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Transgenerational cardiology: One way to a baby's heart is through the mother

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ABSTRACT

Despite decades of progress, congenital heart disease remains a major cause of mortality and suffering in children and young adults. Prevention would be ideal, but formidable biological and technical hurdles face any intervention that seeks to target the main causes, genetic mutations in the embryo. Other factors, however, significantly modify the total risk in individuals who carry mutations. Investigation of these factors could lead to an alternative approach to prevention. To define the risk modifiers, our group has taken an "experimental epidemiologic" approach via inbred mouse strain crosses. The original intent was to map genes that modify an individual's risk of heart defects caused by an *Nkx2-5* mutation. During the analysis of >2000 *Nkx2-5*$^{+/-}$ offspring from one cross we serendipitously discovered a maternal-age associated risk, which also exists in humans. Reciprocal ovarian transplants between young and old mothers indicate that the incidence of heart defects correlates with the age of the mother and not the oocyte, which implicates a maternal pathway as the basis of the risk. The quantitative risk varies between strain backgrounds, so maternal genetic polymorphisms determine the activity of a factor or factors in the pathway. Most strikingly, voluntary exercise by the mother mitigates the risk. Therefore, congenital heart disease can in principle be prevented by targeting a maternal pathway even if the embryo carries a causative mutation. Further mechanistic insight is necessary to develop an intervention that could be implemented on a broad scale, but the physiology of maternal-fetal interactions, aging, and exercise are notoriously complex and undefined. This suggests that an unbiased genetic approach would most efficiently lead to the relevant pathway. A genetic foundation would lay the groundwork for human studies and clinical trials.

1. Introduction

Congenital heart disease afflicts ~1% of all live births, half of which involve a moderate or severe malformation (Hoffman and Kaplan, 2002). In absolute terms, ~20,000 of the 4 million children born each year in the United States will require medical attention or surgery for their heart defect. Although technical innovations in the latter decades of the 20th century improved survival dramatically, morbidity and mortality remain unacceptably high. For example, hypoplastic left heart syndrome, in which the right ventricle must function as the sole pumping chamber, was lethal before a palliative operation was developed in the 1980's (Norwood et al., 1983). By 2010, transplantation-free survival to one year of age had reached 70% (Ohye et al., 2010). The clinical progress has led to two trends in the current era. First, the rate of improvement in surgical outcomes is decelerating (Erikssen et al., 2015). Technical solutions may be approaching the physiological limits of how well a palliated heart can function. Second, the burden of congenital heart disease is shifting into adulthood. More than 1 million adults in the United States have congenital heart disease, which recently surpassed the number of children (Gilboa et al., 2016). Based on current trends, adults will comprise the majority of hospitalized, congenital heart

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disease patients in a few years (O'Leary et al., 2013). Prevention could have the greatest impact on these trends. On what basis then can a strategy be developed?

Prevention typically addresses the root causes of a disease. The major causes of congenital heart disease are inherited and *de novo* genetic mutations in the embryo. The first genetic mutations, affecting *NKX2-5* and *TBX5*, were discovered in the 1990's (Basson et al., 1997; Li et al., 1997; Schott et al., 1998). By 2014, mutations of several dozen genes were known (Andersen et al., 2014). Just two years later, the authors of a whole-exome sequencing study of 1213 parent-offspring trios estimated that *de novo* mutations of 392 genes contribute to the pathogenesis of congenital heart disease (Homsy et al., 2015). Etiologic heterogeneity limits the impact that a prevention strategy focused on one gene could have. Chromosomal structural variation or copy number variants (CNV) contribute to ~10% of cases (Lander and Ware, 2014). Deleterious CNV duplications and deletions of the same genomic interval highlight the critical role of gene dosage for normal cardiac development (Thorsson et al., 2015). The narrow range over which gene dosage can safely vary suggests that a method that modulates the activity of a gene would have to be precise.

The functions of the genes pose a different set of challenges. Causative mutations typically involve transcription factors, epigenetic regulators and cellular signaling. They disrupt developmental pathways in the heart and often other organs (Andersen et al., 2014). Mutations of the same gene can thus cause either an isolated heart defect or a broader syndrome. For example, heterozygous loss-of-function mutations of *NOTCH1* cause isolated heart defects (Garg et al., 2005; Kerstjens-Frederikse et al., 2016) or Adams-Oliver syndrome in rare individuals. Features of the syndrome include scalp cutis aplasia and transverse terminal limb defects in addition to the heart defect (Stittrich et al., 2014). Why two individuals who carry the same mutation can have either an isolated heart defect or multiple anomalies is not understood. The unpredictability of phenotype suggests that the manipulation of a developmental pathway in the hope of a normal heart would be fraught with risk elsewhere in the body.

The benefits might outweigh the risks of an intervention for some individuals, but it is hard to predict who is at risk. Despite the clear genetic basis, most congenital heart defects occur sporadically; family history is not a sufficiently sensitive or specific predictor of risk (Oyen et al., 2009). Furthermore, a defect forms early during embryonic development. Cardiac morphogenesis is complete by the tenth week of gestation when the heart is about 5 mm in diameter (Dhanantwari et al., 2009). Fetal echocardiography can reveal a defect at 17–18 weeks gestation when the heart is 13–15 mm in Early embryonic abnormalities that progress to severe defects are microscopic. The identification of embryos that could benefit from an early intervention is a giant hurdle.

When viewed solely from the perspective of how and when cardiac development goes awry, the prevention of congenital heart disease on a large scale appears unattainable. A narrow focus on the pathogenic mechanisms only seems to magnify the challenges. On the other hand, individuals who carry deleterious genetic mutations or chromosomal abnormalities commonly have normal hearts. For example, Down and DiGeorge syndromes are strongly associated with congenital heart disease, but one half and one quarter of affected individuals, respectively, have a normal heart (Freeman et al., 2008; Ryan et al., 1997). A rational approach to prevention may be suggested by investigating factors that modify risk in the presence of a genetic susceptibility.

2. The multifactorial basis of a congenital heart defect

Decades before the advent of molecular genetics, Nora sought to explain patterns of congenital heart disease in families and twins who appeared to have a heritable, but not simple Mendelian basis. He reasoned that multiple factors beside the cause modify the quantitative risk of a heart defect in an individual. The multifactorial hypothesis, he argued, provided a useful conceptual framework to investigate and hopefully prevent congenital heart disease (Nora, 1968). Nevertheless, scientists in the following decades mainly focused upon the proximate, monogenic causes. Their tools — from linkage analysis to whole-exome sequencing — are better suited to discovering mutations that have large effect. As the nature of the mutated genes became clear (and their potential for targeting in a prevention strategy arguably less so), Nora's hypothesis has attracted renewed attention. To define the modifying factors, investigators have compared groups of affected and normal individuals, both of whom share the same genetic cause for congenital heart disease.

The human studies to date have mainly focused upon polymorphisms that affect risk in Down (Ackerman et al., 2012; Li et al., 2012; Ramachandran et al., 2015a; 2015b) or DiGeorge (Goldmuntz et al., 2009; Guo et al., 2015; Mlynarski et al., 2015) syndrome. The studies typically include several hundred cases and controls. The sample sizes are large for a study of congenital heart disease but small for human genetic association analysis. Genetic polymorphisms in the VEGF pathway and a couple candidate genes have been implicated as Down syndrome modifiers (Ackerman et al., 2012; Li et al., 2012; Robinson et al., 2003). Common genetic variants do not have a detectable effect, but large, rare CNV deletions may increase risk in Down syndrome (Ramachandran et al., 2015a; 2015b). In DiGeorge syndrome, rare, deleterious variants of histone-modifier genes and CNVs that duplicate SLC2A3 may increase risk (Guo et al., 2015; Mlynarski et al., 2015). The genetic modifiers found so far do not suggest a means to reduce risk. There is conflicting evidence for an effect of maternal genetic polymorphisms in the folic acid pathway and DNA methylation in Down syndrome, but whether folic acid supplementation would reduce risk is unclear (Coppede, 2015). Folic acid supplementation probably does not reduce the risk of congenital heart disease in the general population (Leirgul et al., 2015).

Numerous issues in human studies, such as small sample size, genetic heterogeneity, and unknown environmental factors, limit the ability to detect rare modifiers of large effect or common modifiers of small effect, let alone establish consistent results between studies. Our group has used inbred mouse strain crosses to circumvent these issues, taking advantage of the well-described variability of mutant cardiac malformation phenotypes between genetic backgrounds (Bruneau et al., 2001; Sakata et al., 2006; Rajagopal et al., 2007). We chose *Nkx2-5*$^{+/-}$ mice as the model. Mutations of *NKX2-5* were first discovered in humans to cause congenital heart defects of diverse types (Benson et al., 1999; McElhinney et al., 2003; Schott et al., 1998). *Nkx2-5*$^{+/-}$ mice develop similar defects (Biben et al., 2000; Tanaka et al., 2002; Winston et al., 2010). Naturally occurring genetic polymorphisms in mice account for their strain-dependent phenotypes. Within an inbred strain all mice are genetically identical. In a cross between two strains, only one or two alleles of a gene exist. Systematic crosses can ensure that the frequency of an allele in the population is exactly one-half or one, so rare alleles are not confounding. In addition, the environment in the mouse colony is held constant. Variation in diet or lifestyle, which frequently clouds the interpretation of human studies, does not exist.

We expected that the enumeration of modifiers would require the analysis of thousands of mice. For a project this large, we had to solve logistical challenges related to physiology and anatomy. First, congenital heart defects are normally compatible with survival *in utero*, but certain defects cause death in the newborn period. In the