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A Population-Based Study on the Frequency of Additional Congenital Malformations in Infants with Congenital Hypothyroidism: Data from the Italian Registry for Congenital Hypothyroidism (1991–1998)

A. OLIVIERI, M. A. STAZI, P. MASTROIACOVO, C. FAZZINI, E. MEDDA, A. SPAGNOLO, S. DE ANGELIS, M. E. GRANDOLFO, D. TARUSCIO, V. CORDEDDU, M. SORCINI, AND THE STUDY GROUP FOR CONGENITAL HYPOTHYROIDISM*

National Institute of Health (A.O., M.A.S., C.F., E.M., S.D.A., M.E.G., D.T., V.C., M.S.), Catholic University of Sacred Heart (P.M.), and Institute of Social Medicine (A.S.), 00161 Rome, Italy

In the last decade a high frequency of other congenital anomalies has been reported in infants with congenital hypothyroidism (CH) detected by neonatal screening. In the present study the occurrence of additional congenital malformations (CM) in the population of CH infants detected in Italy between 1991 and 1998 (n = 1420) was investigated. In Italy all of the centers in charge of screening, treatment, and follow-up of CH adhere to the Italian National Registry of infants with CH.

In this study a high prevalence of additional CM (8.4%), more than 4-fold higher than that reported in the Italian population (1–2%), was found in the population of CH infants. Cardiac anomalies represented the most frequent malformations associated with CH, with a prevalence of 5.5%. However, a significant association between CH and anomalies of nervous system, eyes, and multiple CM was also observed. In conclusion, the significantly higher frequency of extrathyroidal congenital malformations reported in the CH infants than in the general population represents a further argument supporting the role of a genetic component in the etiology of CH. Investigations of the molecular mechanisms underlying developmental events of formation of thyroid and other organs represent critical steps in the knowledge of CH etiology. (*J Clin Endocrinol Metab* 87: 557–562, 2002)

CONGENITAL HYPOTHYROIDISM (CH) is one of the most common preventable causes of mental retardation. Because few signs or symptoms are present in the neonatal period, routine screening is the only means of detection. The aim of the screening program is the prevention of mental retardation by early diagnosis and therapy (1–4). The neonatal screening program for CH started in Italy in 1977 and then progressively developed covering the entire neonatal population. The Italian National Population-Based Registry of Infants with CH (INRCH) was established in Italy in 1987 as a program of the Health Ministry and is coordinated by the National Institute of Health (5). In Italy the incidence of CH is about 1 in 3000 live births, an incidence slightly higher than that observed in other countries where iodine prophylaxis is carried out (6).

Most permanent cases of CH (80–85%) are due to thyroid dysgenesis resulting from alterations in thyroid development during embryogenesis. Recently several cases of thyroid dysgenesis have been shown to be associated with mutations in genes (TTF1, TTF2, PAX8, and TSHR) involved in the development of thyroid follicular cells (7, 8). However, thyroid organogenesis is a complex process, and other genes are expressed during thyroid gland formation. In the last decade a high frequency of other congenital defects, mostly cardiac, has been reported in infants with CH detected by neonatal screening (9–13). It is becoming clearer that studying additional congenital malformations (CM) in CH infants has important implications for understanding the etiology of CH.

In the present study the occurrence of additional CM in the population of CH infants detected in Italy between 1991 and 1998 was investigated. The aims of the present study were 1) to estimate the prevalence of additional CM in the Italian population of infants with CH recorded in the INRCH, 2) to compare the frequency of additional CM in the CH population with that observed in the general population, 3) to verify whether the occurrence of additional CM may affect the effectiveness of the neonatal thyroid screening program in Italy.

Subjects and Methods

In Italy neonatal screening for CH is performed by 26 regional or interregional centers. Positive results of screening tests are confirmed by definitive tests of thyroid function on serum. Confirmational tests include TSH, T_4, and/or free T_4. Infants with confirmed primary CH were referred to the follow-up centers of their own region for starting replacement therapy (5). At present in Italy replacement therapy is started at median age of 19 d.

Babies with transient hyperthyreotropinemia on the basis of spontaneous normalization of TSH between screening and diagnosis were not recorded in the Registry. According to international guide lines (14, 15), when the definitive diagnosis of hypothyroidism is not established in the neonatal period and a suspicion of transient primary hypothyroidism (TH) is present, reevaluation of CH diagnosis is performed at

Abbreviations: ASD, Atrial septal defects; CH, congenital hypothyroidism; CI, confidence interval; CM, congenital malformations; INRCH, Italian National Registry of infants with congenital hypothyroidism; O/E ratio, observed/expected ratio; TH, transient primary hypothyroidism.

the age of 2–3 yr. At that time, after a 4-wk withdrawal period of L-T_4 treatment, T_4, free T_4, and TSH levels are measured, and ultrasound imaging, scintigraphy, and clinical evaluation are performed to establish the definitive diagnosis. In this study infants with ascertained TH were considered separately.

The birth registration form filled in at diagnosis includes anonymous data concerning CH infants (5). Since 1991 the Register has been requesting specific data on the occurrence of congenital anomalies (detected during neonatal period) other than those of the thyroid gland by using a specific reporting form.

The written descriptions of all congenital malformations reported in the Registry were reviewed in the original form by the Director of the International Center for Birth Defects (http://www.icbd.org), which has been established to coordinate the activities of the International Clearinghouse for Birth Defects Monitoring System (16). Mild and minor malformations, such as cryptorchidism, patent foramen ovalis and patent ductus arteriosus in low birth weight infants, and congenital hip dysplasia, were not considered. All other anomalies were classified as major malformations and coded with the *International Classification of Diseases*, ninth revision. All of the reported forms of infants with two or more malformations were reviewed once again to detect similarities in their association pattern. All major malformations were counted regardless of whether they were isolated, and their birth prevalence per 100 was calculated.

Comparison with other registries

As the infants of general population are not so deeply examined as infants with CH, the possibility of an underestimated birth prevalence of CM in the general population may not be excluded. For this reason the available data of many registries participating in the International Clearinghouse for Birth Defects Monitoring System were screened, and those with the highest prevalence figures, including terminations and based on a large number of births, were chosen for comparing data.

The main categories of congenital malformations observed in the population of CH infants were compared with data from Congenital Malformation Registry of Victoria State-Australia. This Registry was chosen after screening of a large number of congenital malformation registries because it showed the highest figure available (3.1/100 births) computed on a large number of births (>750,000) in a 12-yr period without variations over the years. It includes terminations of pregnancy and tabulates data of anomalies.

Data concerning the frequency of cardiac malformations in the CH population were compared with data from eight different registries (Florence, Baltimore, Tyrol, Strasbourg, Dallas, Indagine Policentrica Italiana Malformazioni Congenite, and Australia) (17–22). In the Florence and Baltimore registries a 1-yr follow-up of the recruited infants was obtained. For each cardiac anomaly the highest figure reported in one of these eight registries was used as the expected value to compare with that observed in the Italian population of CH infants.

Statistical analysis

A *t* test was used to evaluate differences between mean values of variables. The log-normal transformation was used for data that was not normally distributed. Differences in the proportion of CH infants with and without major CM were examined statistically by χ^2 test. Observed/expected (O/E) ratios and 95% confidence intervals (CI) were calculated according to the Poisson distribution for rare events.

Results

Between 1991 and 1998, 1420 CH infants were identified among 4,284,981 live births screened in Italy in that period. One hundred and fifty-four of the 1420 CH infants were reevaluated at the age of 2–3 yr because of the high suspicion of TH. The diagnosis of TH was established in 33 infants (18 males and 15 females), resulting in an incidence of permanent CH of 1:3089 (n = 1387).

Infants with confirmed CH

Among the 1387 infants with CH, 15 babies with Down syndrome (1.1%) were excluded from the analysis because of the known association of this syndrome with transient thyroid dysfunction (23). One hundred and sixty-nine of the remaining 1372 CH infants had additional birth defects. Among these babies, 115 had major anomalies, with a prevalence of 8.4%.

Thyroid scan with ^{99}Tc was performed before the onset of therapy in 57.2% of infants with isolated CH and in 58.2% of infants with major CM (Fig. 1). No significant differences were observed in the frequency of normal gland, hypoplasia, hyperplasia, and agenesis between the groups. As a higher prevalence of CM has been reported in infants with TH than in those with permanent CH (24), in our study the prevalence of major CM was also estimated in a group of CH infants with thyroid ultrascan diagnosis indicating permanent CH (ectopy, hypoplastic gland, and agenesis confirmed by ultrasound findings). This was to avoid a possible overestimation of additional CM due to inclusion in the study of TH infants not yet reevaluated. Thirty-four of the 490 CH infants with diagnosis of permanent CH had major CM with a prevalence (6.9%) not significantly different from that found in the remaining population of CH infants (9.2%, 81 of 882).

Figure 2 shows the frequency of the main categories of congenital anomalies observed in the CH population. A total of 136 major malformations were counted. The same individual was included in more than one malformation category if he had more than one malformation. Twenty-three of the 1372 infants with CH (1.7%) showed more than 1 major CM: 19 had 2 CM, and 4 had 3 or more CM. Among these 23 patients, 17 had cardiac anomalies (73.9%). The written description of infants with 2 or more CM recorded in the Registry did not show any 2 infants with a similar pattern.

The prevalence of babies with major CM observed in the population of CH infants (8.4%) was much higher than that observed in the Italian population (1.0–2.0%, including Down syndrome). Also, the prevalence of multiple congenital anomalies was greatly higher (1.7%) than that reported in the general population (0.13%) (16).

Table 1 presents O/E ratios for the main categories of major CM. The expected values were obtained from the Victoria Congenital Malformation Registry (1983–1994). The O/E ratio analysis showed that anomalies regarding nervous system (O/E ratio, 2.7; 95% CI, 1.4–4.7), eyes (O/E ratio, 5.5; 95% CI, 2.0–11.9), heart (O/E ratio, 5.5; 95% CI, 4.3–7.0), and multiple (3+) congenital anomalies (O/E ratio, 7.2; 95% CI, 2.2–20.6) were significantly more frequent in the CH infants than in the reference population.

Cardiac anomalies represented the most frequent malformations associated with CH with a prevalence of 5.5% (76 of 1372). Preterm babies with isolated patent ductus arteriosus were excluded from the study because of the association of this defect with preterm delivery and/or low birth weight. The most frequent cardiac malformations were represented by the atrial septal defects (ASD) with a rate of 13.8 of 1000; this was different from that found in the general population, in which the most frequent cardiac anomalies (3 of 1000) are represented by ventricular septal defects (25). In contrast to

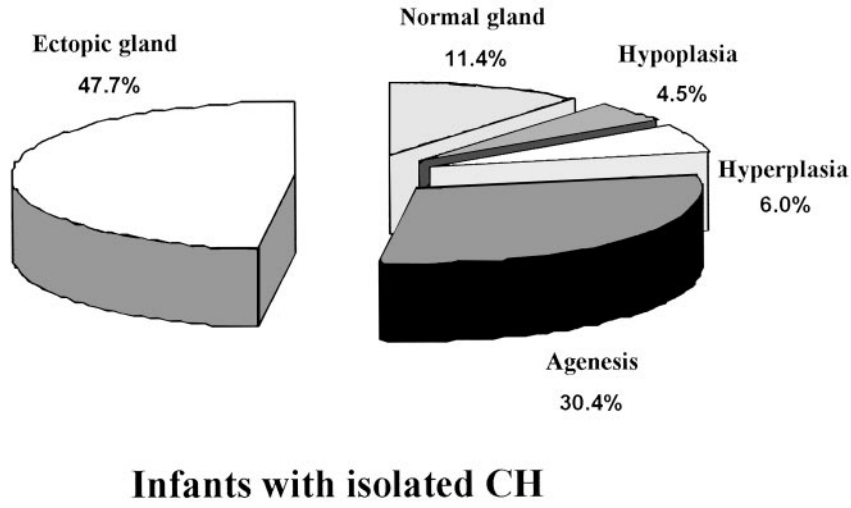
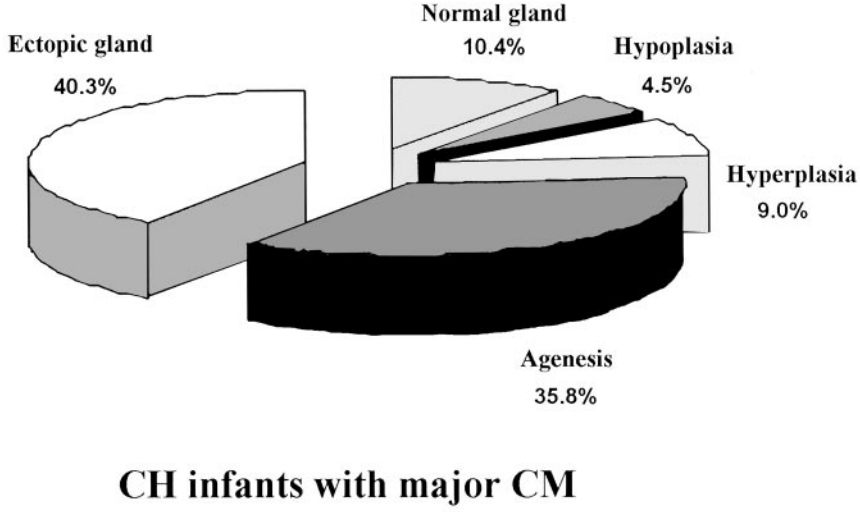


FIG. 1. Scintigraphic diagnosis of infants with CH.



the findings of in other studies (26), in our population of CH infants isolated septal defects were found not only in babies with ectopic thyroid (n = 12) or athyreosis (n = 9), but also in 10 babies with eutopic thyroid (8 normal and 2 hypoplastic).

The O/E ratios for cardiac defects are shown in Table 2. For each cardiac anomaly the highest figure reported in one of these eight considered registries was used as the expected value. The O/E ratio analysis showed that tetralogy of Fallot (O/E ratio, 8.6; 95% CI, 3.1–18.7), ASD-ostium II (O/E ratio, 10.6; 95% CI, 6.4–16.5), and pulmonary stenosis (O/E ratio, 7.8; 95% CI, 3.1–16.0) are significantly more frequent than those observed in the reference population.

Neonatal features of infants with CH

Mean levels of T_4 and TSH at screening and confirmation and some neonatal features observed in the infants with isolated CH were compared with those of infants with major additional CM. Significantly lower serum T_4 levels at screening were observed in the group with major CM than in the infants with isolated CH (3.1 ± 2.4 *vs.* 4.0 ± 2.8 μg/dl; $P = 0.04$). However, no difference between groups was found at confirmation of the diagnosis. Birth weight was significantly lower in CH infants with major CM than in those with isolated CH (3027 ± 732 *vs.* 3225 ± 697 g; $P = 0.01$). Comparison of mean values of gestational age and female/male ratio did not show any significant difference between the groups. Also, the frequency of twins was similar in the two groups: 3.0% in CH infants with major CM and 3.6% in those with isolated CH.

Reevaluated infants with TH

Five (3 females and 2 males) of the 33 infants with ascertained TH had additional congenital anomalies. Two of them (6.1%) had Down syndrome with cardiac septal defects. In the 3 remaining TH infants the following anomalies were observed: ventricular septal defect, persistent fetal circulation, and clubfoot. The prevalence of additional CM in the TH infants, with exclusion of the 2 babies with Down syndrome,

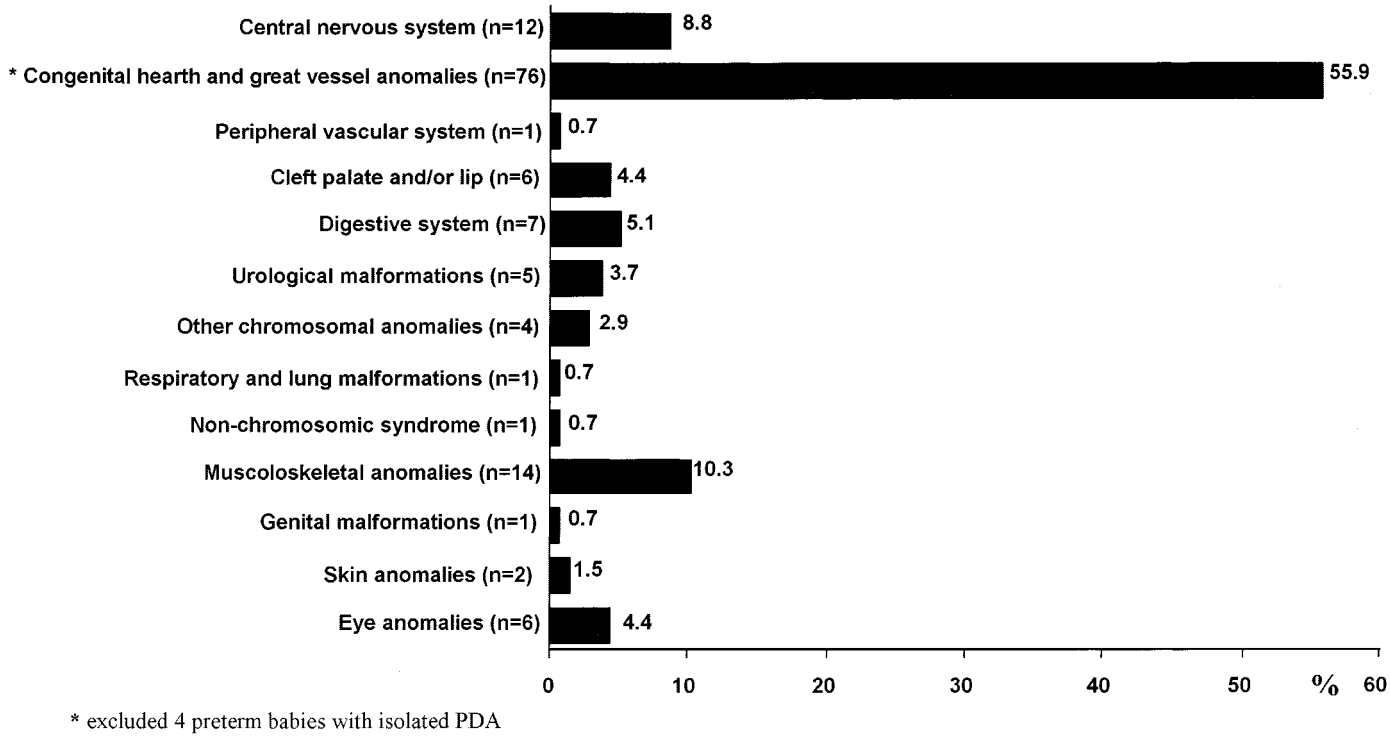


FIG. 2. Distribution of the main categories of major CM observed in CH infants.

TABLE 1. Observed and expected frequencies of major CM among 1372 infants with CH

ICD-9-code	Malformation categories	Obs	Exp	O/E	95% CI
740-2	Nervous system	12	4.5	2.7	1.4–4.7
743	Eye	6	1.1	5.5	2.0–11.9
744	Ear face and neck	0	1.3		
745-6	Heart	66	11.9	5.5	4.3–7.0
747	Circulatory system	1	5.9	0.2	0–1
748	Respiratory tract	1	1.9	0.5	0–2.9
749	Oral clefts	6	2.3	2.6	1–5.7
750-1	Digestive system	7	4.9	1.4	0.6–2.9
752	Genital organs	1	4.8	0.2	0–1.2
753	Urinary system	5	3.7	1.4	0.4–3.2
755	Limbs	2	4.3	0.5	0.1–1.7
7545/6	Musculoskeletal	12	10.3	1.2	0.6–2.0
757-9	Others	7	5.3	1.3	0.6–2.6
Multiple congenital anomalies (2 +)		23	1.1	20.9	13.3–31.4
Multiple congenital anomalies (3 +)		4	0.5	7.2	2.2–20.6
Total anomalies		126	62.2	2.0	1.7–2.4
Total infants with any BD		115	42.5	2.7	2.2–3.3

Expected figures from Victoria CMR 1983–1994.

was not significantly different from that found in the population of infants with confirmed CH (9.7% and 8.4%, respectively).

Effects on effectiveness of the Italian screening program

Although a significant delay in collection of screening specimens was observed in CH infants with major CM (age at screening, 6.6 ± 6.7 *vs.* 4.7 ± 3.5 d; $P = 0.01$), no significant differences between groups were found in the mean age at the start of therapy.

Discussion

In this study a high prevalence of additional CM (8.4%), more than 4-fold higher than that reported in the Italian population, was found in the population of CH infants diagnosed in Italy between 1991 and 1998 and recorded by the INRCH. This finding confirms the high prevalence of additional CM previously reported in other series of infants with CH (9–13). The wide variation in the frequencies of additional CM reported in CH infants (range, 7.3–24.0%) can be due to the size of the sample, to the criteria of inclusion of cases in the study, and to the different geographical location

TABLE 2. Observed and expected frequencies of cardio-vascular malformations among 1372 infants with CH

ICD code	Cardiac malformations	Obs	Exp	O/E	95% CI
745.1	TGV	2	0.6 Fl	3.3	0.4–12.1
745.2	Tetralogy of Fallot	6	0.7 Ty	8.6	3.1–18.7
745.4	VSD	11	9.1 Fl	1.2	0.6–22
745.5	ASD, ostium II	19	1.8 Str	10.6	6.4–16.5
745.6	ECD	2	1.0 Fl	2.0	0.2–7.2
746.0	Pulmonary stenosis	7	0.9 Str	7.8	3.1–16.0
747.0	PDA	7	2.9 Fl	2.4	1.0–5.0
747.1	Coartation of aorta	3	0.7 Str	4.3	0.9–12.5
745–747	Total	66	18.2	3.6	4.3–7.0

Baseline rates from various registries (Florence, Tyrol, Strasbourg).
TGV, Transposition of great vessels; ECD, endocardial cushion defect.

of the considered population. Although there are no doubts on the prevailing view that CH is associated with other malformations, it has been reported that a high frequency of additional CM is more often present in infants with transiently elevated TSH than in those with definitive CH (24). In the our study the population of CH infants included neither babies with transient hyperthyreotropinemia showing a spontaneous normalization of TSH between screening and diagnosis nor TH infants identified after reevaluation of the diagnosis. Moreover, the estimated prevalence of major CM in this CH population was not significantly affected by the possible presence of not yet reevaluated children with TH. In fact, the prevalence of major CM observed in the subgroup of the 490 CH infants with scintigraphic diagnosis indicating permanent CH was not significantly different from that found in the remaining population of CH infants (6.9% and 9.2%, respectively).

The question of whether additional CM may influence the effectiveness of the Italian thyroid screening program has been also considered. This study showed that no delay at the start of therapy is observed in the group of CH infants with additional CM compared with the infants with isolated CH.

Several studies have supported that genetic factors may be involved in the pathogenesis of CH. It has been reported how mutations in the genes encoding transcription factors (TTF1, TTF2, and PAX8) or the TSH receptor cause thyroid dysgenesis in humans or in mouse models (6, 7). Moreover, a familial clustering, including athyreosis and ectopic thyroid gland, has been recently reported (27). This finding strongly suggests the potential involvement of genetic factors, potentially including other genes that are as yet unknown. Again, the significantly higher frequency of extrathyroidal congenital malformations reported in the CH infants than in the general population represents a further argument supporting the role of a genetic component in the etiology of CH. Our results obtained from a large population of CH infants confirmed a strong association between CH and additional CM, mostly for multiple anomalies and for anomalies regarding nervous system, eyes, and heart. The high frequency of multiple congenital anomalies found in the population of CH infants strongly suggests a very early impairment in the first stages of embryo development with a consequent involvement of different organs and structures. The hypothesis that hypothyroidism *per se* (especially in the first 10 wk of fetal life) may play a role in the occurrence of CM cannot be excluded. This hypothesis is also supported by the fact that CH babies with CM showed significantly lower T_4 levels at screening than babies with isolated CH. However, the significant association between CH and anomalies of nervous system, eyes, and heart (representing precocious structures in the developing embryo) fits with results obtained in studies conducted in experimental models. In these studies anomalies occurring in organs that are dependent on neural crest cells for their development were hypothesized to result from a disturbance of the proliferation and migration of the neural crest cells in the early phases of embryo development (28–31). These cells represent a migratory cell population derived from the dorsal neural tube that contributes to a wide variety of tissues throughout the embryo. Studies of avian and mouse embryos demonstrated that a neural crest subpopulation, termed cardiac neural crest, plays a vital role in the normal heart morphogenesis and contributes cells to the aortic arches, thymus, thyroid, and parathyroids (31, 32). Ablation studies in the chick have also demonstrated the occurrence of cardiac outflow tract defects frequently in association with defective or absent thymus, thyroid, and parathyroids (28, 33).

In this study cardiac anomalies were the most frequent CM observed in the CH infants. The O/E analysis also showed a significant association of CH with ASD-ostium II, tetralogy of Fallot, and pulmonary stenosis. It is important to consider the fact that embryonic thyroid development is closely associated with the developing heart. The thyroid is pulled to its position near the base of the neck as a consequence of the continuing descent of the heart during the early stages of thyroid formation (34). It has been reported that mutations in developmental control genes are associated with some cardiac congenital malformations (35). Many point mutations have been identified in *NKX2.5* transcription factor in families with atrial septal defects (36). Sporadic mutations of *NKX2.5* have also been found in patients with tetralogy of Fallot (37). Isolated pulmonary stenosis and tetralogy of Fallot have been associated with Jagged-1 mutations (38). Again, recent studies conducted in experimental models have implicated the homeobox gene *Hex* in the development of the cardiovascular system and the thyroid gland (39, 40). In the light of this evidence, investigations of the molecular mechanisms underlying developmental events of heart and thyroid formation and understanding how perturbations of possible common developmental control genes may result in thyroid dysgenesis and in different forms of congenital heart

disease represent critical steps in the knowledge of CH etiology.

In conclusion, many pathogenic mechanisms may be involved in the increasing frequency of additional congenital anomalies in infants with CH. Elucidation of genetic-environmental networks and mechanisms underlying the development of thyroid and other organs opens up the possibility of understanding the etiology of CH and provides hope that at least some types of thyroid dysgenesis may be prevented by modulating these cofactors. In this view epidemiological studies are relevant because they can promote and orient future molecular studies to more precise targets.

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Address all correspondence and requests for reprints to: Dr. Antonella Olivieri, Laboratory Metabolismo e Biochimica Patologica, Istituto Superiore di Sanità, Viale Regina Elena, 299, 00161 Rome, Italy. E-mail: olivieri@iss.it.

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* R. Altamura (Brindisi), U. Angeloni (Rome), R. Antonetti (Foggia), I. Antonozzi (Rome), M. Baserga (Catanzaro), R. Berardi (Siena), S. Bernasconi (Parma), G. Bona (Novara), M. Burroni, E. Cacciari (Bologna), R. Caldarera (Messina), A. Cassio (Bologna), L. Cavallo (Bari), L. Chiovato (Pisa), G. Chiumello (Milano), B. Ciannamea (Lecce), A. Cohen (Savona), G. V. Coppa (Ancona), C. Corbetta (Milano), R. Cordova (Potenza), P. Costa (Rome), F. Dammacco (Bari), F. De Luca (Messina), C. De Santis (Torino), C. Del Carpio (Palermo), S. Di Maio (Napoli), G. F. Fenzi (Napoli), R. Gastaldi (Genova), R. Gaudio (Taranto), G. Giovannelli (Parma), D. Gullo (Catania), L. Lasciarrea (Bari), A. Lelli (Rome), A. Liotta (Palermo), D. Lojodice (Napoli), R. Lorini (Genova), F. Monaco (Chieti), L. Moschini (Rome), G. C. Mussa (Torino), N. Oggiano (Ancona), S. Pagliardini (Torino), L. Palillo (Palermo), G. Parlato (Catanzaro), E. Pasquini (Firenze), S. Piazzi (Bologna), A. Pignero (Napoli), A. Pinchera (Pisa), C. Pintor (Cagliari), R. Puggioni (Cagliari), A. Rizzo (Lecce), C. Romano (Genova), G. Saggese (Pisa), D. Sala (Napoli), C. Salerno (Napoli), R. Salti (Firenze), L. Sava (Catania), V. Stoppioni (Fano), F. Tancredi (Napoli), L. Tatò (Verona), R. Vigneri (Catania), N. Vizzini (Caltanissetta), C. Volta (Parma), G. Weber (Milano), and E. Zammarchi (Firenze).

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