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An interspecies heart-to-heart: Using *Xenopus* to uncover the genetic basis of congenital heart disease

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Abstract

Purpose of review—Given the enormous impact congenital heart disease has on child health, it is imperative that we improve our understanding of the disease mechanisms that underlie patient phenotypes and clinical outcomes. This review will outline the merits of using the frog model, *Xenopus,* as a tool to study human cardiac development and left-right patterning mechanisms associated with congenital heart disease.

Recent findings—Patient-driven gene discovery continues to provide new insight into the mechanisms of congenital heart disease, and by extension, patient phenotypes and outcomes. By identifying gene variants in CHD patients, studies in *Xenopus* have elucidated the molecular mechanisms of how these candidate genes affect cardiac development, both cardiogenesis as well as left-right patterning, which can have a major impact on cardiac morphogenesis. *Xenopus* has also proved to be a useful screening tool for the biological relevance of identified patient-mutations, and ongoing investigations continue to illuminate disease mechanisms.

Summary—Analyses in model organisms can help to elucidate the disease mechanisms underlying CHD patient phenotypes. Using *Xenopus* to disentangle the genotype-phenotype relationships of well-known and novel disease genes could enhance the ability of physicians to efficaciously treat patients and predict clinical outcomes, ultimately improving quality of life and survival rates of patients born with congenital heart disease.

Keywords

Xenopus; congenital heart disease; left-right patterning; heterotaxy; CRISPR/Cas9

Introduction

Congenital malformations, or birth defects, pose a serious threat to global health and the welfare of infants and children. Roughly 8 million infants are born with congenital

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Compliance with Ethics Guidelines

Conflict of Interest

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Human and Animal Rights and Informed Consent

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malformations each year, affecting approximately 6% of births worldwide. These malformations are the leading cause of infant mortality and rank among the leading causes of all pediatric deaths (1–3). Congenital heart disease (CHD) is the most common and the most life-threatening class of birth defects, affecting 9 out of every 1000 live births, or 1.3 million newborns, annually worldwide (4–7). CHD encompasses an array of cardiovascular malformations ranging in severity from critical abnormalities detectable at birth to mild defects which may go undiagnosed into adulthood (8). About 25% of CHD cases are critical, requiring surgical intervention within the first year of life (9). Advances in clinical therapies for CHD have increased the survival rate of patients born with cardiovascular malformations over the last 60 years (10) permitting a continuing increase in the number of CHD patients living on into adulthood (11). This changing demographic presents new challenges for healthcare providers and scientists as adult patients often present with a wide range of extracardiac congenital anomalies (12–14). Given the enormous impact congenital disorders have on global health, it is imperative that we improve our understanding of its genetic etiology and molecular pathogenesis to increase the efficacy of genetic counseling and therapeutics.

Epidemiological evidence suggests that genetic factors are an important cause of CHD; however, our understanding of the genetic landscape of CHD remains regrettably incomplete (15–17). Less than 20% of CHD cases can be attributed to chromosomal defects and Mendelian single-gene disorders (5), suggesting there are vast numbers of unidentified genes which will likely be implicated in CHD. Recent advances in high-throughput human genomic analyses have expanded the bottleneck of genetic information relevant to CHD(14,18–25) and identified over 350 candidate genes that may cause CHD. These studies are efficiently expanding our potential to define CHD by its diverse genetic and molecular causes. However, the scope of these studies is limited by the size of the cohorts analyzed, making it difficult to define disease causality when most candidate genes have only one identified allele in a single subject. In addition, many of the candidate genes identified are novel to cardiac development, embryonic development in general, or simply have no assigned function. Therefore, connecting patient to gene to disease pathogenesis remains unrealized. Developing an *in vivo* screening method to functionally characterize putative disease genes will greatly benefit the research and medical communities. Here, we discuss the merits of the frog model, *Xenopus,* as an outstanding organism in which to study human development and diverse cardiac disease mechanisms. *Xenopus* is also well equipped to serve as a rapid, *in vivo* screening model for the large number of CHD candidate genes identified through patient driven gene discovery.

The current landscape of human CHD genetics

Building a heart during embryonic development is extremely complex and requires a large repertoire of genes whose expression levels must be temporally and spatially regulated, which necessarily means that the genetic etiology of CHD is extremely heterogeneous. Defects in heart development can occur in any of the highly regulated steps of cardiogenesis, such as cardiac progenitor specification, heart tube formation, outflow tract looping, chamber differentiation, valve formation, and atrial/ventricular septation. This heterogeneity makes it difficult to validate candidate genes as disease causing, as it is rare to find multiple

patients with mutations in the same genes with the same specific phenotypes, especially with the relatively modest cohorts analyzed to date. Thus, it is not surprising that our catalogue of CHD genetics is not yet complete.

Even well-defined genetic causes of CHD are associated with a myriad of cardiac phenotypes, making it difficult to define genotype-phenotype relationships. Tremendous variability of patient symptoms associated with the same specific mutations also points to the difficulty of predicting clinical outcomes for patients whose disease mutations are well characterized. For example, a single point mutation in the transcription factor TBX5 can produce a range of cardiac phenotypes from mild Atrial Septal Defects (ASD) to complete atrioventricular canal defects (CAVC) associated with Holt-Oram Syndrome (26). Other well studied genes important for cardiac cell specification and heart morphogenesis, e.g. transcription factors NKX2.5, Gata4, and other T-box family proteins, have also been implicated in an assortment of CHD conditions in human patients such as ASD, DiGeorge Syndrome, Tetralogy of Fallot, and many others (27–30).

In addition, CHD is not only associated with disruptions in genes required for cardiac morphogenesis but also those required for early steps in embryonic left-right (LR) patterning. LR patterning is dependent on cilia function as well as multiple temporally and spatially regulated signaling cascades (14,19,31,32). Here, we will present evidence that *Xenopus* is an excellent model for human development that can help us unravel the genetics of individual cardiac phenotypes associated with CHD, as well as the underlying LR patterning processes required for cardiac morphology. Using a model system to disentangle the genotype-phenotype relationships of well-known and novel disease genes will enhance the ability of physicians to efficaciously treat patients and predict clinical outcomes.

Advantages of *Xenopus* as a model organism

The frog model *Xenopus* is a convenient, reliable, and efficient *in vivo* system for studying human disease. The two species most often used are *Xenopus laevis and Xenopus tropicalis.* Where *X. laevis* is allotetraploid, *X. tropicalis* is a true diploid organism, making it a convenient genetic background in which to perform loss of function studies by gene depletion using antisense morpholino oligonucleotides (MOs) or gene editing approaches. *X. laevis*, however, is unmatched for gain-of-function studies since they tolerate cooler temperatures which slows early cleavages enabling additional time for microinjection and manipulation. Both species of *Xenopus* are useful models for disease genetics, especially as the human and *Xenopus* genomes have long range synteny and a high degree of orthology (33–35).

Although each *Xenopus* species has individual merits, many of the major experimental advantages that have made this system a favorite of embryologists over the last century are shared between them. *Xenopus* breeding is possible year-round (36,37), with large clutch sizes, up to 2000 eggs per day per frog, and easy *in vitro* fertilization providing researchers with a large continuous set of developmentally synchronized embryos (38). These embryos develop externally and are therefore easily visible and accessible for microinjection-based genetic manipulations. The husbandry and housing of these animals is simple, making

Xenopus both a convenient and affordable model system with which to perform large scale experiments including the screening and functional characterization of candidate genes involved in human disease (38).

Xenopus as a model for LR-dependent cardiac morphology and heterotaxy

The establishment of a proper LR axis during early embryogenesis is critical for the positioning and morphogenesis of the heart and other internal organs. Therefore, disruptions in the LR patterning pathway, a syndrome known as heterotaxy, can lead to a myriad of congenital heart defects (39,40). Left-right asymmetry persists throughout heart development, including OFT looping, such that the left and right sides of the heart have very different structures that are essential for its function. The multiple signaling cascades controlling LR patterning are shared across vertebrates, making *Xenopus* a practical model in which to study LR signaling events that dictate organ situs and contribute to CHD (41–47).

During the establishment of the vertebrate body plan, antero-posterior and dorso-ventral patterning define a bilaterally symmetric embryo. This bilateral symmetry is broken due to signaling events in the Left-Right Organizer (LRO). The LRO is a transient structure containing monociliated cells that sits at the posterior tip of the late-gastrula embryo (45,47–51). There, cilia movement drives extracellular fluid flow leftward, which determines proper patterning along the LR axis (48,52,53). This flow inhibits the Nodal antagonist Cerl2 on the left, which activates Smad2 on the left that is transmitted to the left lateral plate mesoderm (54). Nodal signaling at the LRO is required for asymmetric gene expression where it induces a cascade of genes required for situs specification, including Pitx2 (55–60). This patterning cascade is shared from mammals to *Xenopus,* making the frog a convenient model in which to study LR patterning and its effects on cardiogenesis and morphology.

Xenopus as a model for CHD

There are many advantages of using *Xenopus* to model heart development and cardiac diseases. In general, the events of frog heart development show striking similarities to the equivalent events in human heart development (discussed below). In addition, the *Xenopus* embryo does not depend on blood circulation during the first few days of development, enabling phenotype analysis of mutations that in mice result in embryonic lethality. Heart defects are also extremely convenient to phenotype, as the ventral surface of the tadpole is transparent and the heart easily visible just three days post fertilization. Therefore, simple, rapid, and efficient screening for cardiac looping and cardiac morphogenesis is possible in *Xenopus*.

The deep embryological infrastructure already established for studying *Xenopus* development also helps in studying cardiac development. *Xenopus* embryonic development has been studied extensively and its stages are well defined (61). There is also a well-established fate map of *Xenopus* development which facilitates targeted microinjections so precise cell lineages can be exposed to genetic manipulations in the embryo. This fate map makes it possible to characterize and manipulate initial heart induction in the early embryo,

as the two blastomeres that will give rise to the adult heart can be identified by 3 hours post fertilization (62–64). In fact, injection in one cell of the two-cell stage embryo can target either the right or left sides of the embryo making Xenopus unique for studying LR patterning(45). Importantly, many molecular and cellular pathways required for LR patterning and cardiac morphogenesis are shared among vertebrates (discussed in more detail below). Therefore, *Xenopus* emerges as an excellent model in which to study the diverse etiology of congenital heart disease including the genetics of LR patterning (30,44,46).

Current models of cardiac development and CHD in *Xenopus*

As CHD is a disorder resulting from abnormal heart development, it is likely that failure in the genetic control of cardiac initiation and development account for a large percentage of CHD cases. As mentioned above, early stages of frog heart development bear a strong resemblance to corresponding events in mammalian development, and significant advances in understanding of vertebrate heart development have been made using *Xenopus* (62–65). Here we will discuss the parallel events in *Xenopus* and mammalian cardiac development and genetic models that have elucidated explicit phenotype-genotype relationships for genes required for heart development.

Conserved vertebrate cardiac development

Vertebrate cardiogenesis begins at the onset of gastrulation with the specification of cardiac progenitors (66–71). As gastrulation proceeds, the cardiac progenitors migrate to the ventral midline. The fused heart precursors then form a bilayered simple linear tube, with the myocardium rounding up to cover the endocardium. After the formation of the tube, the heart undergoes the looping and remodeling processes of cardiac morphogenesis. After the heart begins to beat, septation and outflow tract (OFT) looping occur. This septation separates the atrium into the left and right halves (68). Just after septation, the chambers begin adopting distinct morphological features such as the thickening of the ventricular myocardium and trabeculation (68,69,72–74). Finally, the outflow valve and AV valve mature, and the fully developed embryonic heart is complete (68,69).

Specific Xenopus models for cardiogenesis-related CHD

Foundational studies disentangling the genetics of abnormal heart morphogenesis and CHD have long been performed using *Xenopus* to great effect. Most of the work in *Xenopus* has been carried out using genetic knockdown techniques such as MOs and overexpression of mRNA of well-known disease genes in the developing embryo. Recent use of *Xenopus* models has expanded our understanding of the molecular etiology of specific CHD syndromes and cardiac phenotypes, enhancing our understanding of elusive phenotype-genotype relationships (75–79). Here we will briefly describe a subset of these *Xenopus* models; however, a more detailed description can be found in the excellent review sited here (30).

NKX2.5 and Gata4—Atrial septal defects (ASDs) are the most common forms of CHD, accounting for ~10% of all congenital heart defects (11). ASDs are characterized by a failure

of the atrial septum to separate the left and right atrial chambers, leading to mixing of oxygen-rich and oxygen-poor blood in the heart. ASDs are often associated with other cardiac abnormalities; however, human patients with non-syndromic ASD have been found to have mutations in the cardiac transcription factor Nkx2.5, implicating Nkx2.5 specifically in cardiac septation during cardiac development (27,28).

Nkx2.5 is a highly conserved homeodomain transcription factor which is required for cardiac progenitor specification but, as was shown in an overexpression model in *Xenopus*, it is not sufficient to initiate cardiogenesis (80,81). Interestingly, overexpression of patient-derived truncated forms of Nkx2 recapitulates the human ASD phenotype, as well as previously described atrioventricular canal and conduction system defects (27,77), supporting the notion that Nkx2 functions in particular aspects of heart development, and that mutation in this gene can lead to predictable cardiac malformations. Early work suggested Nkx2.5 acts in concert with other transcription factors including its co-factor Gata4 (82). Like Nkx2.5, Gata4 is required for proper cardiogenesis but is not sufficient for cardiac specification (82). In accordance with its role in Nkx2.5-mediated heart development, *Xenopus* models of Gata4 knockdown via MO showed a strong reduction in heart precursor cell number during cardiac specification and cardia bifida, which results in later defects in cardiogenesis and morphology (76). These *Xenopus* models have advanced our understanding of explicit roles such major cardiac transcription factors have during cardiac development, allowing for better predictability of patient phenotypes associated with mutations in these genes.

Tbx5 and Tbx20—Members of the T-box family of transcription factors also play an important role in proper development of the heart. Accordingly, mutations in this family of genes have long been associated with a range of CHD phenotypes(83–86). The precise developmental requirements for any singular family member and the mechanism by which each T-box gene functions during development is still unknown, however, ongoing research is beginning to define specific temporal and spatial requirements of each. For example, Tbx5 is required for cardiac specification and septation, (87–89) and *Xenopus* models have helped delineate the effects of disrupting Tbx5 throughout development on different aspects of heart development. In Xenopus, overexpression of a dominant negative Tbx5 results in the loss of heart tube formation and of heart development (89), while knocking down Tbx5 leads to heart-looping defects, a reduction in cardiac mass and pericardial edema, independent of problems with early specification or differentiation of cardiac tissue (87). This suggests that Tbx5-associated CHD patient phenotypes may be dosage-dependent or dependent on specific alterations of Tbx5 function.

The expression of Tbx20, another member of the T-box family, is critical for regulating gene expression in the developing heart (87). Both loss- and gain-of-function mutations in Tbx20 have been found in patients with CHD (90–92) emphasizing that diverse disease alleles of the same gene are associated with various CHD phenotypes. Tbx20 MO knockdown in *Xenopus* results in cardiac looping defects and pericardial edema, and these animals, like the Tbx5 morphants, correctly expressed markers of early cardiac specification, underlining a potential overlap in function of Tbx5 and Tbx20. Additionally, Tbx20 and Tbx5 are known to directly interact (87), underlining the possibility that they act together.

However, the roles of these factors in cardiac development are not uniform. For example, other studies in *Xenopus* have shown that enforced expression of Tbx20a results in the expression of endodermal and mesodermal lineage markers, indicating that this factor regulates both lineage specifying gene expression and later cardiac morphology. Understanding what is required for each of the specific functions of these factors will allow us to better understand the variations in patient phenotypes. One possibility is that the different functions of these factors are dependent on spatially and temporally regulated interactions with one another or other cofactors (87,93,94).

Ets1—Other recently developed *Xenopus* models have elucidated the role of the gene Ets1, a member of the ETS transcription factor family, in specific tissues required for proper cardiac development, specifically in the cardiac neural crest and mesoderm(95,96). Ets1 has long been implicated in CHD due to its location on the region of chromosome 11 that is deleted in Jacobsen syndrome, a rare condition associated with many common CHDs (97,98). Recently, tissue-specific MO knockdown of Ets1 in *Xenopus* show severely disrupted cardiac morphology (96). Specific depletion of gene expression in neural crest tissue results in shrunken and malformed OFTs, whereas specific disruption in cardiac mesoderm led to delays in heart tube formation resulting in the development of single chamber hearts lacking proper ventricular trabeculation and septation in the aorta. The MO-knockdown of Ets1 in the mesoderm resulted in the loss of a proper endocardium. Trabeculae formation was presumably disrupted since their formation require signals from the endocardium(96). The authors hypothesized that the above described morphological phenotypes could be secondary to a disruption in endocardial specification. Thus, they examined the expression levels of specific factors required in the endocardium, including Tbx20, and found them reduced. These results indicate that Est1 has a specific function in endocardial development required for proper cardiogenesis and morphogenesis, and distinct functions in the neural crest cells in which it is expressed (96).

These findings enhance the growing awareness in the field that the effects of genetic aberrations in well-known loci required for heart development are more complex than initially expected. It is somewhat unsurprising, as the work with Ets1 exemplifies, that many of these genes most likely have significant and varied roles throughout cardiac development.

Modeling Patient driven gene discovery candidates associated with CHDs in *Xenopus*

It is clear from the comorbidity of CHD and laterality disease that the developing heart is extremely sensitive to disturbances in LR patterning. For example, a recent forward genetic screen in mice for CHD disease genes identified many cilia-related candidates, demonstrating their importance for proper cardiac development (31). Although the frog has already proved invaluable in implicating the Nodal cascade in laterality signaling (60,99–101), mutations impacting less well studied factors in LR patterning are just beginning to be explored.

With the advent of inexpensive human genomics platforms, disease gene discovery has become highly efficient in human patients, especially *de novo* mutations. Consequently,

longer and more inclusive lists of putative disease genes are being generated, and *Xenopus* is emerging as a useful model in which to functionally analyze and screen these disease gene candidates (14,18,19). Many genes emerging from these studies are implicated in ciliogenesis and LR patterning events, underscoring the fundamental relationship between CHD and LR patterning, but other processes have been highlighted as well, including a chromatin remodeling and transcriptional regulation(102–104).

Using high-resolution genotyping of 262 heterotaxy patients with CHD and 991 controls, we identified copy number variants (CNVs) affecting 61 different genes. Of those that had *Xenopus* orthologues, 7 genes were found to be expressed at the LRO. When disrupted via MO knockdown, 5 of the 7 genes (NEK2, ROCK2, TGFBR2, GALNT11, and NUP188) induced severe cardiac looping defects in *Xenopus* (19). This study was the first to demonstrate that genes outside the classic candidates can be associated with heterotaxy and CHD. Furthermore, this work opened the door to explore novel disease mechanisms of candidate disease genes, which was a first step in expanding the field's thinking about causes of heterotaxy and related CHD. The Pediatric Cardiac Genomics Consortium has been expanding this work and has enrolled more than 10,000 patients for whole exome sequencing and trio analysis (parental and offspring sequence comparison) (18). These are CHD patients with a variety of cardiac defects, not only those specifically associated with heterotaxy. The goal of this research is to elucidate the genetic etiology of CHD. This group recently analyzed 362 probands for potentially disease causing *de novo* mutations and identified many candidates, including many mutations in genes with roles in chromatin remodeling, specifically in histone methylation or ubiquitination (18).

Finally, a more recent and expansive analysis of human patient samples via exome sequencing of 1213 CHD parent-offspring trios has identified an even more extensive list of protein damaging *de novo* mutations in CHD patients. Many of these genes are involved in previously implicated processes including chromatin modification, as well as expected processes such as cardiac morphogenesis and transcriptional regulation. Importantly, findings from this study revealed shared genetics of CHD and neurodevelopmental defects, as well as other extracardiac congenital anomalies often associated with CHD (14).

These patient-driven gene discovery studies provide new depths of understanding of congenital heart disease genetics. As the genetic complexities, underlying heart development are illuminated, the spectrum of syndromic and isolated CHD appears more continuous. The importance of identifying genetic etiology of congenital disorders comes in to even sharper focus in order to understand genotype-phenotype relationships.

Xenopus models of patient-driven gene discovery Heterotaxy candidates

The discovery of a diverse set of genes associated with CHD inspired the analysis of some of these genes for developmental mechanism. In nearly all cases, a mechanism to connect the candidate gene with cardiac development was simply unknown highlighting the need to investigate the mechanism of these candidate genes. For example, a copy number deletion of the N-acetylgalactosaminyltransferase 11 (GALNT11) gene was discovered in the cohort of heterotaxy patients. Through genetic manipulation in *Xenopus* we defined the mechanisms relating this gene to LR patterning and cardiac development(102). We showed that Galnt11

mediates Notch1 signaling and is essential for the proper specification of motile versus immotile cilia in the LRO. When Galnt11 or Notch1 was depleted the relative number of motile cilia increased, while overexpression of Notch decreased this number. These defects in cilia type lead to expected LR patterning defects including improperly looped hearts. This work exemplified the effectiveness of our model for identifying novel molecular mechanisms that drive cardiac and LR patterning starting from patient-driven gene discovery (102).

As another example, we endeavored to define the disease mechanism of a second CHD/Htx candidate gene, Nup188 (19). Depletion of Nup188 led to abnormal heart looping in *Xenopus*, recapitulating the patient phenotype in our frog model. However, the connection to LR patterning was unclear. We showed an unexpected localization of this nuclear pore complex protein Nup188 and its binding partner, Nup93, to the basal bodies of cilia and that MO knockdown results in a loss of cilia at the LRO. These findings suggest that Nup188 at the cilia base is essential for cilia function, LR patterning, and normal cardiac looping. Additionally, this work provides another case where genes identified in patients can help uncover unexpected disease mechanisms that lead to heterotaxy and CHD.

The future of the frog in CHD genetics: Screening disease candidates in *Xenopus* with CRISPR/Cas9

While the studies cited above have been remarkable in identifying potential disease genes, the size and scope of the list presents researchers with the significant challenge of separating CHD candidate genes that affect cardiac development and those that do not. Typically, the identification of different alleles of the same gene in unrelated patients is used as evidence for disease causality, however the vast majority of currently identified genes have only one associated patient allele. This is unsurprising, as LR patterning and cardiac morphogenesis are complex processes which require a diverse repertoire of genes so that high heterogeneity in a patient cohort is expected. Identifying second alleles will require the analysis of massive populations of CHD patients. Such a sequencing endeavor would be a massive undertaking made challenging by the effort to recruit so many patients and the cost in sequencing. However, discovery of mechanisms of cardiac development can still proceed provided the cost for screening these candidate genes is not prohibitive (as it would be in murine models). By utilizing the inexpensive gene editing tool, CRISPR/Cas9, for F0 genetic manipulation of *Xenopus*, we can quickly screen disease candidate genes for phenotype recapitulation in the frog at a cost basis that is amenable to screening hundreds of genes.

Several recent studies have shown that the CRISPR/Cas9 system is highly efficient for producing mutations in both species of *Xenopus* (105–108), and a recent report from our group demonstrates that this system can be used to rapidly and cost effectively reproduce CHD patient phenotypes in F0 *Xenopus tropicalis* embryos(109). Importantly, this report also showed that using CRISPR with Cas9 protein induces gene editing quickly enough to identify early embryonic phenotypes in F0 embryos. This work, along with ongoing large-scale screening of patient disease genes using CRISPR/Cas9 is quickly advancing and

accelerating our understanding of the genetic basis for cardiac development using patient driven gene discovery as a powerful platform.

Conclusions

Considering the consequences of birth defects on global child health and healthcare costs, it is clear there is an urgent need to make diagnostic and treatment technologies more efficient, accessible, and affordable. To do this, we must greatly enhance our understanding of the causes and pathogenic mechanisms of these birth defects. Current advances in human genomics are providing inroads to their genetic underpinnings of CHD; however, these advances must be combined with powerful disease models, such as the frog model *Xenopus*, to flesh out the molecular mechanisms contributing to the occurrence of birth defects. The studies described here of GALNT11 and NUP188, highlight how complex signaling pathways for LR development and cardiac morphogenesis can be better understood when patient driven gene discovery is paired with disease mechanism discovery in an efficacious animal model such as *Xenopus*. Specific models that pick apart genotype-phenotype association with more classic cardiac genes such as Tbx20 and Ets1 also enhances our understanding of specific genotype-phenotype relationships in CHD. As our understanding of the molecular disease mechanisms of well-known and novel disease genes expand, the benefits will be felt not just by those in the research community striving to better understand developmental processes, but by physicians caring for patients living with congenital heart defects.

One ultimate future goal is to have a list of CHD disease genes with related molecular mechanisms and expected outcomes that can be used to annotate sequencing results from patients born with CHD. If we had a complete list of CHD disease genes, we would have a genetic test for CHD. Exome sequencing technology is in hand and is already cost-effective for clinical testing. Further cost gains will only make this even more apparent. However, we currently do not know, which genes cause CHD, and patient-based sequencing often leads to the identification of “variants of unknown significance,” precluding effective genetic counseling.

If we understood the disease mechanisms of CHD-associated genes, we could use this information in clinical practice. Clinicians are well aware that patients who share a specific form of CHD have extremely variable outcomes before, during, and immediately after surgical correction. It is unclear, however, how to best predict these outcomes. Presently, clinicians group patients by phenotype; however, sequencing results suggest that patient genotypes are likely to be diverse within phenotype groups. Therefore, it is possible that categorization of patients based solely on phenotypes underlies clinical difficulties in predicting patient outcomes. With patient genotype information and a reference catalogue of CHD-associated genes, disease mechanisms, and related phenotypes, clinicians could anticipate and adjust medical interventions to reduce the ill impact on the child improving patient outcomes.

Finally, we must engage in new collaborative efforts to truly capitalize on the promise of the human genomics revolution. This collaboration requires an interface between currently

disparate fields of biomedicine. Clinicians see patients and geneticists analyze the exomes from those patients. However, the developmental biologists that can test sequence variants in their model systems to determine their developmental mechanisms are often academically removed from the work of the others. For patient variants to become tested in model systems, we need to foster close collaborations between these groups. This requires breaking down barriers that separate such spheres of biomedicine and enhancing the synergy across them. By enhancing the interface between these spheres, patients seen by clinicians could be enrolled for exomes analysis by the geneticists who could then provide novel genes or variants for testing in model systems by developmental biologists. Functional analysis of patient-derived genes could inform our understanding of pathogenic mechanisms of disease as well as the basic biology of embryonic development, which in turn could provide insights into patient care. We look forward to a time when currently disparate fields of biomedicine work together to drive patient gene discovery forward, enhancing our understanding of cardiac development and evolving effective patient care for infants and children who are the ultimate inspiration for the studies described here.

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