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Editorial Comment

Searching for order among disorders of laterality

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With a number of notable exceptions, understanding the genetic causes of congenital cardiovascular diseases in humans has proven to be a vexing quest. Among the difficulties in dissecting the genetics of congenital cardiac disease are the paucity of obvious single gene defects, highly variable penetrance, and substantial phenotypic heterogeneity. Compounding these molecular genetic limitations is the problem of taxonomy, in other words, how anatomic cardiovascular anomalies are defined and grouped. Congenital cardiac malformations frequently occur as constellations of abnormally formed cardiovascular structures, which may overlap in variable combinations. As a result, complex cardiovascular anomalies are rarely singular. For no type of congenital cardiac disease is this more true than for anomalies associated with disorders of laterality, often referred to broadly as "heterotaxy".

The early embryonic process of formation of the right-left axis of the body, in which primordial left-sided structures are differentiated from right-sided structures, and according to which the subsequent process of asymmetric organogenesis unfolds, has been extensively characterized in various experiemental systems.[1,2] When genes essential to specification and formation of the left-right axis are mutated or deleted in animal models, it is possible to find phenotypic features consistent with disorders of laterality as seen in the human. Among the array of genes implicated in disordered formation of the left-right axis, and associated with "heterotaxy" phenotypes in mutant mice, many have been screened for mutations in humans with laterality disorders, including *PA26*, *CRELD1*, *ACVR2B*, *LEFTY A*, *LEFTY B*, *CFC1*, *ZIC3*, *N0DAL*, and *INV*.[3–11] In almost all of the cited reports, mutations in the gene or genes studied have been detected in some subjects, but usually no more than a small percentage of them. These findings may be viewed as breakthroughs that offer support for translational investigation of humans with congenitally malformed hearts on the basis of phenotypes observed in genetically modified mice. On the other hand, they may be read as accumulating evidence that, like most forms of congenital cardiac disease, complex congenital cardiovascular anomalies associated with disorders of visceral laterality are unlikely to be caused by single gene abnormalities.

In this issue of *Cardiology in the Young*, Selamet Tierney et al.[12] report the results of a study that is similar in the scope of its findings to those mentioned above, supporting the conclusion that investigations into the genetics of laterality disorders in humans demand both a more complex vision and a more consistent, unified taxonomy of congenital cardiovascular malformations. The authors screened a cohort of individuals with assorted abnormalities of laterality for mutations in the *CFC1* gene.[12] *CFC1* is a human homologue of the murine *cryptic* gene, which encodes an epidermal growth factor-related protein involved in left-right axis formation during early embryogenesis. Mice that are homozygous for deleted *cryptic* alleles (*cryptic*$^{-/-}$) essentially all have right pulmonary isomerism, hyposplenia or asplenia, and abnormally related great arteries, while approximately half have inverted visceral situs, cardiac malposition, abnormal position of the inferior caval and azygous veins, and abnormal branching or sidedness of the aortic arch.[13,14]

As in several previous studies in which investigators tested for mutations of *CFC*1 in patients with congenital cardiovascular malformations, Selamet Tierney et al. found several missense mutations that may be pieces in a more complex polygenetic puzzle, but ultimately, no compelling evidence that mutations of

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CFC1 are a prevalent cause of congenital heart disease in humans with disorders of laterality.[10–12,15] The coding variations reported by Selamet Tierney et al. in individuals with laterality disorders were almost all found in deoxyribonucleic acid from phenotypically normal control subjects as well, although with differences in prevalence according to racial background, and there was apparent linkage disequilibrium between several of the reported coding changes, including C-25T and A61C, A61C and T63C, and T63C and G140A, suggesting that these nucleotide changes may be polymorphic variants.[12] Among the individuals with defects of laterality in whom the coding variations of *CFC1* were found, both in this and prior studies, a multitude of cardiovascular anatomic features and constellations were present, in contrast to the relatively consistent phenotype of *cryptic*$^{-/-}$ mice.[10,12–14] As Bamford et al.[10] acknowledged in their manuscript, "the phenotypes observed in patients with *CFC1* mutations are consistent with heterotaxy, but are not completely concordant with the right-isomerism observed in *Cfc1*$^{-/-}$ mice". This is not to say, however, that *CFC1* is not critical in the normal formation of the left-right axis in humans, or that mutations in *CFC1* do not contribute in an important manner to the spectrum of defects of laterality that occurs in humans. Indeed, some of the mutations found by Selamet Tierney et al.,[12] and in the prior reports by Bamford et al.[10] and Goldmuntz et al.,[15] produce a functionally deficient CRYPTIC protein, the product encoded by *CFC1*, with abnormal cellular localization in transfected cells, and functionally defective protein in a zebrafish rescue assay.

Given that mice with only a single mutated *cryptic* allele are phenotypically normal, it is not surprising that heterozygous mutations in *CFC1*, in isolation, have not proven to be a prominent cause of laterality disorders in humans. The same is true for most other genes that have been implicated in the etiology of laterality disorders, except for *ZIC3*, which is located on the X chromosome, and is associated with X-linked phenotypes, as well as sporadic cases of heterotaxy.[5–9] Ultimately, as several investigators have suggested, a plausible hypothesis for the genetic causes of disorders of laterality is that mutations or polymorphisms in genes involved in the regulation of left-right determination may modify the probability of disordered lateralization, and that involvement of multiple genes may ultimately be required. Environmental and other epigenetic factors may also be important.[16,17] Even in individuals with putative disease-causing mutations, variable penetrance and phenotypic variability support the search for more complex networks of disordered patterning.

Aside from the complexities inherent in dissecting the molecular genetics of laterality disorders in humans, the process of understanding the causes of such defects is complicated by problems of taxonomy and anatomic definition. Supposing that the mutations identified in the studies by Selamet Tierney et al.,[12] and other investigators, are pathogenic for the cardiovascular defects in their subjects, the question remains as to how the specific genetic anomalies relate to the specific cardiovascular anomalies in the affected individuals.

One of the fundamental difficulties in the genetic analysis of complex human malformations is how to group and analyze separate segmental anatomic features. In cases of complex anatomy associated with abnormal laterality, the question is whether to link genetic anomalies with specific cardiovascular anomalies, or with the often "syndromic" constellation of anatomic features. This is a particularly confounding problem when the underlying genetic abnormalities are associated with highly variable penetrance and phenotypic variability. Take *CFC1*, for example. Goldmuntz et al.[15] identified functionally important mutations in *CFC1* in two individuals with abnormally related great arteries but normal visceral arrangement, and hence normal laterality.[15] Does this support the hypothesis that abnormally related great arteries represent a "forme fruste" of disordered laterality, as the authors suggested? Or is anomalous development of the cardiac outflow tracts simply one variable component of the syndromic constellations typical of disordered laterality that may be affected by mutations of *CFC1*, independent of more fundamental perturbations of left-right patterning, possibly according to genetic background or other modifying factors? With respect to cardiovascular anomalies associated with disorders of laterality, this conundrum is particularly relevant to the cardiac anatomic feature most distinctive of such disorders, namely, the morphology of the atrial appendages.

Congenital cardiovascular anomalies associated with disorders of laterality can be among the most complex and clinically challenging defects encountered by the paediatric cardiologist. Among both clinicians and developmental biologists, disorders of laterality are frequently categorized as mirror-imaged arrangement, often called situs inversus, and heterotaxy, alternatively referred to as isomerism or "situs ambiguous". Heterotaxy, in turn, is often reduced to bilateral right-sidedness, which may be termed the asplenic variant or right isomerism, and bilateral left-sidedness, which is also known, alternatively, as the polysplenic variant or left isomerism. Clearly, there are instances in which disordered lateralization and subordinate anatomic anomalies occur according to a relatively consistent pattern, as a result of a defined genetic defect, as in the case of *cryptic*$^{-/-}$ mice.[13,14] In humans, and many other animal models, there is

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usually substantially greater phenotypic variability, and the dichotomous classification of defective lateralization into syndromic variants may be clinically useful on occasion, whether simply as a convenience, or in the service of connoting clinically important distinctions. The scientific validity of a binary classification of heterotaxy into syndromes of left isomerism and right isomerism, however, is suspect. Indeed, such a convention may even stand in the way of deeper understanding. For many years, it has been recognized that human malformations characteristic of mirror-imagery, left isomerism or polysplenic variant, and right isomerism or asplenic variant can occur in different patterns within the same family, suggesting that these apparently distinct "syndromes" may on occasion have a common genetic etiology.[18–22] Is it, then, a mistake to characterize phenotypic patterns as "polysplenia syndrome" or "asplenia syndrome", or even as "left isomerism" or "right isomerism"? Not necessarily, but shorthand classification along these lines is no substitute for specific description of the individual anomalies that are present in a given case. Despite the typical clustering of cardiovascular defects associated with disorders of laterality into syndromic patterns, it is almost always possible to define the anatomy of each cardiovascular segment or structure, whether normal or abnormal, and regardless of the underlying syndromic pattern. Like the term "situs ambiguous", reliance on simple categorization of constellations of cardiovascular defects into syndromic patterns, may do more to perpetuate the impression of ambiguity than to alleviate it. In this respect, it is also the case that description separately of the variously malformed systems of organs is sufficient to remove any perceived ambiguity in "situs ambiguus", particularly when linked with appropriate description of all the lesions within the heart, starting with the arrangement of the atrial appendages.

Unfortunately, it is rarely the case that such details are given of all the relevant structures. Among the various genetically modified murine models characterized by disrupted lateralization, several are notable for isomerism of the bronchopulmonary tree and bronchial-arterial relationship, and, in some cases, of the atrial appendages.[13,14,23] In other cases, these anatomic details are not clearly specified, and it is unclear whether such features were absent or simply not described. Indeed, two of the limitations of the literature describing experimental models of disordered lateralization and studies into the genetics of laterality disorders in humans are the inconsistent nomenclature employed to describe cardiovascular phenotypes, and uncertainty regarding the completeness of cardiovascular anatomic detail. In the experience of Yildirim et al.,[24] also chronicled in this issue of *Cardiology in the Young*, the morphology of the atrial appendages was characterized in only a minority of patients. In the remaining patients, they based the definition of left or right isomerism on the relationship between the aorta and the inferior caval vein, or the azygos or hemiazygos vein. While acknowledging that the morphologies of the atrial appendages are the features most specific for left and right isomerism, and that the position and intactness of the inferior caval vein is not always concordant with, and certainly not pathognomonic of, isomerism of the atrial appendages, lack of data on the morphology of the atrial appendages necessitated the heuristic reliance on the anatomy of the abdominal great vessels for categorization of left and right isomerism. Nonetheless, the conclusion that "the detailed mapping of both cardiac and abdominal morphology in the setting of abnormalities of lateralization, therefore, remains essential", with which they conclude their report, endorses a strict morphologic approach to the definition of laterality disorders.

Despite efforts to classify constellations of cardiovascular anomalies associated with disorders of laterality into discrete syndromic patterns, such definitions will inevitably be approximations, sometimes closer and sometimes more remote, of the complex reality. Understanding the genetic causes of disorders of laterality in humans is a complex process that is progressing slowly, but may be in need of a new paradigm of investigation. A critical component of any strategy that aims to elucidate the genetic and epigenetic causes of complex congenital cardiac disease associated with disorders of laterality will be a compulsive and consistent documentation of the individual cardiovascular anatomic features in patients who are studied. This task will depend not only on progressive molecular genetic and epidemiologic techniques, but on taxonomy, rigorous attention to elemental phenotyping, and statistical approaches tailored to the multivariable nature of the problem. The findings of the studies by Selamet Tierney et al.,[12] and Yildirim et al.,[24] and prior similar studies, are important steps in the quest to find order among disorders of laterality, in no small part because they substantiate the complexity of the task.

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