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Pattern of Cardiovascular Anomalies Associated with Esophageal Atresia: Support for a Caudal Pharyngeal Arch Neurocristopathy

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ABSTRACT

Patients with cephalic neurocristopathy (an abnormality of neural crest differentiation) present a striking pattern of associated cardiovascular anomalies (CVA). Therefore, to support the hypothesis that esophageal atresia (EA) may be related to a defective contribution from the cephalic neural crest, we studied the pattern of CVA associated with EA. Medical records of 99 patients with isolated EA, 101 with isolated anorectal malformations (ARM) and 15 with both EA and ARM, consecutively admitted to our unit, were reviewed. The prevalence and pattern of CVA associated with isolated EA or isolated ARM were compared on the assumption that the cranial or caudal location of a major malformation is related to a different regional patterning of associated anomalies. The prevalence of CVA was 39% in patients with isolated EA and 7% in those with isolated ARM ($p < 0.01$). Neural crest–related CVA (aortic arch anomalies, conotruncal defects, and superior vena cava malformations) accounted for 72% of all CVA in patients with isolated EA *versus* 14% in those with isolated ARM ($p < 0.02$). In patients with isolated EA, anomalies of the fourth and sixth aortic arch derivatives accounted for 75% of all neural crest related CVA. The present pattern of CVA in infants with EA supports the concept that EA may be related to an abnormal contribution from caudal portion of cephalic neural crest. **(*Pediatr Res* 50: 565–568, 2001)**

Abbreviations
CVA, cardiovascular anomalies
EA, esophageal atresia
ARM, anorectal malformations

Nearly all infants with EA present with clinical manifestations of a maturational dysautonomia (1). The autonomic disturbances are similar to those found in infants with Pierre-Robin or Di George syndromes, which are considered pharyngeal arch syndromes related to a cephalic neurocristopathy (an abnormality of cephalic neural crest differentiation) (2). In addition, >90% of patients with EA present with one or more minor facial anomalies, which are considered markers of an abnormal developmental activity of the cephalic neural crest (3). These findings, *i.e.* the association with maturational dysautonomia and facial anomalies, suggest that EA should be included among the pharyngeal arch neurocristopathies (3).

To test this hypothesis, we studied, in a large series of patients with EA, the prevalence of aortic arch and conotruncal heart anomalies, which are considered the most distinctive CVA of Di George syndrome (4–6). These defects appear to be related to an abnormal participation of the cephalic neural crest, inasmuch as ablation of cardiac neural crest, in chick embryos, invariably results in aortico-pulmonary septation defects associated with anomalies of the great arteries (7–10).

METHODS

The records of 114 patients with EA and 116 with ARM, consecutively admitted to our unit between 1967 and 1998, were retrospectively reviewed. We decided to compare the prevalence and the pattern of CVA associated with EA with those associated with ARM, on the assumption that these two abnormalities should have a different pattern of associated anomalies. Actually, abnormal development of the cephalic neural crest may result in face, thymus, thyroid, parathyroid, and heart anomalies (4–10), whereas a dysmorphogenetic event affecting the caudal region is related to "caudal regression syndrome" that includes sacral, anorectal, urogenital, and lower limb anomalies (11–14). This concept is supported by experimental studies on vertebrates indicating that the ho-

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meobox gene complexes that play a key role in embryonic development of the foregut are different from those expressed in the developing hindgut (15). Because many patients may have an "axial mesodermal dysplasia spectrum" (12), which is a feature both of the pharyngeal arch and caudal regression syndromes, we divided the patients in three groups as follow: 1) 99 with isolated EA; 2) 101 with isolated ARM; and 3) 15 with both EA and ARM.

CVA were diagnosed by means of echocardiography, cardiac catheterization, intraoperative, and, when available, postmortem findings. When diagnosed only on the basis of physical examination, chest roentgenogram, or ECG, CVA were considered as not ascertained. In patients with two or more defects, CVA was considered as the most embryogenetically distinctive or hemodynamically significant defect. No defect was counted twice.

CVA were classified as neural crest–related and non-neural crest–related. The former included aortic arch and conotruncal anomalies (4–10). We classified ventricular septal defect as membranous or muscular. The membranous part of the interventricular septum is derived from the cardiac neural crest, whereas the muscular part is derived from the mesenchyme, which is not related to the cardiac neural crest (16). Among the neural crest–related CVA we included not only the membranous ventricular septal defect but also patent ductus arteriosus inasmuch as the cephalic neural crest cells play a role in its mechanism of closure (17). Finally, we considered the anomalies of superior vena cava as possibly neural crest related because, recently, the cephalic neural crest was reported to contribute to the media of anterior cardinal veins (18).

The differences in prevalence and pattern of CVA were tested by means of χ^2 test, applying Yate's correction.

RESULTS

Prevalence of CVA. CVA were found in 39 out of 99 (39%) and in 7 out of 101 (7%) patients with isolated EA and isolated ARM, respectively ($p < 0.01$). Of the 15 patients with EA and ARM, 8 presented with CVA (53%).

Pattern of CVA. The pattern of CVA found in 39 patients with isolated EA, in 8 with EA and ARM, and in 7 with isolated ARM are shown in Table 1.

In patients with isolated EA, neural crest–related CVA accounted for 72% of all CVA, whereas in those with isolated ARM they represented 14% of all the defects ($p < 0.02$). In patients with both EA and ARM, neural crest–related CVA were 75% of all CVA.

In patients with isolated EA, anomalies of the fourth and sixth aortic arch derivatives accounted for 75% of all neural crest–related CVA; the most common aortic arch anomaly was a patent ductus arteriosus (43%). The aortic arches anomalies were not associated with conotruncal defects.

Table 1. *CVA in 39 patients with isolated EA, in 8 with axial mesodermal dysplasia, and in 7 with isolated ARM*

	EA	EA + ARM	ARM
Aortic arch anomalies	21	2	1
PDA			
Isolated	4	—	1
+ PFO	4	—	—
+ ASD	1	—	—
RAA			
Isolated	6	—	—
+ Dextrocardia	1	—	—
IAA	1	—	—
Vascular ring	—	1	—
ASCA	4	1	—
Conotruncal	4	3	—
POF	—	1	—
TOF	1	1	—
TGA	1	—	—
Dextrocardia	1	—	—
VSD (membranous)	—	1	—
VSD (membranous) + LSVC + RAA + PFO	1	—	—
LSVC	3	1	—
Isolated	2	1	—
+ PFO (without shunt)	1	—	—
Non-neural crest related	11	2	4
Not ascertained	—	—	2

PDA, patent ductus arteriosus; PFO, patent foramen ovale; ASD, atrial septal defect; RAA, right aortic arch; IAA, interrupted aortic arch; ASCA, aberrant subclavian artery; POF, pentalogy of Fallot; TOF, tetralogy of Fallot; TGA, transposition of great arteries; VSD, ventricular septal defect; LSVC, left superior vena cava.

DISCUSSION

Prevalence of CVA. In the present series, the prevalence of CVA in patients with EA, a malformation located in the cephalic region, was found to be significantly higher than that in patients with ARM, a malformation located in the caudal region.

The reported prevalence of CVA in patients with EA ranges between 11% and 49% (19–24). The prevalence in our series (39%) is rather high, probably because we also included the less significant CVA, such as aberrant subclavian artery or left superior vena cava. These latter CVA are not important from a hemodynamic point of view but may be embryogenetically related to an abnormal development of the cephalic neural crest derivatives. An analysis of a series of patients with ARM reported in the literature documented a prevalence of CVA ranging from 3% to 22% (25, 26). The rather low prevalence of CVA in our series of patients with ARM is probably due to the fact that we have considered separately those cases with ARM associated with EA.

The difference in prevalence of CVA in those patients with isolated EA and isolated ARM supports the assumption that a cephalic or caudal location of an early embryonic defect results in a region-specific prevalence of CVA.

Pattern of CVA. Classifying the CVA found in the 215 patients in our series according to their embryogenesis, we found that about 72% of all cases of CVA in patients with isolated EA were neural crest related.

The correlation between pattern of CVA and neurocristopathy in patients with EA had not been revealed by previous studies, mainly because CVA had been classified on an anatomical basis, indicating each individual defect (19–24). However, retrospective classification of the CVA in those series according to an embryogenetic rather than an anatomic scheme revealed a prevalence of neural crest–related CVA around

70%. In only one study (21) was the calculated prevalence of neural crest–related CVA much lower (36%) than that found in the present series of patients. The explanation for this discrepancy is that minor abnormalities, such as persistent left superior vena cava or aberrant subclavian artery or right aortic arch, were not listed and were therefore probably included in the group of "others." Furthermore, in the review of previous series, we always considered ventricular septal defect (VSD) as not related to the neural crest, inasmuch as the distinction between a membranous defect and a muscular defect was not reported.

The prevalence of neural crest–related CVA is probably underestimated also in the present study, because minor abnormalities are virtually undetectable unless angiography or postmortem examinations are performed. For instance, in a study on heart-lung specimens (27), a common origin of carotid arteries has been found in about 50% of infants with EA. A similar prevalence of common origin of carotid arteries has been found in infants with Di George syndrome (27).

The high prevalence of neural crest–related CVA in our series of patients with EA is similar to that reported in series of patients with Di George syndrome (4–6), suggesting a common etiology. This hypothesis appears feasible inasmuch as rats with experimental EA, induced by adriamycin, show a similar prevalence of neural crest–related CVA associated with thymus and parathyroid anomalies (28). This cluster of defects, which mimics Di George syndrome, is similar to that induced by ablation of the cardiac neural crest (9).

The EA patients in the present series, in comparison with patients with Di George syndrome (third and fourth pharyngeal arch syndrome), show a similar prevalence but a different pattern of neural crest–related CVA. Indeed, in a collected series of 151 patients with Di George syndrome, 61 out of 136 patients with neural crest–related CVA presented with fourth and sixth aortic arch anomalies (6). This percentage (45%) is much lower than that (75%) found in the present series of patients with EA ($p < 0.01$). In particular, 9 out of 28 patients with neural crest–related CVA presented with a patent ductus arteriosus *versus* 5 of 136 patients with Di George syndrome ($p < 0.001$).

In the present patients with EA, unlike those with Di George syndrome, aortic arch anomalies were not associated with a conotruncal defect, indicating that development of aortic arch artery and heart morphogenesis can be quite independent. These findings were unexpected inasmuch as ablation of the cardiac neural crest invariably causes conotruncal and aortic arch anomalies (10). However, similar results were obtained in chick embryos by antisense treatment of the cardiac neural crest to inactivate paralogous groups of Hox messages with their anterior expression domains in the third, fourth, and sixth pharyngeal arches (10). A possible explanation is that the message blockade acts over a limited period of time and may no longer be effective when the neural crest cells reach the outflow tract. Alternatively, the role of cardiac neural crest cells may be patterning in the development of the pharyngeal arches but not patterning in the development of the heart (10).

All pharyngeal arches receive neural crest cells emanating from the cardiac neural crest (somite 1 to 3). However, the sixth arch, which gives rise to the ductus arteriosus, has a unique identity because only the sixth arch retains Hox 2.1 (Hoxb-5) expression (29, 30). This restricted population of Hoxb-5 positive neural crest cells is not present in the mouse cardiac neural crest but is restricted to the cell population in the sixth arch. In this region, positive cells also reach a mesenchymal cell population on the lateral aspect of the foregut, which is continuous with the pharyngeal arch 6 region (29). These data suggest that an anomalous contribution of this restricted population of Hoxb-5 positive neural crest cells may play a role in the pathogenesis of EA and caudal aortic arch anomalies. Actually, many structures of the proximal esophagus are derived from the caudal pharyngeal arches, including striated muscle, recurrent pharyngeal branches of the vagus, and inferior thyroid artery (31).

This hypothesis is further supported by genetically engineered mice experiments. A disruption of Hox 1.5 (Hoxa-3), results in a phenotype, which includes thymic, thyroid, parathyroid, craniofacial, and aortic arch anomalies (including patent ductus arteriosus) (32). In this animal model, classic conotruncal defects were not seen, whereas a disorganization of the muscle surrounding the esophagus was found. Furthermore, Hoxc-4 disruption, associated with alterations in transcription of both Hoxc-5 and Hoxc-6, may result in a phenotype characterized by partial or complete obstruction of the esophageal lumen, disorganization of esophageal striated muscle, and malformations of thoracic vertebrae (33). No cardiac defects were found in this animal model.

We conclude that, because the Di George syndrome is considered a neurocristopathy of the third and fourth pharyngeal arches, EA and associated CVA should be considered the result of a fourth and sixth pharyngeal arches neurocristopathy. The present data suggest the need for further studies to specifically investigate the role played by the cephalic neural crest in the pathogenesis of EA.

REFERENCES

1. Cozzi F, Myers NA, Madonna L, Drago S, Fiocca G, Piacenti S, Pierro A 1991 Esophageal atresia, choanal atresia and dysautonomia. J Pediatr Surg 26:548–552
2. Couly G, Lagrue A, Griscelli G 1983 Le syndrome de Di George, neurocristopathie rhomboencephalique exemplaire. Rev Stomatol Chir Maxillofac 84:103–108
3. Cozzi F, Myers NA, Piacenti S, Orfei P, Cozzi DA, Bonanni M, Madonna L 1993 Maturational dysautonomia and facial anomalies associated with esophageal atresia: support for neural crest involvement. J Pediatr Surg 6:798–801
4. Freedom RM, Rosen FS, Nadas AS 1972 Congenital cardiovascular disease and anomalies of the third and fourth pharyngeal pouch. Circulation 46:165–172
5. Conley ME, Beckwith JB, Mancer JFK, Tenckhoff L 1979 The spectrum of the Di George syndrome. J Pediatr 94:883–890
6. Van Mierop LHS, Kutsche LM 1986 Cardiovascular anomalies in Di George syndrome and importance of neural crest as a possible pathogenetic factor. Am J Cardiol 58:133–137
7. Le Douarin N 1980 Migration and differentiation of neural crest cells. Curr Top Dev Biol 16:31–85
8. Kirby ML, Gale TF, Stewart DE 1983 Neural crest cells contribute to normal aorticopulmonary septation. Science 220:1059–1061
9. Bockman DE, Kirby ML 1985 Neural crest interactions in the development of the immune system. J Immunol 136:766–768
10. Kirby ML, Hunt P, Wallis K, Thorogood P 1997 Abnormal patterning of the aortic arch arteries does not evoke cardiac malformations. Dev Dyn 208:34–47
11. Duhamel B 1961 From the mermaid to anal imperforation: the syndrome of caudal regression. Arch Dis Child 36:152–155
12. Russel LJ, Weaver DD, Bull MJ 1981 The axial mesodermal dysplasia spectrum. Pediatrics 67:176–182
13. North K, Mc Credie J 1987 Neurotomes and birth defects: a neuroanatomic method of interpretation of multiple congenital malformations. Am J Med Genet 3(suppl):29–42

14. Duncan PA, Shapiro LR 1993 Interrelationships of the hemifacial microsomia-VATER, VATER, and syrenomelia phenotypes. Am J Med Genet 47:75–84
15. Beck F, Tata F, Kallayanee C 2000 Homeobox genes and gut development. Bioessay 22:431–441
16. Waldo K, Miyagawa-Tomita S, Kumiski D, Kirby ML 1998 Cardiac neural crest cells provide new insight into septation of the cardiac outflow tract: aortic sac to ventricular septal closure. Dev Biol 196:129–144
17. Waldo K, Kirby ML 1993 Cardiac neural crest contribution to the pulmonary artery and sixth aortic arch artery complex in chick embryos aged 6 to 18 days. Anat Rec 237:385–399
18. Bergwerff M, Verberne ME, De Ruiter MC, Poelmann RE, Gittenberger-de Groot AC 1998 Neural crest cell contribution to the developing circulatory system: implications for vascular morphology? Circ Res 82:221–231
19. Mellins RB, Blumenthal S 1964 Cardiovascular anomalies and esophageal atresia. Am J Dis Child 107:96–100
20. Greenwood RD, Rosenthal A 1976 Cardiovascular malformations associated with tracheoesophageal fistula and esophageal atresia. Pediatrics 57:87–91
21. Louhimo I, Lindahl H 1983 Esophageal atresia: primary results of 500 consecutively treated patients. J Pediatr Surg 18:217–229
22. Bishop PJ, Klein MD, Philippart AI, Hixson DS, Hertzler JH 1985 Transpleural repair of esophageal atresia without a primary gastrostomy: 240 patients treated between 1951 and 1983. J Pediatr Surg 20:823–828
23. Chittmittrapap S, Spitz L, Kiely EM, Brereton RJ 1989 Oesophageal atresia and associated anomalies. Arch Dis Child 64:364–368
24. Mee RBB, Beasley SW, Auldist AW, Myers NA 1992 Influence of congenital heart disease on management of oesophageal atresia. Pediatr Surg Int 7:90–93
25. Greenwood RD, Rosenthal A, Nadas AS 1975 Cardiovascular malformations associated with imperforate anus. J Pediatr 4:576–579
26. Teixeira OH, Malhotra K, Sellers J, Mercer S 1983 Cardiovascular anomalies with imperforate anus. Arch Dis Child 58:747–749
27. Wells TR, Landing BH, Shankle WR 1993 Syndromal association of common origin of the carotid arteries. Pediatr Pathol 13:203–212
28. Otten C, Migliazza L, Xia H, Rodriguez JI, Diez-Pardo JA, Tovar JA 2000 Neural crest-derived defects in experimental esophageal atresia. Pediatr Res 47:178–183
29. Kuratani SC, Wall NA 1992 Expression of Hox 2.1 protein in restricted populations of neural crest cells and pharyngeal ectoderm. Dev Dyn 195:15–28
30. Bergwerff M, DeRuiter MC, Gittenberger-de Groot AC 1999 Comparative anatomy and ontogeny of the ductus arteriosus, a vascular outsider. Anat Embryol 200:559–571
31. Moore KL, Persaud TVN 1998 The Developing Human: Clinically Oriented Embryology. WB Saunders, Philadelphia, pp 215–256
32. Chisaka O, Capecchi MR 1991 Regionally restricted developmental defects resulting from targeted disruption of the mouse homeobox gene hox-1.5. Nature 350:473–479
33. Boulet AM, Capecchi MR 1996 Targeted disruption of hoxc-4 causes esophageal defects and vertebral transformations. Dev Biol 177:232–249