Published in final edited form as:

Heart Lung Circ. 2017 June ; 26(6): 612–618. doi:10.1016/j.hlc.2016.09.013.

A Novel Mechanism for Human Cardiac Ankyrin-B Syndrome due to Reciprocal Chromosomal Translocation

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Abstract

Background—Cardiac rhythm abnormalities are a leading cause of morbidity and mortality in developed countries. Loss-of-function variants in the *ANK2* gene can cause a variety of cardiac rhythm abnormalities including sinus node dysfunction, atrial fibrillation and ventricular arrhythmias (called the “ankyrin-B syndrome”). *ANK2* encodes ankyrin-B, a molecule critical for the membrane targeting of key cardiac ion channels, transporters, and signalling proteins.

Methods and Results—Here, we describe a family with a reciprocal chromosomal translocation between chromosomes 4q25 and 9q26 that transects the *ANK2* gene on chromosome 4 resulting in loss-of-function of ankyrin-B. Select family members with ankyrin-B haploinsufficiency due to the translocation displayed clinical features of ankyrin-B syndrome. Furthermore, evaluation of primary lymphoblasts from a carrier of the translocation showed

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Disclosures: The authors declare no conflicts of interest.

altered levels of ankyrin-B as well as a reduced expression of downstream ankyrin-binding partners.

Conclusions—Thus, our data conclude that, similar to previously described *ANK2* loss-of-function “point mutations”, large chromosomal translocations resulting in *ANK2* haploinsufficiency are sufficient to cause the human cardiac ankyrin-B syndrome. The unexpected ascertainment of *ANK2* dysfunction via the discovery of a chromosomal translocation in this family, the determination of the familial phenotype, as well as the complexities in formulating screening and treatment strategies are discussed.

Keywords

Ankyrin-B; *ANK2*; Chromosome 4 translocation; Cardiac arrhythmia; Long QT syndrome type 4; Ankyrin-B syndrome

Introduction

The regulation of the cardiac action potential is a highly coordinated event, mediated by the spatio-temporal expression and function of numerous ion channels, pumps, and accessory proteins. Precise physiological activity of cardiac ion channels and transporters is tightly regulated by the defined biophysical characteristics, expression, and localisation properties of those proteins within the specific cardiomyocyte membrane domains. Within the last two decades, the role of the ankyrin family of coordinating proteins have been more fully elucidated, giving rise to new loci for cardiac arrhythmia syndromes. Ankyrins are a family of cytoskeletal adaptor molecules with both structural and modulatory roles in the cardiac myocyte. Specifically, ankyrins localise and regulate a number of cardiac ion channels to specialised membrane domains. Loss-of-function variants in *ANK2* (ankyrin-B), have been demonstrated to underlie the “ankyrin-B syndrome”, a multi-faceted spectrum of cardiac dysfunction phenotypes, including bradycardia, sinus node dysfunction, atrial fibrillation, prolonged QT interval and predisposition to torsades de pointes and sudden cardiac death [1]. It is notable that to date, *ANK2*-linked disease has been limited to single ‘point mutations’ [2].

ANK2 is one of three similar, yet distinct ankyrin genes (*ANK1*, *ANK2*, and *ANK3*). *ANK2* is located on the long arm of chromosome 4 (4q25-q26) [3]. A number of *ANK2* human variants have been identified [4]. Here, we report a family with the unexpected finding of an *ANK2* transection in chromosome 4. Specifically, the proband has a balanced translocation between the long arms of chromosomes 4 and 9. *In vitro* work with lymphoblasts isolated from an affected individual demonstrate a decrease in ankyrin-B expression, along with significant decreases in the expression of known ankyrin-B binding partners. Furthermore, we describe the cardiac phenotypes in a number of family members with the translocation. Finally, we discuss an approach to cardiac screening in this family.

Methods

Ethics Committee Approval

Approval for this study was obtained from the Royal Children's Hospital Human Research Ethics Committee (HREC project number 28097).

Chromosomal Karyotype and Microarray Analysis

Conventional G-banded chromosome analysis on 72 hr synchronised peripheral blood lymphocyte cultures was carried out using standard cytogenetic techniques. Microarray analysis was carried out on DNA isolated from chorionic villi obtained from products of conception. Initial analysis was undertaken on NimbleGen Multiplex 135K v2.0 CGH array (Roche, Australia) using the manufacturer's protocol and the data interpreted using SignalMap software v1.9.0.5 (Roche, Australia). For high resolution analysis, microarray was undertaken on the same DNA sample using Affymetrix 2.7M array (Affymetrix, Santa Clara, CA, USA) according to the manufacturer's protocol as described previously [5].

Determination of Transection Breakpoints

Translocation breakpoints were determined by microarray analysis on DNA from products of conception. The unbalanced translocation enabled breakpoints to be determined by ascertaining the transition between probes on the array with normal copy number (Log_2 ratio = 0.0) and those with an amplified signal representing the duplication (theoretical Log_2 ratio = 0.58). The initial breakpoints were determined using NimbleGen 135K v2.0 CGH array and refined further using high resolution Affymetrix 2.7M array.

Lymphoblast Culture

Lymphoblasts were cultured in RPMI-1640 + 2 mM L-glutamine (supplemented 20% fetal bovine serum, 0.25 μM amphotericin B and 0.1% penicillin-streptomycin) at 37 °C and 5% CO_2 . Cells were passaged every three to four days into T75 flasks until ready for protein extraction.

Cell Lysate Preparation and Immunoblotting

Lymphoblast cultures were centrifuged at 1000 rpm at room temperature and the supernatant replaced with 1 ml homogenisation buffer (0.025 M Tris, 0.15 M NaCl, 0.001 M EDTA, 1% NP-40, 5% glycerol, protease inhibitors, PMSF, pH 7.4). Cells were allowed to homogenise on ice for 30 mins and centrifuged at 14,000 rpm for 10 mins at 4 °C. Protein was quantitated and 100 μg of protein were separated on a 4–12% bis-acrylamide gel. Proteins were transferred to nitrocellulose and incubated in the appropriate antibody solution (in 5% non-fat dry milk/Tris-buffered saline + 0.5% Tween-20 (TBST)) overnight at 4 °C. Following washes in TBST, the blots were incubated in secondary antibody for two hours at 4 °C and developed using the Pierce SuperSignal ECL kit.

Antibodies

GAPDH (Fitzgerald Industries), ankyrin-B (Covance), NKA (ThermoFisher), NCX (Swant), donkey anti-rabbit HRP (Jackson), and donkey anti-mouse HRP (Jackson).

Statistics

Immunoblot data are presented as a mean $\pm$ SD and statistical significance was assessed using the Student t test. The null hypothesis was rejected for $p < 0.05$.

Cardiac Assessment

All family members with the translocation underwent clinical cardiac assessment, baseline electrocardiogram (ECG), baseline echocardiogram, exercise stress test and a 24-hour Holter monitor assessment.

Case Presentation

A 33-year-old gravida 3 para 1 Caucasian woman (II.5) with no known pre-existing medical conditions was referred at 12 weeks gestation due to multiple fetal anomalies detected on antenatal ultrasound. She had had one previous first trimester miscarriage and one healthy child. Her pregnancy was the result of a natural conception. She underwent termination of pregnancy at 16 weeks. The fetus was found to have alobar holoprosencephaly, hypertelorism, arrhinia, median cleft lip and palate and multiple cardiac malformations including a large atrioventricular septal defect. Molecular karyotyping was undertaken on the products of conception which revealed duplications of chromosomes 4q25-qter and 9pter-q31.1.

Conventional G-banded chromosomal karyotyping in both parents of the affected fetus (III.5) revealed a balanced translocation between the long arms of one chromosome 4 and one chromosome 9 (46,XX,t(4;9)(q25;q31.1)) in the mother (II.5, proband) and a normal karyotype in the father. Therefore, the genomic imbalance in the fetus was the result of a maternal 3:1 tertiary trisomy malsegregation event at meiosis. The chromosome 4 translocation breakpoint lies within the GRCh37/hg19 genomic co-ordinates chr4:113,787,994 and 113,791,816 (Figure 1). The breakpoint is located between the first and second exon of *ANK2* isoform 3 with isoforms 1 and 2 being unaffected.

Testing was performed in other family members and five individuals were found to also have the balanced translocation (Figure 2).

Cardiac Assessment of Balanced Translocation Carriers

The proband was assessed at the age of 35. Her echocardiogram revealed a right ventricle size at the upper limit of normal, with normal systolic function. She had normal resting and exercise QT dynamics. Her Holter monitor showed episodes of nocturnal bradycardia with the slowest being 36 beats per minute and the longest episode lasting 32 beats. There were also rare isolated ventricular and atrial ectopic beats. These changes are considered to be within physiological limits.

The proband's mother (I.2) also carries the translocation and underwent cardiac screening at the age of 63. She was found to have mild cardiomegaly with mild left ventricular dysfunction. This woman also had a history of miscarriage at seven weeks gestation as well as an ectopic pregnancy. Investigations at the time of a previous fractured ankle revealed marked bradycardia with her heart rate as low as 34 beats per minute. A 24-hour Holter

monitor demonstrated frequent ventricular ectopy and periods of bigeminy. The cause of her frequent ventricular ectopy is unclear although it may be related to her ventricular dysfunction. The QT dynamics in both her resting ECGs and exercise ECGs were normal (Figure 3). She is under further investigation and monitoring for possible dilated cardiomyopathy.

An exercise stress test was performed for a brother (II.3) of the proband at age 40. He had normal QTc (400 msec) at rest (Figure 4). There was, however, atrial tachycardia during the recovery period. During the tachycardia, he had a 2:1 atrioventricular block, however, the QTc remained normal (Figure 5). A Holter monitor revealed 581 episodes of morning bradycardia with the slowest being 32 beats per minute and the longest bradycardic period lasting 52 beats. He also had isolated polymorphic ventricular ectopic beats and rare atrial ectopics.

Another brother (II.2) who also had the translocation reported an episode of loss of consciousness after strenuous cycling at the age of 41. Cardiac assessment after that episode revealed some signs of sinus node dysfunction and sinus arrhythmia during exercise stress test. His resting ECG before the exercise test was normal with a QTc of 440 msec in supine position. Standing QTc was 420 msec and on exercise his QTc was 460 msec at one stage. His investigations did not demonstrate any evidence of cardiac dysfunction and the loss of consciousness was not thought to be due to an arrhythmic episode.

The proband's youngest brother (II.4), another translocation carrier, was admitted to the hospital with an arterial embolic event. Apart from rare premature atrial complexes on the Holter monitor, his cardiac investigations were normal. He was 38 years old at the time of assessment.

The proband's daughter who carries the translocation (III.4) has had serial screening between the ages of three and eight, without any definitive evidence of long QT syndrome on baseline ECG, exercise stress ECG and Holter recordings. Non-diagnostic prominent U waves were noted on her most recent Holter monitor (Figure 6). She has had chest pain assessed as musculoskeletal and will continue to be followed. She has been advised on principle to avoid QT prolonging medications.

There is no history of sudden/ unexpected death nor long QT syndrome in the immediate or extended family.

To evaluate the effect of the translocation on ankyrin-B expression and function, we isolated and cultured lymphoblasts from one of the affected family members. Immunoblotting of proteins isolated from the lymphoblasts demonstrated a significant reduction in the expression of ankyrin-B, as well as the known ankyrin-B binding partners Na/Ca exchanger, Na/K ATPase, and Kir6.2 (Figure 7). These results are consistent with demonstrated findings in a model of ankyrin-B haploinsufficiency [2].

Discussion

Reciprocal chromosome translocations occur due to recombination errors in the meiotic phase of cell division and can generate balanced and unbalanced gametes. Unbalanced gametes can result in miscarriage or a surviving child often with polymalformation and intellectual disability. Children who inherit the balanced translocation unaltered from a parent generally do not have increased rates of phenotypic impact as the overall genetic material is no different to the normal parent cell [7]. However, if the translocation transects a coding gene, then the function of this gene could be affected and may manifest as a genetic disorder depending on the gene that is transected [8].

The transected gene in this family, *ANK2*, is located on the long arm of chromosome 4 within the genomic co-ordinates chr4:113,787,994 and 113,791,816 (UCSC Genome Browser GRCh37/hg19). The ankyrin-B molecule is comprised of an N-terminal membrane-binding domain (MBD), a central spectrin-binding domain (SBD), a death domain (DD) with ill-defined functions, and a variable C-terminal regulatory domain [9–12]. Variants identified in the C-terminal regulatory domain are linked with arrhythmia and sudden cardiac death [13]. The different ankyrin-B domains do not work independently, but connect with each other through intramolecular interactions [2].

The *ANK2* gene encodes a member of the ankyrin family of proteins, ankyrin-B, whose function is to localise and regulate ion channels and transporters to excitable cell membranes [14]. While most inherited arrhythmia syndromes are caused by disruptions to ion channels, ankyrin-B is the first non-ion channel subunit implicated in inherited arrhythmia [13]. Ankyrin-B is involved in the cellular organisation of the Na/K ATPase, the Na/Ca exchanger, Kir6.2, and inositol-1,4,5-trisphosphate receptors [15, 16]. Functional studies in engineered mouse models have demonstrated that ankyrin-B haploinsufficiency due to point mutations in *ANK2* results in a cardiac phenotype similar to humans with ankyrin-B loss-of-function variants [2].

Work from ankyrin-B^{+/-} murine myocytes has identified that the indirect effect on these ankyrin-associated channels/transporters can bring about reduction in extracellular calcium export via the sodium/calcium exchanger and therefore increased retention of calcium in the sarcoplasmic reticulum [13]. It is predicted that this increase in calcium within the sarcoplasmic reticulum may have no adverse effect on the cardiomyocyte or may even result in increased contractility under basal conditions [17]. However, inappropriate calcium export from the sarcoplasmic reticulum following catecholaminergic stimulation may result in extrasystoles and resultant arrhythmias [13].

While ankyrin-B was initially identified as a locus for type 4 long QT syndrome, not all variant carriers with cardiac dysfunction display QTc prolongation. Moreover, cardiac phenotypes are not limited to defects in ventricular repolarisation and may include phenotypes ranging from sinus bradycardia, atrial fibrillation to idiopathic ventricular fibrillation, catecholaminergic polymorphic ventricular tachycardia and sudden death [1] depending on the specific variant and genotype/environment variables. Thus, this wide range of phenotypes has been dubbed the human “ankyrin-B syndrome” to more appropriately

convey the disease heterogeneity [18]. Notably, like most other cardiac arrhythmia genes, *ANK2* variants have also been identified in asymptomatic family members of clinically affected individuals, demonstrating incomplete penetrance. It has been previously demonstrated that point mutations causing functional *ANK2* haploinsufficiency can give rise to a varied phenotype ranging from individuals with no symptoms to ventricular arrhythmias and sudden cardiac death, even within the same family [2]. This highlights the broad phenotypic variability seen in *ANK2* loss-of-function. It is difficult to speculate on the aetiology of this variation although many factors such as genetic and environmental modifiers may be playing a part. This represents a future important area of research.

It is likely that the multiple abnormalities identified in the fetus were due to the duplication of parts of chromosome 4 and 9. It remains unknown if the haploinsufficiency of *ANK2* could have played a role in the congenital heart defect.

Bradycardia noted on screening of the family members described here is consistent with the milder end of spectrum of the ankyrin-B syndrome [19]. The proband's mother's (I.2) cardiomyopathy as well as the finding of the right ventricular size at the upper limit of normal in the proband (II.5) needs further exploration and may or may not be directly related to the *ANK2* transection. Ankyrin-B is expressed in atrial and ventricular cardiomyocytes as well as the conduction system; therefore, ankyrin-B loss-of-function is predicted to affect multiple aspects of cardiac function. Moreover, ankyrin-B expression is not restricted to the heart, with the protein also being expressed in the beta cells of pancreas [20] [21] [22], the nervous system, [23] [24] [25] and the retina [26]. Therefore, a wider phenotype is not unexpected in those with loss-of-function *ANK2* mutations. Striking loss and remodelling of ankyrin-B and ankyrin-B associated partners in the myocardial infarct border zone have also been demonstrated in animal models of coronary artery occlusion, suggesting a role for ankyrin-B not only in germline genetic human disease, but also in acquired human disease [27].

The management of the translocation carriers in this family is not straightforward. It is difficult to predict the severity in affected persons. In the absence of clear guidelines for surveillance and monitoring in ankyrin B syndrome, we have recommended annual electrocardiogram, 24-hour Holter monitoring and echocardiograms in all translocation carriers to assess any evidence of bradyarrhythmia or cardiomyopathy. Individuals with sinus bradycardia have been recommended to undergo an exercise stress test. If QT prolongation is detected during screening, management will be similar to long QT syndrome including avoidance of QT prolonging drugs. There is currently no indication for loop recorders in this family as all members remain asymptomatic. However, if they have any symptoms suggestive of underlying arrhythmias, a loop recorder will be advocated.

Acknowledgments

Funding: NIH Grants HL083422 HL084583; American Heart Association

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Figure 1.

Diagram demonstrating the breakpoint transecting the *ANK2* gene. UCSC GRCh37/hg19 genome browser view that depicts the breakpoint region on chromosome 4q25 [6] (<http://genome.ucsc.edu/>). Solid red arrow indicates region of transection between the first and second exon of *ANK2* isoform 3, as determined by microarray analysis on DNA from products of conception.

Figure 2.
Pedigree

Figure 3.
Resting supine ECG from proband's mother (I.2) demonstrating a normal QTc of 455ms and ventricular ectopics.

Figure 4.
Resting supine ECG from proband's brother (II.3) which shows normal QTc of 400 msec at rest.

Figure 5. ECG during recovery phase of an exercise test in proband's bother (II.3) demonstrating atrial tachycardia with 2:1 atrioventricular block. However, the QTc remained normal.

Figure 6.
Prominent U waves recorded in the proband's daughter (III.4). Recorded at the minimum heart rate overnight. These U waves however are not diagnostic of ankyrin-B syndrome.

Figure 7.

Lymphoblasts isolated from the proband (Patient 2) demonstrated significant decreases in the expression of ankyrin-B (44% decrease; $p < 0.05$), Na/Ca exchanger (NCX; 25% decrease; $p < 0.05$), Kir6.2 (42% decrease; $p < 0.05$), and Na/K ATPase (NKA; 24% decrease; $p < 0.05$) compared to a control patient (Patient 1)