

ANKYRIN-B SYNDROME AND ANKYRIN-G BRUGADA SYNDROME: TWO ARRHYTHMOGENIC FATAL CARDIAC ARRHYTHMIC ENTITIES

Andrés Ricardo Pérez Riera¹; Edgardo Schapachnik² Sergio Dubner³

- 1) Chief of the Sector of Electro-Vectocardiography of the Discipline of Cardiology, School of Medicine, ABC Foundation – Santo André – São Paulo – Brazil.
- 2) Chief of the Department of Chagas Disease of the Dr. Cosme Argerich Hospital, Buenos Aires, Argentina.
- 3) Director of Arrhythmias and Electrophysiology Service, Clinical and Maternidad Suizo Argentina, Buenos Aires, Argentina.

Correspondence:

Sebastião Afonso, 885 CEP: 04417-000
Jardim Miriam – São Paulo - Brazil
Phone: (11) 5621-2390
Fax: (11) 5506-0398
E-mail: riera@uol.com.br

Key words: Ankyrins – Sudden Cardiac Death – Brugada Syndrome – Ankyrin-B Syndrome

The **coordinated** activity of ion channels and transporters in **the** cardiac muscle is critical for normal excitation-contraction coupling and cardiac rhythm.

Human gene variants, which alter ion channel biophysical properties, have been linked with fatal cardiac arrhythmias.

Ankyrins are intracellular polypeptides required for the biogenesis and maintenance of membrane domains in both excitable and non-excitable cells. Additionally, they are membrane adaptor molecules that play important roles in coupling integral membrane proteins to the spectrin-based cytoskeleton network.

Ankyrin family polypeptides have been implicated in the targeting and stabilization of membrane proteins including ion channels, transporters,

exchangers and cell adhesion molecules in diverse tissues and cell types including erythrocyte, kidney, lung and brain.

Ankyrin is a cytoskeletal adaptor protein that controls Ca^{2+} efflux at inositol 1, 4, 5-trisphosphate receptors (IP(3)R) on the endoplasmic reticulum.

Dysfunction in ankyrin-based pathways has previously been linked to abnormalities in vertebrate physiology including hereditary spherocytosis, anemia, ataxia and axonal degeneration. Ankyrin deficiency is one of the most common causes of hereditary spherocytosis in humans¹. Recent findings have illuminated the importance of ankyrin-based pathways in excitable cells of the heart. Human mutations of ankyrin genes lead to severe genetic diseases such as fatal cardiac arrhythmias syndromes. Specifically, two ankyrin gene products, 220-kDa ankyrin-B also known as ankyrin 2 and 190-kDa ankyrin-G, have been implicated in the targeting of structurally diverse membrane ion channels and transporters to excitable membrane domains in cardiomyocytes.

Ankyrin-B is a spectrin-binding protein that is required for localization of inositol 1,4,5-trisphosphate receptor and ryanodine receptor in neonatal cardiomyocytes. There is an interaction between ankyrin-B and beta(2)-spectrin in these cells. Ankyrin-B and beta(2)-spectrin are colocalized in an intracellular striated compartment overlying the M-line and distinct from T-tubules, sarcoplasmic reticulum, Golgi, endoplasmic reticulum, lysosomes, and endosomes. Beta(2)-Spectrin is absent in ankyrin-B-null cardiomyocytes and is restored to a normal striated pattern by rescue with green fluorescent protein-220-kDa ankyrin-B.

Ankyrin-B and ankyrin-G are recognized constituents of the heart that target diverse ion channels/pumps/transporters to physiologic sites of action in cardiomyocytes. Mutations of ankyrin-B cause a newly defined cardiac arrhythmia syndrome associated with abnormal calcium homeostasis in a mouse model.

Ankyrin-G associates with the principal voltage-gated Na channel in the heart, and loss of this interaction due to mutation of Nav1.5 results in Brugada syndrome².

Mohler et al. **identified** a human mutation (E1053K) in the ankyrin-binding motif of Na(v)1.5 that is associated with Brugada syndrome, a fatal cardiac arrhythmia caused by altered function of Na(v)1.5. The E1053K mutation abolishes binding of Na(v)1.5 to ankyrin-G, and also prevents accumulation of Na(v)1.5 at cell surface sites in ventricular cardiomyocytes. Ankyrin-G and Na(v)1.5 are both localized at intercalated disc and T-tubule membranes in cardiomyocytes, and Na(v)1.5 coimmunoprecipitates with 190-kDa ankyrin-G from detergent-soluble lysates from rat heart.

These data suggest that Na(v)1.5 associates with ankyrin-G and that ankyrin-G is required for Na(v)1.5 localization at excitable membranes in cardiomyocytes. Together with previous work in neurons, these results in cardiomyocytes suggest that ankyrin-G participates in a common pathway for localization of voltage-gated Na(v) channels at sites of function in multiple excitable cell types³.

Figures 1, 2 and 3 **show** ankyrin structure, its diverse localizations and functions and the spectrum of human ankyrin mutations.

Findings in humans and mice have determined the critical nature of ankyrin-based pathways for normal cardiac excitability.

Cai and Zhang **demonstrated** that duplication of ankyrin genes occurred at two different stages. The first duplication resulted from an independent evolution event specific in Arthropoda after its divergence from Chordata. Following the separation from Urochordata, expansion of ankyrins in vertebrates involved ancestral genome duplications. The authors did not find evidence of coordinated arrangements of gene families of ankyrin-associated membrane

proteins on paralogous chromosomes. In addition, evolution of the 24 ANK-repeats strikingly correlated with the exon boundary sites of ankyrin genes, which might have occurred before its duplication in vertebrates. Such correlation is speculated to bring functional diversity and complexity. Moreover, based on the phylogenetic analysis of the ANK-repeat domain, they put forward a novel model for the putative primordial ankyrin that contains the fourth six-ANK-repeat subdomain and the spectrin-binding domain. These findings will provide guides for future studies concerning structure, function, evolutionary origins of ankyrins, and possibly other cytoskeletal proteins⁴. Reduction of 220-kDa ankyrin-B expression levels in mice or the presence of ankyrin-B loss-of-function mutations in humans leads to cause a new cardiac arrhythmia syndrome named ankyrin-B syndrome, a cardiac disease with a spectrum of clinical presentations that include:

- 1) Bradycardia with sinus node dysfunction;
- 2) Ventricular tachycardia;
- 3) Idiopathic ventricular fibrillation;
- 4) Catecholaminergic Polymorphic Ventricular Tachycardia;
- 5) Sudden cardiac death in response to catecholaminergic stimuli.

The syndrome initially was classified as type 4 long QT syndrome. The new cardiac arrhythmia syndrome is distinct from long QT syndromes (LQTS). A prolonged rate-corrected QT interval is not a consistent feature, indicating that ankyrin-B dysfunction represents a clinical entity distinct from classic LQTS. Mohler et al. have uncovered a role for ankyrin-B, a cytoskeletal "adaptor" protein or a non-ion channel protein, in type IV LQTS. In this new syndrome calcium **signaling** is altered, and the functions of several channels and pumps that normally interact with wild-type ankyrin-B are impaired in the presence of mutant ankyrin-B. The authors suggest that by disrupting the functions of these channels, a new mechanism has been uncovered that can lead to cardiac myopathy⁵.

Ankyrin-B mutation results in elevated calcium transients in cardiomyocytes (all mutations abolish ability of ankyrin-B to restore abnormal Ca^{2+} dynamics) accompanied by loss of cellular targeting of ankyrin-binding proteins Na/K

ATPase, Na⁺/Ca²⁺ exchanger, and InsP₃ receptor to cardiomyocyte membrane domains. 220-kDa ankyrin-B is required for coordinated assembly of Na/Ca exchanger, Na/K ATPase, and inositol 1,4,5-trisphosphate receptor at transverse-tubule/sarcoplasmic reticulum sites in cardiomyocytes⁶. Pulse-chase biosynthesis experiments demonstrate that reduction or loss of ankyrin-B in ankyrin-B (+/-) or ankyrin-B (-/-) neonatal cardiomyocytes leads to approximately 3-fold reduction in half-life of newly synthesized InsP(3)R. Furthermore, interactions with **ankyrin-B required** for Inositol triphosphate (IP₃) receptors stability as abnormal InsP(3)R phenotypes, including mis-localization, and reduced half-life in ankyrin-B (+/-) cardiomyocytes can be rescued by green fluorescent protein (GFP)-220-kDa ankyrin-B but not by GFP-220-kDa ankyrin-B mutants, which do not associate with InsP(3)R. A molecular partner required for early post-translational stability of InsP(3)R⁷. IP₃ receptors are integral SR membrane proteins that mediate the efflux of Ca⁺² from this intracellular store. Additionally IP₃ receptors may have a role in the maintenance of diastolic function and in the physiological modulation of contractility in response to a variety of drugs and hormones. IP₃ receptors **are** produced by hydrolysis of the membrane phospholipids phosphatidylinositol-biphosphate, catalyzed by the enzyme phosphatidylinositol-biophosphatase, catalyzed by the enzyme phospholipase C in the heart.

Mohler et al. identified two mutants (A1000P and DAR976AAA) located in the ZU5 domain which eliminate spectrin binding activity of ankyrin-B. Ankyrin-B mutants lacking spectrin binding activity are normally targeted but do not reestablish beta(2)-spectrin in ankyrin-B(+/-) cardiomyocytes. However, both mutant forms of ankyrin-B are still capable of restoring inositol 1,4,5-trisphosphate receptor localization and normal contraction frequency of cardiomyocytes. Therefore, direct binding of beta(2)-spectrin to ankyrin-B is required for the normal targeting of beta(2)-spectrin in neonatal cardiomyocytes. In contrast, ankyrin-B localization and function are independent of beta(2)-spectrin. Interaction between members of the ankyrin and beta-spectrin families previously established in erythrocytes and axon initial segments also occurs in neonatal cardiomyocytes with ankyrin-B and beta(2)-spectrin. There is a functional hierarchy in which ankyrin-B determines the localization of beta(2)-

spectrin and operates independently from beta(2)-spectrin in its role in organizing membrane-spanning proteins⁸.

The principal voltage-gated Na⁺ channel in heart, Nav1.5, is directly associated with ankyrin-G, which is encoded by a distinct gene from ankyrin-B. Mutation of Nav1.5 causing loss of binding to ankyrin-G results in Brugada syndrome and loss of targeting of Nav1.5 to the cell surface of cardiomyocytes.

Ankyrin-G is required for expression of the major cardiac voltage-gated Na(v) channel, Na(v)1.5, at specialized cardiac membrane domains. Human variants in SCN5A (encodes Na(v)1.5) that block Na(v)1.5 interaction with ankyrin-G lead to loss of Na(v)1.5 membrane expression and Brugada syndrome. Together, these recent findings in the heart reinforce the importance of ankyrin-based pathways for normal vertebrate physiology and raise exciting new questions regarding the cellular roles for ankyrin polypeptides in cardiac and other excitable cells. While ankyrins have only been recently identified in heart, our current understanding suggests that elucidating the roles of ankyrins in organizing and targeting protein complexes to excitable membrane domains will yield important insights into the molecular basis of cardiac arrhythmias⁹. In the past decade, human gene variants, which alter ion channel biophysical properties, have been linked with fatal cardiac arrhythmias. Ankyrins are a family of "adaptor" proteins, which play critical roles in the proper expression and membrane localization of ion channels and transporters in excitable and nonexcitable cells. Recent findings demonstrate a new paradigm for human cardiac arrhythmia based not on gene mutations that affect channel biophysical properties, but instead on mutations that affect ion channel/transporter localization at excitable membranes in heart. Human ANK2 mutations are associated with "ankyrin-B syndrome" (an atypical arrhythmia syndrome with risk of sudden cardiac death). Human gene mutations, which affect ankyrin-G-based pathways for voltage-gated Na(v) channel localization, are associated with Brugada syndrome, a second potentially fatal arrhythmia. Together, these data demonstrate the importance of the molecular events involved in the cellular organization of membrane domains in excitable cells. Moreover, these data define an exciting new field of cardiac "channelopathies" due to defects in proper channel targeting/localization¹⁰.

Conclusion

A new paradigm for human cardiac arrhythmia based not on gene mutations that affect channel biophysical properties, but instead on mutations that affect ion channel/transporter localization at excitable cardiomyocyte membranes. Abnormalities in cellular anchoring protein cause fatal heart syndrome. Human ANK2 mutations are associated with a new syndrome named "ankyrin-B syndrome" with a spectrum of clinical presentations that include bradycardia with sinus node dysfunction, ventricular tachycardia, Idiopathic ventricular fibrillation, catecholaminergic polymorphic ventricular tachycardia and sudden cardiac death in response to catecholaminergic stimuli. Human gene mutations, which affect ankyrin-G-based pathways for voltage-gated Na(v) channel localization, are associated with Brugada syndrome, a second potentially fatal arrhythmic syndrome. These data define a new hopeful field of cardiac "channelopathies" due to defects in proper channel targeting/localization. Ankyrin-based cardiac arrhythmias are a new class of channelopathies due to loss of cellular targeting.

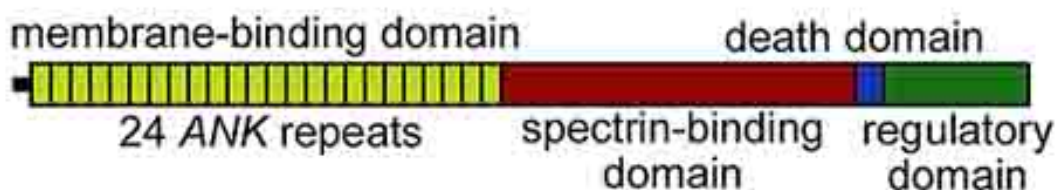
References

- 1) Birkenmeier CS, Gifford EJ, Barker JE. Normoblastosis, a murine model for ankyrin-deficient hemolytic anemia, is caused by a hypomorphic mutation in the erythroid ankyrin gene Ank1. *Hematol J.* 2003;4: 445-449.
- 2) Mohler PJ, Bennett V. Ankyrin-based cardiac arrhythmias: a new class of channelopathies due to loss of cellular targeting. *Curr Opin Cardiol.* 2005; 20:189-193.
- 3) Mohler PJ, Rivolta I, Napolitano C, LeMaillet G, Lambert S, Priori SG, Bennett V. Nav1.5 E1053K mutation causing Brugada syndrome blocks binding to ankyrin-G and expression of Nav1.5 on the surface of cardiomyocytes. *Proc Natl Acad Sci U S A.* 2004; 101:17533-17538.
- 4) Cai X, Zhang Y. Molecular evolution of the ankyrin gene family. *Mol Biol Evol.* 2006; 23:550-558.

- 5) Yong S, Tian X, Wang Q. LQT4 gene: the "missing" ankyrin. *Mol Interv.* 2003; 3: 131-136.
- 6) Mohler PJ, Splawski I, Napolitano C, Bottelli G, Sharpe L, Timothy K, Priori SG, Keating MT, Bennett V. A cardiac arrhythmia syndrome caused by loss of ankyrin-B function. *Proc Natl Acad Sci U S A.* 2004; 101:9137-142.
- 7) Mohler PJ, Davis JQ, Davis LH, Hoffman JA, Michaely P, Bennett V. Inositol 1, 4, 5-trisphosphate receptor localization and stability in neonatal cardiomyocytes requires interaction with ankyrin-B. *J Biol Chem.* 2004; 279:12980-12987.
- 8) Mohler PJ, Yoon W, Bennett V. Ankyrin-B targets beta2-spectrin to an intracellular compartment in neonatal cardiomyocytes. *J Biol Chem.* 2004;279:40185-93.
- 9) Cunha SR, Mohler PJ. Cardiac ankyrins: Essential components for development and maintenance of excitable membrane domains in heart. *Cardiovasc Res.* 2006; 71:22-29.
- 10) Mohler PJ. Ankyrins and Human Disease: What the Electrophysiologist Should Know. *J Cardiovasc Electrophysiol.* 2006 Jun 27; [Epub ahead of print]

Figure 1

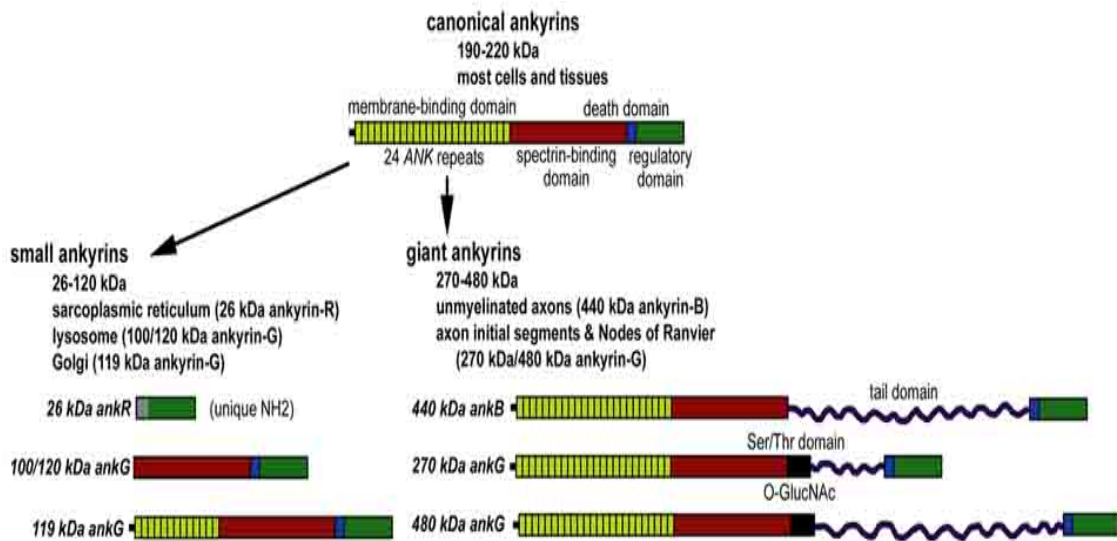
ANKYRIN DOMAIN ORGANIZATION



Canonical ankyrin domain organization. Ankyrins have a large membrane-binding domain comprised of 24 ANK repeats (yellow boxes), a spectrin-

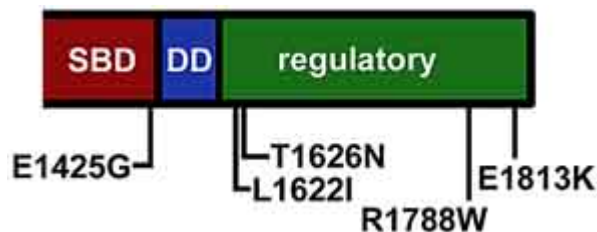
binding domain (red), a death domain of unknown function (blue), and a C-terminal regulatory domain (green).

Figure 2



Alternative splicing of ankyrin genes produce a number of ankyrin polypeptides with diverse localizations and functions.

Figure 3



Spectrum of human mutations in *ANK2* spectrin-binding domain and C-terminal regulatory domain associated with ventricular arrhythmia and sudden cardiac death. Abnormalities in Cellular Anchoring Protein Cause Fatal Heart Syndrome