Unraveling De Novo Copy Number Variants in Congenital Heart Defects
The Bottom of the Iceberg Is Under Attack
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During the 1980s, it was commonly accepted that congenital heart defects (CHDs) were secondary to multifactorial components. Nora and Nora\(^1\) presented a graph of a Gaussian curve with a vertical bar. Individuals of a general population were under the curve with individuals on the left side of the Gaussian curve carrying the least amount of CHD genetic predisposing factors and individuals on the right side carrying the greatest amount of genetic predisposing factors. The vertical bar symbolized the environment. Among the population, only those who were on the right of the environmental bar had a CHD. The bright side of this presentation was that you could easily explain any case of CHD whether sporadic or familial. The negative side of the concept was that it was depressing for those who envisaged deciphering CHD predisposing factors because the large number of environmental and genetic factors suggested by the graph meant that each of them had a small effect.

Contrary to what this diagram suggested, not all cases of CHD are always secondary to multiple small-effect factors. By focusing on exceptional familial cases in the past decades, it has become possible to identify genetic factors that are strong enough to result in an inheritance close to Mendelian inheritance.\(^2,3\) One conceptual prerequisite to this progress was to admit that a single (strong) genetic factor could result in a variety of CHD types, including an incomplete penetrance.

In this issue of *Circulation: Cardiovascular Genetics*, Sanchez-Castro et al.\(^4\) presented the result of a study where 316 trios (unaffected parents and an affected child) had a high-resolution array comparative genomic hybridization, including 76 aortic coarctation, 159 transposition of the great arteries, and 81 tetralogy of Fallot. De novo deletions or duplications were found only in aortic coarctation (2 deletions and 1 duplication) and tetralogy of Fallot (3 deletions and 2 duplications) cases but never in the cases of transposition of the great arteries. Other less appealing copy number variants (CNVs) were reported on the grounds that they contained a coding region and that they were rare (<1% in public databases) but were inherited from a normal parent. One major difficulty in malformations, cardiac and noncardiac, is to be able to infer a causality link between a genomic anomaly and a malformation. Genomic anomalies appearing in an affected child of unaffected parents support a causality link between the 2, but one can also imagine that this novel genomic anomaly has no deleterious effect. It would be presumably wrong to disregard a rare CNV observed in an affected child because it was inherited from a normal parent. Of course, it is less convincing because one has to assume an incomplete penetrance. However, the causality presumption is strengthened when this genomic region is involved in several independent, unrelated cases. Indeed, in the study by Sanchez-Castro et al.\(^4\) overlapping CNVs were observed in 3 genomic regions: 10q24.32, 11p11.2, and 20p11.23 each with 2 patients. Moreover, by searching databases and publications, they found 4 additional CNVs that were overlapping with affected children of their series.

One surprising finding of this study is the fact that among the 15 aortic coarctation patients with an identified CNV, 14 contained a putative binding site for the FOXC1 gene (93%). Altogether, 77% of identified CNVs included at least 1 putative FOXC1-binding site (20/29 in transposition of the great arteries and 19/25 in tetralogy of Fallot). FOXC1 encodes a transcriptional binding protein and has never been involved in nonsyndromic CHD so far but in Axenfeld–Rieger syndrome, a syndrome with anterior segment dysgenesis of the eye leading to glaucoma. Interestingly though, FOXC1 and PITX2 physically interact, colocalized in a common nuclear subcompartment and PITX2A (an isoform of PITX2) can function as a negative regulator of FOXC1 transactivity.\(^5\) PITX2 is involved in many developmental processes, including lateralization. In addition, mice null for the *Pitx2* gene have arrested embryonic rotation and right pulmonary isomerism,\(^6,7\) circumstances where CHD are frequently observed. Could PITX2 and FOXC1 have adjacent DNA-binding sites? Could tetralogy of Fallot, transposition of the great arteries, and aortic coarctation be considered as heterotaxy restricted to the heart field?

This study and others screening for de novo CNVs\(^8,9\) in addition to studies screening for de novo point mutations\(^10,11\) are unraveling a whole new aspect of genetic predisposing factors. It was actually unexpected that de novo mutations could account for such a substantial percentage of CHD cases. Even in familial CHD cases, de novo mutations were...
reported.\textsuperscript{12} Altogether, de novo CNV and point mutations could account for as much as \(\approx 20\%\) of CHD. This percentage is impressive, but what is even more notable is the fact that these discoveries concern essentially so-called sporadic CHD cases that represent the bottom of the iceberg because familial cases are estimated to account for only 4% to 9% of CHDs.\textsuperscript{13} These discoveries offer a new perspective to the understanding of CHD. The prevalence of a genetic disease is influenced both by cases corresponding to new mutations and cases in which patients have survived long enough to be able to transmit their mutations to the next generation. The most deleterious mutations are exclusively de novo, such as in progeria or osteogenesis imperfecta, whereas milder mutations are either recessive or less deleterious dominant mutations, such as founder mutations. On this wide spectrum, CHD is probably located toward the side of severe mutations resulting in a highly pojarative effect on reproductive selection—\textemdash as a consequence—numerous cases secondary to de novo mutations. On a human scale, the development of the medical and surgical care provided to CHD patients is relatively recent (one generation), and even today, the negative effect of selection imposed to CHD patients is strong either prenatally or postnatally.

Of course, the economic feasibility of the screenings done by Sanchez-Castro et al\textsuperscript{9} and Zaidi et al\textsuperscript{10} is currently out of reach in clinical practice. However, if a decreased cost of next-generation sequencing becomes possible, it can be envisioned that performing exome sequencing and CNV on trios for sporadic CHD cases becomes economically feasible. The goal of this process would be to counsel parents on the risk of recurrence for future pregnancies with a much more accurate prediction than by using the average recurrence risk. However, the gap between discovering genomic anomalies and inferring their role in the genesis of a cardiac malformation to the point that it can be used in genetic counseling will take much time to fill.

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**References**

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