Guiding Cardiac Conduction With GATA

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Despite remarkable progress in the past few years, the gene regulatory networks underlying formation and function of the cardiac conduction system (CCS) remain incompletely understood. Transcription factors such as Nkx2.5 and Tbx2/3/5 that control various aspects of heart development have emerged as key regulators of cardiac conduction gene expression and function. By showing alterations in the structure of the atrioventricular node (AVN) and the electrophysiological parameters of mice harboring a mutated GATA-binding factor 6 (GATA6) protein, Liu et al.1 add a new player to the growing list of transcription factors involved in cardiac rhythm regulation. This finding provides insight that will help advance efforts to elucidate the pathogenesis of cardiac rhythm disturbances.

In human, cardiac rhythm disturbances are a major cause of mortality and morbidity from fetal to adult life. They can develop in response to numerous conditions, such as electrolyte imbalance, cardiovascular disease including ischemia and pressure overload, structural heart malformations, or as undesirable drug side effects. Arrhythmias and conduction defects can also be because of inherited mutations in genes that affect generation or propagation of the electric impulse of the heart, as exemplified by the long QT Syndrome. How these genetic or acquired factors influence cardiac rhythm is not yet fully understood. In the past years, great strides were achieved for treatment of these important disorders from drug and ablation therapy to implantable devices. However, all treatments have significant short falls and none is curative. A better understanding of the molecular mechanism underlying normal development of the CCS will help unravel the pathogenesis of rhythm disturbances and the development of effective therapies.

Proper heart contraction and relaxation processes are controlled by the CCS, a specialized component of the heart responsible for initiating and orchestrating the propagation of the electric signal required for optimal blood delivery into the circulation. The CCS is composed of slow and fast conducting structures forming the proximal and distal CCS. The pacemaker sinoatrial node and the AVN compose the proximal component of the CCS while the interatrial conduction tracts the His bundle, bundle branches, and Purkinje fibers make up the distal component. Small perturbations in any of the CCS components can lead to drastic outcomes ranging from heart arrhythmias, heart block to sudden death. Understanding the mechanisms of CCS pathophysiology is, therefore, critical.

Our knowledge of CCS development has come largely from studies of transgenic and conditional knockout mouse model, which helped to identify developmental regulators of various CCS components. Human genetic studies, including more recent genome-wide association studies and exon sequencing of candidate genes have also contributed to identifying genes and genetic loci associated with rhythm disturbances. The emerging genetic circuits involved in the formation and function of the pacemaker node include several transcription factors that play crucial roles in many other aspects of heart development. They include Nkx2.5 as well as Tbx5, the gene mutated in Holt–Oram syndrome.2 These same genes also contribute to AVN formation as first suggested by the discovery that familial mutations in Nkx2.5 and Tbx5 transcription factors are associated with atrioventricular conduction defects.3,4 Mouse models with haploinsufficiency in either factor recapitulate the human phenotypes: Nkx2.5 haploinsufficiency results in defective central and peripheral conduction systems along with absence of the AVN subdomain.5 Similarly, Tbx5 haploinsufficiency results in PR interval prolongation6 (the period between the onset of atrial depolarization and the beginning of ventricular depolarization) along with defective patterning of the right and left bundle branches.7 The role of other Tbox proteins Tbx2/3 as well as NOTCH signaling in AVN formation and specification have also been documented.2

Evidence for an important role for the GATA family of zinc finger proteins, and more specifically the cardiac subfamily Gata4, 5, and 6 has been accumulating. First, all the 3 proteins are expressed in cardiomyocytes and cardiac structures and cell types that are important for conduction, including atrioventricular canal myocardium, cardiac neural crest, and cardiac fibroblasts. Therein, GATA proteins regulate many conduction relevant genes, such as those for gap junction and ion channels. They also physically and functionally interact with Nkx2.5 and Tbx2/3/5 to modulate their activity on target genes; as such they act as genetic modifiers of arrhythmia causing genes. Interestingly, mutations in all the 3 proteins have been reported in association with human atrial fibrillation.8–10 The most extensively studied GATA factor in relation to cardiac conduction is Gata4 which was shown to activate Cx30.2,11 as well as Cx40, and several atrioventricular canal enhancers.12,13 Consistent with this, mice heterozygote for Gata4 were reported to have shorter PR.11

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No studies, to date, have directly linked GATA6 with regulation of the CCS but previous studies showed that an enhancer region upstream of the GATA6 promoter is specifically active in the Atroventricular conduction structure. GATA6 was also shown to activate the NCX1 and Kv4.2 promoters. Localized on chromosome 18 in human and in mice, GATA6 is expressed in myocardial, neural crest, and endocardial cells as well as in vascular smooth muscle cells. Consistent with an important role for GATA6 in heart morphogenesis and outflow tract development, many mutations in the human gene were reported in association with a large spectrum of congenital heart diseases, including atrial and ventricular septal defects, Tetralogy of Fallot and Patent Ductus Arteriosus.

In this issue of *Circulation Cardiovascular Genetics*, Liu et al report that in addition to its crucial role in heart morphogenesis, intact GATA6 is required for normal development and functional maintenance of the AVN. GATA6 was found to be abundantly expressed in the proximal CCS at E12.5 to E16.5 and its expression overlapped with TBX3, a marker of the atroventricular conduction system, and with the transcriptional repressor ID2. To determine if GATA6 contributes to CCS, a mouse model with myocardial-specific deletion of the carboxyl zinc finger domain of Gata6 under the control of the myosin light chain 2v (MLC2v) promoter, was generated resulting in truncation of GATA6 in the ventricular myocardium, right ventricular outflow tract, and atroventricular annulus. ECG analysis performed in young animals revealed prolonged PR intervals and AVN defects in these mutant mice. The myocardial-specific mutation of GATA6 led to defects affecting only the proximal atroventricular conduction but not the infranodal CCS; these defects included AVN hypoplasia, probably because of reduced cell-cycle exit of TBX3+ myocytes in the atroventricular myocardium and defective myocyte differentiation. Myocardial-specific deletion of the carboxyl zinc finger domain of Gata6 was also shown to lead to downregulation of the transcriptional repressor ID2 in the proximal CCS of mutant embryos, as well as in the AVN and lower nodal region of adult mutant hearts. Transfection experiments confirmed that the Id2 promoter is directly activated by GATA6.

This work provides, for the first time, experimental evidence linking GATA6 to AVN formation and function and represents an important contribution to our evolving understanding of CCS in development and disease.

Although the work clearly shows that intact GATA6 is essential for proper AVN development, many questions about the cellular and molecular mechanisms underlying this effect remain to be answered. A role for GATA6 in the AVN is in line with its involvement in atroventricular canal development as demonstrated by the use of the same floxed allele to delete myocardial Gata6 more broadly using Nkx2.5-cre mice. This led mostly to ventricular septal defect, whereas mice with MLC2v-cre–mediated deletion did not apparently have any such structural cardiac defects. Given that Nkx2.5 is expressed in the proximal CCS, this raises interesting questions about temporal as well as spatial GATA6 function. The exact cell type(s) contributing to GATA6-dependent AVN defects will likely need to be directly addressed through additional cell-specific deletion of Gata6 including in CCS cells.

Equally intriguing is the absence of rhythm disturbances in Gata6 heterozygote animals. Whether this reflects dosage sensitivity or whether truncation versus loss of GATA6 leads to differing phenotypes will need to be ascertained. Such possibility might be of great relevance for understanding the effect of different GATA6 genotypes on their associated phenotypes.

Finally, the mechanisms by which loss of the C-terminal half of GATA6 alters AVN size and gene expression requires further investigation. The remaining truncated GATA6 protein contains functional domains, including the N-terminal activation domain and the N-zinc finger. By analogy to GATA4, such protein would have a nuclear localization, maintain the ability to interact with critical cardiogenic cofactors, including BAF60c and other GATA proteins or GATA regulators such as FOG 2. This, in turn, will affect the activity of such important AVN regulators as Nkx2.5 and TBXs. Thus, the observed mouse phenotype may reflect the combined role of GATA6 and that of other transcription factors in CCS cells. Similar manipulations using another available Gata6 floxed allele would help to clarify the exact role of GATA6 in cardiac conduction.

Notwithstanding these mechanistic considerations, the finding that intact GATA6 is required for proper AVN function represents an important contribution to understanding the intricate regulation of the cardiac pacemaker. Prolonged PR intervals may indicate first degree heart block and are an undesirable side effect of several widely used drugs including calcium channel and β-blockers. Prolonged PR interval doubles the risk of atrial fibrillation and triples the risk of needing a pacemaker device according to the Framingham Heart Study. The identification of a new regulator of the PR interval has potential applications in many sectors including pharmacogenetics where much work is still needed.

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**References**


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