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Genetics and Sinus Node Dysfunction

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Introduction

Sinus node dysfunction (SND) is commonly encountered in the clinic. The clinical phenotype ranges from asymptomatic sinus bradycardia to complete atrial standstill. In some cases, sinus bradycardia is associated with other myocardial conditions such as congenital abnormalities, myocarditis, dystrophies, cardiomyopathies as well as fibrosis or other structural remodeling of the SA node.[1-8] Although there are many etiologies for symptomatic slow heart rates, the only effective treatment available today is the implantation of a pacemaker. The predominant ion channel currents contributing to the pacemaker activity in the sinoatrial node (SAN) include currents flowing through hyperpolarization-activated, cyclic nucleotide-gated (HCN) channels,[9] L- type Ca, T-type Ca,[10] delayed rectifier K,[11,12] and acetylcholine (ACh)-activated[13,14] channels. However, their relative contribution remains a matter of debate and the cellular mechanisms contributing to abnormal sinus node function leading to bradycardia are not fully elucidated. Sodium channel current (INa), encoded by SCN5A, is responsible for the cardiac action potential (AP) upstroke and therefore has an important role in initiation and propagation of the cardiac action potential. Although it is largely absent in the sinus node, it plays an important role at the periphery of the sinus node in transmitting electrical activity from the sinus node to the rest of the atria.

Mutations in genes encoding structural anchoring proteins (ANK2, Caveolin- 3, AKAP9) have been associated with the development of atrial as well as ventricular arrhythmias.[15, 16, 17] Sinus node dysfunction has been associated with a variety of atrial tachyarrhythmias, atrial fibrillation (AF) in particular. In recent years, numerous publications have focused on the genetic basis for ion channels and structural protein remodeling, providing further insights in the mechanisms of sinus node dysfunction and its role in AF. In this review, we will focus on the genetic aspects of the various forms of sinus node dysfunction and their relation to AF.

HCN4

Mutations in the gene encoding the HCN4 ion channel have been shown to be associated with inherited sinus bradycardia.[18-21] HCN4 encodes the protein that contributes to formation of If channels, which participate in spontaneous diastolic membrane depolarization of sinoatrial node cells.[22, 23, 24, 25] Modulation of these channels by cAMP is believed to be responsible for acceleration of heart rate.[23] Four HCN gene family members have been cloned, three of which are present in heart (HCN1, HCN2, HCN4). HCN4 is the most prominent HCN transcript in the atria, whereas HCN2 is the dominant transcript in the ventricle.[9, 26] SA cells from knock out mice lacking HCN4 have 75% less If and SA cells from mice lacking HCN2 have 25% less HCN current.[27, 28, 29, 30] Of note

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in humans, HCN2 and HCN4 were found to be the dominant mRNA transcripts.[31]

To date, five HCN4 mutations have been reported in humans. Two described symptomatic patients with malignant syncope.[19, 32] One of these patients also suffered from bouts of AF.[19] In this case, the patient had a stop codon resulting in the deletion of the cyclic nucleotide binding domain (CNBD), making the mutant channels insensitive to cAMP. In the second case,[33] a missense mutation in HCN4, affecting trafficking of the mutant channel, segregated among family members with a prolonged QTc. The proband had an episode of torsade de pointes (TdP). The basis for association of a prolonged QTc with a decrease in HCN4 current amplitude is unclear, since a loss of function of If is not expected to prolong the QT interval, other than through a reduction in heart rate. Studies in both humans[34, 35] and mice[36] have not observed prolongation of the QTc in response to If blockers.

Two large families with mutations in HCN4 causing asymptomatic bradycardia have been reported by us and others.[18, 21] A missence mutation (S672R) was found by Milanesi et al.[18] to be associated with asymptomatic bradycardia. Despite its location in the CNBD, this mutation did not affect the binding properties of cAMP, but changed the biophysical properties of the channel. Mutant channels deactivated slower and the voltage-dependence of activation shifted in the hyperpolarizing direction, leading to a decrease in If , responsible for the slowing of the heart rate.[18, 37]

We described[21] a family with asymptomatic sinus bradycardia with no extracardiac abnormalities, managed conservatively during long term followup (14±11years). All affected family members were asymptomatic with normal exercise capacity during long-term follow-up. Electrophysiological testing performed on 2 affected family members confirmed significant isolated sinus node dysfunction. Genetic analysis revealed a missence mutation (G480R) in the HCN4 channel pore. Our in vitro expression studies suggested that sinus bradycardia in affected family members was likely due to combined synthesis and trafficking defects as well as altered biophysical properties of the mutant HCN4 channels.

We recently[38] identified 2 families with symptomatic bradycardia. Affected members presented with a history of presyncope, except for one subject who had a poorly documented event of loss of consciousness with apparent cardiopulmonary arrest, which resolved following basic CPR; he recovered without defibrillation. There were no documented events of syncope and all had a normal exercise test. Sequencing of the HCN4 gene in the probands of these families revealed a new heterozygous A485V missense mutation within the pore-forming region of the channel. A485 is a conserved residue not found in 50 controls. We are currently extending the genetic analysis to exclude other genetic variations.

The common feature of these familial bradycardia cases is a relatively benign prognosis and lack of chronotropic incompetence. These findings are in partial agreement with a study conducted in the adult HCN4 knockout mouse model reported by Herrmann et al.[39] Like the families described, the knockout mice had no impairment of heart rate response during exercise. However, instead of bradycardia, they displayed sinus pauses. Taken together, the available animal and human data suggest that while If may be a major contributor to diastolic depolarization at rest, its contribution to the positive chronotropic response of sympathetic stimulation is less clear.

One patient with an HCN4 loss of function mutation had documented AF.[19] It is not clear whether this association is merely a coincidence or whether this genetic defect can predispose to AF. From a theoretical point of view, a loss of function of HCN4 channel current should depress phase 4 of the sinus node action potential as its principal effect and would not be expected to cause any type of atrial arrhythmia. Indeed, over expression of HCN4 in the atria can lead to atrial ectopy leading to initiation of AF. In a canine heart failure model,[40] HCN4 channels were found to be downregulated in the SN but up regulated in the right atrium. The authors suggested that atrial HCN4-up regulation may contribute to the increased incidence of atrial arrhythmias in heart failure patients.

SCN5A

Mutations in SCN5A, the gene that encodes the

α subunit of the cardiac sodium channel, have been associated with several rhythm disorders including Brugada syndrome,[41] long QT syndrome type 3 (LQT3)[42] and cardiac conduction disease. Although SCN5A does not play a prominent role in sinus node activity, loss of function mutations may lead to bradycardia[43-49] by reducing excitability and impairing conduction of impulses generated in the sinus node into the atria. Recent studies using Tetrodotoxin (TTX) a selective Na+ current inhibitor have demonstrated that TTX can abolish the action potential upstroke in the periphery of the SA node but not in the center of the SA node.[50] These studies point to the lack of participation of NaV1.5 in the conduction of the electrical impulse through the SA node proper.

Loss of function mutations in SCN5A can lead to a widening of the of the P- wave, prolongation of PR interval as well as widening of the QRS interval in the surface ECG.[43-49] Changes in these parameters are often encountered in patients with SCN5A-mediated Brugada syndrome[51] and are due to depression of INa-mediated parameters. In some cases the same mutation has been shown to lead to both LQT3 and Brugada[52] in the same family or to a combination of progressive cardiac conduction disease and Brugada syndrome[53] or LQT3, Brugada ECG and sinus node dysfunction.[44] This overlap of three syndromes was reported to be the result of a 1795insD SCN5A mutation, which is associated with an increase in late INa , responsible for the LQT3 phenotype, and a negative shift of the voltage-dependence of inactivation, which causes a loss of function of INa responsible for the Brugada and sinus node dysfunction phenotypes.[44] Using a mathematical model of an SA node action potential, the authors argued that the persistent late INa coupled with the negative shift in the voltage-dependence of inactivation caused a prolongation of APD and a slowing of phase 4, which together are responsible for the bradycardia or sinus node dysfunction. Interestingly, the sea anemone toxin, ATX-II, a compound known to increase late Ina, has been shown to induce a prolonged P-R interval and SA node recovery time as well as LQT3 in intact mouse hearts.[50]

In another report,[40] compound heterozygous SCN5A mutations were found to be associated with sick sinus syndrome (SSS). Mutation carriers exhibited symptomatic sinus bradycardia progressing to atrial inexcitability, necessitating permanent pacing. Affected carriers also had prolonged HV and QRS intervals. Biophysical characterization of the mutant sodium channels in a heterologous expression system demonstrated loss of function or significant impairments in channel gating (inactivation) that predicted a reduced myocardial excitability. Here again, sinus bradycardia may be the result of failure of the impulse to conduc into adjacent atrial myocardium.[40] Another possibility may be that INa has a direct effect on the SN as suggested by Veldkamp et al.[44]

AF and other supraventricular tachycardias (SVT) have been shown to be associated with an SCN5A mutation (D1275N) segregating among family members with conduction disease.[44, 54, 55] In 2 of these reports, in addition to the above, affected members also presented with a dilated cardiomyopathy. Dilated cardiomyopathy was preceded by AF, SN dysfunction and conduction block.[55] The extent to which SCN5A mutations are related with AF is not clear. It is possible that AF is secondary to structural changes associated with SCN5A mutations, in addition to direct reduction of INa. Interestingly, Brugada patients are also known to have a relatively high incidence of atrial arrhythmias, AF in particular[56-60, 61] whether or not the syndrome is caused by SCN5A mutations.[62] In the SCN5A-related Brugada syndrome cases, as in SCN5A-mediated sinus node dysfunction, AF may be in part related to an abnormal "substrate" in the form of fibrosis as well as the "electrical" impairment.

Recent studies have reported major difference in the characteristics of the sodium channel between atrial and ventricular cells in the canine heart.[63, 64] These studies showed that steady-state inactivation of the sodium channels was 9 to 16 mV more negative in atrial vs. ventricular myocytes. This distinction coupled with the more positive resting membrane potential of atrial cells, suggests that a large fraction of sodium channels are inactivated and unavailable at the normal resting membrane potential of atrial cells. Consequently, the impact of some SCN5A mutations may be much greater in atria than in ventricles, predisposing to the development of arrhythmias more readily in the atria vs. ventricles.[61]

Connexin

Connexins are gap junction proteins responsible for electrical communication between cells. Connexin (Cx) 40 (encoded by GJA5) is specific to the atria, whereas Cx43 (GJA1) and Cx45 (GJA7) are also expressed in the ventricle.[65] Cx40 defects were found to be associated with AF [66] and atrial standstill (AS).[41] In a study[66] involving patients with idiopathic AF, 4 out of 15 patients were found to have mutations in GJA5. Interestingly, 3 of them had tissue specific mutations and in only one was the mutation also present in lymphocytes, indicating that the first two are somatic mutations. Analysis of the expression of mutant proteins revealed impaired intracellular transport or reduced intercellular electrical coupling. This gives rise to regions of heterogeneous conduction, providing a substrate for atrial arrhythmias. Of note, none of these patients had a prolonged P wave during sinus rhythm. There are no data on the PR interval in these patients. All of them had a normal QRS interval, which is consistent with the fact that Cx40 is not present in the ventricle. In 2003, a large family with progressive atrial standstill was reported.[41] Symptoms started in the late twenties to thirties and progressed to total AS necessitating implantation of a pacemaker. Genetic analysis revealed that affected individuals in the family inherited both the D1275N missense mutation in SCN5A. In addition, two closely linked polymorphisms were identified in the regulatory regions of the gene for connexin40 (Cx40) leading to a loss of function. The D1275N SCN5A mutant channels shifted the activation curve to more positive voltages, predicting a loss of function of INa and consequently reduced excitability. It is noteworthy that family members with D1275N alone or the rare Cx40 genotype alone were not clinically affected. Thus, familial AS in this case was associated with the occurrence of a cardiac sodium channel mutation and rare polymorphisms in the atrial-specific Cx40 gene. Although the functional effect of each genetic change is relatively benign, the combined effect of the genetic variants led to the development of AS. Prior to AS, 2 of the 4 affected family members had atrial arrhythmias. Thus, at present evidence exists that mutations in Cx40 can cause AF, but there is no evidence indicating that Cx40 mutations alone can result in AS.

KCNQ1

KCNQ1 encodes the α subunit of the voltagegated slowly activating delayed rectifier K+ channel responsible for IKs. Mutations in this gene have been linked to LQT1 (loss of function),[67, 68] Short QT (SQT) 2 (gain of function)[69] and AF (gain of function).[70] In 2005, Hong et al. reported a case in which a de novo mutation in KCNQ1 (V141M) was responsible for AF and SQT.[71] Interestingly, the child carrying this mutation was born with severe bradycardia. In a computerized model, this mutation caused a shortening of APD. The abbreviated APD in the ventricle explains the SQT phenotype while the abbreviated APD in the atrium can explain the AF phenotype. In addition, the enhanced outward IKs in sinoatrial cells, could lead to a shift of resting membrane voltage to more negative potentials. The authors speculated that this might slow or halt spontaneous firing of the SN cells in vivo causing AS. Of note, this is the only KCNQ1 mutation reported to affect SN cell activity. The absence of P waves associated with irregular heart rate in this child theoretically may actually be due to AF and not to AS. Accordingly, the slow ventricular response may be due to a diseased AV conduction rather than slowed SN activity.

ANK2

Protein encoded by the ANK2 gene is a member of the ankyrin family of proteins that link the integral membrane proteins to the underlying cytoskeleton. Ankyrins play a role in activities such as cell motility, activation, proliferation, contact and the maintenance of specialized membrane domains. Ankyrin-B (ANKB or ANK2) is a membrane adaptor protein. Mutations in this gene have been identified as the cause of LQT4.[15, 72, 73] Mutations in this gene increase the total intracellular calcium by reducing the expression of Na/ K ATPase and Na/ Ca exchanger in the face of unchanged Ca 2+ entry by ICa. This increase is most probably responsible for the early after depolarization (EAD) and delayed after depolarization (DAD) seen in knockout AnkB+/- mice leading to polymorphic ventricular tachycardia.[15] LQT4 has several distinctive characteristics compared to other LQT syndromes. AnkB is located in both atria and ventricular cells,[72] therefore it is not surprising that patients carrying mutations in this gene exhibit a variety of symptoms, including sinus bradycardia, sinus arrhythmia, catecholaminergic ventricular tachycardia, SCD and AF.

In the first large family described, 12 out of 25 affected family members had AF.[15] In this family, all of the affected members as well as all of the knock-

out AnkB +/- mice displayed sinus bradycardia. Interestingly, QTc prolongation is not as severe as in other LQT syndromes[15] and in some the QTc interval is within normal range.[72] Recently, two families with sinus node dysfunction and AF have been associated with mutation in ANKB.[74] Heart rate was lower than 50 bpm in all affected adult individuals. In one family, the rhythm originated in the SN in 7/ 25 affected family members, from the coronary sinus in 7/ 25 and as junctional escape in 11/ 25. Thirteen of them had AF. In the second family, the rhythm originated from the SN in 10/ 13 and from the coronary sinus in 3/ 13 members. Three of them had AF. The prevalence of AF in both families increased with age. The precise mechanism underlying ANKB mutationmediated sinus bradycardia and AF is not known. One possibility is that the EADs and/or DADs observed in the ventricles of knockout AnkB +/- mice may also develop in the atria. EAD and DAD have been shown to initiate AF in animal models.[75-77] Another possibility may be that both AF and SN dysfunction are a consequence of conduction delay providing the substrate for micro-reentry.

If one takes in account the different interactions ANKB can have with a wide variety of ion channel proteins and transporters, the potential for modulating cardiac function and dysfunction is great and may explain the widely varied phenotypes seen in patients carrying ANKB mutations.

EMD

EMD encodes the nuclear membrane protein emerin. Mutations in this gene and lamin A can lead to Emery-Dreifuss muscular dystrophy (EDMD). These nuclear proteins are thought to take part in maintenance of the nuclear envelope structure and in regulation of gene expression.[78, 79] EDMD is characterized by early contractions of elbows, neck extensors and Achilles tendons, rigid spine, slowly progressive humero- peroneal muscle wasting and weakness, and cardiomyopathy with AV conduction block.[78, 79] AF has been associated with EMD in a large family with EDMD.[78, 79] In this family, a mutation (Lys27del) in EMD was sufficient to produce the cardiac phenotype that involved conduction abnormalities in all affected individuals and AF in most. Those exhibiting the full EDMD phenotype had an additional mutation in lamin A. Recently a Lys27del in EMD was associated with SN dysfunction and AF.[80] Four males presented with SSS and subsequently developed AF. All of them had symptoms of bradycardia, including syncope, in their teenage years. Four asymptomatic females were found to have only non-sustained supraventricular tachycardia events and sinus bradycardia. While all males received a permanent pacemaker at their fourth to eighth decade of life, none of the affected female members needed pacing. This discrepancy between males and females is explained by the fact the EDMD is an X-linked recessive trait. Interestingly, although in affected males there was a near total lack of emerin staining in buccal epithelial cells, none of them developed contractures or muscle weakness. Thus, Lys27del- EMD has for an unknown reason a cardio-selective effect. Emerin is involved not only in maintaining nuclear membrane integrity[81] but it was also found to be associate with the intercalated disk in cardiac muscle.[82] This structural role may provide the substrate for AF and conduction disease.[83]

Conclusion

Genetic defects in ion channels as well as structural proteins have been shown to contribute to sinus node dysfunction. In most cases, there is also a clear association with clinical AF. Even in cases in which one-mutation produces both clinical conditions, the mechanisms responsible may not always be the same. In most of the cases discussed, the clinical presentation of SN dysfunction and AF do not appear concurrently. One typically precedes the other by several years, pointing to multiple pathways by which a genetic mutation may affect the electrical system of the heart. Although, SN dysfunction and AF are both very common clinical conditions, the precise mechanism responsible for each remains a matter of some debate. Recent molecular genetic findings have provided new insights into the pathophysiological basis for atrial arrhythmias and SN function and dysfunction that hopefully will guide us to improved diagnosis and approaches to therapy.

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