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REVIEW TOPIC OF THE WEEK

Advances in the Genetics of Congenital Heart Disease

A Clinician's Guide

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CME Objective for This Article: After reading this article, the reader should be able to: 1) identify patients with congenital heart disease who will benefit from referral to a clinical genetics service; 2) explain the genetic basis of congenital heart disease to affected patients and their families; and 3) compare available genetic technologies and their respective applications.

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ABSTRACT

Our understanding of the genetics of congenital heart disease (CHD) is rapidly expanding; however, many questions, particularly those relating to sporadic forms of disease, remain unanswered. Massively parallel sequencing technology has made significant contributions to the field, both from a diagnostic perspective for patients and, importantly, also from the perspective of disease mechanism. The importance of de novo variation in sporadic disease is a recent highlight, and the genetic link between heart and brain development has been established. Furthermore, evidence of an underlying burden of genetic variation contributing to sporadic and familial forms of CHD has been identified. Although we are still unable to identify the cause of CHD for most patients, recent findings have provided us with a much clearer understanding of the types of variants and their individual contributions and collectively mark an important milestone in our understanding of both familial and sporadic forms of disease. (J Am Coll Cardiol 2017;69:859–70)

CURRENT STATUS OF GENETIC RESEARCH IN CONGENITAL HEART DISEASE

Since the first report specifically addressing the genetics of congenital heart disease (CHD) in the 1950s (1), much research has been devoted to understanding the heritable nature of this condition, including the notable work of James Nora on multifactorial inheritance in the 1960s (2), as well as the landmark Baltimore-Washington Infant Study assessing the epidemiology of CHD in the 1980s (3) (**Figure 1**). Familial forms of CHD have provided most of the genetic information on structural heart disease to date because of their suitability to early research techniques, including linkage analysis and candidate gene approaches. Indeed, many of the genes associated with CHD, including *NKX2-5*, *GATA4*, *TBX5*, *NOTCH1*, and *TBX20*, were identified using these early genetic techniques (4–8); however, a prerequisite to the use of linkage analysis is the existence of large families with multiple affected individuals segregating disease according to Mendelian principles, which is rare in CHD (9).

The development of chromosomal microarray (CMA) technology, including array comparative genome hybridization and single-nucleotide polymorphism (SNP) arrays, in the early 2000s provided a new tool for research in the field (**Figure 1**). Using CMA, a novel candidate gene, *TAB2*, was identified after the identification of an 850-kb deletion on chromosome 6q that was shared among 12 patients with CHD (10). Since then, a number of studies have used CMA to locate novel candidate genes involved in heterotaxy (10), isolated tetralogy of Fallot (TOF) (11), and left-sided CHD (12), as well as novel genomic regions of interest (13). Comparative genome hybridization has largely replaced routine karyotyping in clinical practice as part of the initial assessment of newborns with important CHD. Although CMA has many uses, particularly in the clinical and diagnostic setting, such as excluding diagnoses of trisomy 21, 22q11 deletion syndrome, and other major chromosomal abnormalities, it is fairly limited from a research perspective.

The contribution of somatic mutations as a potential genetic mechanism emerged after the discoveries of the Reamon-Buettner and Borlak research group and the Leipzig heart collection (14,15). This was a highly attractive hypothesis that could explain the clinical presentation of many of the isolated, sporadic forms of CHD. A number of other research teams attempted to replicate these findings using fresh-frozen tissue (as opposed to formalin-fixed hearts) but did not identify any important somatic mutations (16,17). Although subsequent studies suggest that somatic mutations are not a common cause of CHD, there is a possibility that they may play an as yet undetermined role in disease development in a

polygenic or multifactorial setting. Other novel mechanisms of disease have been proposed recently, including the concept of "off targets" of mutant transcription factors, relevant in experimental models of CHD, as well as cancer (18,19).

It was realized, through application of linkage analysis and CMA, that most of the identified mutations were family specific and could not account for the majority of presenting CHD cases, and so the focus of CHD genetic research shifted to common variants. At the time, it was postulated that multiple common variants with small effects most likely underlie common diseases such as CHD, and this became known as the common disease-common variant hypothesis (20). This theory was adopted into our understanding of complex CHD and, after the application of a more advanced study design known as genome-wide association studies (GWAS), led to the identification of associations between a number of genomic regions and CHD risk, including for atrial septal defects (21), TOF (22), and left-sided CHD (23). Associations between variants in specific genes (*ISL1*) and CHD risk were also observed (24). In GWAS, significant associations are identified between a marker SNP in a genomic region and the CHD trait, yet in most cases, we have little understanding of which variants in close proximity to the marker SNP that segregate with a haplotype have a causal role. Furthermore, the identification of a statistical association between a genetic variation and disease does not explain the underlying biology, which leaves many questions regarding the mechanisms involved unanswered. These associations could be highly significant from a statistical perspective but have limited clinical relevance, with low odds ratios. Another limitation of GWAS is the inability to transfer findings to other populations because of allele frequency differences between ethnic groups. One of the biggest limitations, however, is that the SNP genotypes identified by GWAS have only explained a small percentage of the population-attributable risk, for example, only 9% for atrial septal defects (21). As a result, although they contribute to our overall understanding of CHD development, GWAS have failed to explain the majority of genetic variation observed in complex disease, giving rise to the term *missing heritability* (25). After this, the theory emerged that the missing heritability might be explained by rare variants with larger effects acting in conjunction with common variants and environmental factors (26).

ABBREVIATIONS AND ACRONYMS

AVSD = atrioventricular septal defect

CHD = congenital heart disease

CMA = chromosomal microarray

CNV = copy number variations

ECA = extracardiac congenital anomaly

GWAS = genome-wide association studies

MPS = massively parallel sequencing

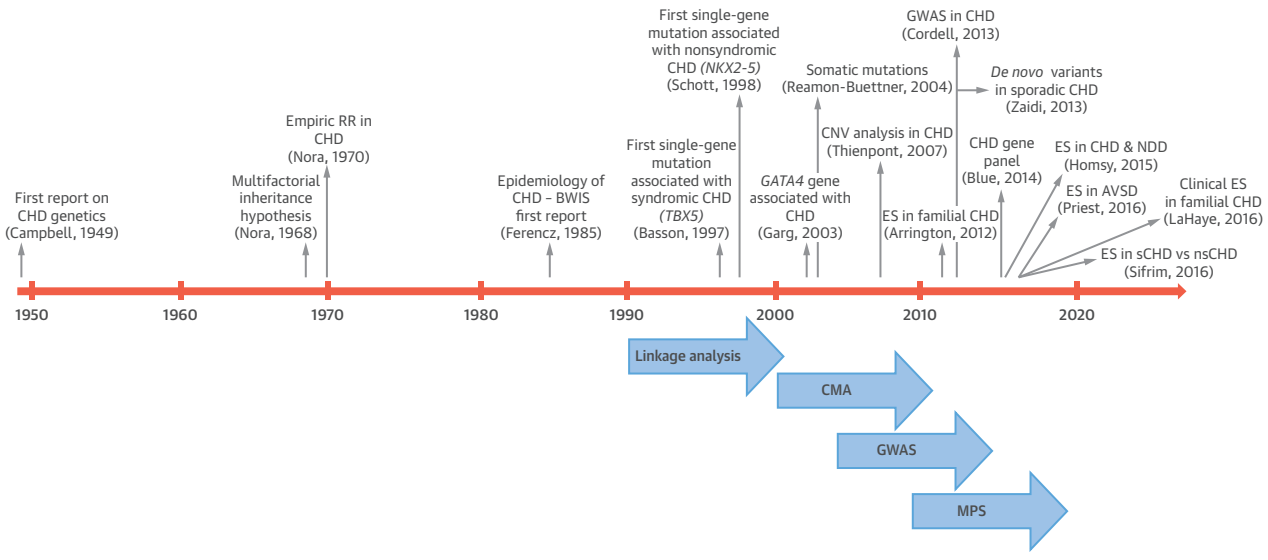
NDD = neurodevelopmental disabilities

SNP = single-nucleotide polymorphism

TOF = tetralogy of Fallot

WGS = whole-genome sequencing

FIGURE 1 Timeline of CHD Genetic Discoveries and the Genetic Technologies and Study Designs Used



Genetic technologies/study designs are indicated by blue arrows and mark the approximate time when the technology was developed and used. AVSD = atrioventricular septal defect; BWIS = Baltimore-Washington Infant Study; CHD = congenital heart disease; CMA = chromosomal microarray; CNV = copy-number variation; ES = exome sequencing; GWAS = genome-wide association studies; MPS = massively parallel sequencing; NDD = neurodevelopmental disabilities; nsCHD = nonsyndromic CHD; RR = recurrence risk; sCHD = syndromic CHD.

TABLE 1 Summary of Recent MPS Study Findings on Sporadic CHD

First Author (Ref. #)	Yr	Cohort	Primary Findings	Comments
Zaidi et al. (34)	2013	Sporadic nonsyndromic CHD (including cases with NDD/ECA)*	• Significant excess of damaging de novo variants in HHE and chromatin-modifying genes • De novo variants implicated in 10% of sporadic nonsyndromic CHD	• Cohort combines cases with isolated CHD and CHD + NDD/ECA • Chromatin-modifying pathway implicated
Homsy et al. (35)	2015	Sporadic nonsyndromic isolated CHD vs. CHD + NDD vs. CHD + NDD + ECA*	• Significant excess of damaging de novo variants in HHE and across all genes • Significant excess of damaging de novo variants in CHD + NDD + ECA and CHD + NDD but not isolated CHD • Shared genetic contributions to CHD, NDD and ECA	• Cohort incorporates cohort from study by Zaidi et al. (34) • Replicated findings from Zaidi et al. (34) • Chromatin-modifying pathway implicated
Sifrim et al. (37)	2016	CHD + ECA/facial gestalt (referred to as syndromic CHD) vs. nonsyndromic isolated CHD	• Significant increase in damaging de novo variants in CHD + ECA/facial gestalt in HHE genes • Significant increase in damaging inherited variants in nonsyndromic CHD in HHE genes and across all genes	• Different cohort with different participant definitions • Confirms findings from Homsy et al. (35) re CHD + NDD/ECA • Additional pathways implicated

*Excluding patients with an established genetic diagnosis/syndrome.

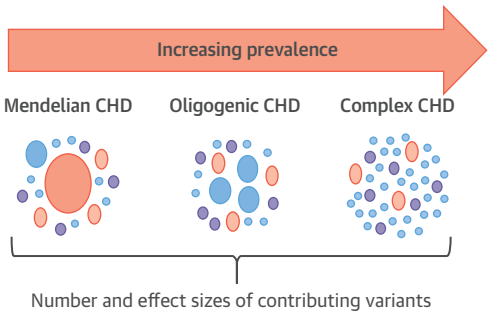
CHD = congenital heart disease; ECA = extracardiac congenital anomaly; HHE = high heart expression; MPS = massively parallel sequencing; NDD = neurodevelopmental disabilities.

Advances in technology provided a potential tool to explain the missing heritability. Collectively termed *massively parallel sequencing* (MPS), this tool can either be targeted (exome sequencing and disease-specific gene panels) or nontargeted (whole-genome sequencing [WGS]). Through its unbiased approach, many sources of variation, including common and rare variants, indels (insertions and deletions), and copy-number variations (CNVs), can be identified with MPS, providing a detailed picture of genomic variation compared with SNP-only data in GWAS (27). Furthermore, by supporting the analysis of individual variants, MPS has enabled research to be directed back to affected individuals and their families, as opposed to the population-applicable results of GWAS. The advent of this technology marked a change in focus from large cohorts with similar phenotypes and ethnic background to studies of well-phenotyped families, more akin to the clinical workflow.

Since its development, MPS technology, specifically exome sequencing, has been applied to both familial and sporadic forms of CHD. Initially, familial forms were expected to be easily "solved" on the basis of the presumed Mendelian inheritance of the heart defects. Although causal variants have been identified in some families (28-32), in practice, there are considerable challenges in applying this approach. Variant interpretation can be challenging, and it is not always possible to firmly establish the pathogenicity of a variant. This is particularly so in small, dominant families, in which the number of candidate variants can be such that it is impossible to filter down to a single, strong candidate. In a recent example, no high-effect coding variants were identified in a multigenerational family with bicuspid aortic valve and other forms of CHD, despite the use of 3 commonly used variant selection strategies and linkage analysis in combination with exome sequencing (33). In some settings, yield can be improved by combining exome sequencing with other techniques, such as homozygosity mapping in consanguineous families (31,32). Together, these studies highlight the difficulties with MPS technology, even in cases with seemingly Mendelian inheritance, in which affected/nonaffected status is easily discerned.

Exome sequencing has also been extended to sporadic forms of CHD (**Table 1**). The first study to apply this tool to sporadic CHD using parent-offspring trios identified a significant increase (odds ratio: 2.53) in de novo coding variants in ~4,000 genes highly expressed in the developing heart in more than 300 cases of severe CHD (34). Enriching for deleterious de novo mutations by first removing missense mutations at weakly conserved sites, followed by removing those at highly conserved sites, thereby leaving only nonsense, splicing, and frameshift mutations, significantly increased the association with disease to odds ratios of 3.60 and 7.50, respectively ($p = 0.001$). A subsequent study in which a significant excess of damaging de novo variants was identified across all genes (enrichment = 1.4), as well as in genes highly expressed in the developing heart (enrichment = 2.4) (35), replicated these findings. The subsequent study also presented important genetic evidence to support the previously suggested genetic link between heart and brain development in nonsyndromic CHD (36). A more recent study applied exome sequencing to both syndromic and nonsyndromic forms of CHD (37). In many of these studies, the term *syndromic* was used to describe clinical perceptions rather than clinical

FIGURE 2 The Number and Effect Sizes of the Contributing Genetic Variants for the Different Inheritance Modes Observed in CHD in Conjunction With Disease Prevalence



Each spot represents a variant contributing to the phenotype and the size of the circle is representative of the effect of the variant on the phenotype. Adapted with permission to CHD from Marian (20). CHD = congenital heart disease.

diagnoses. As such, in this study, syndromic cases were defined as patients with CHD presenting with an extracardiac congenital anomaly (ECA) or a distinct facial gestalt, and not people with an established genetic diagnosis or syndrome. A significant excess of de novo protein-truncating variants was identified in the syndromic cases, whereas the nonsyndromic cases exhibited a significant excess of inherited protein-truncating variants. Exome sequencing has also been applied to specific types of CHD, namely, atrioventricular septal defects (AVSD) (38,39).

Other targeted MPS methodologies, such as targeted gene panels, have been applied to CHD. The first CHD-specific gene panel identified the cause for the heart defects in 31% of the cohort comprising familial, nonsyndromic CHD (40). Interestingly, in just over one-half of those diagnosed, the causal gene was associated with a specific syndrome; however, the phenotypic presentation was primarily cardiac based. In a subsequent similar study, likely causal variants were identified in 46% of the cohort, also comprising familial, nonsyndromic CHD (41). Although gene panels are an attractive alternative in that they mitigate some of the issues associated with exome sequencing, such as inconsistent coverage, lengthy analyses, and large data storage requirements, this comes at the expense of novel gene discovery. "CHD panels" are now available commercially alongside cardiomyopathy and arrhythmia panels. With the declining costs of exome and genome sequencing, opportunities to create "virtual panels" focusing on genes of interest, without specific analysis of unrelated genes, is being realized (42).

Taken together, the studies discussed in the preceding text demonstrate the success of MPS application in CHD; however, they also highlight that for many of these individuals and families, the answer is likely to lie beyond the exome and in the genome, such as in the noncoding regulome (43). It also raises the possibility that some familial forms of CHD, particularly in small families, are oligogenic/polygenic, requiring the additive effects of 2 or more variants for disease manifestation. To date, no published studies have applied WGS to CHD, and it is likely that this more comprehensive analysis will provide further insights into the genetic architecture of both familial and sporadic forms of disease.

CONTRIBUTIONS FROM MPS STUDIES

Aside from the potential personal benefits to affected individuals and their families, MPS studies have also significantly contributed to our understanding of the genetic architecture of CHD. First, they suggest that not all seemingly Mendelian forms of CHD are due to single gene mutations, but could be oligogenic or polygenic. This is evident in families such as the large family segregating bicuspid aortic valve in a seemingly dominant inheritance pattern, noted previously, in which no likely causal variants were identified (33). Although this could be due, among other reasons, to the causal variant residing in the noncoding region of the genome, in which case it could potentially be identified through the application of WGS, the idea that not all seemingly Mendelian forms of CHD are due to a single genetic mutation is an attractive explanation for the reduced penetrance and variable expressivity that often accompany familial CHD.

Second, a novel and common theme among some of the more recent studies is the suggestion of an underlying burden of genetic variation in CHD development. As demonstrated by these studies, individuals with CHD have identifiable additional genetic variation in genes expressed during heart formation, irrespective of whether they are considered complex or Mendelian (34,35,37,39,40). The distinction between Mendelian and complex forms of disease is becoming increasingly blurred and eventually might be viewed as a continuum apart from single-gene disorders (**Figure 2**). As mentioned, this is evident clinically in the reduced penetrance and variable expression often present in familial CHD (8), and it is also highlighted in the many families with enough affected family members for them not to be considered sporadic, but with too few to be considered familial.

Another interesting discovery arising from these studies is the contribution of de novo variants in sporadic CHD development (**Table 1**) (34,35,37). The findings by Zaidi et al. (34) implicate de novo variants in key heart-expressing genes in the pathogenesis of ~10% of sporadic cases. However, although this finding marks a novel and significant contribution to our overall understanding of disease mechanisms for the majority of presenting cases in the clinical world, it focuses attention on the remaining 90% of sporadic CHD that arises from causes other than or in addition to de novo variation in known pathogenic genes. Similarly, Homsy et al. (35) and Sifrim et al. (37) identified a significant excess of damaging de novo variants in CHD cases with neurodevelopmental disabilities (NDDs) or ECA in genes highly expressed in the developing heart and brain. These findings, therefore, implicate de novo variations not only in isolated CHD but in developmental disorders in general.

Finally, the previously suggested genetic link between heart and brain development was demonstrated (35). This is a timely and important discovery, because CHD physicians and surgeons have demonstrated that the likelihood of neurodevelopmental issues in association with complex neonatal CHD depends more on pre-existing factors and less on the patient's clinical course, including specific operative techniques (such as deep hypothermic circulatory arrest) that were previously thought to be highly contributory (44,45). The idea that common genetic factors contribute to both CHD and NDD (36) is also supported by the many genetic syndromes in which both cardiac and NDD occur, such as Williams syndrome, Alagille syndrome, Noonan syndrome, and 22q11 deletion syndrome, among others (46). However, the findings from Homsy et al. (35) provided the important evidence that such a link exists, through the identification of a significant enrichment of damaging de novo variants among published NDD genes with high heart expression in CHD cases with NDD. The marked difference in de novo mutation burden between syndromic and nonsyndromic CHD identified by Sifrim et al. (37) further confirms this finding, because NDD is the most commonly associated extracardiac malformation. Not all cases with CHD have NDD and vice versa, which suggests that in addition to exhibiting pleiotropic effects, these genetic variants also display variable expressivity.

BURDEN OF GENETIC VARIATION MODEL

The concept of *additional genetic burden* in specific genes is evident in a number of diseases, including autism and peripheral neuropathy. Autism is considered a multigenic disorder, with current estimates implicating ~1,000 genes in disease causation, including both rare and common genetic variants (47). In peripheral neuropathy, a significant increase in rare variants in 58 relevant genes in case versus control subjects was demonstrated, with the combined effect of rare variants contributing to mutation burden and phenotypic variability, including disease severity (48). The first evidence of genetic burden in CHD was presented by Zaidi et al. (34). In their study on sporadic CHD, they identified a significant excess of de novo variants in genes involved in histone 3 lysine 4 methylation. Subsequently, an excess of damaging de novo variants was identified in heart-expressing genes, as well as across all genes (35). Since then, a number of other studies have reported additional burden of de novo or rare variants in specific genes in patients with CHD. Gene burden testing on patients with nonsyndromic AVSD identified a significant enrichment of rare variants in the gene *NR2F2* after the analysis of 9 genes with verified de novo variants in 13 trios (38). Using a rare-disease inheritance model in genes previously associated with CHD, Priest et al. (39) identified an increase of inherited and de novo variants in 16 genes in patients with AVSD compared with control subjects. Sifrim et al. (37) proposed a similar model for nonsyndromic CHD, identifying a significant excess of inherited and de novo protein-truncating variants in CHD-associated genes. Interestingly, the significant excess of rare inherited variants was still apparent after the removal of genes associated with CHD and other developmental disorders, which suggests contributions from novel CHD-associated genes. The findings of these studies therefore suggest a combination of de novo and inherited rare variants in disease causation.

Although the focus of the studies mentioned previously was on sporadic forms of disease, genetic burden has also been reported in syndromic CHD patients, specifically trisomy 21 and 22q11 deletion syndrome (49,50). These studies identified an increase in additional variation in syndromic patients presenting with a heart defect compared with those with no heart defects. Surprisingly, additional genetic variation has also been reported in familial forms of CHD. In addition to achieving a clinically actionable molecular diagnosis in 31% of the cohort comprising familial forms of CHD, Blue et al. (40) identified a significant increase in rare and low-frequency variants (minor allele frequency <0.05) in familial CHD case subjects compared with control subjects. Importantly, this difference was observed after the causal variants in 31% of the CHD cases were

removed, demonstrating the presence of additional genetic variation, even in families in which a presumed single causal variant was identified. This surprising finding supports the hypothesis regarding the putative burden of this variation; however, the relative contribution of these additional variants to the development of the heart defect, especially in those individuals or families in which the pathogenic variant was identified as being the sole cause, is unclear at present. One possibility is that these additional variants contribute to the final presentation of the heart defect and could therefore explain in part the variable expression and penetrance often evidenced in families affected by CHD. Furthermore, the burden of genetic variation model for CHD represents a plausible explanation for the occurrence of CHD through the existence of a threshold level of variation, above which other nongenetic factors, such as teratogens and other environmental factors, including stochastic effects, might exert a significant effect.

Additional genetic burden has also been identified in CHD cases with NDD or ECA (35). As noted previously, exome sequencing in 559 CHD cases with NDD or ECA identified a 3-fold enrichment of damaging de novo variants of genes with high heart expression compared with controls and a 4.7-fold enrichment in genes with high heart expression in 138 CHD cases with both NDD and ECA. Importantly, genes with high heart expression were identified in embryonic mouse hearts, not humans, in this study. Although additional genetic burden has been reported in other neurological disorders, such as autism and peripheral neuropathy (47,48), the significant enrichment identified in this study was observed in genes highly expressed in the heart, which suggests that these de novo variants have pleiotropic effects, as well as the potential to affect development of the heart, brain, and other organs. The concept of burden of genetic variation is therefore likely to be a common theme for congenital disorders in general, and further research is required to understand these complex developmental processes and interactions.

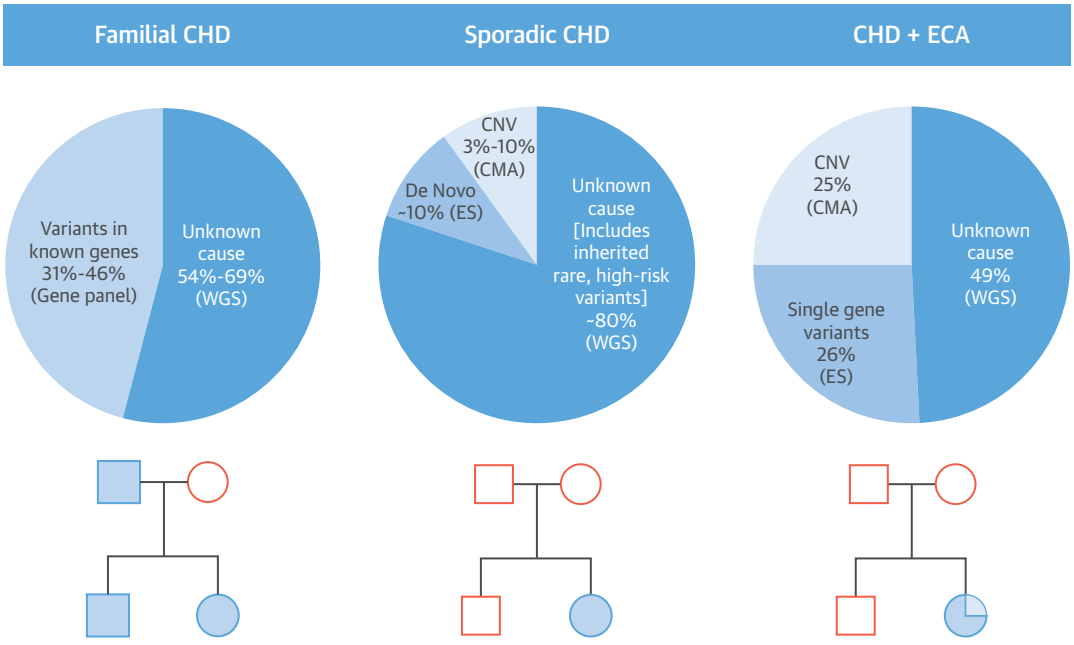
PATIENT GENOTYPES AND CLINICAL OUTCOMES

Understanding the genetic basis of CHD is important for affected individuals and their families in regard to family planning and further clinical management; however, there may be other, wider implications for the affected individual. With the majority of patients born with CHD surviving to adulthood, the focus of research has shifted to improving outcomes post-surgery, treatment, and quality of life of patients. Much focus has been directed to the best ways of providing high quality and consistent care through improving surgical techniques and perioperative care, but variations in patient outcomes remain poorly understood. Chromosomal abnormalities and syndromes are well-described risk factors for mortality and morbidity in neonatal cardiac surgery (51), but subtler genetic variation is also likely to be relevant.

Among the first studies highlighting the importance of patient genotype on post-surgical outcomes in CHD were studies investigating polymorphisms in apolipoprotein E. These studies identified an association between the apolipoprotein E ε2 allele and adverse neurodevelopmental outcomes (52), as well as impaired weight gain (53) post-cardiac surgery in infancy. Since then, a number of other genotypes have been associated with various surgical outcomes. Examples include the carbamoyl-phosphate synthetase I Thr1405NAsn genotype and its association with increased post-operative pulmonary artery pressure (54) and the angiotensin-converting enzyme insertion/deletion polymorphism and the associated increased risk of post-operative tachycardia (55). Cardioprotective effects have also been described, such as the common mitochondrial aldehyde dehydrogenase-2 Glu504Lys genotype, which results in increased tolerance to ischemic and reperfusion injury (56). Similarly, protective variants in hypoxia-inducible factor 1α have been associated with preservation of right ventricular function, less right ventricular dilation, and fewer reinterventions in patients with TOF, which suggests that pre-operative adaption to hypoxia may influence post-operative right ventricular phenotype (57).

Other forms of genetic variation have been associated with post-surgical outcomes. The study by Carey et al. (58) identified a significant association between pathogenic CNVs and poor linear growth, as well as poor neurocognitive outcomes in patients with single-ventricle heart defects. More recently, Kim et al. (59) found that CNVs present in children with isolated CHD were associated with a 2.55-fold increased risk of death or transplantation after surgery. An earlier study by the same group identified a 16-fold increased risk of death or heart transplantation post-surgery in infants homozygous for both the *VEGFA* and *SOD2* SNPs, rs833069 and rs2758331, respectively (60). Collectively, these studies suggest that CNVs, as well as specific genotypes, are important modifiers of post-surgery survival and heart transplantation.

CENTRAL ILLUSTRATION **Genetics of CHD: Percentages of Known and Unknown Causes of the Different Forms of Presenting Nonsyndromic CHD Patients**



Blue, G.M. et al. J Am Coll Cardiol. 2017;69(7):859–70.

Percentages are based on current published reports (34,35,37,40–42,64,66). The molecular technologies commonly used or recommended to identify the various genetic causes are indicated in parentheses. Pedigrees below provide examples of the inheritance modes expected for the different forms of presenting nonsyndromic CHD patients. **Blue circles/squares** represent individuals affected by CHD, and **light blue** represents ECA. CHD = congenital heart disease; CMA = chromosomal microarray; CNV = copy-number variation; ECA = extracardiac congenital anomalies; ES = exome sequencing; WGS = whole-genome sequencing.

The identification of specific genotypes or alleles associated with improved long-term surgical care is a growing field of research, not to mention a field with significant implications for direct clinical management. The ability of MPS to identify both rare and common variants is likely to uncover many more associations between genotype and long-term outcomes, thereby enabling the provision of individualized care and ensuring optimal treatment.

IMPLICATIONS FOR PATIENTS

On the basis of everything we have learned from linkage analysis, GWAS, and more recently, MPS technology, we have a much clearer picture of CHD causation than ever before. Although we cannot yet pinpoint the genetic variant(s) causing heart defects in every patient, we have a much better understanding of the types of variants involved and the roles they play (**Central Illustration**).

In patients with accurately phenotyped familial CHD, we know that there is a 31% to 46% chance of identifying the causal genetic variants and that these very often reside in known CHD genes (40,41). Familial CHD is therefore well suited to targeted gene panels comprising genes commonly associated with CHD as an initial test. Although genetic testing in familial forms of CHD is currently not routine, the chance of achieving a molecular diagnosis with MPS technology is now comparable to that of other genetic conditions (61). Providing a molecular diagnosis not only has implications for psychosocial well-being and future family planning but can also significantly influence the clinical management of some patients with variants in specific genes known to be associated with the future development of conduction defects or cardiomyopathies, such as *NKX2-5*, *TBX5*, and *TBX20* (8,62,63). Although novel or rare variants with large effects are regarded as the "major players" in familial CHD, the final presentation of disease (including the

presence, severity, or type of heart defect) may be determined by additional genetic variation, such as other rare and common variants with smaller effects (33,40). However, we also need to be aware of the possibility that some familial forms with a seemingly Mendelian inheritance might in fact be oligogenic or polygenic in nature.

In patients with sporadic CHD, we know that de novo coding variants in known and novel CHD genes account for a small proportion of cases (~10%) (34,35). Many of these de novo changes were identified in genes involved in the production, reading, or removal of histone 3 lysine 4 methylation, thereby implicating this chromatin-modifying pathway as an important contributor to CHD development (34,35). There is an over-representation of genes associated with gene ontology terms relating to protein phosphorylation, as well as neural tube and cardiac development, in addition to chromatin modification, thereby implicating these as additional pathways of interest (37).

We also know that approximately 3% to 10% of isolated CHD cases are caused by CNVs (64). MPS technologies and chromosomal microarrays are therefore appropriate tools for initial assessment of isolated CHD cases; however, it is important to remember that compared with familial forms of CHD, the chance of identifying a causal variation (including a gene mutation or CNV) is low (up to 10%).

For the remaining ~80% of isolated CHD cases, the inheritance is presumed to be multifactorial, implicating many genetic and environmental factors in disease causation. We know that this genetic component includes a significant excess of inherited rare, damaging variants (37) and that these most likely act in conjunction with other more common variants conferring a small effect, which cumulatively gives rise to the heart defect (**Figure 2**). Environmental and other factors will, of course, also make a contribution. Although WGS is the most suitable tool for assessment, because it covers the entire spectrum of genetic variation, interpretation of findings, particularly in noncoding regions, remains a significant obstacle. For this group of patients, participation in research studies to identify the types of variants and pathways involved in disease development is currently the best approach.

It is well known that patients with CHD, including those with isolated CHD, are at an increased risk of ECA, such as neurological, genitourinary, and craniofacial malformations, among others (65). In patients presenting with ECA in addition to the heart defects, the diagnostic rate of exome sequencing is increased significantly (26%) and comparable to that of other genetic conditions (29.5%) (66). The detection of causal CNVs is also significantly increased (up to 25%) in patients with CHD and ECA (64). For this group of patients, CMA and MPS can therefore be considered as good options from a diagnostic perspective (10,67).

As demonstrated by Homsy et al. (35), we know that some genetic variants can predispose an individual to the development of both heart and brain abnormalities. The significant enrichment in damaging de novo mutations in genes highly expressed in the heart and brain in the CHD plus NDD cohort and the lack thereof in the CHD-only cohort are strongly suggestive of the development of NDD in CHD infants comprising this group of variants. Specifically, this study showed that patients with damaging de novo mutations in 69 genes implicated in both CHD and NDD had a significantly increased risk of NDD compared with control subjects. In particular, damaging mutations in genes involved in chromatin modifiers conferred the highest risk for NDD. Collectively, this information is beginning to pinpoint distinct genes and genetic variants associated with an increased risk of NDD development in CHD patients. The ability to conduct a risk analysis to identify which CHD patients are at increased risk of developing NDD on the basis of their genotypes is fast becoming a reality.

In the study by Homsy et al. (35), the investigators also identified a significant enrichment of genes highly expressed in the heart in CHD cases presenting with ECA other than NDD, including craniofacial, skeletal, and genitourinary malformations. More recently, a genetic link between congenital renal defects and nonsyndromic CHD has been identified (68), thereby providing further evidence for the genetic mechanisms underlying the observed increase in ECA in children with nonsyndromic CHD. Although this was not clarified by the investigators, the renal abnormalities identified in this study could be indicative of syndromic CHD.

The studies discussed describe a new understanding of the role of genetic variation; however, we must be cautious not to over-call the likelihood of a genetic diagnosis on the basis of bioinformatics analyses alone, because there is often little evidence that a variant identified is the cause of disease. By way of example, of the first 15 genes identified in Table 2 by Zaidi et al. (34) as candidate CHD risk genes, 4 have heart defects in null mice, 5 have no heart defect in null mice, and 5 have no supporting mouse data. Bioinformatics predictions of deleterious effects could be wrong in up to 40% of cases (69), particularly when functional analyses or animal models have been established. Requirements for interpretation of

research-generated data, a belief in "big genomics," and affected families' thirst for information are placing increasing demands on clinical genetics services.

CONCLUSIONS

There are likely to be ~400 genes involved in the causation of CHD, many of which are yet to be identified (34,35). Rare inherited variants in genes not previously associated with CHD or associated extracardiac manifestations, including NDD, are likely to be highly relevant (37). MPS technology is therefore likely to reveal many more coding genes associated with and contributing to the intricate developmental network of cardiogenesis. Although WGS is currently still in its infancy, and further refinements in information analysis are required, it is likely to identify contributions of the noncoding regions of the genome to CHD causation, including from regulatory elements and microRNAs (43,70). Furthermore, advances in bioinformatics tools will enable more sophisticated analyses and, together with larger collaborative efforts, provide an opportunity to assess the burden of variation predisposing toward defects in heart development in complex forms of disease.

Epigenetic factors, as well as the interplay between genetic and environmental contributions, such as hypoxia during embryogenesis, are likely to be important contributors (71); however, studies to investigate these factors, particularly the interaction between genetic and environmental contributors, are complex and could require large-scale collaborative efforts. The greatest bottleneck, however, will be in making sense of the information and effects of individual mutations, as well as how they result in or predispose an individual to CHD.

The translation of this information into the clinical workflow and patient care will need to be promoted and refined. A recent scientific statement addresses the requirements for the effective integration of core competencies in genetics and genomic knowledge relevant to specialists in the field (72). For an individual with isolated, sporadic CHD, genetic testing is, at present, a low-yield exercise, but major advances are expected in coming years. However, we can now provide a genetic diagnosis to many more patients with familial and syndromic CHD than ever before, and we have a framework and the technology to evaluate the polygenic burden in common forms of CHD. We now have a unifying hypothesis that may account for many instances of NDD in children with CHD, further establishing the role of genetics in CHD as a relevant clinical focus and not just a scientific work in progress.

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PERSPECTIVES

COMPETENCY IN PATIENT CARE: With advances in genetic technology, molecular diagnostic yield in patients with nonsyndromic familial CHD, as well as CHD and extracardiac congenital anomalies, is comparable to that of other genetic diseases and should be considered. The establishment of a molecular diagnosis has important implications for patient management and family planning.

TRANSLATIONAL OUTLOOK: A genetic and molecular diagnosis is becoming a reality for many patients, particularly those with known syndromes, additional birth abnormalities, or neurodevelopmental delay. Defining the burden of variation underlying complex, sporadic forms of disease is the next horizon.

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