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ECLAMC: The Latin-American Collaborative Study of Congenital Malformations

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Key Words

Birth defects · Birth prevalence · Environmental risk factors · Genetic risks · Hospital network · Molecular markers · Population structure · Risk factors · Secular trends · South American population

Abstract

Definition: ECLAMC ('Estudio Colaborativo Latino Americano de Malformaciones Congénitas') is a program for the clinical and epidemiological investigation of risk factors in the etiology of congenital anomalies in Latin-American hospitals, using a case-control methodological approach. It is a voluntary agreement among professionals lacking institutional base as well as designated budgets. ECLAMC has been usually funded by research-funding agencies rather than public health ministries. The National Research Councils of Argentina and Brazil have been the main sources of support during its 36 years of existence. Since vital and health statistics are unreliable in South America, ECLAMC collects all the information required for the denominators in a hospital-based sample of births. ECLAMC can be defined as a continental network of persons interested in research and prevention of birth defects. ***History and Evolution:*** From the institutional point of view, ECLAMC has had headquarters in diverse centers of Argentina and Brazil, but always as an independent research project, without a defined administrative link. ECLAMC began operating in 1967, as an investigation limited to the city of Buenos Aires, Argentina, and it gradually expanded until covering all the 10 countries of South America as well as Costa Rica and the Dominican Republic. Even though ECLAMC has maintained essentially the same original experimental design since 1967, due to the data accumulated by the program, the increasing experience as well as the development in science, technical modifications occurred including a DNA bank and a fully informatized data handling system. Since 1974 ECLAMC has been a founder member of the International Clearinghouse for Birth Defects Monitoring Systems; since 1994 a WHO Collaborating Center for the Prevention of Congenital Malformations, and since 2000 a collaborating member of the NIH Global Netwok for Women's and Children's Health Research. ***Methodology:*** The maternity hospital network of ECLAMC examines around 200,000 births per year. All major and minor anomalies diagnosed at birth in infants weighing 500 g or more are registered according to a manual of procedures. The next non-malformed baby of the same sex born in the same hospital is selected as a

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control subject for each case. Thus, a one-to-one healthy control group matched by sex, time and place of birth is obtained. As a system of epidemic surveillance, ECLAMC systematically observes the fluctuations in the frequencies of different malformations and, in the case of an alarm for a probable epidemic of a given malformation, at a given moment, and given area, it acts to identify its cause. As termination of pregnancy has severe legal restrictions in South America, prevention of birth defects should concentrate on primary, preconceptional and tertiary measures. Tertiary measures aim to avoid complications of the affected patients from the medical, psychological, and social standpoints.

Definition and Philosophy

ECLAMC is the Spanish acronym for 'Estudio Colaborativo Latino Americano de Malformaciones Congénitas' (Latin-American Collaborative Study of Congenital Malformations) [1]. ECLAMC is a program for the clinical and epidemiological investigation of risk factors in the etiology of congenital anomalies in Latin-American hospitals, using a case-control methodological approach. Since more than half of malformations have an unknown cause [2], the main objective and the strategy of ECLAMC are that of prevention through research. Further information can be obtained at: www.eclamcnet.net.

ECLAMC is a voluntary agreement among professionals dedicated to the study of congenital anomalies in Latin-America. The spirit of collaboration, guided by a set of operational regulations contained in a manual of operations, ensures the uniformity of approaches, necessary for the comparability of the data registered in the different hospitals. Being an agreement among people, ECLAMC lacks both institutional base and designated budgets. Its voluntary basis and continuous process of quality control guarantee the quality of the information.

In contrast to most developed countries, ECLAMC cannot count on reliable baseline information about the populations under investigation in South America. Neither demographic data nor hospital records are usually available. Therefore, ECLAMC is forced to collect all the information required, making their report forms and file data system highly complex.

Therefore, ECLAMC can be defined as a continental network of persons interested in research and prevention of birth defects.

History

Institutional Evolution

From the institutional point of view, ECLAMC has had headquarters in diverse centers of Argentina and Brazil, but always as an independent research project, without a defined administrative link. Since 2000, in order to enlarge the use of available resources, and facilitated by the available electronic ways of communication, the coordination of ECLAMC has decentralized, maintaining two offices in Brazil, at the Departments of Genetics of the Oswaldo Cruz Institute (Ministry of Health) and of the Federal University of Rio de Janeiro, and two offices in Argentina, at the Multidisciplinary Institute of Cellular Biology and at the 'Centro de Estudios Médicos e Investigaciones Clínicas' (Center of Medical Studies and Clinical Investigations). Further details are available at www.eclamcnet.net.

Regional Evolution

ECLAMC began operating in 1967, as an investigation limited to the city of Buenos Aires, Argentina. Two years later, the program had already extended to hospitals in several cities of Argentina, Chile and Uruguay. During the first annual meeting of the program, in December of 1969, the name ECLAMC was chosen for the present and the future. In 1973, ECLAMC was extended to seven Latin-American countries: Brazil, Ecuador, Peru and Venezuela besides the three already mentioned, and in 1990 to all the ten countries of South America as well as Costa Rica and the Dominican Republic (fig. 1).

Functional Evolution

Even though ECLAMC has essentially maintained the same original experimental design since 1967, due to the data accumulation by the program, the increasing experience as well as the development in science, some modifications were introduced, their main landmarks being:

- 1967 (July 1): Initiation of the Malformation Registry in Buenos Aires
- 1969: Election of 'ECLAMC' as the name of the group
- 1970: Approval of the current manual of operation in a plenary session
- 1977: Start of the quarterly epidemiology surveillance
- 1978: Inclusion of stillborns ≥ 500 g
- 1982: First personal computer – replacement of the large-frame computer by personal computers
- 1982: Increase in birth coverage from 50,000 to 200,000 births annually

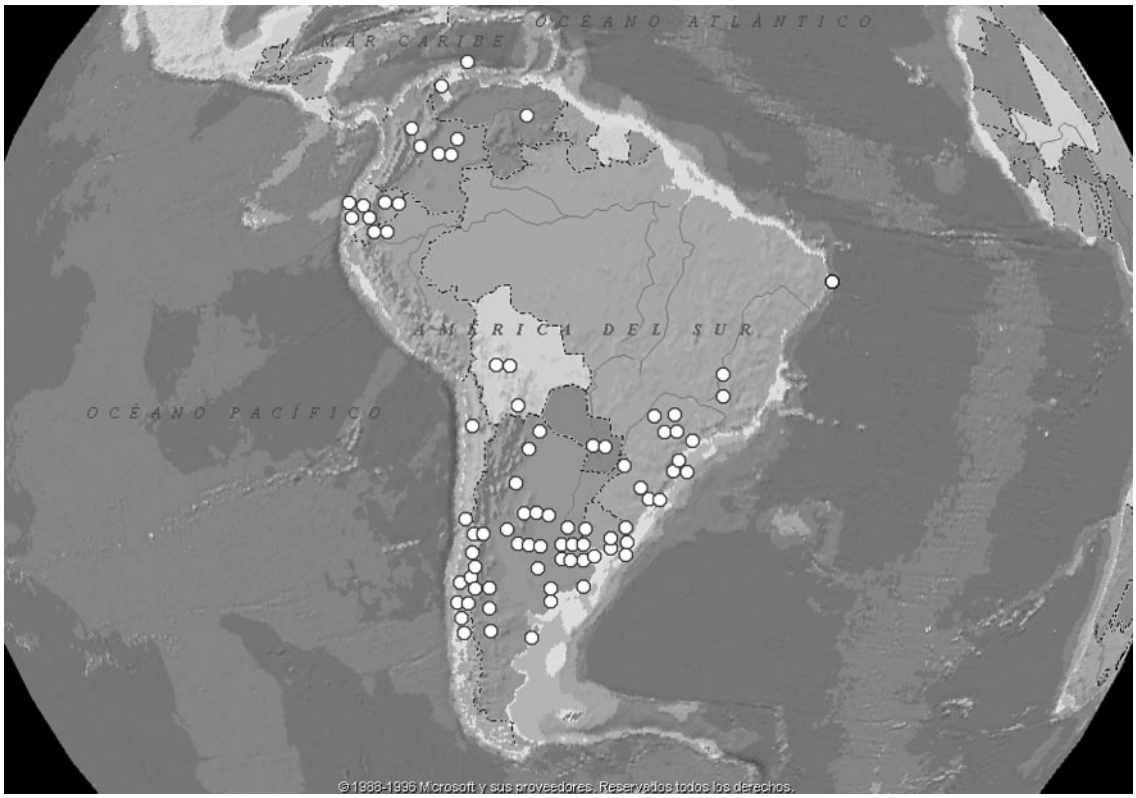

Fig. 1. ECLAMC hospital network in South America.

- 1994: Obligations in health actions for primary prevention
- 1997: Initiation of a bank of DNA and cells: Moleclamc
- 1997: Advancement of electronic data transmission: Cybereclamc
- 1998: Blood collection for oral clefts only
- 1999: Meeting of the DNA and Cell Bank Steering Committee in Buenos Aires
- 2000: Decentralization of the coordination: four institutions in two countries
- 2000: First US funding of the Argentinean (Conicet) and Brazilian (CNPq) funds sustaining ECLAMC since its inception
- 2000: Blood collection of all major congenital anomalies (MOD funded)
- 2001: Start of the ORIENT project for community outreach
- 2002: Start of the tertiary prevention intervention project (Global-NIH funds)
- 2003: Handheld electronic data collection; paper forms being abandoned

International Recognition

- 1974: Founder member of the International Clearinghouse for Birth Defects Monitoring Systems (ICBDMS)
- 1994: Designated Collaborating Center for the Prevention of Congenital Malformations by the WHO-Human Genetic Program
- 2000: Collaborating member of the Global Network for Women's and Children's Health Research; Site 04: Birth Defects Treatment and Prevention Program, through sub-contract with the University of Iowa (Principal Investigator: Prof. Jeffrey Murray).

Organization and Funding

Due to the strategy of prevention through research adopted in ECLAMC as well as the lower impact of congenital anomalies on health indicators in the developing than in the developed countries (see Research), ECLAMC had been usually funded by research-funding agencies rather than public health ministries. The National Re-

search Councils of Argentina and Brazil have been the main sources of support during the 36 years of existence of ECLAMC. Other current sources of support are the March of Dimes, the National Institutes of Health and the Centers for Disease Control. Research projects developed using ECLAMC data and ECLAMC hospital-net expertise are supported, but not ECLAMC as such. International organizations as the WHO and its regional branch, PAHO (Promoting Health in the Americas), only occasionally provided assistance, mainly in the form of temporary advisers.

Methodology

Hospital Based: Hospital Network

The maternity hospital network of ECLAMC examines between 150,000 and 200,000 births per year. All major and minor anomalies diagnosed at birth in infants weighting 500 g or more are registered according to a manual of procedures.

All detected congenital anomalies are described according to preestablished protocols and documented with photos and radiographs whenever possible. Each malformation is coded at the central level using a standard 6-digit code. For infants with more than one malformation, all diagnosed anomalies are coded and later on classified as isolated or associated.

Case-Control Studies

The next non-malformed baby of the same sex born in the same hospital is selected as a control subject for each case. Thus, a one-to-one healthy control group matched by sex, time and place of birth is obtained.

The same reporting form and definitions are used for cases and controls concerning 50 variables of possible risk factors. They include sex, twinning, parental ages, parity, prenatal vaginal bleeding, maternal illnesses and drug intake, other parental exposures, parental educational and occupational levels, parental subfertility, race, place of birth and family history including parental consanguinity and familial occurrence and/or recurrence of congenital anomalies. Gestational length, fetal presentation, birth weight, and perinatal survival data are collected to improve phenotype definition of specific defects. Information is directly collected from the mother by a trained pediatrician during the puerperium. A pedigree tree is depicted in patients with a positive family history for parental consanguinity or congenital defects in any family member.

Data Base

The collected information from the malformed newborn infants, healthy matched controls and total births examined is stored in DBE (Data Base ECLAMC), a simple dbase file system composed of a series of relational files, manageable by means of Fox-Pro. It is exactly the same for the paper as for the handheld operational modes. The speed in data transmission and handling increases substantially in the handheld model because manual data organization is automated, and manual coding of congenital anomalies and reported pedigrees is reduced to only 20% of the total burden (fig. 2).

Record Layout and Variables

In table 1, the record layout lists all the 80 variables available for cases and controls. Most of these 80 variables are self-explanatory. However, a few of them need to be explained and/or justified.

Hospital. The first of the 3 digits identifying the hospital indicates the country: 1 = Uruguay; 2 = Chile; 3–9 = Argentina; A = Brazil; B = Bolivia; C = Peru; D = Paraguay; E = Ecuador; F = Venezuela; G = Colombia; H = Costa Rica; J = Dominican Republic.

Difficulty to Conceive. This is a direct question intended to indicate subfertility. Two alternatives: yes and no. The latter is further subdivided in: any studies performed (yes, no), and any treatments performed (yes, no).

Malformation, Complex, Polymalformed. All anomalies are verbatim described. Each *malformation* is coded according to a 6-digit coding system developed by ECLAMC, but easily convertible by program to ICD-X-BPA codes. Even though the record layout has capacity for up to eight different anomalies, there is no maximum limit because cases with more than eight anomalies described and coded are entered into a second record. Anomalies that are interrelated identify a *complex* (e.g. sequence, association and spectrum). Up to three complexes can be coded for a given case. When all the anomalies of a given case fit into one complex, it is placed in the Polymalformed field. The *Polymalformed* field includes the diagnosis that explains the whole case. It can be a syndrome, a complex, or an unknown multiply-malformed entity. A case having micrognathia, glossoptosis, cleft palate, esophageal atresia, rocker-bottom feet, clenched fingers, and a 47,XY +18 karyotype, for example, will have codes for six Malformations, one Complex (Pierre Robin), and one Polymalformed field (trisomy 18).

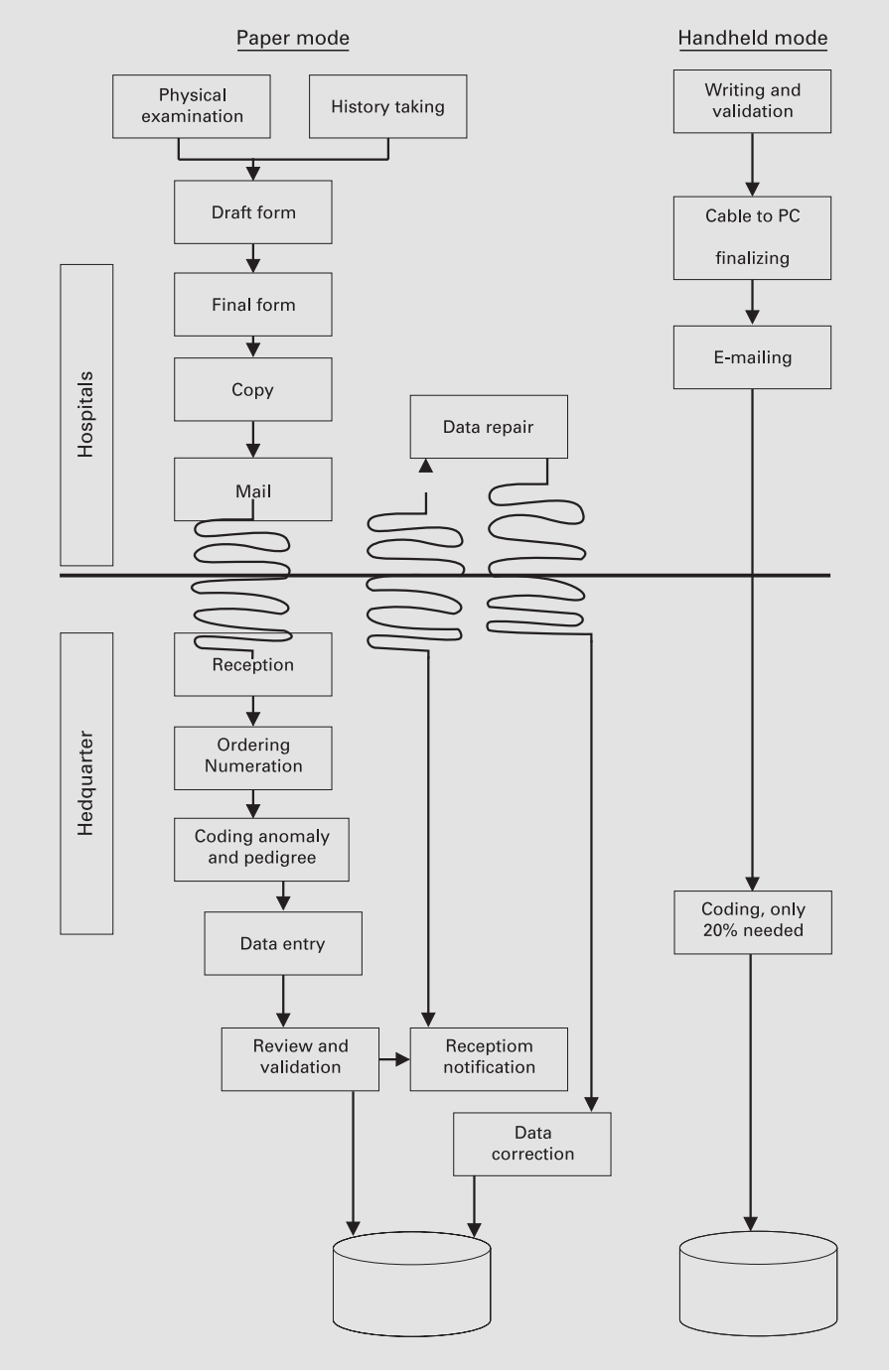

Fig. 2. Data flow through two systems: paper and electronic modes.

Gestational Age, by Dates. It is the interval between the last menstrual period and the birth date (in days).

Gestational Age, Clinical. According to one of several methods common in South America, there are three alternatives: SGA equals small, AGE adequate and LGE large for gestational age by dates.

Ethnic Descent/Background. Eight different ethnic groups are checked, which produce 256 possible combinations. The eight groups are: 1 = Latin European; 2 = Non-Latin European; 3 = Jews; 4 = Native (Amerindian plus Latin European); 5 = African black; 6 = Arab; 7 = Oriental; 8 = other.

Table 1. ECLAMC record layout for cases and controls

Area	Variable	Values	Bytes
ID code	Hospital	ECLAMC code	3
	Case	3 digit consecutive	3
	Birth year	yy	2
Birth date	Birth month	mm	2
	Birth day	dd	2
Delivery	Fetal presentation	Cephalic, podalic, transverse	1
	Delivery type	Normal, forceps, cesarean	1
	Birth weight	g	4
	Gestational order	n	2
	Status	Liveborn, stillborn	1
	Sex	Male, female, intersex	1
	Twinning	No, yes	1
	Hospital discharge	No, yes	1
	Age at discharge	Days	3
	Age at diagnosis	Days	3
	Last menstrual period	dd mm yy	6
	ABO Rh – newborn	ECLAMC codes	1
	ABO Rh – mother	ECLAMC codes	1
	ABO Rh – father	ECLAMC codes	1
	Umbilical cord	Knots, twists, other	1
	Placenta weight	g	3
	Placenta characteristics	Normal, abnormal	1
	Difficulty to conceive	No, yes	1
Malformations	Diagnostic evidences	Clinical, X-ray, surgical, etc.	1
	Malformation – 1	6 digit ECLAMC code	6
	Malformation – 2	6 digit ECLAMC code	6
	Malformation – 3	6 digit ECLAMC code	6
	Malformation – 4	6 digit ECLAMC code	6
	Malformation – 5	6 digit ECLAMC code	6
	Malformation – 6	6 digit ECLAMC code	6
	Malformation – 7	6 digit ECLAMC code	6
	Malformation – 8	6 digit ECLAMC code	6
	Complex – 1	3 digit ECLAMC code	3
	Complex – 2	3 digit ECLAMC code	3
	Complex – 3	3 digit ECLAMC code	3
	Polymalformed	5 digit ECLAMC code	5
	Documents	Photo, X-ray, etc.	1
Twin	Birth order	1, 2	1
	Sex	Male, female, intersex	1
	Status	Liveborn, stillborn, abortion	1
	Fetal presentation	Cephalic, podalic, transverse	1
	Delivery type	Normal, forceps, cesarean	1
	Umbilical cord vessels	Number: 3, 2, other	1
	Birth weight	g	4
Prenatal care	Prenatal care	No, yes	1
	Prenatal visits	n	1
	From – to	Weeks of gestation	2
	Prenatal visits – Place	This hospital, other place	1
	Ultrasonography	No, yes	1
	Last ultrasonography date	dd mm yy	6
	Ultrasonography	n	1
	Gestational age, by dates	Days	3
	Gestational age, clinical	Small, adequate, large	1

➤

Table 1 (continued)

Area	Variable	Values	Bytes
Prenatal history	Maternal illnesses	ICDX code	4
	Maternal vaccines	ECLAMC codes	1
	Radiation exposures	ECLAMC codes	1
	Drugs – medicaments	ATC codes	7
	Drugs – social	ECLAMC codes	1
	Maternal smoking	ECLAMC codes	1
	Maternal alcohol drinking	ECLAMC codes	1
	Vaginal bleeding	No, yes	1
Family history	Ethnic descent/background	260 combinations of 8 groups	3
Parents	Maternal residence	Municipality	1
	Marriage date	dd mm yy	2
	Maternal age	Years	2
	Paternal age	Years	2
	Maternal education, level	8 point score	1
	Paternal education, level	8 point score	1
	Maternal occupation, level	8 point score	1
	Paternal occupation, level	8 point score	1
	Maternal occupation, activity	WLO code	4
	Parental consanguinity	150 degrees and types	2
	Maternal birthplace	ECLAMC codes	9
	Paternal birthplace	ECLAMC codes	9
	Grandparents: country of birth	ECLAMC codes	8
Sibs	(for each sib)		0
	Product	Liveborn, stillborn, miscarriage	1
	Sex	Male, female, intersex	1
	Birth date	dd mm yy	6
	Same father	1 – yes, 2 – no	1
Malformed	(for each affected relative)		0
	Relation	No, yes	2
	Malformation	6 digit ECLAMC code	6
			216

ID = Identification; WLO = World Labor Organization International Codes for Occupations; ATC = Anatomical Therapeutic Chemical Classification.

Maternal/Paternal Education, Level. It indicates the sociocultural status. There are eight levels: 1 = illiterate; 2 = reads and writes, no school; 3 = incomplete grammar school; 4 = complete grammar school; 5 = incomplete high school; 6 = complete high school; 7 = incomplete university; 8 = complete university; 0 = unspecified.

Maternal/Paternal Occupation, Level. It indicates the sociocultural status: 1 = unemployed; 2 = housewife; 3 = unskilled labor; 4 = skilled labor; 5 = independent labor; 6 = manager; 7 = clerk (white collar); 8 = professional, university; 0 = unspecified.

Maternal Occupation, Activity. Indicates occupational risks or exposures. Described and coded according to the WLO (World Labor Organization International Codes for Occupations).

Same Father. Indicates full and half sibs on the maternal side. Yes or no.

Denominators

These data are provided monthly for each participating hospital. Information is obtained from the delivery room logbook, including the following variables: hospital, month and year. Independently for live births and stillbirths (stillbirths weighing ≥ 500 g), the births were listed in each of the following categories: sex – male, female, intersex, unspecified; weight – 500-gram categories; death

Table 2. ECLAMC birth prevalence rates for 59 congenital anomaly types in the 1982–2001 period (total births: 3,574,609 including 62,375 stillbirths)

ICDX	Congenital anomaly	n	Rate	ICDX	Congenital anomaly	n	Rate
Q79.2	Omphalocele	920	2.6	NEC	Polydactyly, other types	447	1.3
Q79.3	Gastroschisis	512	1.4	NEC	Syndactyly, 2–3 toes	682	1.9
Q00	Anencephaly	2,481	6.9	NEC	Syndactyly, other types	1,402	3.9
Q05	Spina bifida	2,892	8.1	Q71.0	LRD, amelia	74	0.2
Q03	Hydrocephalus	2,853	8.0	NEC	LRD, transverse terminal	932	2.6
Q01	Cephalocele	3,498	9.8	NEC	LRD, hypoplasia	355	1.0
Q02	Microcephaly	235	0.7	NEC	LRD, transverse intercallary	171	0.5
Q11	An-/microphthalmia	383	1.1	NEC	LRD, preaxial	433	1.2
Q16.0 Q17.2	An-/microtia	1,318	3.7	NEC	LRD, postaxial	142	0.4
NEC	CHD: conotruncal	1,358	3.8	NEC	LRD, axial	84	0.2
Q21	CHD: septal	3,498	9.8	NEC	LRD, other types	18	0.1
Q23.4	CHD: left heart hypoplasia	235	0.7	NEC	LRD, combined	89	0.2
Q25.0	CHD: PDA	383	1.1	Q65.5	Hip, subdislocation	5,331	14.9
NEC	CHD: other	1,318	3.7	Q65.2	Hip, true dislocation	474	1.3
Q24.9	CHD: unspecified type	1,358	3.8	Q74.3	Arthrogryphosis	659	1.8
Q35	Cleft palate	1,361	3.8	Q79.0	Diaphragmatic hernia	853	2.4
Q36 q37	Cleft lip ± palate	4,017	11.2	Q65.2	Abdominal muscular hypoplasia	273	0.8
Q39	Esophageal atresia	1,040	2.9	Q79.82	Pectoral muscular defect	208	0.6
Q41.0	Duodenal atresia	358	1.0	Q79.80	Constrictive band	289	0.8
Q41.9	Jejuno-ileal atresia	204	0.6	Q90	Down's syndrome	5,841	16.3
Q42.3	Anal atresia	1,556	4.4	Q89.4	Conjoined twins	92	0.3
Q43.3	Intestinal malrotation	143	0.4	Q87.03	Cyclopia	116	0.3
Q56	Ambiguous genitalia	696	1.9	Q89.80	Sirenomelia	76	0.2
Q54	Hypospadias	3,042	8.5	NEC	Acardiac acephalus	34	0.1
Q60	Renal agenesis	541	1.5	NEC	Syndrome, pathogenetic	4,003	11.2
Q61	Polycystic kidney	865	2.4	NEC	Syndrome, etiologic	2,757	7.7
Q62	Hydronephrosis	1,361	3.8	NEC	Multiply malformed	10,017	28.0
Q66.0	Pes equinovarus	5,875	16.4	NEC	Other defects	82,559	231.0
Q66.4	Pes talovalgus	2,352	6.6				
Q69.-2	Postaxial polydactyly	5,749	16.1		Total	99,336	277.9
Q69.-0	Preaxial polydactyly	966	2.7				

CHD = Congenital heart defect; PDA = patent ductus arteriosus; LRD = limb reduction defects.

before discharge (only for live births) – yes, no; malformed – yes, no; autopsies (only for stillborns and dead liveborns); maternal age – 5-year categories; gestational order – 1, 2, 3, 4, 5, 6, 7, 8 and 9+; twinning – yes, no, and twins – sex of twin pairs.

For the data available for all the births examined there is no need to compare cases with controls, and relative risks for those variables as risk factors can be estimated.

Available Data

Obviously, the amount and complexity of available data in the ECLAMC database are immense. In table 2, recorded birth prevalence rates for 59 congenital anomaly types in the period from 1982 to 2001 are presented. Birth prevalence rates were calculated from 3,574,609 total births (62,375 stillbirths included).

This illustrative table excludes the initial phase of ECLAMC from 1967 to 1981, because of the large differences in ascertainment during that pre-ultrasonography era, as well as because stillborns have begun being considered by ECLAMC since 1978, seriously affecting the recorded prevalence rates for highly lethal anomalies as anencephaly.

ECLAMC birth prevalence rates for most congenital anomaly types fit well into the normal interpopulation variation expected for a worldwide data set, as the one gathered by ICBDMS [3].

From Clinical Epidemiology to State-of-the-Art Technology

Even keeping its essence in philosophy, organization, and methodology, ECLAMC kept pace with scientific and technical evolutions in the last 3 decades. ECLAMC started as a clinical-epidemiological program, extending later on into molecular biology and electronic ways of data collection, transmission, storage, retrieval, and analysis.

Clinical Epidemiology

ECLAMC, a clinical-epidemiological research project initiated in 1967, maintains a complex data bank comprising more than 100,000 registered malformed newborns and an equal number of matched controls ascertained from about 5 million examined births occurring in selected maternity hospitals from different South American countries. Quality control of the collected information is routinely performed, and the accumulated data are highly reliable. Several joint investigations were performed in association with American and European registries [4–7]. The quality of any clinical-epidemiological program is necessarily limited by the accuracy of the clinical diagnoses. Within ECLAMC, their accuracy is ensured in three ways:

(a) The annual meeting of ECLAMC, which has taken place without interruption for the last 36 years, provides an important point of reference and exchange. A number of eminent clinical dysmorphologists regularly attend these meetings and participate in the discussion of individual case studies, which occupy 50% of the meeting time. These discussions provide important training for the less experienced physicians from participating hospitals.

(b) Over the past 36 years of the existence of ECLAMC, most cases for which there was a question concerning the diagnosis were photographed and X-rayed. Clinical descriptions and documentation are then forwarded to a Clinical Review Committee, which meets weekly to review all diagnoses. If this committee is still unable to resolve uncertainties, the cases are then placed in the private section of the ECLAMC web page, and opinions from other clinical geneticists are solicited.

(c) An integral part of the diagnostic procedures within ECLAMC involves the collection of family histories, and where possible and relevant, examination of affected relatives. Such information, which may reveal the existence of consanguineous unions, often provides additional checks of the accuracy of the diagnoses [8, 9].

Moleclamc; Cell and DNA Banking

As the new millennium approached, in 1997, ECLAMC assembled a steering advisory committee to design a strategy to evolve the epidemiological network for birth defects of ECLAMC into a molecular epidemiological surveillance system and cell bank. This committee first met in June 1999, under the auspices of the Rockefeller Foundation, as a team residency for 2 weeks at the Bellagio Study and Conference Center in Italy, with the specific aim to create a pan-Latin-American molecular epidemiological program to analyze birth defects.

The recommendations included the creation of a bank of biological material. The proposed creation of a cell and DNA bank of malformed newborn infants for the molecular study of congenital anomalies did not include the testing of a specific hypothesis. The proposal was founded on the proposition that the identification of genetic and environmental factors for a given specific defect is possible with presently available techniques in molecular biology and genetics. In addition, good clinical and epidemiological definitions of the defects permit their subdivision into less heterogeneous groups, thereby increasing the possibility of identifying candidate genes as well as environmental determining factors.

Blood collection in ECLAMC started with oral clefts in 1998, extended to holoprosencephaly in 1999 and to all major congenital anomalies in 2000. The first 2 years served as a feasibility study, as well as to adjust technical and laboratory routines.

Oral clefts were selected because of their common and conspicuous anomaly, for which the etiology remains largely unknown, mainly for the most common non-syndromic types [10], considered to be secondary to complex gene/gene or gene/environment interactions [11].

Holoprosencephaly is the more frequent defect in the brain and face, with a precise diagnosis, despite its clinical variability. The normal development of the brain is perturbed during a short period of time during gastrulation by teratogens or genetic factors, making the study of holoprosencephaly a model for the approach to the complex genetics of brain development [12]. These studies could serve as a paradigm for the study of other congenital defects with complex causes.

Molecular studies performed by ECLAMC had already produced 7 of the 29 papers ECLAMC has published since 2000 [13–19]. This start had been possible due to the fruitful collaboration with the laboratories led by Dr. Maximilian Muenke (NIH) and Prof. Jeffrey Murray (University of Iowa, USA).

A bank of DNA extracted from dried blood, following standards given by Loffredo and Ewing [20] in 1997, was created by ECLAMC, and put into action in January 1999 under the following specifications.

Bank-Specific Aims

- To collect, maintain and store biological material of relevance for research in the causes of birth defects.
- To inform the scientific community on the contents of the collections through updated catalogues.
- To act as an advisory center for the scientific community and to offer teaching and service facilities for the development of similar collections in other areas, especially in Latin-America.

The congenital anomaly case sample is selected from the newborn infants registered in ECLAMC-participating maternity hospitals, distributed in different South American countries.

Two types of biological material are stored at the ECLAMC Cell and DNA Bank, immortalized lymphoblastic cell lines, and DNA from dried blood spots and whole fresh blood.

Control Samples

For population control, random cord blood samples were collected from at least 100 anonymous consecutive births at each participating hospital, aiming to have at least 300 samples from each participating city. Therefore, when a single hospital in a given city was a member of the ECLAMC network, 300 consecutive newborn cord blood samples were collected.

Case Samples

All major congenital anomalies are selected for dry blood spot collection. As for cell immortalization, these criteria are reviewed every year by the Steering Committee. This broad definition provides 1 case in every 150 consecutive births, thus expecting about 650 cases per year.

These cases include the low-frequency neural tube defects [21], and the high-frequency microtia [22] at high altitudes; the high rate of hypospadias in subfertile couples [23] and the low frequency of esophageal atresia in Venezuela [7]. It will also comprise anomalies for which specific diagnoses or subtypes are available in the ECLAMC material: limb reduction defects [24] and syndactyly [25].

ECLAMC provides collaborating hospitals with IsoCode® filter papers, which are identified and dried before enveloping them. Those samples are sent monthly to the ECLAMC coordination together with the usual reporting forms. The study coordinator then sends the sample and the appropriate data to the Rio de Janeiro Center. One filter paper card is prepared per case with several blood drops per paper.

During its first 2 years of operation, the bank accumulated over 10,000 blood spot samples from consecutive normal control newborns, and samples from more than one thousand dyads of malformed newborn infants and their mothers. At the ECLAMC headquarters, blood samples are properly stored, and DNA extraction performed as required.

Cell Immortalization

The inclusion of immortalized cells to the bank did not start yet because it implies a substantial budget not available yet. Fresh blood for cell immortalization could be collected from a subset of the 30 best-equipped hospitals, capable to deliver the fresh blood samples to one of two central laboratories at the ECLAMC headquarters in Buenos Aires and Rio de Janeiro in less than 72 h. These 30 hospitals cover around 100,000 births yearly. Cell immortalization will be reserved for cases with a strong genetic component, based on knowledge or family history, while blood spots will be taken for other congenital anomaly types as described below. Thus, a genetic etiology will be the main selection criterion.

Blood Spots

The main advantage of blood spots in a hospital network as ECLAMC, which is scattered over 10 countries, is the facility for sending the material to the coordination center via mail. The main drawback is the small DNA yield. Several efforts are being taking to increase the DNA result, such as the use of the DOP-PCR (degenerate oligonucleotide primed-polymerase chain reaction) technique. Instructions such as: do not to surpass the filter paper absorption capacity, wait at least 4 h before enveloping to facilitate drying or keep the envelopes in a box with silica gel until extraction, are helpful to avoid DNA degradation and contamination.

Identifiers

Coded specimens and the disclosure of the codes are restricted to only three members of the ECLAMC coordinating group. Original reporting forms are kept under locked file cabinets. No identifying information, other than hospital, and month and year of birth are kept in the computer database. Thus, the laboratories receiving materials from the Bank are unable to identify the persons

from whom the blood samples were drawn. These rules were presented and accepted by the ethics committees of each of the participating hospitals, after being approved by the ECLAMC Ethics Committee. Special studies dealing with useful information on the family, as those studying monogenic diseases, provide individual results to the referring pediatricians.

Requested Information

It includes all the information available for each case at the ECLAMC database (Record Layout and Variables).

Cybereclamc

The widespread availability of personal computers and Internet connections even in backward areas of South America moved ECLAMC to develop and implement electronic ways for data handling.

Electronic Reporting Forms by Handheld Devices

A mobile system for data collection and transmission at the bedside started being developed for ECLAMC in 2000, and it was extended to 40 hospitals in 2003, helping to reduce operating costs. ECLAMC reporting personnel were provided with specially configured handheld devices to be used for data collection, and minimum specifications of hardware and software were provided.

Data collected by each research unit (hospital) are transmitted to the Data Center (Coordination) over the Internet using software provided by the Data Center. The data transfer software system currently provided by the Data Center uses BLAST DataPump® from BLAST (www.blast.com). BLAST DataPump runs under Microsoft Windows 2000/NT/XP/ME/98/95 workstation or server. (Other versions of BLAST are available for Unix, Linux, and VMS operating systems.)

The Data Center currently uses COMP/IP® software from Tactical Software (www.tactical-sw.com) to enable BLAST DataPump on a data center server to receive data over the Internet. COMP/IP software runs under Microsoft Windows 2000/NT/XP/ME/98/95 workstation or server.

Clinical Photographs

Electronic reporting of cases systematizes and in some way limits the description of the congenital anomalies present, which increases the need of suitable photographs to illustrate the clinical picture.

Participating members are provided with digital cameras, and a special e-mail address was created to receive those pictures. All pictures are identified by a series of 14 characters specifying hospital, birth date, patient initials, photograph number, dot and extension (e.g. jpg, gif, tif or cam).

Iconographic documentation, whether electronic or not, whether clinical pictures or X-rays, is reviewed weekly at a special clinical session by at least two trained dysmorphologists of the coordinating group. Interesting cases are included in the ECLAMC Quarterly Newsletter (Boletrim), and the top interesting cases, mainly undiagnosed malformation syndromes, are yearly presented and discussed at the annual ECLAMC meeting.

Webpage

A webpage for ECLAMC was created in April 1998.

An open sector contains valid information for the community, as well as for interested professionals. This public sector, presented in three languages – English, Portuguese and Spanish – includes the following pages: the history of ECLAMC; what is ECLAMC; a list of participants with addresses; a map of the hospital network of ECLAMC in South America; the curriculum vitae of ECLAMC; publications; links of interest and a list with more than 500 parent/patient organizations in South America.

A restricted sector, accessible with a password, limited to authorized professionals (mostly ECLAMC members), includes the instruments to be downloaded: programs, forms, manuals, as well as more specific and intimate information. Boletrims (Quarterly newsletter), private history of ECLAMC, journal trip (a bimonthly literature review on congenital anomalies), Leo Salgado's notes (short stories in some way connected to ECLAMC), a manual of operations and forms (to be downloaded), Minieclamcs (forthcoming regional meetings), Moleclamc (available DNA material in the bank), ongoing projects, RAE (Annual Meetings, proceedings from previous meetings, and announcement of the next meeting), ECLAMC tasks and surveillance. The last page includes three ECLAMC routines: monitoring, rumor and focus (see Surveillance).

Surveillance

The thalidomide disaster of the late 50s stimulated the creation of a series of monitoring programs around the world: in 1961 the Congenital Malformation Monitoring Program of the Czech Republic; in 1963, the Finnish Register of Congenital Malformations; in 1964, the National Congenital Anomaly System of England and Wales; in 1964, the Swedish Registry of Congenital Malforma-

tions and the Medical Birth Registry, and, in 1966, the Alberta Congenital Anomalies Surveillance System. The British Columbia Health Status Registry Congenital Anomalies Surveillance Program existed already since 1952. In the year 1967, three birth defect monitoring programs started: the Metropolitan Atlanta Congenital Defects Program, the Medical Birth Registry of Norway and ECLAMC [3]. Since 1974, the sharing and dissemination of information between these and other programs has been coordinated by ICBDMS, nowadays located in Rome [26].

Searching for Positive Alarms

ECLAMC, as a system of epidemic surveillance, systematically observes the fluctuations in the frequencies of different malformations and, in the case of an alarm for a probable epidemic of a given malformation, at a given moment, and given area, it acts to identify its cause. Quarterly analyses are produced and reports disseminated within the ECLAMC hospital network, as well as to other fellow surveillance programs through ICBDMS [26].

Searching for Negative Alarms

As a complex early alarm system, ECLAMC is capable to identify trends, time and geographic clusters, as well as sudden changes in the birth prevalence rates of all congenital anomaly types. ECLAMC is not only suitable to detect alarms for epidemics, but also decreases the frequency of a given anomaly, in a given place, for a given period. This fortunate situation was recently created in one South American country, Chile, where an essential food staple, wheat flour, has been fortified with folic acid since January 2000 [27].

Two years after the beginning of the fortification, preliminary data from ECLAMC were published, showing a significant reduction in the birth prevalence rate only for neural tube defects, among three selected congenital anomaly types, and only in Chile among five South American countries [28]. However, the assumption of a direct cause-effect relationship between the two events could be a flaw. For instance, the absence of historical data could not rule out that this is not a part of a preexisting decreasing trend. Likewise, within a hospital-based system as ECLAMC, entering and leaving the surveillance system by participating hospitals, which are the actual observational units, as well as selective hospital transfer of prenatally diagnosed neural tube defects, could mask or simulate changing trends over an observational period of several years. Thus a second study was performed 1 year later on births which occurred during 2 years after fortification, using a historical time series of 21 years and adjusting observations by hospital [29]. This second analysis proved the decrease in the frequency of neural tube defects was neither part of a preexisting decreasing trend nor random fluctuation, establishing a relationship to the fortification with folic acid.

This was possible because of the following characteristics of ECLAMC: (1) monitoring of birth defects has been continuous and uninterrupted in Chile since 1969, offering a long-term historical baseline; (2) the same monitoring has been performed in other South American countries with no folic acid fortification; thus comparative trends were available; (3) monitoring has not been restricted to neural tube defects, including all major and minor anomalies, and (4) expertise in monitoring birth defects has been well established since 1967.

Searching for Geographic Clusters

FOCUS is a project directed to the detection, evaluation, and registry of geographic clusters for congenital anomalies and genetic disorders.

A geographic cluster is easily defined in epidemiology by a prevalence rate higher than expected (as derived from comparable population data), for a given disease, in a population living in a defined geographic area, over a long period of time. However, for genetic diseases, this definition may be different, as the diseases in question usually are very rare, expected prevalence rates are commonly unknown, and because of asymptomatic heterozygotes for recessive conditions, and non-penetrants for dominants, what actually has to be considered is the frequency of genes and genotypes rather than that of phenotypes. As ECLAMC demonstrated for ataxia-telangiectasia in Aicuña and the Bloom syndrome in San-Luis-del-Palmar, clusters for autosomal recessive, uncommon, inconspicuous conditions may be defined with only one affected individual. For example in the rural and primitive Aicuña, affected homozygous ataxia-telangiectasia cases died undiagnosed from common respiratory infections within an area with a very high infant mortality rate. However, the availability of a long-standing pedigree enabled the identification of future births being at high risk to suffer from this disease, subjected to a community-based diagnostic program [30].

Among key geographic clusters of congenial anomalies, high frequencies of microtia in Quito (Ecuador) and Cordoba (Argentina), hypospadias in Brazil and cleft lip in La Paz (Bolivia) were never perceived before the actual counting of cases was carried out in South America by ECLAMC.

Evaluating Rumors

Definitions: Rumors, Alarms and Epidemics

'Rumor' is the systematic and scientific evaluation of any account of unusual occurrence of a given congenital anomaly or risk factor exposure. A lay person's anecdotal description of many cases of a given malformation in a given village is a rumor; a newspaper reporting dioxin contamination of the soil in a given area is a rumor.

Rumor is a birth defect surveillance methodology, employed for the early detection of new teratogens in the general population. As such, rumor is not a replacement but a complement to monitoring and other ways of surveillance.

ECLAMC, as most birth defect monitoring systems, only surveys a limited sample of the whole population, thus a new teratogen affecting an uncovered segment of the population will go unnoticed. Rumors compensate for this limitation by extending the coverage to the entire population. Rumors are very sensitive and poorly specific. They even precede a working hypothesis. However, rumors need to be answered in an objective and convincing manner, and must be evaluated step by step, with a minimal investment of time and resources in the beginning.

There are no specific definitions for rumors, alarms and epidemics because they are different levels of a single continuous scale of certainty. Nevertheless, a set of operational definitions could be advanced for practical, not epistemological, purposes. The underlying idea is that any level of suspicion must be systematically evaluated, and that the invested time and effort in the evaluation should be proportional to its reliability.

Rumors. Any kind of account, oral or written, about an unusual occurrence of a given congenital anomaly or risk factor exposure is a rumor. Rumors are groundless, and most of them will prove to be false.

Alarms. An unusual frequency for a given congenital anomaly in a given place and time is an alarm. Alarms are based on some kind of statistical testing, and most of them are due to random variations or to operational changes.

Epidemics. An epidemic is an unusual frequency, as described above, which has been proven to be real. Obviously, all conclusions about epidemics have started from a working hypothesis: either a rumor or an alarm.

Methods for Rumor Evaluation

For the systematic evaluation of rumors at ECLAMC, action is taken in five consecutive steps:

Case Listing: Data Collection. A reporting form is provided, containing the following column headings, each line corresponding to one case: identification, birthplace, birth date and the type of anomaly. This form can be completed by anybody, either health provider or lay person.

Case Listing: Analysis and Report. For observed prevalence rates, the above data are taken as numerator, while denominators, either birth series or inhabitants, are estimated from demographic data sources. Expected values are derived from the ECLAMC database. This step evaluates the rumor on statistical grounds. A report is produced for the rumor source. Over 90% of rumors prove not to be real. If not discarded, the next step is taken.

Site Visit and Data Collection. This is a short, 1- or 2-day site visit, and a site visit reporting form is filled up by a member of the ECLAMC network. Funds are available from the normal budget of ECLAMC for this action.

Research Project. A research project is developed and performed, according to the situation under investigation.

Research Project Conclusions. Research project conclusions and recommended measures are transmitted as soon as possible to the local health authorities and also published in a mainstream journal. Two examples of this activity developed at ECLAMC are those involving the ongoing adverse pregnancy outcomes due to thalidomide in South America [31], and the teratogenicity of misoprostol due to its inappropriate use as an abortifacient [32].

Other rumor-evaluating programs operate in California [33] and in New Zealand [34].

Research

It has been estimated that half of all birth defects are preventable [2], and 75% of them are of unknown etiology, as long as 'multifactorial' is not considered as a known cause [35]. Furthermore, in recent years it has become clear that most birth defects have a complex etiology, which is both genetic and environmental in nature. Thus, the genetic makeup of an individual may increase susceptibility to environmental insults resulting in birth defects. It is now well known, for example, that a deficiency in folic acid during pregnancy increases the likelihood of neural tube defects. However, mutations at loci coding for methionine synthetase and 5,10-methylene-tetrahydrofolate reductase may also increase the likelihood of neural tube defects. As much as 10% of the population may have significantly increased folic acid requirements because of

a mutation in the gene coding for 5,10-methylene-tetrahydrofolate reductase, which renders it less active [36].

These facts gave grounds to the concept of preventing congenital anomalies through research. Furthermore, in developing countries, where congenital anomalies are not a leading cause of infant morbidity and mortality, public health authorities are not much concerned with this subject. However, although research councils are usually based on the principle that public health should be mainly concerned with the present, research should include predictable future situations.

Community Outreach

As termination of pregnancy has severe legal restrictions in South America, prevention of birth defects should concentrate on primary, preconceptional and tertiary measures. The latter aims to avoid complications of the affected patients from the medical, psychological, and social standpoints. ECLAMC initiated extramural activities directed at the prevention of birth defects in 1994, and the following three projects fit into this scope.

Decalogue for Primary Prevention

ECLAMC developed and widely publicized a decalogue for the primary prevention of birth defects, which was disseminated through the following channels: a *manifest* aimed at public health authorities; a *book* aimed at health providers specialized in birth defects [37], and *a lecture*, placed on the web, aimed at health providers in general (http://www.pitt.edu/~super1/lecture/lec0212P/index.htm).

The decalogue centers on the avoidance of the following risk factors, and it is worded according to the target population: (1) unintended pregnancy, (2) advanced maternal age, (3) deficient prenatal controls, (4) rubella, (5) self-medication, (6) alcohol, (7) smoking, (8) malnutrition, (9) occupational risks and (10) poor health care.

ORIENT: Program of Parent Guidance

A correct neonatal guidance is an action of tertiary prevention of congenital anomalies which acts on affected persons avoiding the complications of the disease through adequate rehabilitation and correction. Its timing is mainly postnatal; avoiding complications, it has a high cost/benefit ratio. It requires moderate planning, and, compared with prenatal diagnosis and pregnancy termination, it is widely accepted by most societies. Treatment and rehabilitation fits into this level of prevention, reducing complications of congenital anomalies, since an early diagnosis and treatment increases the probability of a better survival, as well as better health and increased quality of life.

ORIENT, a project developed by ECLAMC, aimed at informing parents of a newborn with a congenital anomaly about the appropriate treatment specialists and centers in order to abridge the period of confusion and suffering that follows the knowledge of the baby' s problem. For this purpose, ECLAMC is providing health providers within the South American network of about 100 maternity hospitals, involving more than 300 pediatricians, obstetricians and medical geneticists, with a framework of general principles and tools, which can be adjusted to the characteristics and possibilities of each community.

The ORIENT strategy includes four integrated actions described in the following.

Early Information to Parents. Orientation guidelines, containing basic information about a given congenital anomaly type and the addresses of local recommended centers for treatment and/or rehabilitation, are produced by ECLAMC and distributed to pediatricians taking care of the newborn babies. Initially, four different orientation guides were produced, focusing on the following congenital anomaly types: congenital anomalies in general, spina bifida, oral clefts and Down's syndrome.

Identification of Excellence Centers. ECLAMC participants identify the excellence centers for treatment and rehabilitation of each congenital anomaly type in the area, and their addresses and phone numbers are manually added to the brochure. A close, personal communication with those centers is also established.

Patient Transfer. ECLAMC pediatricians and the perinatology team are asked to develop the best possible channels for patient transfer, including social workers, voluntary personnel and available support organizations to accompany the parents of an affected newborn baby to the appropriate treatment center.

Integrating Maternity Hospitals and Support Organizations. A directory including more than 500 patient/parent organizations in South America was produced by ECLAMC and placed on its website for the general population (www.eclamcnet.net; available in English, Spanish and Portuguese).

Tertiary Prevention by Systematic Pediatric Care

Craniofacial anomalies and cleft lip with or without cleft palate (CL/P) can serve as a model for the impact of birth defects on fetal and neonatal health directly and on maternal health indirectly. Clefts are a common form of

birth defects ranging in prevalence from 1 in 500 in most Asian populations through 1 in 1,000 in most Caucasian/Hispanic/Latino populations to 1 in 2,500 in African populations. The etiology of CL/P is complex. Hundreds of individually rare syndromes cause approximately 30% of orofacial clefting. Seventy-five percent of orofacial CL/P cases are non-syndromic (NSCLP) with no associated structural or cognitive anomalies. Current evidence suggests that these non-syndromic forms are secondary to a combination of genetic and environmental etiologies. Researchers are just beginning to discover and understand the genetic etiologies of clefting. However, environmental contributions to the cause of clefting are clearer. Maternal smoking and alcohol exposure contribute to an increased frequency of clefts, and evidence also supports a role for maternal malnutrition, in particular folate and B_6 deficiency. Because clefts are usually easy to identify it is possible to obtain occurrence and recurrence risks from population-based surveys and to recruit individuals and families for prevention studies. Similarly, because clefts are relatively common and require long-term multispecialty involvement for their care even in underdeveloped countries, families who have children with clefts may have repeated medical contacts. Even when not enrolled formally in care programs, untreated infants and children are readily recognized in home communities, and their ascertainment for studies of prevention and recurrence is made easy by their overt physical appearance.

In this Tertiary Prevention by Systematic Pediatric Care study we use CL/P as a model sentinel birth defect to study the impact of birth defects in general on maternal, fetal, and neonatal health. Systematic pediatric intervention over the 1st month of life will be used in an attempt to reduce the 28-day mortality rate. Systematic pediatric intervention over 2 years will be used to improve neurodevelopment and growth in children with NSCLP. The aims of this study will not only be to gather direct knowledge of ways to minimize the burden of CLP, but also a determination of methodologies to minimize the burden of birth defects in general. Birth defects in developed countries now make up the single most important contributor to neonatal morbidity and mortality. As developing countries attempt to diminish the burden of other causes of maternal and infant mortality, e.g. malnutrition and infection, birth defects will play an increasingly important role in infant mortality.

The first goal is to determine if there is a significant decrease in neonatal mortality in children born with CL/P or CPO (cleft palate only) following a high intensity program of pediatric care in the 1st month of life. The second objective is to measure the impact of having a child born with NSCLP on subsequent infant/family health over 2 years and to reduce that impact by intervention using a systematic pediatric care strategy.

Uniqueness

The Region

For disorders of complex etiology such as the birth defects, South America offers four main unique characteristics that favor the study of gene-environment interactions: environmental diversity, ethnic diversity, population structure and a high rate of environmental exposures.

Environmental Diversity

The physical environments in Latin-America are among the most diverse found on the planet. They range from the tropical (the Amazon regions of Bolivia, Brazil, Colombia, Ecuador and Peru) to the temperate (the pampas region of Argentina, Brazil, and Uruguay) to the Antarctic (Tierra del Fuego in Argentina and Chile). Some of the most arid zones of the world lie in Latin-America. Finally, it should be pointed out that three capital cities in Latin-America lie 7, 000 feet (2,000 m) above sea level. La Paz, Bolivia, at 11,910 feet (3,630 meters) above sea level, Quito at 9,200 feet (2,800 meters) and Bogotá at 7,870 feet (2,400 meters) offer large population samples covered by the ECLAMC program. No other continent has such large population concentrations at such altitudes. (Himalayan populations are primarily organized into small rural groups.) Recently, an increased prevalence of ear and other branchial arch derivatives and a reduced prevalence of neural tube defects associated with altitude has been reported by ECLAMC [21].

Ethnic Diversity

As well as being very diverse environmentally, the population of Latin-America is also very diverse ethnically. Against the background of Amerindian genes there has been an inflow of European and African genes that started with the conquest of America and has continued until the present. The predominance of genes of different ethnic origin varies sharply from region to region in Latin-America. It is well known that the variation in the prevalence of certain genetic diseases varies according to the ethnic background. Thus, for example a form of cystic fibrosis associated with stunted growth and produced by a native-American-specific mutation has been reported in Pueblo

and Navajo tribes [38]. Conversely, myotonic dystrophy and fragile-X syndrome are almost nonexistent in Amerindians probably due to low basal levels of repetition and peculiarities of structure in the trinucleotide domains responsible for these diseases [39, 40]. These examples illustrate the correlation between ethnicity and prevalence of genetic anomalies. Possibly, the same correlation between population gene structure and congenital malformations also exists.

Population Structure

A large proportion of the population of Latin-America is distributed in small, rural, semi-isolated communities with little immigration and, until the last 50–100 years, little emigration. Consequently, these communities are inbred, often to quite high levels [41], and, as a result, often have elevated frequencies of rare recessive diseases. Religious isolates such as the Amish in North America have been important in the analysis of rare genetic diseases for the same reason [42]. In comparable outbred communities, such genetic diseases occur only sporadically and at frequencies which are usually too low to allow for their study.

Exposure to Teratogenic Agents

It is an unfortunate fact that exposure to teratogenic agents is higher in many communities in Latin-America than in other parts of the world. Legislation protecting workers in basic manufacturing industries continues to be significantly more lax than in other countries, resulting in exposure to noxious chemicals, that are either ingredients or by-products of manufacturing processes, at levels that are considered hazardous to human health in other countries. Furthermore, environmental protection laws are also more lax or, even worse, frequently contravened, with seeming impunity. It suffices to mention the town of Cubatão, in the outskirts of Sao Paulo, Brazil, which has been described as the one of the most-polluted communities in the world. Inhabitants of Cubatão have a greatly increased incidence of health problems, and a significantly higher incidence of child mortality compared to similar less-polluted communities [43].

The Design

ECLAMC is unique amongst the other monitoring programs in two main aspects. (i) It monitors birth defects throughout the entire South America, while the few other programs in the area are limited to one country. (ii) Participation in the program is voluntary, and participating hospitals and pediatricians are considered to be all co-investigators and co-directors of the program, with equal access to the data gathered. This egalitarian model of organization is undoubtedly responsible for its continued existence and success in a continent where, over the last 30 years, the political climate has been notoriously unstable, experiencing terrorism, military dictatorships, civil wars, political persecutions, and near-government bankruptcies. It is hardly surprising that in this climate, funding for scientific research and public health has been unpredictable and evanescent.

The most outstanding features of ECLAMC are the following.

Long-Standing Surveillance. ECLAMC, recording congenital anomalies with the same ascertainment methodology for over 36 year, is able to study long-range secular trends. Very few surveillance systems are as old as ECLAMC, usually those conducted in Europe, e.g. Sweden, Norway and the Czech Republic [3]. This attribute facilitated the study of trends in neural tube defects [44] capable of masking the effect of folic acid supplementation [28], of limb reduction anomalies [24] to identify thalidomide syndrome epidemics [31] or of gastroschisis to disclose the still unsolved puzzle of its worldwide rising trend [unpublished].

Verbatim Description. Congenital anomalies, as many as are observed in a given infant, are verbatim described following standardized rules. Simple naming of an anomaly, as gastroschisis, phocomelia, or myelomeningocele, are not accepted by ECLAMC, and data validation is requested. This permitted to study actual microtia, instead of ear anomalies, mixing together different anomalies as ear pinnae deformation and malformation [22], and specific types of limb reduction defects, even of complex types [24]. Detailed descriptions did also enable the study of rare syndromes, as achondrogenesis-II with polydactyly [45], or the acrocallosal syndrome [46], as well as the definition and delineation of congenital anomaly associations, such as VATER [47].

Consideration of Minor Anomalies. The inclusion of minor as well as major anomalies enabled ECLAMC to study the previously unknown epidemiology of subtle anomalies, as healed cleft lip [48], the etiopathogenetic relationship between microtia and pre-auricular tags [22], and of very minor defects as pigmented naevi [49, 50]. Interestingly, the two papers published over 20 years ago on the epidemiology of a birthmark as pigmented naevus are the ones with the highest impact on the whole scientific production of ECLAMC [51].

Parental Consanguinity. For each malformed and healthy control newborn infant, a complete pedigree is

drawn including first- and second-degree relatives, plus grandparents. This permits to analyze precise types and degrees of parental consanguinity, resulting in several publications [8, 9, 52]. One of them identified Brazil and Venezuela as being the most inbred countries in South America. However, while the largest contributor to inbreeding in Brazil is close consanguinity, in Venezuela inbreeding is mainly based on remote consanguinity [8].

Family Occurrence. As the proband in ECLAMC is by definition a newborn infant, the presence of other affected relatives should be considered as familial pre-occurrence, rather than as recurrence. This information, as that of parental consanguinity, is derived from the above-mentioned detailed pedigrees, and it has led to several publications on complex mechanisms of inheritance in congenital anomalies. They include paternal imprinting for postaxial polydactyly [53], complex segregation analysis of cleft lip and palate [54], previously unrecognized male-to-male transmission of supernumerary nipples [55], lack of evidence for a major gene producing postaxial polydactyly [56] and evidence for a major gene producing cleft palate [19]. A more general paper on the heritability of malformations was also produced [57].

Ethnic Extraction. Races in South America are not as stratified as in the USA and Europe. Most South American populations are di- or tri-hybrids, involving Latin-European Caucasians, Amerindians and African blacks in different degrees of admixture. For this reason, neither race nor ethnicity can be taken as an attribute for the individual person. However, race is an important genetic variable in the study of congenital anomalies, and ECLAMC decided to register the ethnic groups recognizable in the ancestry of the proband (see Record Layout and Variables). In several studies, the complexity of ethnicity was determined, and it contributed to the investigation of causality. Thus, Non-Latin-European descent was shown to be associated with second-/third-toe syndactyly [25], ethnicity was proved not to be an etiological factor, but a confounder, for Down's syndrome [58], and Amerindian descent was proven by molecular studies of the Y chromosome to be related to cleft palate [16].

Conclusions

Strengths and Pitfalls

The unique features of the ECLAMC network which provide its strength, namely its multinational nature and its voluntary, cooperative organization, also create challenges which are not faced by other programs. Although the skills of physicians and facilities in hospitals in large metropoles such as São Paulo and Santiago (Chile) are excellent and compare well to similar ones found in the USA and Western Europe, the same cannot be said for those in smaller cities and other countries in Latin-America. A challenge which faces us is how to incorporate this quite heterogeneous network into a multinational molecular epidemiological program. Ten different countries are involved, and the cooperation of the public health agencies in the different countries, states and provinces are required.

We have to deal with the following issues. (i) A sensitive political issue may involve the routine transference of biological material to be banked outside most countries. (ii) There also are differences in the ethical and legal aspects of biological material collection (e.g. informed consent rules), and its use for research purposes. We endeavor to follow international guidelines. (iii) We have to adapt the training and scientific procedures to the tasks of a heterogeneous network. An additional problem is that in some areas, the first language of these workers is neither Spanish nor Portuguese. The generation of training manuals and scientific protocols in Spanish and Portuguese will be relatively straightforward given the expertise of members of the team. However, we would need to evaluate the necessity of manuals in such languages as Quechua. (iv) Costs and reliability of transportation from the more isolated hospitals and clinical centers may influence the organization of the network.

As it was revealed by the monitoring of folic acid fortification in Chile (see Searching for Negative Alarms), the main epidemiological pitfall of ECLAMC is its hospital-based mode of ascertainment of birth defects. The selective transfer of pregnancies with prenatally conspicuous birth defects to high complexity hospitals, which are more likely to participate in a voluntary research project as ECLAMC, may lead to bias of ascertainment.

An Exportable Model

Because of its proven efficiency and sustainability, ECLAMC can be taken as a model, feasible for other parts of the world, particularly the so-called developing world. At least six birth defect monitoring systems have been patterned after ECLAMC, all of them are active members of the ICBDMS:

(1) *ECEMC:* 'Estudio Colaborativo Español de Malformaciones Congénitas', Spain (Spanish Collaborative Study of Congenital Malformations), hospital based, created in 1976.

(2) *RYVEMCE:* 'Registro y Vigilancia Epidemiológica de Malformaciones Congénitas Externas' (Mexican Registry and Epidemiological Surveillance of External Congenital Malformations), Mexico, hospital based, created in 1978.

(3) *RECUMAC:* 'Registro Cubano de Malformaciones Congénitas' (Cuban Registry of Congenital Malformations), Cuba, population based, created in 1985.

(4) *REDOMAC:* 'Registro Dominicano de Malformaciones Congénitas' (Dominican Registry of Congenital Malformations), Dominican Republic, hospital based, created in 1988.

(5) *CREC:* 'Centro de Registro de Enfermedades Congénitas' (Congenital Diseases Registry Center), Costa Rica, population based, created in 1987.

(6) *RRMC-SSM:* 'Registro Regional de Malformaciones Congénitas del Maule' (Maule Regional Registry of Congenital Malformations), Chile, population based, created in 2001.

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