significant contribution to this work, by defining which early measurements are most likely to be predictive.

Despite the challenges of international collaboration, it will be essential in the field of craniofacial anomalies to continue to pursue large inter-centre collaborative research initiatives and to encourage close working relationships between clinicians and laboratory scientists.

### 7.3 GENETIC INVOLVEMENT IN CRANIOFACIAL ANOMALIES

Genes that contribute to complex traits such as cleft lip and/or palate can be identified by using a combination of family collections, careful phenotyping, high-throughput genotyping, robust analytical strategies, fine structure mapping and mutation characterization. Carinci et al. (2003) described the contemporary situation with respect to orofacial clefts in a review of recent developments, stating that, because of the complexity of the genetics of non-syndromic cleft lip and/or palate, differences between cleft lip and/or palate and cleft palate, the heterogeneity of each group, the number of genes involved, the type of inheritance and interaction with environmental factors, he advocated use of several approaches: epidemiological studies, animal models, human genetic studies and in-vitro studies.
**Genotype–phenotype correlation**

It will be increasingly important in this field to have accurate phenotypic descriptions of craniofacial abnormalities and clefts. Therefore, the involvement of expertise in clinical genetics and dysmorphology is crucial. This will be particularly important as new discoveries in genetics provide the potential to improve the sensitivity and specificity of genotype–phenotype correlations.

Precise definitions are needed of what constitutes non-syndromic cleft lip and palate, particularly in the light of evidence that apparently non-syndromic clefts might be caused by a single gene. In such cases, it is inevitable that incomplete penetrance and variable expression will complicate the genetic picture.

Subdivision of clefts into two major categories, cleft lip and/or palate and isolated cleft palate, has been useful in the epidemiology, ascertainment, treatment and etiology where differences are apparent. Nevertheless, genetically, a number of overlaps in the etiology have emerged, which have significant implications for future research into the pathogenesis of both primary and secondary palatal clefting.

It will be important in future research to be able to sub-phenotype subjects with different types of clefts into homogeneous subsets, particularly for the rarer abnormalities. As no single centre will be able to accumulate a large enough group of subjects, multicentre, international collaboration will be essential.

**Identification of candidate genes**

The existing strategies for investigating gene involvement in cleft lip and palate are likely to continue to be of use in the future. These include:

- animal models, particularly mouse and chick;
- studies of relevant populations through linkage disequilibrium;
- genetic linkage and association studies;
- chromosome rearrangements; and
- studies of monozygotic twin discordance.

**Multidisciplinary, multicentre collaboration**

In combination with the above, interdisciplinary research involving epidemiologists, statisticians, developmental biologists and molecular biologists will make important discoveries more likely. Establishing connections and working closely with clinical colleagues for the collection of data and biological samples is essential for genetic and gene–environment interaction studies.

**Role of WHO**

The role of the WHO group on craniofacial anomalies is to raise awareness and to establish the place of research on this topic alongside other genetic birth defects and public health issues. The WHO group should also coordinate work on candidate genes, markers and analytical approaches in animal models. The group should optimize collaboration through the website and disseminate information for the initiation of research; it should also facilitate research by establishing protocols and guidelines for countries wishing to become involved.
7.4 GENE–ENVIRONMENT INTERACTIONS IN CRANIOFACIAL ANOMALIES

A comment in a recent review of the etiology of orofacial clefts (Wong & Hagg, 2004) summarizes the current situation in relation to genetic and environmental factors: “Determining the relative risk of cleft lip and palate, on the basis of genetic background and environmental influence, including smoking, alcohol use, and dietary factors, will aid in genetic counselling and the development of future preventive measures.”

Administrative aspects

- Support international collaborative efforts to improve knowledge about the etiology, diagnosis, prevention and treatment of craniofacial anomalies.
- Assist WHO in conducting international collaborative meetings on craniofacial anomalies research and registration of birth defects and subsequent dissemination of the recommendations.
- Provide support for and regular updates to the directory of resources set up by WHO. This directory will include information about researchers, institutions, funding bodies and other agencies concerned with craniofacial anomalies research.
- Support efforts to develop the WHO craniofacial anomalies website, thus making the directory of resources available on the internet.
- Continue the WHO registry database (International Database on Craniofacial Anomalies), composed of surveillance data from around the world and agreed common data elements for craniofacial anomalies.

Research aspects

- Assist WHO in its stated objectives with regard to craniofacial anomalies research, in particular maintenance of global research networks in gene–environment interactions and in surveillance of craniofacial anomalies.
- Continue to encourage international research on craniofacial anomalies in specific ethnic groups and communities, with special emphasis on gene–environment interactions in the etiology of craniofacial anomalies.
- Initiate and sustain a global network of collaboration on research into gene–environment interactions in craniofacial anomalies.
- Coordinate research efforts according to future research needs, including research infrastructure and methods necessary for meeting those research needs and protocols for exemplary projects.
- Encourage the development of databases of information and biobanks of genetic and biochemical data for future research.
- Encourage active involvement in international initiatives such as the Cochrane Collaboration and the Human Genome Network.
- Keep abreast of latest developments in bioinformatics, genetics and statistical methods that might benefit future research on gene–environment interactions.
Coordination

An innovation that could be coordinated by the WHO craniofacial anomalies group is formation of a global network to facilitate meta- and pooled analyses of gene–craniofacial anomalies associations and related gene–environment and gene–gene interactions. The following advantages to such a network were identified:

- An initiative analogous to the Cochrane Collaboration is needed for the appraisal and synthesis of evidence on gene–environment interactions in the etiology of craniofacial anomalies and of evidence on gene–gene interactions and associations between clefts and specific genetic polymorphisms (for which the principle of Mendelian randomization might be considered to corroborate evidence about associations with specific exposures).

- The collaboration that has begun within the WHO initiative on craniofacial anomalies would serve as an excellent basis for such an initiative.

- There is an opportunity for collaboration with the Human Genome Epidemiology Network: http://www.cdc.gov/genomics/hugenet/default.htm.

- A register of data and sample collections is needed to minimize publication bias and to facilitate large-scale collaborative work. The register could be derived in part from the EUROCRAN register of resources.

- When a lead is suggested by a meta-analysis (or other work), the collaboration and register would allow pooled analysis of data on individuals, possibly with additional genotyping. This could save the resources that would be spent in setting up new studies.

- Collaboration could be established with biobanking initiatives in which pregnant women are being followed. Many of these (e.g. in Ottawa, Canada, and the Avon Longitudinal Study of Parents and Children in the United Kingdom) are too small for studies of congenital anomalies, but the data on exposure are potentially of high quality, and combination studies should be encouraged.

- In any trial of interventions for the prevention of congenital anomalies, craniofacial anomalies should be included, and biological specimens should be collected, to allow testing of gene–intervention interactions (potentially by a case-only analysis).

7.5 Prevention of Craniofacial Anomalies

Enough is already known about the risk factors for a range of birth defects, including orofacial clefts, to make public health recommendations for pregnancy planning and to improve reproductive safety. Further research is still needed, however, on both environmental and genetic factors, to improve understanding and risk assessment. A key approach to discovering the roles of environmental factors in the etiology of orofacial clefts and other craniofacial anomalies will be a concerted global effort to form a network of registries and researchers in both industrialized and developing countries. It would be useful to extend this approach throughout the emerging WHO craniofacial anomalies global network.

Maternal nutrition:
Several nutrients, including folate, vitamin B₆, zinc and vitamin A, have been associated with the risk for orofacial clefts, and many other nutrients and dietary patterns have been implicated. Case–control studies with innovative use of biomarkers of nutritional status, genetic markers and histories of illness and exposure to medications during pregnancy are the best approach for studying the nutritional causes of orofacial clefts and are best done with representative population samples from broad geographical areas.

Randomized clinical trials are useful in research on the prevention of birth defects, as exemplified by the study in the United Kingdom on folic acid supplements and neural tube defects and the trial on recurrence that is under way in Brazil. There is more scope for large, well-designed randomized clinical trials for testing specific nutrients or dietary patterns for the prevention of orofacial clefts. The design of such trials should be based on data from initial observational studies. Both observational and experimental studies require well-functioning birth defects surveillance systems.

**Maternal lifestyle**

Maternal cigarette smoking remains the best-studied environmental risk factor for orofacial clefts, and evidence continues to accumulate that maternal exposure to tobacco smoke increases the risk for orofacial clefts by an average of 30%. The time has come for concerted public health action to raise the profile of evidence that exposure to tobacco smoke is a cause of orofacial clefts and to use these findings directly in campaigns to reduce smoking among women of reproductive age.

While there is less evidence that maternal alcohol drinking is a cause of orofacial clefts, the role of alcohol use during pregnancy in fetal alcohol syndrome is well known. Strong, worldwide public health campaigns are warranted to reduce alcohol drinking during pregnancy.

**Maternal occupational exposure, illness and drug use**

Maternal occupational exposure to glycol ethers, aliphatic aldehydes and lead compounds is implicated in cleft lip and palate, and exposure to biocides, antineoplastic drugs, trichloroethylene and others in cleft palate. Glycol ethers are found in a wide range of domestic and industrial products used in women’s work environments. Waste disposal sites, a potential hazard to health in general, are also implicated in increasing the risk for congenital anomalies. Certain medications, such as anticonvulsants, accutane and folate antagonists, have been implicated in the past. Reliable information on maternal recreational drug use is notoriously difficult to obtain, and many of the studies that have identified chemical teratogenesis are small and of low power; nevertheless, they indicate the need for more research. Only when large multicentre studies with standardized methods are conducted will the consistency of associations clarify which are true reproductive toxins.

### 7.6 FUNDING OF COLLABORATIVE RESEARCH ON CRANIOFACIAL ANOMALIES

Ultimately, the success of the craniofacial anomalies project will be measured by the ability to make research on this topic a priority. Access to funding from governmental and nongovernmental agencies will depend on their motivation. This document is intended to raise awareness and to provide information on the value and benefits of investing in research on craniofacial anomalies and clefts.
Future applications for research funding should address well-defined, important questions raised by groups of individuals, and the responsibilities for the research protocols should be devolved to persons who have the expertise and are familiar with the latest developments in their respective disciplines.

This report provides evidence, broad strategies and detailed protocols and also practical advice on mobilizing resources for research in developing countries. This type of information will be updated regularly in our directory of resources [http://www.who.int/genomics/anomalies/cfaproject/en/#mtg](http://www.who.int/genomics/anomalies/cfaproject/en/#mtg).

In the field of craniofacial anomalies, research subjects that will receive funds, even in a competitive setting, are innovative ways of identifying candidate genes, new methods and genes in research on gene–environment interactions, collection of information from birth defects surveillance systems and registries, statistical and analytical methods, particularly for research on gene–environment interactions, and optimizing health-care protocols (surgical and non-surgical) in the management of craniofacial birth defects. Tissue engineering and wound healing are promising areas of research. Standardized, accurate measures of treatment outcome, including three-dimensional morphometrics, and recognition of psychosocial aspects are also important.

A new strategy for the future of research into craniofacial anomalies is as a complement to research on other birth defects and addressing public health needs of countries, particularly developing countries. Issues such as smoking, alcohol intake and nutrition are highly relevant to craniofacial birth defects, yet are not addressed strategically as public health issues. For instance, in spite of convincing evidence of the effects of smoking on reproductive health (including an association with orofacial clefts), smoking continues to be regarded as a public health problem only in relation to cancer and cardiovascular and respiratory diseases. Leading experts in all areas of research on craniofacial health care, from diagnosis to etiology to treatment and prevention, must bring the issue to the attention of local, national and international governmental and nongovernmental funding agencies. Researchers in craniofacial anomalies should also be made aware of parallel problems with other birth defects, such as congenital heart defects and club foot, and with obstetric issues such as maternal mortality, infertility, low birth weight, prematurity and developmental delay.

### 7.7 OVERALL CONCLUSION

Birth defects are an increasing problem, particularly in the developing world, and this report provides evidence for health ministries around the world that it is possible and cost-effective to address this problem. A significant percentage of birth defects involve the craniofacial structures. The birth prevalence, the burden of care and the quality of care for these defects in different countries can be estimated. Furthermore, it is possible to make recommendations on treatment with multidisciplinary surgical and non-surgical care, and on primary prevention through changes in lifestyle, nutrition and pregnancy planning. Intensive research efforts are under way on care and prevention, and country-specific efforts will help to establish sustainable, technologically appropriate interventions.

The four themes addressed in this report—epidemiology, treatment, etiology (genetics and gene–environment interactions) and prevention—should be carried forward separately but collaboratively and simultaneously, in close collaboration with the global community that supports and activates proposals. It was agreed that the main products of the WHO craniofacial anomalies project that should be promoted are:

- the International Database on Craniofacial Anomalies,
- the WHO Human Genetics programme website on craniofacial anomalies,
• international promotion of common protocols for research and
• networks of collaborators across all of the above areas.

In collaboration with WHO and with the involvement of leading international experts in a range of disciplines, the evidence base for craniofacial anomalies research is now available, and recommendations for future research have been made. This report will encourage, enable and empower research personnel throughout the world to contribute to the necessary global collaborative effort in the field of craniofacial anomalies. Developing countries, particularly those with greatest need such as India and the Philippines and countries of Africa, South-East Asia and South America, are establishing research infrastructure and are set to play an increasingly significant role in research in surveillance, etiology and treatment.

Our collective aim is to address the challenge of craniofacial anomalies, to alleviate suffering and to ensure that affected children can grow up to live productive, healthy lives. International collaboration and cooperation will shorten the time-scale for achieving this goal.
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10. APPENDICES

Appendix 1. Birth defects registry project: Data collection form
### Appendix 2. Registries contributing to the International Clearing-house for Birth Defects Surveillance and Research

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<tr>
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<td>Norway</td>
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<td>Russian Federation</td>
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<tr>
<td>Moscow Regional Registry of</td>
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<tr>
<td>Congenital Malformation</td>
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<td>South Africa</td>
<td>David Bourne</td>
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<td>South America</td>
<td>Maria Luisa Martinez Frias</td>
</tr>
<tr>
<td>Latin American Collaborative</td>
<td>Goran Anneren</td>
</tr>
<tr>
<td>Study of Congenital</td>
<td></td>
</tr>
<tr>
<td>Malformations</td>
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<tr>
<td>Spain</td>
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<tr>
<td>Spanish Collaborative Study</td>
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</tr>
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<td>of Congenital Malformations</td>
<td></td>
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<tr>
<td>Sweden</td>
<td></td>
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<tr>
<td>Swedish Registry of</td>
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<td>Congenital Malformations and</td>
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<td>Medical Birth Registry</td>
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<tr>
<td>Defects Program</td>
<td>Mark Canfield</td>
</tr>
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<td>Congenital abnormality study</td>
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</tr>
<tr>
<td>group</td>
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<tr>
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</tr>
<tr>
<td>Division</td>
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<td>United States, California</td>
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### Appendix 3. Malformations reported to the International Clearing-house for Birth Defects Surveillance and Research

<table>
<thead>
<tr>
<th>Malformation</th>
<th>ICD9</th>
<th>ICD10</th>
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<tbody>
<tr>
<td>Anencephaly</td>
<td>740.0–740.1</td>
<td>Q00.0–Q00.1</td>
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<tr>
<td>Spina bifida</td>
<td>741</td>
<td>Q05</td>
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<tr>
<td>Encephalocele</td>
<td>742</td>
<td>Q01</td>
</tr>
<tr>
<td>Microcephaly</td>
<td>742.1</td>
<td>Q02</td>
</tr>
<tr>
<td>Arhinencephaly / holoprosencephaly</td>
<td>742.26</td>
<td>Q04.1–Q04.2</td>
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<tr>
<td>Hydrocephaly</td>
<td>742.30 742.31 742.38 742.39</td>
<td>Q03–Q07.0</td>
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<tr>
<td>Anophthalmos</td>
<td>743</td>
<td>Q11.1</td>
</tr>
<tr>
<td>Microphthalmos</td>
<td>743.1</td>
<td>Q11.2 Q11.3</td>
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<tr>
<td>Anotia</td>
<td>744.01</td>
<td>Q16.0</td>
</tr>
<tr>
<td>Microtia</td>
<td>744.21</td>
<td>Q17.2</td>
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<tr>
<td>Transposition of great vessels</td>
<td>745.1</td>
<td>Q20.3–Q20.5</td>
</tr>
<tr>
<td>Tetralogy of Fallot</td>
<td>745.2</td>
<td>Q21.3</td>
</tr>
<tr>
<td>Hypoplastic left heart syndrome</td>
<td>746.7</td>
<td>Q23.4</td>
</tr>
<tr>
<td>Coarctation of aorta</td>
<td>747.1</td>
<td>Q25.1</td>
</tr>
<tr>
<td>Choanal atresia</td>
<td>748</td>
<td>Q30.0</td>
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<tr>
<td>Cleft palate without cleft lip</td>
<td>749.00–749.07</td>
<td>Q35, excluding Q35.7</td>
</tr>
<tr>
<td>Cleft lip with or without cleft palate</td>
<td>749.1</td>
<td>Q36–Q37</td>
</tr>
<tr>
<td>Oesophageal atresia / stenosis with or without fistula</td>
<td>750.30–750.34</td>
<td>Q39.0–Q39.3</td>
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<tr>
<td>Small intestine atresia / stenosis</td>
<td>751.1</td>
<td>Q41</td>
</tr>
<tr>
<td>Anorectal atresia / stenosis</td>
<td>751.2</td>
<td>Q42.0–Q42.3</td>
</tr>
<tr>
<td>Undescended testis (37 weeks of gestation or later)</td>
<td>752.50–752.52</td>
<td>Q53</td>
</tr>
<tr>
<td>Hypospadias</td>
<td>752.6</td>
<td>Q54 excluding Q54.4</td>
</tr>
<tr>
<td>Epispadias</td>
<td>752.61</td>
<td>Q64.0</td>
</tr>
<tr>
<td>Indeterminate sex</td>
<td>752.7</td>
<td>Q56</td>
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<tr>
<td>Renal agenesis</td>
<td>753</td>
<td>Q60</td>
</tr>
<tr>
<td>Cystic kidney</td>
<td>753.1</td>
<td>Q61</td>
</tr>
<tr>
<td>Bladder extrophy</td>
<td>753.5</td>
<td>Q64.1</td>
</tr>
<tr>
<td>Polydactyly, pre-axial</td>
<td>755.01 + 755.02</td>
<td>Q69.1–Q69.2</td>
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<tr>
<td>Limb reduction defects</td>
<td>755.2–755.4</td>
<td>Q71–Q73</td>
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<tr>
<td>Diaphragmatic hernia</td>
<td>756.6</td>
<td>Q79.0–Q79.1</td>
</tr>
<tr>
<td>Omphalocele</td>
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<td>Q79.2</td>
</tr>
<tr>
<td>Gastrochisis</td>
<td>756.71</td>
<td>Q79.3</td>
</tr>
<tr>
<td>Prune belly sequence</td>
<td>756.72</td>
<td>Q79.4</td>
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<tr>
<td>Trisomy 13</td>
<td>758.1</td>
<td>Q92</td>
</tr>
<tr>
<td>Trisomy 18</td>
<td>758.2</td>
<td>Q91</td>
</tr>
<tr>
<td>Down syndrome, all ages (including age unknown)</td>
<td>758</td>
<td>Q90</td>
</tr>
</tbody>
</table>
Appendix 4. International Database on Craniofacial Anomalies and International Perinatal Databases of Typical Orofacial Clefts

Aim of this appendix
To involve your organization in the International Perinatal Databases of Typical Orofacial Clefts.

Aims of the International Perinatal Databases of Typical Orofacial Clefts

- to build up validated, broad information on the current epidemiology of typical oral clefts, including birth prevalence, time trends, differences among populations and sex ratio;
- to persuade researchers to use the data collected to generate and test hypotheses; and
- to persuade researchers to plan ad-hoc research on risk factors to be tested in a set of validated cases.

Support and coordination of the International Perinatal Databases of Typical Orofacial Clefts

- Supporting organization: WHO Human Genetics programme Responsible officer, Dr Victor Boulvyjenkov. e-mail: boulvyjenkovv@who.ch
- Coordinating centre: International Centre on Birth Defects. e-mail: www.icbd.org
- Principal investigators: Pierpaolo Mastroiacovo and Elisabeth Robert-Gnansia
- Advisor: Eduardo E. Castilla

Requested data

Case by case information is collected on typical oral clefts. The minimum dataset collected for each case comprises:

- identification code: key available only locally;
- type of cleft: any coding system, preferably ICD 9 or ICD 10, and extension plus, if available, verbatim description;
- associated anomalies: any coding system, preferably ICD 9 or ICD 10, and extension plus, if available, verbatim description;
- final clinical diagnosis: isolated, with other malformations, syndrome. This field is proposed for addition to your database. Staff of the International Centre on Birth Defects will check this field to reach a standardized, common definition of isolated and multiple congenital anomalies and syndromes.
- month of birth;
- year of birth;
- status: livebirth, stillbirth, termination of pregnancy;
- sex: male, female, undetermined;
- birthweight, in grams;
- gestational age, in weeks;
- twinning status (single, twin, triplet, etc.);
- sex and malformation(s) of co-twin(s) (if available); and
- maternal age.

Period
You are invited to send cases of typical oral clefts seen since January 2001, 2002, 2003 or 2004, as is most suitable.

Denominators
You are invited to send the number of births (livebirths plus stillbirths plus terminations of pregnancy for any birth defect) by year and by maternal age, single year or 5-year period

The definition of births is the total number of livebirths plus stillbirths plus terminations of pregnancy performed for any birth defect.

Validation of data
All information will be reviewed centrally by the staff of the International Centre on Birth Defects. The final diagnosis (isolated, with other malformations, syndrome) of each case will be checked and changed if necessary, according to the rules and definitions given elsewhere.

A validation score will be prepared on the basis of:
• the description of data collection (e.g. hospital-based, population-based, multiple sources, active ascertainment);
• the rate of other malformations; and
• a specific validation study.

Appendix 4 (contd)

How to send data
Case-by-case data
The format for transmitting data is flexible. Any commonly used formats can be used, although Excel files are preferred and are the easiest for data processing. It is important thing to indicate the record layout and the meanings of the codes used.

Denominators
The total number of births (livebirths, stillbirths, terminations of pregnancy for any birth defect), if available by maternal age (single year or 5-year classes of –20, 20–24, 25–29, 30–34, 35–39, 40–44, ≥ 45) should be sent annually.

Validation
In order to build up a validation score for each participating register, you are invited to send the most complete description of your register and data collection and to fill in the questionnaire below.

Deadline
Any problems? Do not hesitate to write to  icbd@icbd.org.

Questionnaire for validating calculated rates

Are the cases to be registered in your register collected actively? If yes, please describe.
Is the source of the cases to be registered single or multiple? If multiple, please fill in a table like the following.

Multiple source of information for all cases sent to the International Perinatal Databases of Typical Orofacial Clefts

<table>
<thead>
<tr>
<th>Source (please specify)</th>
<th>Total number of cases sent to be registered by the named source</th>
<th>Number of cases already known to the registera</th>
<th>Number of new cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
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<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a Describe how multiple notifications of the same case are recognized.

Population-based registers: send a map of the area covered.
Fill in the following table:

<table>
<thead>
<tr>
<th>All births</th>
<th>Births with a typical oral cleft</th>
</tr>
</thead>
<tbody>
<tr>
<td>All deliveries</td>
<td>Residence in the area</td>
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<tr>
<td>In the area</td>
<td>XXXXXXXXXX</td>
</tr>
<tr>
<td>Outside the area</td>
<td>XXXXXXXXX</td>
</tr>
<tr>
<td>Deliveries of typical oral clefts</td>
<td>In the area</td>
</tr>
<tr>
<td>In the area</td>
<td>XXXXXXXXX</td>
</tr>
<tr>
<td>Outside the area</td>
<td>XXXXXXXXX</td>
</tr>
</tbody>
</table>
Hospital-based registers: send data to the International Perinatal Databases of Typical Orofacial Clefts only for the year in which cases were registered.
Please fill in a table like this, in Excel:

<table>
<thead>
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<th>Hospital</th>
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<tr>
<td>7</td>
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</tr>
<tr>
<td>Total</td>
<td></td>
</tr>
</tbody>
</table>

Add rows if necessary
Total

Number of case of selected defects. Please fill in this table:

<table>
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<tr>
<th>Total births</th>
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<th>2002</th>
<th>2003</th>
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<tr>
<td>Oesophageal atresia</td>
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<tr>
<td>Anal atresia</td>
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<td></td>
</tr>
<tr>
<td>Diaphragmatic hernia</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total births, 20–30 years</td>
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<td></td>
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<tr>
<td>Down syndrome, 20–30 years</td>
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</table>
Appendix 5. Coding of craniofacial anomalies and of infants with multiple malformations

Case definitions (ICD 9 code in parentheses)
Any coding system will be accepted, with any extension, provided that the key is given.

Cleft palate (749.0)
A congenital malformation characterized by a closure defect of the hard palate and/or soft palate behind the foramen incisivum without cleft lip. Exclude cleft uvula. In some databases, Pierre Robin sequence is included Pierre Robin sequence (756.03)
A congenital malformation characterized by a closure defect of the palate behind the foramen incisivum, without cleft lip, associated with significant micrognathia (small mandible) with or without a clinically relevant glossophtosis (retroposition of tongue) or respiratory distress.
This is not a multiple malformation but a sequence and therefore may be isolated or associated with unrelated defects or part of a known syndrome.
In some databases, this condition has a distinct code and is differentiated from usual cleft palates.

Cleft lip (749.1)
A congenital malformation characterized by partial or complete clefting of the upper lip. Exclude rare and oblique facial clefts.

Cleft lip and palate (749.2)
A congenital malformation characterized by partial or complete clefting of the upper lip with clefting of the alveolar ridge and/or the hard palate. Exclude rare and oblique facial clefts.

Isolated cases
Any case with only one major defect registered. In this database, only an orofacial cleft.

Multi-malformed infants
Background and definition
Any case with a major defect other than the orofacial cleft. Each infant must have a code for each malformation diagnosed, major or not. The presence of two codes does not necessarily mean that the infant has multiple malformations. Only infants with two or more major unrelated malformations without a specific diagnosis of a known syndrome are considered to be multi-malformed infants.

Major malformation: Any defect that has a relevant impact on the health of the infant and requires medical or surgical treatment. See list of exclusions below.

Unrelated malformation: Malformations are considered unrelated if they occur in different organ systems or at different body sites and are not part of a known embryological sequence or have a common primary defect.

Conditions that should be considered as related entities and counted as one defect:
Defects of the same nature affecting the same organ, organ system or body part:
anophthalmia, microphthalmia
anotia, microtia
renal agenesis, renal dysplasia
oesophageal atresia, tracheo-oesophageal fistula
anencephaly, spina bifida, encephalocoele
multiple cardiac defects
urethral, ureteral stenosis, atresia, renal aplasia or dysplasia
multiple small intestinal atresias
brain defects, defects of retina or visual pathways
syndactyly, reduction defect of corresponding limbs
cleft lip, oblique facial cleft, cleft palate
reduction defect of different limbs

Defects that are components of a malformation sequence or have a common primary defect:
spina bifida, talipes, hydrocephaly, axial skeleton
urethral obstruction (prune belly), hydronephrosis, abdominal muscle deficiency
Pierre Robin, micrognathia, glossophtosis, cleft palate
renal aplasia or dysplasia (Potter), pulmonary hypoplasia, compression facies, limb deformations
omphalocoele, gastrochisis, malrotation of gut, small intestinal atresia
diaphragmatic hernia, pulmonary hypoplasia
Appendix 5 (contd)

- holoprosencephaly, arhinencephaly, cyclopia, midline cleft lip and/or palate, anophthalmia or microphthalmia
- exstrophy of the cloaca, imperforate anus, lumbosacral vertebral defect, meningomyelocele, omphalocele
- vaginal atresia, rudimentary uterus, renal aplasia or dysplasia
- septo-optic dysplasia, absence of septum pellucidum, optic nerve hypoplasia, hypothalamic defect
- sirenomelia, single lower extremity, renal aplasia or dysplasia, absent external genitalia, imperforate anus, pulmonary hypoplasia
- laterality defect, congenital heart defect, situs inversus or ambiguous, asplenia or polysplenia
- thymus aplasia or hypoplasia (Di George), conotruncal heart defects, ear anomalies
- caudal regression, sacral hypoplasia or aplasia, lower limb reduction or deformation

Defects observed in conjoined twinning

Known syndrome: A set of multiple defects (major or not or combination) in which a clinician recognizes a syndrome and gives the clinical picture a name. Often, the picture can be coded with a code from the Online Mendelian Inheritance in Man. When a chromosomal defect is found, the karyotype formula is the code.

Exclusions from the working definition of a major malformation

The following conditions should not be considered major defects when an infant with multiple malformations is to be defined. This is a collaborative epidemiological working list and is not necessarily in agreement with a clinical view or judgement. This list does not pretend to be exhaustive but is simply a long series of examples.

- skin cysts
- nonecavernous single small haemangioma (less than 1 cm in diameter)
- benign skin neoplasms
- naevoid flammemus
- birth mark
- mongolian spots
- cutis marmorata
- café au lait spots
- scalp defects
- lanugo, excessive or persistent
- accessory nipple
- large or small fontanelle
- macrocephaly
- head asymmetry
- dolichocephaly
- facial palsy
- facial asymmetry
- micrognathia
- eso- or exotropia
- nystagnus
- blue sclera
- Brushfield spots
- epicanthal folds
- eye slant (upwards or downwards)
- nasolachrymal duct obstruction
- ear tags
- bat, caulifower, elfin, lop, pointed, posteriorly rotated or low-set ears
- auricular tubercle
- preauricular sinus, cyst or pit
- macrotia
- flat or wide nasal bridge, upturned nose or other minor nose malformation
- deviation of nasal septum
- ankyloglossia
- macroglossia
- microglossia
- natal teeth
- big, wide or small lips
- high-arched palate
- bifid uvula
- redundant neck skin folds
Appendix 5 (contd)

webbing of neck
short neck
patent ductus arteriosus or foramen ovale (birth weight < 2500 g)
mild, trivial or physiological valvular regurgitation
single umbilical artery
hepatomegaly
splenomegaly
ectopic kidney
Meckel diverticulum
anal tags
rectal fissures
pilonidal or sacral dimple
inguinal hernia in males
inguinal hernia in females (birth weight < 2500 g)
umbilical hernia (skin covered)
hydrocoele
imperforate hymen
prominent clitoris
fusion of vulva
vaginal or hymenal tags
cyst of vagina, vulva or canal of Nuck
undescended testicles (birth weight < 2500 g)
small penis
chordee
patent urachus or urachal cyst
skin tags on hands and feet
partial syndactyly of feet
brachydactryly of feet
clinodactryly
camptodactryly
long fingers and toes
nail hypoplasia
enlarged or hypertrophic nails
widely spaced first and second toes
overlapping toes
tibial torsion
genu valgum
genu varum
hallux valgus
hallux varus
cervical rib
simian or Sydney lines
hip subluxation
Appendix 6. Registries participating in the International Perinatal Databases of Typical Orofacial Clefts

<table>
<thead>
<tr>
<th>Country</th>
<th>Register</th>
<th>Coordinator</th>
</tr>
</thead>
<tbody>
<tr>
<td>Argentina</td>
<td>Latin American Collaborative Study of Congenital Malformations</td>
<td>Eduardo E. Castilla</td>
</tr>
<tr>
<td>Australia, Victoria</td>
<td>Victoria Birth Defects Registry</td>
<td>Jane Halliday</td>
</tr>
<tr>
<td>Austria, Styria</td>
<td>Styrian Malformation Registry</td>
<td>Martin Haeusler</td>
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<tr>
<td>Belgium, Antwerp</td>
<td>EUROCAT Registry of Congenital Anomalies</td>
<td>Vera Nelen</td>
</tr>
<tr>
<td>Belgium, Hainaut</td>
<td>Registry of Hainaut-Namur</td>
<td>Yves Gillerot</td>
</tr>
<tr>
<td>Bolivia</td>
<td>Latin American Collaborative Study of Congenital Malformations</td>
<td>Eduardo E. Castilla</td>
</tr>
<tr>
<td>Brazil</td>
<td>Latin American Collaborative Study of Congenital Malformations</td>
<td>Eduardo E. Castilla</td>
</tr>
<tr>
<td>Canada, Alberta</td>
<td>Alberta Congenital Anomalies Surveillance System</td>
<td>Brian Lowry</td>
</tr>
<tr>
<td>Canada, British Columbia</td>
<td>British Columbia Health Status Registry, Congenital Anomalies Surveillance Program</td>
<td>Ron Danderfer</td>
</tr>
<tr>
<td>Chile</td>
<td>Latin American Collaborative Study of Congenital Malformations</td>
<td>Eduardo E. Castilla</td>
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<td>Colombia</td>
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<td>Eduardo E. Castilla</td>
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<td>Croatia</td>
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<td>Czech Republic</td>
<td>Congenital Malformations Monitoring Programme of the Czech Republic</td>
<td>Antonin Sipek</td>
</tr>
<tr>
<td>Denmark, Odense</td>
<td>Registry of Funen County</td>
<td>Ester Garne</td>
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<tr>
<td>Ecuador</td>
<td>Latin American Collaborative Study of Congenital Malformations</td>
<td>Eduardo E. Castilla</td>
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<tr>
<td>France, central-east</td>
<td>Central-East France Register of Congenital Malformations</td>
<td>Elisabeth Robert-Gnansia</td>
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<td>France, Paris</td>
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<td>Lisa Ann Miller</td>
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<td>Hawaii Birth Defects Program</td>
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<td>Latin American Collaborative Study of Congenital Malformations</td>
<td>Eduardo E. Castilla</td>
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Appendix 7. Preliminary guidelines for setting up an international database of craniofacial anomalies seen in surgical departments

15 November 2004

The WHO Human Genetics programme and the National Institute of Dental and Craniofacial Research (United States) are sponsoring a worldwide database called the International Database of Craniofacial Anomalies. The project is coordinated by the International Centre on Birth Defects (principal investigators, Pierpaolo Mastroiacovo and Elisabeth Robert-Gnansia; advisor, Eduardo E. Castilla). More information is available on the website: www.icbd.org.

The International Database of Craniofacial Anomalies (see Appendix 4) centralizes a number of databases, which, together, can be used to answer various questions related to patients with a craniofacial anomaly, their families and their health-care providers. A further activity of the Database is to stimulate the creation of specific databases on craniofacial anomalies. An international database on Moebius syndrome has been planned (see www.icbd.org, powerpoint lectures, for more information), and an international surgical departments database of craniofacial anomalies is to be set up, with information from surgical departments in different cities, regions and countries.

Aims

- to establish a worldwide collaborative structure for the registration and follow-up of persons with craniofacial anomalies treated in surgical departments;
- to establish a number of local databases of craniofacial anomalies in craniofacial surgical departments in various areas, regions, states or countries; and
- to stimulate use of the database for public health or clinical surveys, to evaluate issues such as:
  - quality of life,
  - quality of health services,
  - clinical studies,
  - diagnostic issues,
  - genotype–phenotype correlations,
  - genotypic variation and
  - prevalence estimates.

Eligible participants

Any surgical department for craniofacial anomalies that is

- willing to collaborate to achieve relevant scientific and public health goals,
- possibly linked with a clinical genetics service,
- able to provide the requested information regularly (see ‘Requested information case by case’) and
- able to follow up registered patients directly or through their health-care providers.

Patients to be included in the database

- The database is prospective.
- All persons with a craniofacial anomaly (Box 1) seen since (date), including:
  - new patients, seen for the first time at the department for evaluation, before surgical treatment; and
  - new patients, to be treated surgically or, in the week before registration, treated surgically.
- All persons must have signed an informed consent form to be registered and possibly to be contacted in the future.
Appendix 7 (contd)

Box 1. Craniofacial anomalies to be included in the international surgical departments database of craniofacial anomalies

<table>
<thead>
<tr>
<th>Includes only surgically treated conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any orofacial cleft, typical or atypical</td>
</tr>
<tr>
<td>Craniosynostosis, isolated and syndromic</td>
</tr>
<tr>
<td>Hemifacial microsomia, isolated or syndromic</td>
</tr>
<tr>
<td>Any complex craniofacial anomaly or syndrome (e.g. Treacher Collins, Apert, Moebius)</td>
</tr>
<tr>
<td>Any condition treated surgically in the department (e.g. blepharofimosis; anotia or microtia; facial palsy)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Excludes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Craniofacial tumour</td>
</tr>
<tr>
<td>Encephalocoele, isolated or syndromic</td>
</tr>
<tr>
<td>Haemangiomases</td>
</tr>
<tr>
<td>Hydrocephalus</td>
</tr>
<tr>
<td>Neck and cervical column anomalies</td>
</tr>
<tr>
<td>Vascular malformations</td>
</tr>
</tbody>
</table>

Patients who refuse consent should be registered with the minimum data set and specification in the field ‘patient consent’.

Requested information case-by-case
The types of information requested were classified as essential (minimum dataset), desirable or optional. The minimum dataset comprises:

- date of compilation;
- doctor’s code;
- patient’s code;
- type of registration (new patient or already registered patient);
- date of birth (at least month and year);
- sex;
- description of craniofacial anomaly;
- description of other defects, if present;
- name of syndrome, if assigned;
- evidence of diagnosis, if a syndrome; and
- patient consent (yes or no).

General organization
The structure is based on dedicated coordinators:

- department coordinator: one in each participating department (a surgeon, clinical geneticist or epidemiologist);
- local database coordinator: one in every two or more surgical departments located in the same area;
- general database coordinator: International Centre on Birth Defects; and
- steering committee: that of the International Database of Craniofacial Anomalies plus one or more representatives from surgical departments.

and on continuous interaction among the coordinators, including:

- case-by-case review of diagnoses, with photographs and imaging when feasible (by e-mail);
- active discussion on results as they become available (by e-mail);
- active discussion to prepare common protocols for special studies (by e-mail); and
- annual meetings.
The aggregated results will be contained in a website under the supervision of the WHO Human Genetics programme and the International Centre on Birth Defects.

Appendix 7 (contd)

Strictly anonymous, basic data on each case will be submitted periodically (e.g. every 20 new cases) by the department coordinator, on a worksheet or in any other feasible way, to the local database coordinator and to the International Centre on Birth Defects, which will review all cases and interact with the notifying doctor to achieve the best standardized information.

All department and local coordinators will have access to the aggregated data through a password, which will be changed periodically.

Rules for proposing special studies, authorship and data security will be prepared by the steering committee.

Why should my department participate?

The are two advantages to participating in a prospective database on a specific disease or group of diseases, in our context, birth defects and congenital syndromes:

- contact with colleagues throughout the world working on the same topic; and
- the opportunity to propose, coordinate or participate in studies that can be conducted with the database.

Immediate advantages include:

- consensus on the diagnosis of rare syndromes;
- information about different kinds of care in different settings;
- a larger sample size than that of any single department;
- information about the range of hypotheses to be tested in specific studies and the generalizability of results; and
- the feasibility of conducting specific studies rapidly, as eligible patients eligibility ready available, patients’ consent and collaboration are assured and their diagnoses are validated.

Anticipated questions

What is the definition of ‘database’ in this context?

In this context, a database is simply a collection of cases with a specific craniofacial anomaly, who have given informed consent to be included in the database and to participate in clinical and social studies in an international setting.

Who is the ideal department database coordinator?

The department database coordinator should be a dedicated, interested person such as a surgeon or clinical geneticist, who can interact with other colleagues and participate at least once a year in an international meeting.

Who is the ideal local database coordinator?

In areas where there is a birth defects registry, its coordinator would be the ideal local database coordinator, as he or she could be in direct contact with surgical departments.

We would like to participate, but we have very few cases.

This is not a problem, as the database is very flexible. Please check the notification form to see whether the information shown in green is available.
Appendix 8. Proposed classification of craniofacial anomalies

Craniofacial anomalies comprise a large number of birth defects, reflecting the great complexity of the systems, organs and functions in the cranium and face. These anomalies can affect the oral region, the eye region, the nasal region, the cranium and the brain. They are either isolated or associated with other anomalies within or outside the cephalic pole. One of the most frequent craniofacial anomalies is cleft lip and/or palate, which is seen in 1:700 newborns.

Many sources can facilitate a clinical diagnosis (Gorlin, 2001; Wyszynski, 2002), but it is noteworthy that we still lack a classification that is accepted worldwide. Different groups working on the clinical diagnosis of craniofacial anomalies have been using different classification systems, or different terms for the same anomaly. The classification of oral clefts serves as an example. In Brazil, oral clefts are classified according to the method of Spina (1982), which is based on embryological development, while in other countries classification is based on the surgical technique used in reconstruction.

On behalf of colleagues in São Paulo State, Dr Danilo Moretti Ferreira proposed a classification system based on the method proposed by Nilton Freire-Maia (1985). In this model, numbers have been assigned to each structure involved (teeth, nails, hair and sweat glands). Thus, we suggest that a primary number be assigned to each craniofacial region or organ and secondary numbers be used to further specify the region or organ affected.

Example of this classification are shown below. With this classification system, every patient would have a ‘number’, making the nosological classification of craniofacial anomalies much easier. Furthermore, the numbers could be used to integrate all the currently available and future databases.

A classification system of this magnitude should be implemented by an international group of investigators, a WHO-sponsored task force, who would represent the various opinions and later facilitate use of the new method in their own countries and integration of the currently available databases.

<table>
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<tr>
<th>Examples of classification with the proposed system</th>
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<tr>
<td>1. Oral region</td>
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<tr>
<td>1.1 Lips</td>
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<tr>
<td>1.1.1 Clefts</td>
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<tr>
<td>1.1.1.1 Unilateral</td>
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<tr>
<td>1.1.1.2 Bilateral</td>
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<td>1.1.2 Macrostomia</td>
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<td>1.2 Palate</td>
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<td>1.2.1 Clefts</td>
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<tr>
<td>1.2.1.1 Primary</td>
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<td>1.2.1.3 Submucosa</td>
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<td>2. Nasal region</td>
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<tr>
<td>3. Eye region</td>
</tr>
<tr>
<td>3.1 Eye</td>
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<tr>
<td>4. Cranium</td>
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