Epidemiology and genetics of microtia-anotia: a registry based study on over one million births

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Abstract

The epidemiology and genetics of microtia-anotia (M-A) were studied using data collected from the Italian Multicentre Birth Defects Registry (IPIMC) from 1983 to 1992. Among 1 173 794 births, we identified 172 with M-A, a rate of 1·46/10 000; 38 infants (22·1%) had anotia. Of the 172 infants, 114 (66·2%) had an isolated defect, 48 (27·9%) were multiformed infants (MMI) with M-A, and 10 (5·8%) had a well defined syndrome. The frequency of bilateral defects among non-syndromic cases was 12% compared to 50% of syndromic cases ($p = 0·007$). Among the MMI only holoprosencephaly was preferentially associated with M-A (four cases observed *v* 0·7 expected, $p = 0·005$). No significant variations were identified in the prevalence of non-syndromic cases by geographical area (range 0·62–2·37/10 000 births) or by five month time periods (range 0·21–2·58/10 000 births), nor was there evidence of time trends. When M-A cases were compared to controls, we found that mothers with parity 1 had a higher risk of giving birth to an MMI with M-A, and that mothers with chronic maternal insulin dependent diabetes were at significantly higher risk for having a child with M-A. MMI with M-A had higher rates of prematurity, low birth weight, reduced intrauterine growth, and neonatal mortality than infants with isolated M-A and controls. Babies with isolated M-A had, on average, a lower birth weight than controls; the difference was higher for females. The analysis of pedigrees and familial cases suggests an autosomal dominant trait with variable expression and incomplete penetrance in a proportion of cases, or a multifactorial aetiology. Three cases had consanguineous parents, but the absence of M-A among previous sibs does not support autosomal recessive inheritance.

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Microtia and anotia (M-A) are malformations of the auricle ranging from a measurably small external ear with minimal structural abnormality, to an ear with major structural alteration, to total absence of the ear.[1]

M-A can occur either as an isolated defect or in association with other defects. Only in a minority of cases has a genetic or environmental cause for M-A been found; in these cases M-A is usually part of a specific pattern of multiple congenital anomalies. For instance, M-A is an essential component of isotretinoin embryopathy, an important manifestation of thalidomide embryopathy, and can be also part of the prenatal alcohol syndrome and maternal diabetes embryopathy. M-A has also been reported in a number of single gene disorders, such as Treacher Collins syndrome, or chromosomal syndromes (for example, trisomy 18).

Even when the cause is unknown, M-A has been described in seemingly non-random patterns of multiple defects, such as the oculo-auricolovertebral phenotype (OAV), whose aetiology and pathogenesis, presumably heterogeneous, have yet to be elucidated, and in association with either cervical spine fusion (not necessarily part of the OAV phenotype) or renal malformations.

The epidemiology of M-A has not been adequately studied, with most studies reporting little more than the prevalence at birth,[1–4] or giving data for all external ear defects.[5] Only one study, to our knowledge, has given a complete picture of the classical epidemiological features of M-A as the frequency distribution by time, place, and affected subjects.[6]

We have used the Italian Multicentre Birth Defects Registry (IPMC) data collected on over one million births to describe the epidemiological and genetic features of M-A and to explore potential relationships between environmental, maternal, and infant characteristics and the occurrence of M-A as an isolated defect and in association with unrelated major malformations.

Subjects and methods

We ascertained cases with M-A through the IPIMC, a hospital based birth defects registry, active since 1978, that ascertains defects in the neonatal period. IPIMC monitors 110 000 births annually (20% of births in Italy), from over 100 hospitals located throughout the country. The registry and its methodology are described in detail elsewhere.[7 8] In short, all liveborns and stillborns with an anatomical defect diagnosed in the first days of life are registered. The notification form is completed by the paediatrician or neonatologist in charge of the registry in the hospital where the diagnosis is made and includes detailed demographic, clinical, and diagnostic data, and medical history, including exposure during the prenatal period (for example, drugs, smoking, alcohol, etc). The physician's report may include photographs, *x* rays, drawings, and clinical, laboratory, or pathology reports.

At the IPIMC coordination centre, each form is first reviewed by a paediatrician trained in dysmorphology and then transferred to a computerised file; the description of the defect is entered verbatim. For ease of retrieval, a three digit code is assigned to each defect or sequence. Sequences (pathogenetically related defects) are coded as one defect: examples include the combination of spina bifida plus hydrocephalus and/or limb deformities, and M-A with a combination of preauricular tags, meatal atresia, and ipsilateral mandibular hypoplasia. Multimalformed infants (MMI) are defined as infants presenting two or more major unrelated defects or sequences, not part of a well defined syndrome.

For the present study we reviewed all records of cases with M-A born from 1983 to 1992 among 1 173 794 consecutive births.

The following working classification was used.

Microtia type 1: small auricle with a straightforward abnormal shape, usually associated with meatal atresia (fig 1). This group includes only the severest cases of microtia type I of Marx's widely used classification.[19]

Microtia type 2: severe anomaly of the external ear with a more or less irregular longitudinal mass of cartilage (fig 2). This group includes microtia type II and III of Marx's classification.

Anotia: complete or almost complete absence of the external ear. It includes microtia type IV of Marx's classification.

Affected cases were further categorised as: (1) isolated (only M-A); (2) MMI; (3) syndromic when the cause, environmental or genetic, was identified. Infants with the OAV phenotype were included among the MMI since its aetiology is unknown, its clinical definition is unclear, and the phenotypic spectrum may include isolated M-A.

To identify defects that are associated specifically with M-A (non-random or preferential associations[10]), the observed number of occurrences was compared to the expected number using the observed to expected (O/E) ratio. To compute the expected number of occurrences the method suggested by Khoury *et al*[11] was used.

The temporal and spatial distribution of non-syndromic cases was analysed by five month periods and by 16 geographical places of birth, respectively. The five month periods and the 16 areas were chosen to define temporal or spatial units with nearly 50 000 births.

To study the associations with possible risk factors, all non-malformed infants notified to the registry with minor or trivial defects (for example, single palmar crease, epicanthus, small haemangioma), excluding those with preauricular tags, were used as controls. Overall, the control material did not differ from the general Italian population by sex, birth weight, gestational age, maternal age, and maternal education distributions. For each case four controls were selected, matched by region and year of birth.

The mother's type of residence was defined as urban when the residence was in county towns. Maternal education was used as an indicator of socioeconomic class.[12] Three categories were used: low (less than high school graduates), medium (high school graduates), and high (university graduates). Small for gestational age infants were those with a birth weight under the 10th centile for gestational age.[13]

χ^2 test, Fisher's exact test, and t test were used for statistical analysis.

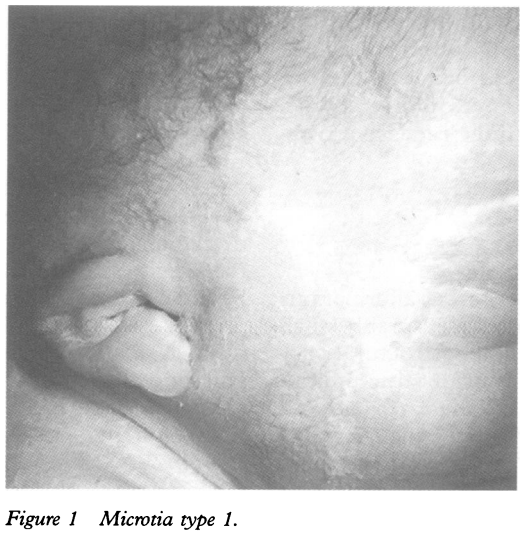

Figure 1 Microtia type 1.

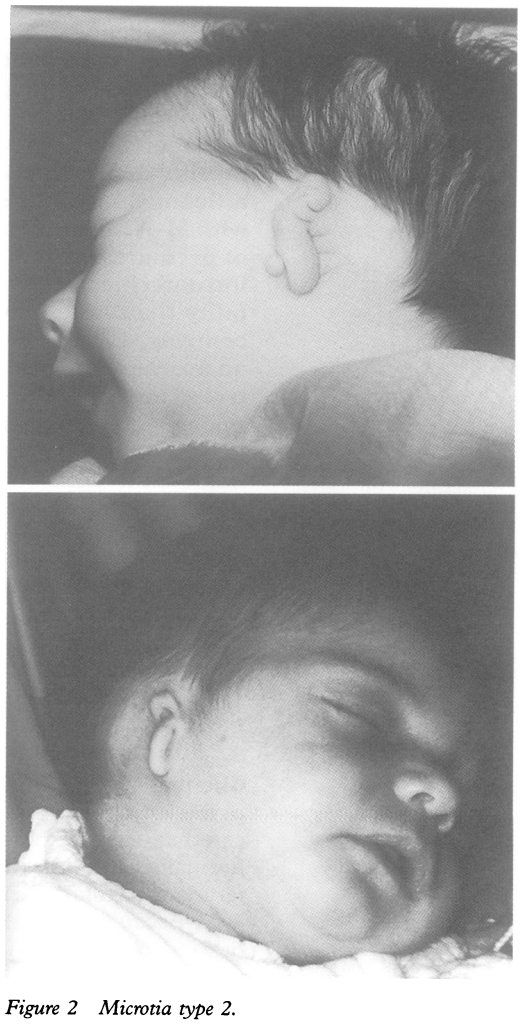

Figure 2 Microtia type 2.

Results

From 1983 to 1992, 172 infants with M-A were ascertained in IPIMC.

TYPE OF M-A AND CLINICAL DIAGNOSIS

Table 1 gives the distribution of cases by M-A and clinical diagnosis. Of the 172 infants with M-A, 36 (20·9%) had microtia type 1, 98 (57·0%) had microtia type 2, and 38 (22·1%) had anotia. By clinical diagnosis, 114 cases (66·2%) had an isolated defect, 10 (5·8%) had a well defined syndrome, and 48 (27·9%) were MMI with M-A. The 10 infants with syndromic M-A had trisomy 13 (two cases), trisomy 18, Treacher Collins syndrome (four cases), Nager syndrome, Fraser syndrome, and epidermolysis bullosa. Of 48 MMI, eight were considered to have the OAV phenotype. The distribution of M-A among the isolated, MMI, and syndromic cases was similar. No statistically significant association between the type of M-A and clinical diagnosis was found.

Table 1 Distribution of M-A by type and clinical diagnosis

	Isolated	*MMI*	*Syndromes*	*Total (%)*
Microtia type 1	26	8	2	36 (20·9)
Microtia type 2	63	28	7	98 (57·0)
Anotia	25	12	1	38 (22·1)
Total	114	48	10	172
(%)	(66·2)	(27·9)	(5·8)	

Table 2 Observed and expected numbers of associations of M-A with other unrelated defects, and its O/E ratio. Ordered by magnitude of O/E ratio. Only occurrence of three or more are given

	Observed	*%*	*Expected*	*O/E*
Holoprosencephaly	4	8·3	0·7	5·4 p=0·005
Cleft palate	8	16·7	4·4	1·8
Preaxial polydactyly	3	6·3	1·8	1·7
Oesophageal atresia	7	14·6	4·3	1·6
Vertebral defects	6	12·5	4·0	1·5
Congenital heart disease	15	31·3	20·6	0·7
Hydrocephalus	3	6·3	4·6	0·7
Anorectal atresia	3	6·3	6·2	0·5
Hypospadias	3	6·3	6·8	0·4

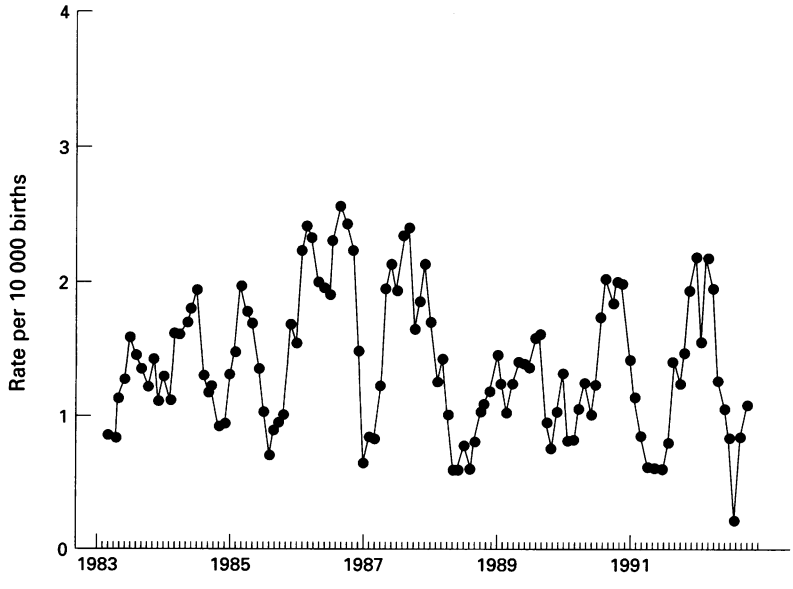

Figure 3 Prevalence at birth of M-A expressed as five month moving average.

LATERALITY

Information on laterality of M-A was available for 156 of the 172 infants. Of the 156 cases, 133 (85·3%) had unilateral M-A (76 or 57·1% on the right side and 57 or 42·9% on the left). Twenty-three cases (14·7%) had bilateral M-A, though the anomaly was not invariably of the same severity on the two sides. Of the 10 syndromic cases, five (50%) had bilateral M-A, compared to 18 of 146 (12%) non-syndromic cases (Fisher p value = 0·007). The five syndromes with bilateral involvement were Fraser syndrome, Nager syndrome, and Treacher Collins syndrome (three of the four cases). Isolated cases and MMI did not differ in the proportion with bilateral involvement.

ASSOCIATED MALFORMATIONS

Of the 172 cases with M-A, 48 (27·9%) were MMI. Of the 48 infants, 28 (58·3%) had two defects, 10 (20·8%) had three defects, and 10 (20·8%) had four or more defects (including M-A). Table 2 shows the occurrence of major malformations associated with M-A. Only defects occurring in at least three infants are shown. The defects most frequently associated with M-A were congenital heart defects (31·3%), cleft palate (16·7%), oesophageal atresia (14·6%), and vertebral defects (12·5%). A high O/E ratio for non-random preferential association was found for holoprosencephaly (O/E ratio 5·4), cleft palate (1·8), preaxial polydactyly (1·7), oesophageal atresia (1·6), and vertebral defects (1·5). Only the association with holoprosencephaly reached statistical significance (four cases observed *v* 0·7 expected; O/E = 5·4; p = 0·005).

There were five infants with associated defects described as part of isotretinoin embryopathy.[14] Of the five infants, only one had the typical combination of microtia, hydrocephalus, and transposition of the great vessels. However, none of the five had been exposed in utero to retinoic acid.

PREVALENCE AT BIRTH BY TIME AND PLACE

The overall prevalence at birth of M-A was 1·46 per 10 000 births (172/1 173 794). The prevalence at birth for non-syndromic cases was 1·38 per 10 000 births (162/1 173 794), with no heterogeneity among the 16 areas (range 0·62–2·37 per 10 000; χ^2 18·3, df 15, p = 0·25) or among 24 five month periods (range 0·21–2·58 per 10 000; χ^2 18·2, df 23, p = 0·75). There was no time trend (fig 3). The highest rate (2·58 per 10 000, 13 cases) was observed in July to November 1986; a review of the information available on the 13 cases failed to identify anything in common with clinical presentation, prenatal exposure, maternal residence, and parental occupation and age.

FACTORS ASSOCIATED WITH M-A

The following analyses were performed only on cases of non-syndromic M-A (isolated and MMI).

Table 3 Parental characteristics of infants with isolated M-A, multiformed infants with M-A, and controls

	Isolated (114)	*MMI (48)*	*Controls (648)*
Maternal age			
Mean (years) (SD)	28·0 (4·9)	26·9 (4·9)	27·9 (5·0)
35+	11·5%	6·4%	10·4%
Paternal age			
Mean (years) (SD)	31·5 (5·2)	30·6 (5·8)	31·5 (5·7)
35+	23·9%	22·2%	27·7%
Residence			
County city	86·0%	73·0%	73·0%
Maternal parity			
Primiparous	55·3%	71·7%	50·5% p=0·016
Spontaneous abortion rate	16·3%	22·7%	22·2%
Induced abortion rate	10·5%	4·5%	9·4%
Maternal education			
Low	54·7%	54·8%	58·1%
Medium	38·7%	38·1%	35·3%
High	6·6%	7·1%	6·5%
Smoking			
During first trimester	19·3%	20·8%	24·0%

Table 4 Main characteristics of infants with isolated M-A and of MMI with M-A compared to controls

	Isolated (114)	*MMI (48)*	*Controls (648)*
Male sex	52·6%	54·2%	52·3%
Stillbirths	0·9%	6·3%	0·3%
Neonatal deaths	0·9%	22·2%*	0·4%
Mean birth weight (SD)	3148 (520)*	2635 (687)*	3300 (492)
Low birth weight	8·9%	37·8%*	5·2%
Mean GA (weeks) (SD)	39·2 (2·0)	37·6 (3·0)*	39·2 (1·7)
Prematurity	6·4%	23·9%*	6·7%
SGA (<10 p)	16·7%	47·7%*	10·5%

* p<0·05.
GA: gestational age; SGA: small for gestational age; SD: standard deviation.

Twinning
Only one of the 162 cases was a twin. This male twin had microtia type 2 and his co-twin, of the same sex, was normal.

Consanguinity
Three cases had consanguineous parents, compared to 0·8 expected. In one case the parents were first cousins and the infant had microtia type 1. In two cases the parents were second cousins: one case had microtia type 1, multiple vertebral defects, and superior eyelid coloboma, classified as probable OAV phenotype, and the other case had isolated microtia type 2.

M-A in family members
None of the 89 previous liveborn sibs of the 162 cases had M-A. A parent was affected only in one family: the proband was female, and the proband's mother and maternal grandmother had microtia type 2 of similar severity. In another four families a relative was affected: only one affected relative was present in each family and the affected members were a paternal cousin, a maternal cousin, a maternal uncle, and a maternal aunt.

Parental characteristics
Table 3 shows the main features of parents of infants with isolated M-A and MMI compared with controls. No statistically significant differences were found among the three groups (isolated, MMI, controls) with the exception of maternal parity 1 that was found in 71·7% of MMI with M-A, more frequently than among cases with isolated M-A (55·2%) or controls (50·5%) (χ^2 8·25, df 2, p=0·016).

Cases with M-A were more likely than controls to have been born to a mother with chronic insulin dependent diabetes mellitus (IDDM). The odds ratio, based on four exposed cases, was 8·7 (95% CI 1·2–96·4). Of these four cases, two were isolated microtia and two were MMI (one associated with VSD and one with holoprosencephaly). The occurrence of M-A was not associated with other chronic maternal diseases, maternal or paternal occupation, maternal drug use, smoking, or alcohol consumption.

Infant characteristics
Table 4 compares the 114 infants with isolated M-A and the 48 MMI to the controls. Compared to controls, infants with isolated M-A had a lower mean birth weight (3148 *v* 3300 g), and the difference was greater among females (mean 3031 *v* 3215 g, a 184 g or 5·6% reduction; p=0·008) than among males (mean 3256 *v* 3378, a 122 g or 3·6% reduction; p=0·08). Compared to controls, MMI with M-A had a higher mortality, a lower birth weight, a lower gestational age, and were more often small for gestational age.

Discussion

In this study we describe the epidemiological features of M-A in a large national data set and explore potential relationships between the occurrence of M-A and environmental, maternal, and infant characteristics. Very few studies are available on this topic. Melnick and Myrianthopoulos[5] studied all external ear defects, including preauricular sinuses and tags. In their study only 16 cases of microtia were available, representing 2·6% of all ear defects. Castilla and Orioli[6] performed a case-control study of M-A in South America. Controls were unaffected infants. Infants were divided in two groups: those born in Quito, where a higher prevalence was found (17·4 per 10 000 births), and those born in other areas of South America covered by the ECLAMC registry. In the Quito series the authors identified several risk factors for M-A, including prenatal drug exposure (in general), high birth order, and increased paternal age, while in the other series from the rest of South America none was found.

In our study the overall birth prevalence rate was 1·46 per 10 000. This rate is lower than some studies,[2 5 6] but it is higher than the figure reported by Stevenson *et al*[15] (1·06 per 10 000) and it is very similar to the median rate among the registries reporting to the International Clearinghouse of Birth Defects Monitoring Systems[4] (1·4 per 10 000), to the rate from the Metropolitan Atlanta Congenital Defects Program[4] (1·5 per 10 000) which actively as-

certains cases up to 1 year of age, and to the rate reported by another Italian registry, Emilia Romagna[4] (1·4 per 10 000). For these reasons we think that major underascertainment of cases, if present, is minimal.

The prevalence rate showed no significant variation among geographical areas and time periods, with no evidence of clusters.

Among infants with M-A, bilateral defects were more frequent in those with a genetic syndrome. This finding is consistent with what has been reported for other defects, for instance, limb defects.

Among MMI, M-A was preferentially associated with holoprosencephaly. This association had already been observed previously in a study of holoprosencephaly[16] in which holoprosencephaly was associated not only with M-A but also with other external ear defects such as preauricular tags. This suggests that this association could represent the mildest form of the spectrum of anomalies of the holoprosencephaly-otocephaly sequence.[16] Other defects were also more frequent than expected among MMI with M-A (table 4), including cleft palate and preaxial polydactyly. However, the excess did not reach statistical significance, perhaps because of the small number of cases available for analysis; a number of MMI at least four to six times greater would have been necessary for these associations to reach statistical significance. An association of M-A with preaxial polydactyly has been described in the LADD syndrome,[17] and the three infants with this association in our study might represent unrecognised cases of LADD syndrome.

We also investigated possible inheritance patterns among infants with non-syndromic M-A. One family showed transmission through three generations suggestive of dominant inheritance. The presence of other affected members in four families could indicate either an autosomal dominant trait with variable expressivity and incomplete penetrance, or a multifactorial mechanism. The finding that none of the 89 previous sibs of infants with M-A were themselves affected does not support autosomal recessive inheritance (though three cases with consanguineous parents were observed), and this is consistent with a recent discussion of familial cases.[1]

MMI with M-A were more likely to have a primiparous mother, compared to infants with isolated M-A and controls. Castilla and Orioli,[6] on the contrary, in their study of the Quito sample found that mothers of parity 3 were more likely to have infants with M-A. A reasonable explanation of these findings is lacking and we cannot exclude that they have arisen by chance.

MMI with M-A had a higher rate of premature birth, low birth weight, reduced intrauterine growth, and neonatal mortality, compared to infants with isolated M-A and controls. This finding does not seem to be specific to M-A in that it is commonly seen in infants with multiple congenital anomalies, with or without M-A.

Infants with isolated M-A had, on average, a lower birth weight than controls. This suggests that the factors that cause M-A, even the isolated forms, probably interfere also with fetal growth; that lower birth weight is the result of decreased growth rather than premature birth is supported by the finding that, compared to controls, infants with isolated M-A are more frequently small for gestational age.

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1 Carey JC. External ear. In: Stevenson ED, Hall JG, Goodman RM, eds. *Human malformations and related anomalies.* Volume II. Oxford: Oxford University Press, 1993:193–204.
2 Aase JM, Tegtmeier RE. Microtia in New Mexico: evidence for multifactorial causation. In: *Numerical taxonomy of birth defects and polygenic disorders. Birth Defects: Original Article Series,* XIII(3A). White-Plains: National Foundation March of Dimes, 1977: 113–16.
3 Smahel Z, Horàk I. Craniofacial changes in unilateral microtia. An anthropometric study. *J Craniofac Genet Develop Biol* 1984; **4**: 7–16.
4 International Clearinghouse for Birth Defects Monitoring Systems. *Congenital malformations worldwide.* Amsterdam: Elsevier, 1991.
5 Melnick M, Myrianthopoulos NC. External ear malformations: epidemiology, genetics, and natural history. *Birth Defects* 1979;**XV**(9).
6 Castilla EE, Orioli IM. Prevalence rates of microtia in South America. *Int J Epidemiol* 1986;**15**:364–8.
7 Mastroiacovo P, Musacchio P, Bertollini R. L'Indagine Policentrica Italiana sulle Malformazioni Congenite: un progetto collaborativo pilota finalizzato alla sorveglianza delle malformazioni congenite. *Prosp Pediatr* 1982;**45**:23–38.
8 Mastroiacovo P. The Italian Birth Defects Monitoring System. Baseline rates based on 183,453 births and comparison with other registries. In: Marois M, ed. *Progress in biological and clinical research.* Volume 114, Part B. *Epidemiology, early detection and therapy, and environmental factors.* New York: Alan R Liss, 1985:17–21.
9 Marx H. Die Missbildungen des Ohres. In: Henke F, Lubarsch O, eds. *Handbuch der Spez Path Aanat Hist.* Berlin: Springer, 1926:620–5.
10 Mastroiacovo P. An ICBDMS collaborative study: monitoring multiformed infants. *Int J Risk Safety Med* 1991;**2**: 255–70.
11 Khoury MJ, James LM, Erickson JD. On the measurement and interpretation of birth defect associations in epidemiologic studies. *Am J Med Genet* 1990;**37**:229–36.
12 Liberatos P, Link BG, Kelsey JL. The measurement of social class in epidemiology. *Epidemiol Res* 1988;**10**:87–121.
13 Parazzini F, Bortolous R. Standard di peso alla nascita in Italia. *Medico e Bambino* 1993; **9**: 624–8.
14 Lynberg MC, Khoury MJ, Lammer EJ, *et al.* Sensitivity, specificity, and positive predictive value of multiple malformations in isotretinoin embryopathy surveillance. *Teratology* 1990;**42**:513–9.
15 Stevenson AC, Johnston HA, Stewart MIP, Golding D. Congenital malformations. A report of a study of series of consecutive births in 24 centres. *Bull WHO* 1996;**suppl 34**:70–1.
16 Mastroiacovo P, Botto LD, Cavalcanti DP, *et al.* Epidemiological and genetic study of holoprosencephaly in 106 cases observed in the Italian Multicentric Registry 1978–1989. In: Mastroiacovo P, Källén B, Castilla EE, eds. *Proceedings of the First International Meeting of the Genetic and Reproductive Epidemiology Research Society (GRERS).* Milano: Ghedini Editore 1992:71–82.
17 Hollister DW, Klein SH, Dejager HJ, *et al.* The lacrimo-auricolo-dento-digital (LADD) syndrome. *J Pediatr* 1973; **83**:438–44.