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Epidemiology underpinning research in the aetiology of orofacial clefts*

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Structured Abstract

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Introduction – Epidemiological information gathered through birth defects surveillance is an important adjunct to carrying out clinical and aetiological research. Information on the incidence in the population, causative risk factors and providing baseline data prior to intervention are all important elements. Under the auspices of the World Health Organisation, it was agreed that a global registry and database on craniofacial anomalies should be created and this, the International Database on Craniofacial Anomalies (ICDFA) was designed to gather information on craniofacial abnormalities from existing birth defects registries and databases around the world to become a resource underpinning research. There are currently 62 registries covering 2 million births per year contributing to a database along with information on the size and type of studies used to collect the information, any variation in ascertainment and on the inclusion of syndromes and associated abnormalities.

Generation of hypotheses – From the epidemiological data collected it is possible to carry out meta-analysis and to search for trends and consistencies in the data that enable hypothesis to be generated. Issues such as geographical distribution, ethnicity, gender, associated abnormalities and clefts in stillbirths can all be examined in a meta-analytical approach. Collection of information on risk factors such as maternal illnesses, medications, lifestyle factors, nutrition and perhaps occupational exposures enables investigation into environmental contribution to causality and genetic predisposition. A range of techniques are currently being used to identify new candidate genes and ultimately it will be necessary to test genetic and environmental hypothesis in the context of human population studies.

Conclusions – It is only by consistency of association between different populations with different gene pools and maternal exposures, lifestyles, nutrition etc that conclusive evidence regarding causality will be found. It is therefore essential, and a major objective of the WHO that international multicentre collaborative studies are setup to gather the appropriate evidence and improve knowledge and the cause of birth defects in general and orofacial clefts in particular, with the ultimate humanitarian and scientific objective of the WHO being primary prevention.

Clinical utility and implications – This IDCFA project fulfils three basic objectives namely to enable global surveillance of CFA; to create online access to those who wish to contribute to the IDCFA, and to develop an online directory of resources on craniofacial anomalies for the support of research and improving quality of care. The

next steps for IPDTOC are to expand the number of participating registries and to actively collect data on other craniofacial birth defects.

Key words: cleft lip; cleft palate; epidemiology; research

Introduction

The United States of America has always been a world leader in healthcare and among its instruments in promoting health is the March of Dimes which was established in 1938 to address the problem of polio. In 1998 the March of Dimes broadened its mission beyond the United States and established an office of programmes and its mission being to improve infant health by preventing birth defects, premature births and infant mortality through research, community services, education and advocacy.

In 2006 March of Dimes published its 'Global Report on Birth Defects: the hidden toll of dying and disabled children'. The following statement is taken from this report 'every year, an estimated 7.9 million children – 6% of total births worldwide are born with a serious birth defect of genetic or partly genetic origin. Birth defects are a global problem, but their impact is particularly severe in middle and low income countries where more than 94% of the births with serious birth defects and 95% of the deaths of these children occur' (1). Furthermore, in a report on congenital anomalies by (2) the burden of lifelong disability, effects on families and society and serious effect on life expectancy was reported. This report also points out that between 44% and 60% of congenital anomalies affect the craniofacial structures and the most common group of craniofacial anomalies is orofacial clefts (OFC).

Role of epidemiology in the study of OFC

The main value of epidemiology is to provide accurate information and data that will underpin research and clinical trials:

- To assess the burden of OFC at all levels in order to plan public health resources and strategies.
- To assess causes of OFC, including genetic, nutritional, infectious, environmental and other factors.
- To provide a scientific basis for evaluating the scope for intervention strategies and in particular prevention.

The idea of producing a global registry and databases on craniofacial anomalies was discussed at a World Health Organisation (WHO) Conference held in Bauru, Brazil in December 2001 – a conference that was financially supported by the National Institute of Health (NIH). This was reported to be a World Health Initiative promoted by the WHO Human Genetics Programme with the primary aim being to establish a single database for the collection of existing and new information on craniofacial anomalies from around the world. Thus the International Database on Craniofacial Anomalies (IDCFA) was created. This multi-source, meta-analytic approach to a particular birth defect has a number of significant advantages over the traditional cumulative and regionalized approach.

The main aims of the IDCFA were to (1) to stimulate existing databases to share their data creating a specific worldwide database dedicated to craniofacial birth defects; (2) to present the collected data in a suitable way or to make available more specific data to stimulate research addressing primary prevention and improved quality of care for craniofacial anomalies; and (3) to stimulate scientific and lay organizations to collect and share relevant data and information on persons affected by a craniofacial anomaly.

This meeting also defined the minimum information dataset and produced the protocols that could be disseminated to the existing registries requesting the appropriate information. The principle of obtaining case by case information with the possibility of linking individual data with the diagnosis and other information relevant to aetiology underpins this initiative. Data protection is ensured by keys for the linking of data being held locally and only subject coded information is forwarded to the central database. It was agreed that this could be housed at the offices of the international centre for birth defects (ICBD) in Rome.

The first step of the IDCFA was to focus on developing the 'International Perinatal Databases of Typical Orofacial Clefts' (IPDTOC). With typical orofacial clefts the database is based on an international collaboration using existing resources and the most reliable data.

Data on typical oral clefts are the most reliable, and the coding systems are well developed. The data can be subjected to meta-analyses of many aspects of descriptive epidemiology, and the resulting information is used to underpin research. Many other craniofacial anomalies present challenges in definition, detection during the perinatal period, diagnosis and coding. The diagnosis may be: (1) isolated oral cleft – with the appropriate code; (2) multiple congenital anomalies – with a code identifying the number of major unrelated defects present; (3) syndrome – with the OMIM or expanded ICD 10 code identifying the syndrome. Standardized definitions for these were agreed upon and made available to all participating registries.

As of March 2006, 62 registries covering 2 million births per year have contributed and with the first initiative concentrating on typical oral clefts it has been possible to record the rates which are presented in Table 1.

Quality issues in descriptive epidemiology

The completeness of ascertainment will inevitably vary between different registries where different methods of ascertainment and number of ascertainment sources are used in the validation of the data collected. Variation in the size and type of studies used to collect the information, variations in ascertainment, criteria and expertise, the recording of still births and induced abortions may vary, whether syndromes and associated abnormalities are consistently diagnosed all make a difference to the quality of the data collected.

Table 1. **Rates of Typical Oral Clefts in 17 areas, ordered by increasing rate**

No.	Registries set	No. of Reg	No. of cases	Rate × 10 000	95% Confidence interval
1	South Africa	1	33	4.76	3.28–6.69
2	Europe Mediterranean	10	856	8.80	8.22–9.41
3	USA East	3	500	10.02	9.16–10.93
4	United Arab Emirates	1	19	11.05	6.66–17.26
5	USA Atlanta	1	215	13.90	12.10–15.88
6	Italy – Sicily, ISMAC	1	47	14.23	10.46–18.93
7	USA – Hawaii	1	75	14.35	11.29–17.99
8	Europe Central – East	9	2,467	14.91	14.32–15.51
9	Europe British Islands	5	613	15.41	14.22–16.68
10	Cen–South America – 1	7	587	16.16	14.88–17.52
11	Europe North – 1	4	300	16.65	14.82–18.64
12	USA Central	5	1176	16.82	15.87–17.81
13	Australia Victoria	1	219	17.49	15.25–19.96
14	North America West	3	994	20.99	19.71–22.34
15	South America – 2	3	220	21.68	19.91–24.74
16	North Europe	4	354	22.64	20.34–25.13
17	Japan	1	644	23.79	21.99–25.71

- A range of measures can be implemented to improve the quality of descriptive epidemiology in the field of orofacial clefts. These include:
- Common core protocols to help 'standardize' data collection.
- Multiple sources of ascertainment.
- Consistent inclusion of stillbirths and earlier foetal losses.
- By diagnostic and classification procedures separate syndromic, non-syndromic and associated anomalies.
- Split OFC into more consistent and homogenous sub-sets for analysis.
- Ethnic grouping/stratification.

Generation of hypotheses

While these variations in quality and varying degrees of inaccuracies and inconsistencies will occur, there are nevertheless trends and consistencies in the data that provide clues to the aetiology, and enable hypothesis to be generated. These, for orofacial clefts are noteworthy trends.

1. Geographical distribution and time trends.
2. Distribution of associated malformations in OFC.
3. Occurrence rates of OFC in live births vs. still births.
4. Ethnicity trends are orofacial clefts.
5. Gender and cleft type.
6. Cleft lip and palate: the issue of laterality.
7. Difference in the ratio of cleft lip to cleft lip and palate.

(1) Geographical distribution and time trends

Descriptive epidemiology using IDCFA data has confirmed the apparent correlation between frequency of orofacial clefts and latitude in Europe ($r = 0.69$), and analysis of the USA data revealed a similar correlation with longitude, i.e. increasing frequency from East to

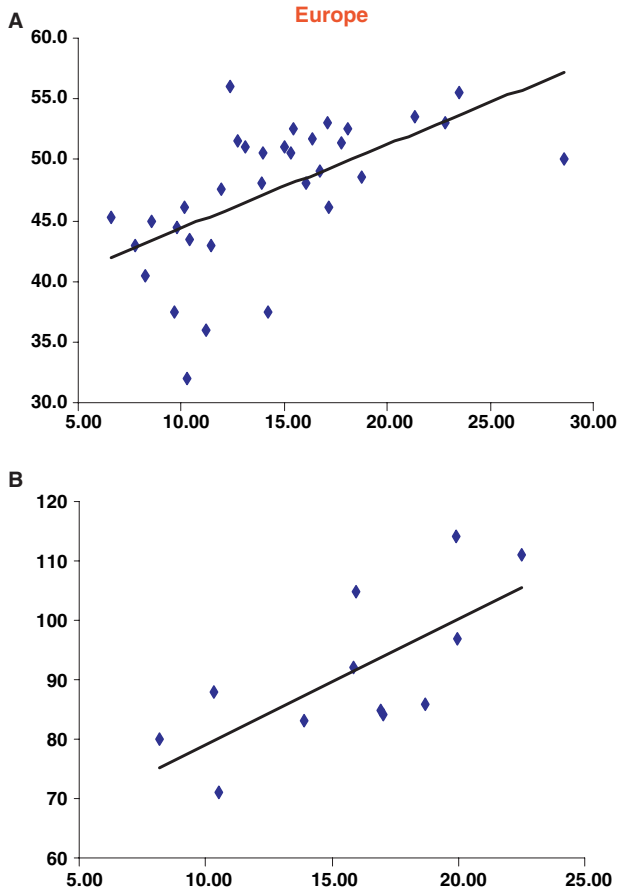

Fig. 1. (A) Correlation between OFC total rates and latitude in Europe. (B) Correlation between OFC total rates and longitude in North America.

West in North America (r = 0.67) (http://www.who.int/genomics/anomalies/idcfa/en/). Fig. 1a, b illustrates these and trends and the correlations for both were found to be statistically significant.

Time trend information is best derived from registries which have been collecting data with reasonably consistent ascertainment, such as ICBD, 1974–1998 (24 years) and Eurocat, 1980-1994 (14 years) (3, 4). From these data, a few trends such as progressive increase in CP and CL/P clefting in Finland and increase in CP clefts in Norway have been noted. Also the overall prevalence of OFC in Denmark has risen from 1:667 LB (1942) to 1:529 LB (1981) (5), and although the figures are derived from a voluntary reporting system, there has been a steady reduction in CP and CL/P clefting in England and Wales.

(2) Distribution of associated malformations in OFC

The frequency of associated malformations in OFC has been found to be greatest for isolated cleft palate (CP) and least for the least severe manifestation of a cleft (CL). For instance (6) in a West of Scotland study reported that 45% of CL(P) cases and 66% of CP cases had associated malformations, while (7) in a Swedish dataset reported frequencies of CP = 46.7%, CLP = 36.8% and CL = 13.6%. Furthermore within the CL/P group (8) reported that frequency of associated malformations was greater in BCLP compared with UCLP.

(3) Live births vs. still births

Occurrence rates of OFC are also found to be greater in still births (SB) than in live births (LB). For example (9) in a study of whites in Iowa found a prevalence of 6.43 per 1000 SB vs. 2.16 per 1000 LB, and in a study of for blacks, Mexicans and whites (10), the rates reported were 2.72 per 1000 SB vs. 0.91 per 1000 LB. It is also noteworthy that in a Hungarian study of SB vs. LB (11), there was a sevenfold increase for CP (2.38 per 1000 in SB vs. 0.36 per 1000 in LB) and a threefold increase for CL/P (3.17 per 1000 in SB vs. 1.15 per 1000 in LB). Krause *et al.* (12) also found that there was a greater risk of associated malformations accompanying clefts in SB.

(4) Ethnicty and OFC

For CL(P) the highest recorded rates are found in Far East, India, Aborigines, Scandinavia, parts of South America and Native Americans, while the lowest rates in Africa, Southern Europe and African Americans, and Kirby et al. (13) reported that in general the trend in the US is for the rate of CLP to be greatest in White races, intermediate in Hispanics and lowest in Blacks. For CP, there is less geographical variation than CL/P, and the highest rates are in Finland, Scotland and Australia, and as a general rule Western rates exceed those in Asia, which in turn exceed the reported African rates (14).

(5) Gender and cleft type

The male predominance in CL/P and female predominance in CP are consistent features reported in datasets across the world, but with some geographical variations noted (14–16). Also the male excess in CL/P is more apparent with increasing severity of cleft (15), and male excess is less apparent when more than one sibling affected (17).

(6) Laterality

Unilateral clefts form 80–85% of all CL(P) cases (8) and two-thirds of these have left-sided clefts regardless of

sex, race and severity of the defect (5, 15, 18–20). A possible explanation that blood vessels, supplying the right side of the foetal head leave the aortic arch closer to the heart and perhaps therefore are better profused by blood than those going to the left side was proposed by Johnston and Brown (21) but this is difficult to verify.

(7) CL vs. CL/P

In descriptive epidemiology it is traditional for cleft lip with cleft palate and cleft lip without cleft palate to be lumped together for analysis and in the past it has been described as 'logical to assume' that these are of similar aetiology as they involve the primary palate. There are distinctive differences between CL(P) and CP, but less evidence regarding CL vs. CL/P differences. Mossey and Little (14) however noted that in those geographical areas where there were higher overall rates of CL(P), there was also a higher proportion of CLP compared with isolated CL, the milder manifestation of primary palate defects (Fig. 2).

Conclusions from descriptive epidemiology

Excluding syndromic orofacial clefts and multiple malformed infants there is evidence for distinctive differences between CLP and isolated cleft palate. Evidence is also emerging that for non-syndromic clefts there may be differences in cleft lip with cleft palate (CLP) and isolated cleft lip (CL), and the model which best describes and explains the descriptive epidemiology reported here is the multifactorial threshold model, and this has been reported in relation to a substantial Californian dataset by Tolarova and Cervenka (22).

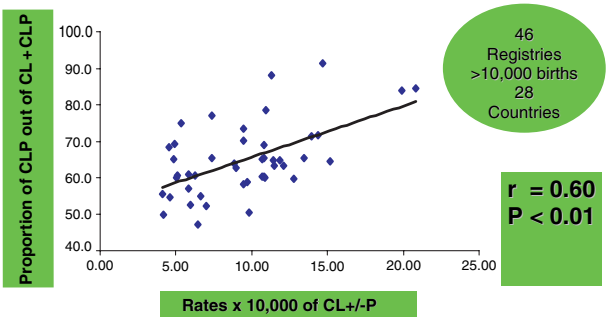

Fig. 2. Correlation between overall rate of CL(P) and proportion of CLP vs. CL.

From epidemiology to research hypotheses

It is accepted that descriptive epidemiology alone will not produce evidence of causality and the modern approach to epidemiology is to select additional case and control information on putative risk factors, both genetic and environmental. The environmental risk factors will include lifestyle factors such as smoking and alcohol consumption, maternal illnesses and medications, nutrition and perhaps occupational exposures. In addition it is important to collect biological information such as DNA which allows and investigation of genetic predisposition.

Evidence for possible environmental influence

Examples of hypothesis generation are exemplified by observations in certain populations of the effect of socio-economic status. In Kuala Lumpur, 65% of clefts from lower class, 28% from middle class and 7% from upper class (23). In 1987, Womersley & Stone (6) reported that the highest rates of clefts were observed in areas or high unemployment, poor housing and unskilled workers, and the lowest rates in affluent areas of the West of Scotland; and in a study of Filipinos in California, Hawaii and Philippines Croen *et al.* (24) observed the highest OFC rates in Philippines, and lowest in California, according to socio-economic status.

The data from the registry of OFC births in Scotland between 1989 and 1998 are shown in Fig. 3, but there are difficulties in defining what exactly contributes to low socioeconomic status. This could be a combination of a known environmental factor, maternal smoking (as there are higher rates among the more deprived social classes), housing conditions with increased maternal illness and infections, alcohol consumption, differences in nutrition, medications or recreational drugs etc. Multi-centre case–control studies will be required to test the association between these various exposures and orofacial clefts.

Genetic epidemiology

In addition to information on environmental exposures, the genetic predisposition is important and requires research on genetic polymorphisms and gene–environment interaction (GEI). In turn this requires biological samples and/or DNA samples from cases and their parents.

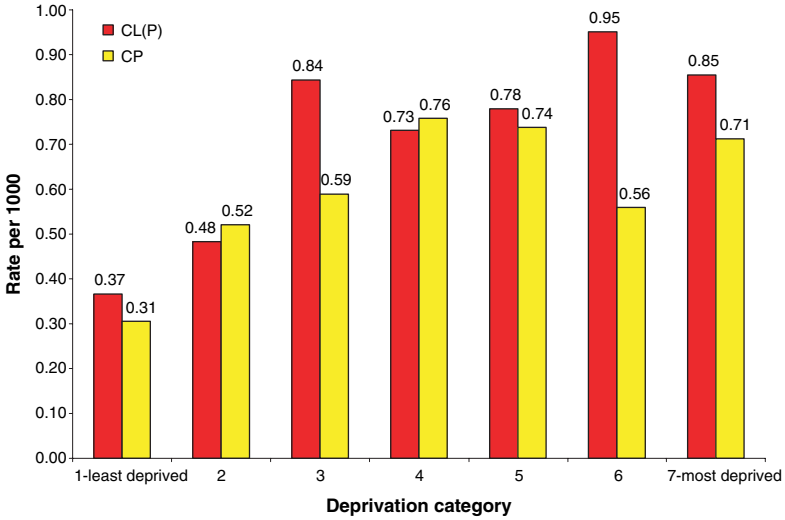

Fig. 3. Prevalenceof clefts in Scotland according to socio-economic status (Depcat index).

Considerable progress has been made in identification of new candidate genes by a number of different approaches, all of which complement each other in the quest for gene discovery. These are listed in table 2 with the highlighted gene loci showing promise in orofacial cleft studies, some of which are carried out in human population samples. The categories listed in this table are transcription factors, growth factors, cell signalling molecules, folate pathway genes and detoxification enzymes.

Many of these are being investigated in larger multicentre collaborative initiatives that will enable an analysis of consistency of association which is the first step towards proof of causality. Another recent technique used for gene discovery is identification of chromosomal breakpoints and deletions, and an example of a recent success with this technique was the discovery of the SATB2 gene is a plausible location for a polymorphism causing isolated cleft palate (25). It may be that, in the future, the new diagnostic tool, comparative genomic hybridization (CGH), which enables detection of microdeletions will facilitate the discovery of genomic loci for cleft predisposing or causing genes. Array-CGH may well become a routine method of genome-wide screening for imbalanced rearrangements in children with CP and perhaps also CL(P).

Table 2. **Candidate Gene classes for OFC**

TF	GF	CS	FP	DE
MSX1	TGFA	PVRL1	MTHFR	CYP1A1
TBX22T	TGFB1	PVRL2	RFC1	NAT1
IRF6	TGFB2	PVR	MTRR	NAT2
LHX8	TGFB3	PTCH	GCP2	GSTM1
TBX10	TP63	GABRB3	CBS	GSTT1
DLX1/2/5/6	SKI1	ARNT2	MTHFD	GSTP1
SATB2	TGFBR1	WNT9A	FOLRA	RARA
RYK	FGFR1	ROR2	BHMT	EPHX1

Where do we go from here?

It is now incumbent on those leading research initiatives in the field of craniofacial anomalies and orofacial clefts to progress what has been started by the WHO initiative in craniofacial anomalies through the consensus meetings in November 2000, May 2001, December 2001 and December 2004. The IDCFA project fulfils three basic objectives namely to enable global surveillance of CFA; to create online access to those who wish to contribute to the IDCFA, and to develop an online directory of resources on craniofacial anomalies for the support of research and improving quality of care. The next steps for IPDTOC are to expand the number of participating registries focussing on parts of the developing world where no registries currently exist and to actively collect data on other craniofacial birth defects.

Examples of developing countries involved in WHO birth defects surveillance and gene–environment interaction (GEI) studies are India, Africa, Thailand, Jordan, Egypt and Nepal.

At the second international conference on birth defects and disabilities in the developing world held in Beijing between 11th and 14th of September 2005, a

manifesto was produced and a memorandum of understanding signed by the participating countries. This stated that:

'We must continue to collaborate to establish and maintain birth defects surveillance and monitoring systems, foster research on the causes and prevention of birth defects and genetic diseases, and establish sustainable, technologically appropriate interventions for the prevention and care of these conditions'. Beijing Manifesto (2005).

In order to realize this, the present collaborations must be continued and strengthened and new collaborations need to be established. The ongoing development will be reported in the website that has been established for this purpose at http://www.who.int

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