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Spectrin-based pathways underlying electrical and mechanical dysfunction in cardiac disease

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Abstract

Introduction—In the heart, pathways that transduce extracellular environmental cues (e.g. mechanical force, inflammatory stress) into electrical and/or chemical signals at the cellular level are critical for the organ-level response to chronic biomechanical/neurohumoral stress. Specifically, a diverse array of membrane-bound receptors and stretch-activated proteins converge on a network of intracellular signaling cascades that control gene expression, protein translation, degradation and/or regulation. These cellular reprogramming events ultimately lead to changes in cell excitability, growth, proliferation, and/or survival.

Areas covered—The actin/spectrin cytoskeleton has emerged as having important roles in not only providing structural support for organelle function but also in serving as a signaling "super highway," linking signaling events at/near the membrane to distal cellular domains (e.g. nucleus, mitochondria). Furthermore, recent work suggests that the integrity of the actin/spectrin cytoskeleton is critical for canonical signaling of pathways involved in cellular response to stress. This review discusses these emerging roles for spectrin and consider implications for heart function and disease.

Expert Commentary—Despite growth in our understanding of the broader roles for spectrins in cardiac myocytes and other metazoan cells, there remain important unanswered questions, the answers to which may point the way to new therapies for human cardiac disease patients.

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Declaration of Interest

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Keywords

ankyrin; arrhythmia (mechanisms); calmodulin dependent kinase II; heart failure; ion channels; spectrin

1.0 Spectrin structure and function

1.1 What are spectrins and why should we care?

The evolution of metazoans from unicellular ancestors required the emergence of cellular systems to support, among other functions, cell adhesion, long-range communication, defense, and membrane integrity in the face of high mechanical stress [1, 2]. Importantly, with multicellular animals arose *de novo* cellular pathways to exert spatiotemporal control over gene programs, often involving redeployment of ancestral genes [1]. Spectrins are cytoskeletal proteins originating with early metazoans that help resolve some of these unique challenges faced by multicellular animals [2].

First discovered as a key constituent of detergent-extracted erythrocytes, or “ghosts,” the spectrin molecule is a long, flexible chain ~200 nm in length formed as a heterotetramer (dimer of anti parallel heterodimers) of α- and β-subunits [2, 3]. The human spectrin family includes two α- and five β-spectrin subunits (expanded from one α- and two β-subunits in invertebrates) encoded by distinct genes with additional diversity through alternative splicing (Table 1). Spectrins are widely expressed in mammalian tissues with α_{II}- and β_{II}-spectrin as the predominant non-erythrocytic isoforms. A characteristic feature of both α- and β-spectrin structure is the presence of multiple triple-helical repeats (spectrin repeats), which confer flexibility to the spectrin molecule and facilitate protein-protein interaction including between α- and β-subunits themselves (involves coupling of incomplete helical repeats) [2]. Canonical α-spectrin consists of 20 spectrin repeats with a src homology domain (SH3) between repeat 9 and 10 and a C-terminal calmodulin-related domain. β-spectrin is comprised of a highly conserved N-terminal actin-binding region, followed by 17 spectrin repeats (except for β_V-spectrin, which has 30 repeats) and a C-terminal domain with interesting and understudied signaling motifs including a pleckstrin homology domain. Repeat 15 in β-spectrin contains a highly conserved motif that facilitates interaction with ankyrins, cytoskeletal proteins that link membrane proteins to the spectrin-actin cytoskeleton [4]. An interaction between spectrin and protein 4.1 likely stabilizes spectrin-actin interaction [5, 6].

Expression of α_I-, β_I-and β_{IV}-spectrins, in addition to α_{II}- and β_{II}-spectrins, has been reported in cardiomyocytes with distinct localization patterns [5, 7–9] that is affected by alternative splicing [10]. α_{II}- and β_{II}–spectrin are the predominant isoforms found at the Z-line and sarcoplasmic reticulum (SR) membranes. In contrast, α_I- and α_{II}- spectrin along with β_I-spectrin account for major spectrin components at the lateral membrane. Finally, α_{II}-, β_{II}-, β_{IV}-spectrin are principal family members localized to intercalated disc (Figure 1) [5, 7, 11–14]. The localization of spectrin isoforms to myocyte membrane domains important for cell-cell communication (e.g. intercalated disc) and contraction (e.g. Z-lines,

SR membranes) suggests important roles in regulating both electrical and mechanical cardiac function.

1.2 Established roles for spectin in membrane stability and ion channel targeting

Spectrin serves as a principal component of the molecular scaffolding linking the plasma membrane and associated proteins to the actin cytoskeleton in numerous tissue types [2, 5, 15]. In both erythrocytes and nucleated cell types, the spectrin-based cytoskeleton is critical in maintaining normal cell morphology, plasma membrane stability, and mechanical properties. A prototypical example of this canonical function is found in the erythrocyte where spectrin forms a polygonal network with actin that links to membrane proteins via ankyrin to support the lipid bilayer [2]. In neurons, super resolution microscopy has revealed a distinct periodic pattern of ring-like actin structures interconnected by spectrin tetramers aligned along the axon shaft [16]. Spectrin isoforms are found not only at the submembrane but also in the nucleus where they contribute to nucleoskeleton flexibility and chromosome stability [17, 18]. Interestingly, in this regard, studies point to an important role for nuclear α_{II}-spectrin as a scaffold for repair of DNA interstrand cross-links [18]. Given its central role in formation of the erythrocyte membrane cytoskeleton, it is not surprising that defects in spectrin lead to erythrocyte membrane fragility, which ultimately may manifest as elliptocytosis and anemia [19]. Similarly, epithelial cells deficient in either β_{II}-spectrin or ankyrin-G fail to maintain normal basolateral and apical membrane area, converting cells from columnar to squamous cell morphology [20].

Through its interaction with ankyrin family proteins, spectrins not only confer membrane stability but also play key roles in localization of ion channels, transporters and exchangers to membrane domains important for cell function. Classic examples of this function are found in the requirement of spectrin for membrane targeting of anion exchanger band 3 in erythrocytes [21, 22] and Na^+/K^+ ATPase in epithelial cells [23–25]. In neurons, β_{IV}-spectrin is highly enriched with ankyrin-G and voltage-gated ion channels at axon initial segments (AISs) and nodes of Ranvier [26–31] while β_{II}-spectrin is found primarily at paranodal regions with ankyrin-B [32]. Defects in β_{IV}-spectrin or ankryin-G result in loss of normal voltage-gated Na^+ (Na_v) channel clustering, abnormal cell membrane excitability and neurological dysfunction (e.g. ataxia) in mice [26, 33–35]. Similarly, spectrin and ankyrin have been shown to be important for organization of ion channels at several membrane domains in cardiac myocytes including the cardiac dyad and intercalated disc (Figure 1). Namely, β_{II}-spectrin is enriched with ankyrin-B at the cardiac dyad, a micro domain integral to cardiomyocyte excitation-contraction coupling [8, 36–38]. Loss of β_{II}-spectrin or ankyrin-B results in abnormal targeting of Ca^{2+} cycling proteins (e.g. Na^+/Ca^{2+} exchanger, ryanodine receptor SR Ca^{2+} release channels), aberrant Ca^{2+} cycling and arrhythmias [8, 37, 39]. In contrast, β_{IV}-spectrin is highly localized with ankyrin-G at the cardiac intercalated disc, a specialized membrane domain important for electrical and mechanical cell-to-cell coupling [7, 40–42]. While it is clear that ankyrin-G is required for proper localization of β_{IV}-spectrin and intercalated disc proteins, including $Na_v1.5$ (primary cardiac Na_v), the role of β_{IV}-spectrin in ion channel targeting is less established [12, 40–43]. It is likely that β_{IV}-spectrin, in fact, plays a more prominent role in regulation of $Na_v1.5$ rather than targeting (discussed in more detail below). However, β_{IV}-spectrin has been

linked to membrane targeting of other cardiac ion channels, specifically, the two-pore K^+ channel, TREK-1 [12, 44]. TREK-1 channels encoded by *KCNK2*, belong to the two-pore-domain background potassium channel protein family (K_{2P}) and are expressed in nervous and cardiovascular systems where they regulate cell membrane excitability and participate in transduction of a variety of environmental stimuli [45, 46]. Interestingly, loss of β_{IV}-spectrin or TREK-1 has been shown to produce arrhythmia in mice characterized by pronounced sinus node dysfunction in response to acute adrenergic stimulation [12, 44]. Interestingly, several groups have identified involvement of spectrin in post-golgi targeting and long range transport of ion channel cargo to the membrane, although its specific role remains controversial [2, 23, 47, 48]. In a similar vein, exciting work has identified a spectrin-based complex involving Mena, VASP and α_{II}-spectrin for regulating actin dynamics [49].

1.3 Emerging roles for spectrins in control of signaling pathways

While the ways in which spectrins provide metazoans with increased membrane support and organization are relatively established, only recently has the field begun to appreciate alternative roles for spectrins in coordinating signaling and gene programs. Studies on mice lacking β_{II}-spectrin (also known as embryonic liver fodrin, ELF) provide an excellent example of how spectrin might have evolved to mediate sophisticated cell signaling pathways [50]. Smad proteins are a group of transcription factors important for mediating TGF-β signaling that evolved with early metazoans [1]. β_{II}-spectrin associates with Smad family members to regulate TGF-β-dependent signaling and β_{II}-spectrin-deficient mice display systemic developmental defects including aberrant cardiac development [50]. More recently, studies have shown that analogous to this β_{II}-spectrin/TGF-β/Smad signaling pathway,β_{IV}-spectrin coordinates spatial and temporal organization of Ca^{2+}/calmodulin kinase II (CaMKII) signaling in cardiomyocytes and neurons [7]. CaMKII is a multifunctional serine/threonine protein kinase with broad tissue distribution and a single ancestral gene, which likely evolved at a point in metazoan evolution just prior to spectrin (found in unicellular eukaryote and metazoan ancestor choanoflagellate, but not plants or yeast) [51–54]. β_{IV}-spectrin organizes a macromolecular signaling complex with ankyrin-G to regulate CaMKII-dependent phosphorylation of the cardiac voltage-gated Na^+ channel, $Na_v1.5$ [7, 41, 55, 56]. This spectrin-based complex is important for CaMKII-dependent regulation of cardiac cell membrane excitability and cardiac function in response to both acute and chronic adrenergic stress [7, 55, 56]. Interestingly, spectrins via their association with ankyrins likely also regulate the negative axis of CaMKII-dependent signaling via targeting of protein phosphatase 2A [57–59].

2.0 Roles for spectrin in disease

2.1 Association between spectrin dysfunction and disease in multiple organ systems

Given the many ways that spectrins support metazoan cell function, the close link between spectrin dysfunction and human disease is not surprising. Mutations in spectrins and ankryins have been identified as the underlying cause of forms of hereditary sperocytosis and hemolytic anemia, as well as spinocerebellar ataxia in mice and humans [15, 19, 35]. In a similar vein, genome-wide association studies have uncovered *ANK3* (encodes for spectrin-associated AnkG) as a susceptibility locus for human bipolar disorder [60] while

mutations in *ANK3* have been linked to broad spectrum neurological disorders including autism [61, 62]. More recently, it has been reported that a homozygous nonsense mutation in *SPTBN4* (encoding β_{IV}-spectrin) is a novel candidate disease gene for congenital myopathy, neuropathy, and deafness in a consanguineous Kurdish family [63].

2.2 Spectrin dysfunction in cardiac arrhythmia and disease

Defects in spectrin-based pathways have been linked to cardiac arrhythmia and disease. Interestingly, spectrin dysfunction has been shown to alter both electrical and mechanical function suggesting that spectrin may be a therapeutic node for effectively treating both arrhythmias and underlying substrate in disease.α_I-spectrin deficiency in mice results in a dilated cardiomyopathy, although the presence of severe anemia complicates the phenotype in these animals [5, 64]. Global deletion of β_{II}-spectrin in mice is embryonic lethal, with profound developemental defects in heart and other organs [50]. In particular, β_{II}-spectrin deficiency leads to a dramatic loss or disorganization of dystrophin, F-actin, α-smooth muscle actin, and tropomyosin, contributing to compromised myocyte contractile function. At the same time, loss of β_{II}-spectrin promotes aberrant expression/activity of myocardial transcription factors Nkx2.5, GATA4, and MEF2c, together with pronounced defects in TGF-β/Smad signaling (addressed above), which likely contributes to loss of the normal myocardial trabeculated pattern and severe thinning of the compact layer by E14.5. Cardiac specific β_{II}-spectrin deletion in mice, although not embryonic lethal, results in pronounced arrhythmia and increased mortality/remodeling in response to chronic pressure overload [8]. Human *ANK2* variants (encoding ankyrin-B) result in a broad spectrum arrhythmia syndrome recapitulated by mice deficient in ankyrin-B [37, 65, 66]. Recently, a novel human *ANK2* variant associated with increased susceptibility to arrhythmias was shown to alter binding to β_{II}-spectrin [67].

Beyond rare, inherited disorders, mounting data support a role for dysfunction in spectrin-based pathways in common forms of cardiac disease. Recent studies have identified downregulation of both spectrin and ankyrin family proteins in animal models and human patients with common forms of acquired cardiac disease [12, 67–69]. Specifically, significant decreases in the levels of β_{II}-spectrin, β_{IV}-spectrin, and ankyrin-B have been reported in human failing hearts [12, 67], which are also observed in animal models of heart failure and myocardial infarction [67, 69]. Similar changes in β_{II}-spectrin,β_{IV}-spectrin, and ankyrin-B expression have been observed in atria of human AF patients and canine model of sinus node dysfunction [44, 66, 67]. In general, chronic stress conditions appear to promote decreased spectrin/ankyrin levels at the protein but not transcript levels, indicating abnormal post-translational processing, linked to elevations in the Ca^{2+} activated protease, calpain [67, 68], a phenomenon also observed in tramautic brain injury [70]. Interestingly, α_{II}-spectrin breakdown products have been suggested as biomarker for brain injury during open heart surgery in neonates with congenital heart disease [71]. Together, these data indicate the highly conserved loss of spectrin across species in response to adverse cardiac remodeling from either pressure overload and/or ischemic stress, suggesting a potentially prominent role in contributing to and driving disease remodeling.

2.3 Molecular mechanisms linking spectrin dysfunction to disease

The molecular mechanisms underlying maladaptive remodeling and arrhythmias in animals with spectrin dysfunction remain unclear, due in part to the lack of appropriate animal models (e.g. cardiac-specific spectrin knockout models like for β_{II}-spectrin). Several possible mechanisms have been touched on already in this review and include defects in ion channel localization/activity and/or aberrant cell signaling, perhaps converging on pathways important for myocyte survival and fibrosis. For example, in the case of cardiac-specific β_{II}-spectrin deletion, there is loss of normal Ca^{2+} homeostasis due in part to a precise defect in the expression and localization of SR ryanodine receptor Ca^{2+} release channels (without global changes in t-tubule structure or $Ca_v1.2$), giving rise to increased frequency of spontaneous Ca^{2+} waves and arrhythmogenic after depolarizations [8]. In addition to the pro-arrhythmic contributions from these changes, the resulting defect in Ca^{2+} handling may drive myocyte loss, hypertrophy, and an accelerated decline in contractile performance through Ca^{2+}-dependent signaling pathways (e.g. CaMKII, calcineurin). At the same time, it is possible that defects in β_{II}-spectrin may disrupt intracellular signaling and gene expression programs directly through its role in coordinating cell signaling networks (e.g. TGF-β/SMAD signaling [50], as discussed in the previous section). In a similar vein, the link between β_{IV}-spectrin and CaMKII signaling provides a potential mechanism underlying pathogenesis induced by spectrin dysfunction. Aberrant CaMKII activity/expression is a common finding across a broad spectrum of cardiac disease states and has been linked to not only altered ion channel activity but also changes in transcriptional, inflammatory, apoptotic and fibrotic pathways [52]. It is interesting to consider the possibility that given its role its association with CaMKII, β_{IV}-spectrin may dually regulate electrical and mechanical function in cardiac myocytes.

3. Expert commentary

Although we have learned a great deal about spectrin since its discovery almost 50 years ago, there remain a host of important unanswered questions, especially related to its role in cardiac physiology and disease. As addressed in this review, spectrin satisfies a number of unique metazoan needs from membrane support to long range signaling, which begs the question: Why has the metazoan (more specifically, cardiac) cell evolved to impart both structural and signaling functions to a single protein family (also observed with integrins, catenins, etc.)? Similarly, how has a single class of proteins evolved to participate in so many different cellular processes? A compelling theory in this regard is that spectrin family members evolved with metazoans to “uncouple” cellular reprogramming from environmental cues, effectively subjugating the individual cell to the needs of the organism [1]. It is interesting to consider the corollary then that loss of an essential support for this uncoupling would effectively cause cells to revert to a “unicellular” ancestral state to the detriment of the organism. Aside from these larger more philosophical questions, further investigation is required to address the more basic but no less important questions related to the roles of spectrin isoforms in controlling cardiac myocyte function, consequences at the organ and organismal level, and ultimately novel ways for repairing the spectrin-based cytoskeleton in the setting of disease. For example, while the structure and function of elaborate spectrin/actin networks have been mapped out in erythrocytes, neurons and other

cells [16, 20, 31, 72], we lack the same level of detailed information in cardiomyocytes. At the same time, intriguing gaps remain in our understanding of the broader roles for spectrin aside from membrane support and ion channel targeting. For instance, how is spectrin able to link distal signals to the nucleus and ultimately altered gene expression? Aside from chromosome repair, are spectrins able to alter cell gene programs via interplay with signaling molecules (e.g CaMKII) and/or transcription factors (e.g. Smads) and what are the consequences for cardiac disease?

Finally, it is important to note that in order to answer these and other pressing questions related to spectrin biology, we must overcome considerable technical challenges in dissecting the multiple aspects of pleiotropic protein function. Conventional biomedical science relies almost exclusively on large system perturbations (e.g. total gene knockout/overexpression) to elucidate function at the molecular level. While such studies have generated important insight across disciplines, their binary “all-or-nothing” nature potentially obfuscates a finer level of detail essential for accurate assessment of the system as a whole. It will be important, going forward, to establish novel paradigms for “molecular sensitivity analysis” to study the more nuanced aspects of the actin/spectrin signaling network discussed here.

4. Five-year view

In light of the recent developments in our understanding of spectrin and other cytoskeleton proteins, it is exciting to consider how our view will continue to evolve over the next five years. First, technological advances (e.g. high throughput CRISPR gene editing, “big data” science) will allow us to develop more nuanced views of protein function over the traditional “all-or-nothing” knockout/overexpression approach. Second, due in part to improved technology, we will develop a more complete view of how specific extracellular stimuli (chronic and acute) lead to very different cell-level responses (adaptive vs. maladaptive) and how cytoskeletal proteins like spectrin help transduce these signals. Finally, we hope that these advances will lead to a more complete grasp of how to manipulate cytoskeletal components to protect cell membrane integrity in the face of chronic adverse conditions without launching a cellular reprogramming that ultimately compromises organ function.

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Key issues

- Spectrins are cytoskeletal proteins originating with early metazoans that help resolve some of the unique challenges faced by multicellular animals.
- Spectrins provide metazoans with increased membrane support and organization, but also help coordinate signaling and gene programs.
- Spectrin dysfunction is associated with multiple human diseases, including hereditary forms of spherocytosis, hemolytic anemia, neuropathy and myopathy.
- Defects in spectrin have been shown to alter both electrical and mechanical cardiac function suggesting that spectrin may be a therapeutic node for effectively treating both arrhythmias and underlying substrate in cardiac disease.

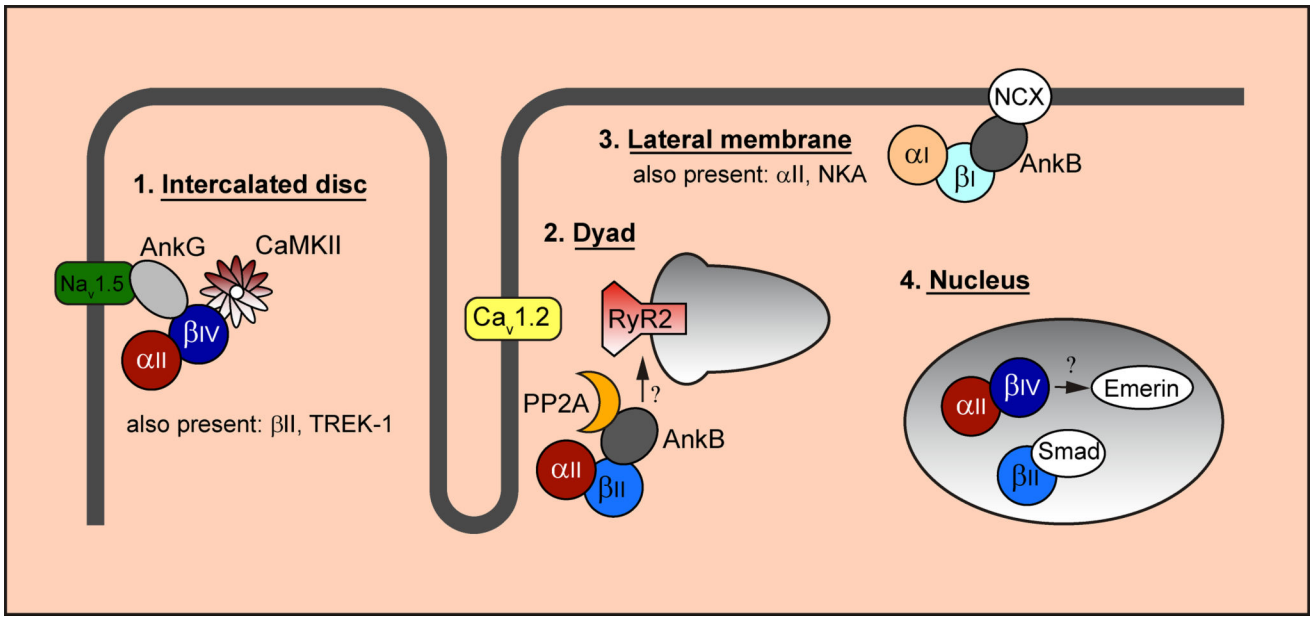

Figure 1. Spectrin isoforms organize macromolecular complexes at distinct membrane domains to regulate myocyte function

α_I-, α_{II}-, β_I-, β_{II}- and β_{IV}-spectrin are all expressed in cardiac myocytes with differential localization to key cellular domains (e.g. intercalated disc, cardiac dyad, lateral membrane and nucleus). α_{II}/β_{IV}-spectrin complexes with $Na_v1.5$ (via ankyrin-G) and CaMKII at the intercalated disc (β_{II}-spectrin and TREK-1 are also expressed in this region, not depicted). At the cardiac dyad, α_{II}/β_{II}-spectrin targets protein phosphatase 2A (PP2A, via ankyrin-B) and regulates expression/localization of sarcoplasmic reticulum ryanodine receptor Ca^{2+} release channels (RyR2). α_I- and β_I-spectrin are found exclusively at the lateral membrane where they likely control membrane targeting of Na^+/Ca^{2+} exchanger (NCX via ankyrin-B) and Na^+/K^+ ATPase (not depicted). Several spectrin isoforms, including α_{II}-, β_{II}- and β_{IV}-spectrin, are found in the nucleus where they are implicated in transcriptional regulation (e.g. by shuttling of Smad proteins into the nucleus) and in nucleoskeleton support via emerin.

Table 1

Characteristics, tissue expression and disease associations of spectrin isoforms

Gene	Isomer	Size (amino acids, full length)	Tissue Expression	Associated diseases
SPTA1	α_I-spectrin	2419	Erythrocytes, neutrophils and lung alveolar lavage	spherocytosis, type 3 pyropoikilocytosis elliptocytosis-2 spta1-related spherocytosis hereditary spherocytosis hereditary elliptocytosis hypophosphatasia
SPTAN1	α_{II}-spectrin	2472	Brain, spinal cord, cardiac muscle, retina, liver	epileptic encephalopathy, early infantile, 5 west syndrome infantile epileptic encephalopathy quadriplegia neonatal lupus erythematosus ohtahara syndrome spastic quadriplegia
SPTB	β_I-spectrin	2137	Skeletal muscle, heart, neutrophils	sptb-related spherocytosis elliptocytosis 3 pyropoikilocytosis hereditary elliptocytosis gnathomiasis otopalatodigital syndrome hereditary spherocytosis
SPTBN1	β_{II}-spectrin	2364	Brain, spinal cord, heart, liver	Williams-Neuren syndrome, neurofibromatosis type 2
SPTBN2	β_{III}-spectrin	2390	Brain, salivary glands, retina and cervix	spinocerebellar ataxia 5 spinocerebellar ataxia, autosomal recessive 14 spectrin-associated autosomal recessive cerebellar ataxia spinocerebellar ataxia ataxia
SPTBN4	β_{IV}-spectrin	2564	Brain, heart, lung, retina and pancreas	Cardiac arrhythmia, myopathy
SPTBN5	β_V-spectrin	3674	Adipocytes, platelets and breast tissue	Macular disorders