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Touch, Tension, and Transduction – the Function and Regulation of Piezo Ion Channels

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Abstract

In 2010, two proteins, Piezo1 and Piezo2, were identified as the long-sought molecular carriers of an excitatory mechanically activated current found in many cells. This discovery has opened the floodgates for studying a vast number of mechanotransduction processes. Over the past six years, groundbreaking research has identified Piezos as ion channels that sense light touch, proprioception, and vascular blood flow, ruled out roles for Piezos in several other mechanotransduction processes, and revealed the basic structural and functional properties of the channel. Here, we review these findings and discuss the many aspects of Piezo function that remain mysterious, including how Piezos convert a variety of mechanical stimuli into channel activation and subsequent inactivation, and what molecules and mechanisms modulate Piezo function.

Piezo proteins: True mechanically activated ion channels?

Piezo proteins are pore-forming subunits of ion channels that open in response to mechanical stimuli, allowing positively charged ions, including calcium, to flow into the cell (Figure 1) [1]. Piezo orthologs have thus far been identified in numerous eukaryotes. Most vertebrates have two channel isoforms, Piezo1 and Piezo2, whereas *Drosophila melanogaster* has a single ortholog (sharing equal homology to Piezo1 and Piezo2) that has also been confirmed to form a channel [1–3]. Genomic analysis predicts single Piezo orthologs in most lower organisms, including *Caenorhabditis elegans*, plants and slime mold, although zebrafish have three and protozoa have up to six predicted isoforms [1, 2, 4–6]. No homologs have been identified in bacteria or yeast, suggesting the evolutionary need for a novel mechanosensor in higher organisms.

Across species, Piezos are very large proteins (2521 and 2752 amino acids for human Piezo1 and human Piezo2, respectively) with numerous (>14) predicted transmembrane (TM) domains per subunit and, strikingly, no homology to other known proteins [1]. In near-physiological solutions, Piezos permeate cations with a single-channel conductance of ~29

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pS and ~24 pS for mouse Piezo1 and mouse Piezo2, respectively [3, 7]. In the presence of a constant stimulus, Piezo-mediated currents decay on a millisecond timescale due to a poorly understood mechanism, likely including channel inactivation (see Glossary), that is subject to genetic and physiological modulation (Box 1 and Figure 1). Piezos fulfill many requirements for true mechanically activated ion channels, as they are pore-forming subunits, confer mechanically-activated currents when expressed in a heterologous system, and are necessary for mechanical responses in many cells [8]. However, no one has yet demonstrated that Piezos undergo mechically-induced (rather than spontaneous or chemically-induced) openings in a purified lipid bilayer, which would be considered as much stronger evidence that Piezos are intrinsically mechanosensitive.

Box 1

inactivation vs. adaptation

In the presence of a constant stimulus, Piezo currents decay with a characteristic time constant that could arise from two distinct mechanisms, inactivation and adaptation (Figure I). Inactivation refers to a process where after initial response and decay to a given stimulus, a further increase in stimulus intensity is not sufficient to elicit an increase in open probability; the stimulus must be completely removed and channels must deactivate in a time-dependent manner and return to a basal state, where they are available for new stimulation. In contrast, adaptation is a process where after an initial response and decay to a given stimulus, a further increase in stimulus intensity increases open probability, even if no time is given for channels to recover.

For Piezo1, the contribution of adaptation was tested using the “stretch” assay: after currents decayed in response to a moderate pressure stimulus, only a small amount of additional current was elicited upon a step to a stronger pressure, which is consistent with inactivation as the process driving the loss of current [67]. Moreover, after removal of a stimulus, both Piezo1 and Piezo2 must recover over a time course of seconds before fully responding to a new stimulus, which again is consistent with an inactivation mechanism [3, 40]. However, the contribution of adaptation has not been directly tested for Piezo2 (or for Piezo1 using other stimulus methods), and therefore more rigorous study is needed. Interestingly, when assayed using the “poke” assay, the decay of rapidly-adapting (Piezo2-mediated) mechanosensitive current in DRG neurons is due to a combination of inactivation and adaptation, suggesting that Piezo2 could undergo both processes [79].

While the molecular mechanism of inactivation and/or adaptation for Piezo channels remains unknown, two common inactivation mechanisms found in other ion channels are potential candidates: “N-type” and “C-type” inactivation, which result from a domain physically occluding the pore and a pore collapse, respectively [80]. The voltage dependence of Piezo inactivation points toward a permeation-dependent C-type mechanism, whereas the extensive extracellular and intracellular domains of Piezo1 hint toward an N-type mechanism.

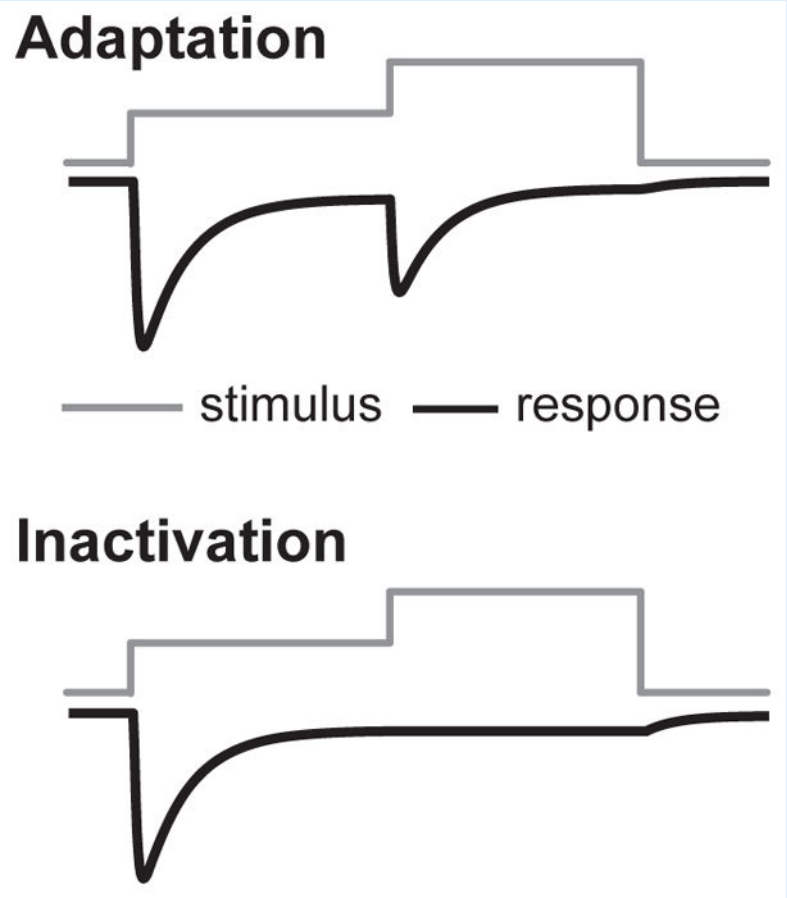

Figure I. Adapting vs. inactivating currents

Idealized currents (black) in response to a two-pulse stimulus protocol (gray), demonstrating the presence of additional current upon an increase in stimulus intensity for an adapting, but not for an inactivating current.

Clearly, mechanistic knowledge is still lacking for many aspects of channel function. Here, we describe what is currently known about the physiological roles of Piezos and their mechanistic function, highlight technological advances that have facilitated the study of these processes, and identify key questions that must be addressed in future work.

Physiology of Piezo mechanotransduction

Piezo expression in tissues and cells

While the two mammalian isoforms are abundantly expressed in a wide range of mechanically sensitive cells, Piezo1 is primarily expressed in non-sensory tissues exposed to fluid pressure and flow (e.g., kidneys, red blood cells), whereas Piezo2 is predominantly found in sensory tissue (e.g., dorsal root ganglia (DRG) sensory neurons and Merkel cells) that respond to touch (Figure 2). This distinct distribution pattern is apparently conserved in other species with multiple isoforms, as Piezo1 is found in erythrocytes and Piezo2 in Rohon-Beard sensory neurons in zebrafish; Piezo2 expression has also been confirmed in

sensory trigeminal ganglion neurons in the star-nosed mole andbirds (with a particular enrichment of Piezo2-expressing neurons in tactile foraging waterfowl) [6, 9–12]. The single *Drosophila* isoform is found both in sensory tissue (including Type 1 ciliated and Type II multidendritic sensory neurons) and in non-sensory tissue (including hindgut, aorta, and trachea), suggesting Piezos may be less specialized in lower organisms [2]. In addition, a few cell types express both Piezo1 and Piezo2, raising the possibility that they could form heteromeric channels with potentially distinct functions [13]. The fact that Piezos were identified as components of mechanically activated ion channels, together with their presence in cells and tissues well-known to be mechanically sensitive, suggested early on that these proteins might play important physiological roles in mechanotransduction.

Piezo physiology – insights from knockout studies and human disease-related point mutations

In vertebrates, expression of Piezo channels is essential for survival. A global knockout of Piezo1 in mouse is lethal during midgestation, owing at least in part to disrupted development of the vasculature system [14, 15]. A smooth muscle cell-specific knockout of Piezo1 (*sm22Cre Piezo1*$^{-/-}$) is viable, but the mice have deficits in arterial remodeling upon hypertension [16]. Consistent with these phenotypes, Piezo1 senses shear stress and cell volume in red blood cells and vascular endothelial cells, and mediates stretch-activated currents in other flow-sensitive cells, including renal epithelial and bladder urothelial cells [9, 14, 15, 17–20]. Piezo1 also senses the local cellular environment (e.g., stochastic nanoroughness, confinement, or substrate stiffness) in neurons and other cells, thereby promoting downstream changes in specific cell-cell interactions, lineage choice, and motility [21–23]. The involvement of Piezo1 in cell motility may explain the link between upregulated Piezo1-mediated activity in the breast cancer line MCF-7 and reduced survival rates in patients with increased Piezo1 mRNA levels in the primary tumor [15].

Consistent with these essential roles in the vasculature and other mechanically sensitive cells, over 25 mutations in Piezo1 have been linked to multiple human disorders (Table 1). Although most mutations have not been characterized in detail, six of these are known to be gain-of-function mutations that slow inactivation rate and are associated with dehydrated hereditary xerocytosis [24–28]. Mechanistically, the mutant channels allow excess calcium influx into red blood cells, leading to downstream activation of a potassium channel and subsequent osmotically-driven dehydration [29]. In contrast, several loss-of-function mutations occur in patients with congenital lymphatic dysplasia [30, 31]. Paradoxically, there appears to be overlap in the symptoms of these two disorders: Some xerocytosis patients also have lymphedema, especially perinatally [28, 32], while red blood cells in lymphedema patients show occasional stomatocytes [31]. The mechanisms by which functionally distinct mutations result in similar pathologies, and how the effects of single mutations in a widely-expressed ion channel are apparently limited to the malfunction of specific organs, remain unknown.

Like for Piezo1, global knockout of Piezo2 in mouse is lethal, with pups dying at birth [33]. Several tissue-specific conditional knockout lines have shown that Piezo2 mediates much of the organism’s response to light, but not harsh mechanical touch. Specifically, Piezo2

channels confer the mechanically sensitive current in Merkel cells; consistent with this, skin-specific knockout of Piezo2 (*Krt14Cre;Piezo2$^{fl/fl}$*) leads to reduced light touch responses [34, 35]. Likewise, an inducible knockout of Piezo2 from dorsal root ganglion (DRG) neurons (*Advil-creERT2;Piezo2^{fl}*), nearly abolishes rapidly-adapting, mechanically activated currents in these cells, causing severe deficits in multiple assays designed to test response to innocuous touch and gait stability [33]. The latter phenotype pointed to a role in proprioception, which was more clearly probed by two mouse models: a proprioceptive-specific DRG knockout (*Pvalb- Cre;Piezo2$^{fl/f}$*), which lead to severely impaired limb coordination, and a knockout from proprioceptive neurons of the mesencephalic trigeminal nucleus, which lead to impairments in coordination and balance [36, 37]. Piezo2 also mediates mechanosensitive currents in enterochromaffin cells from mouse small bowel [38]. Intriguingly, the primarily sensory-specific roles of Piezo2 have not yet been reconciled with the lethal phenotype of the global knockout, indicating that there must be additional functions not yet identified.

Over a dozen mutations in Piezo2 are associated with several arthrogryposis disorders (Table 1) [39–41]. Two of these mutations have been electrophysiologically characterized and destabilize inactivation, leading to an overall increase of calcium influx [40].

While roles of Piezos in some modalities of mechanotransduction are now well-established, other mechanotransduction processes have been shown to be independent of Piezos: Piezo2 ablation does not reduce either intermediately- or slowly-adapting mechanical responses of DRG neurons (to which the protein Tentonin3 was recently proposed to contribute) or responses to harsh mechanical touch in mice [1, 33, 42]; additionally, mechanotransduction in the hair-cells of the inner ear is not dependent on Piezo1 [43].

Together, these studies make clear that Piezo ion channels transduce many types of mechanical inputs, raising the questions of what mechanical forces Piezos sense and how they are transduced into channel activation.

The activation mechanism(s) of Piezos

Activating stimuli of Piezos

Researchers have developed multiple techniques for stimulation of Piezo ion channels *in vitro,* each of which has distinct advantages and disadvantages with respect to ease, number of channels sampled, and quantification of stimulus and response (Figure 3). The most commonly used are “stretch” and “poke” in combination with patch-clamp electrophysiology (Figure 1). In “stretch”, the membrane is stimulated using a high-speed pressure clamp, which results in highly reproducible pressure-response relationships. The use of parallel imaging reveals that “stretch” induces global membrane curvature; using measurements of curvature (patch radius) and pressure, Laplace’s law can be used to calculate the corresponding global membrane tension, one physical stimulus sensed by Piezo1 [44, 45].

In “poke” the membrane is indented with a piezoelectric-driven blunt glass pipette during a whole-cell recording, leading to larger current amplitudes and ease of perfusion of

pharmacological agents. In contrast to “stretch”, however, “poke” recruits varying numbers of channels with each indentation depth, leading to inconsistent stimulus-response relationships and often resulting in a lack of response saturation prior to patch rupture [46]. While this method requires relatively large deflections from above to elicit currents from the soma (~5 μm) or neurites (~500 nm), small deflections (~10 nm) of micropillar arrays supporting cells from below can also activate Piezo-mediated currents with high sensitivity [47]. Similar to poke, the stimulus-response relationship is inconsistent and does not saturate.

While both “poke” and “stretch” stimulation have been instrumental in defining the basic properties of Piezos, it is unclear how either method relates to physiological forces experienced by Piezo-expressing cells. Precise measurements of the force applied to the membrane can be achieved through the use of atomic force microscopy (AFM). For example, mechanical loading forces of ~400 nN compress chondrocytes and elicit Piezo1-mediated currents [13]. However, the membrane geometry induced by the indentation and experienced by the channel is again undefined and likely variable. Shear stress, which occurs upon the flow of blood along walls of arterial cells, also activates Piezo1, and can be experimentally applied by placing a cell next to a superfusion pipette or in a microfluidic chamber [14, 15]. Notably, chronic application (minutes to hours) of shear stress allows probing for long-term effects of mechanotransduction, including changes in gene expression and cell morphology, which is not easily achievable through other techniques [13, 14]. Fluid flow could activate Piezo1 by multiple mechanisms, including the induction of shear wall stress in the membrane or through direct frictional forces on the channel itself.

The chemical agonist Yoda1 specifically activates (and modulates – see next section) Piezo1 channels in Ca^{2+}-imaging assays, providing a simple method for uniform stimulation of a large population of channels [48]. The precise mechanism by which Yoda1 activates Piezo1 remains unknown; the open state stabilized by Yoda1 has an identical single-channel conductance to the tension-gated open state, suggesting both pore open conformations are similar.

One common limitation of the above described techniques is that they cannot probe mechanical sensitivity on a submolecular level (Figure 3). To overcome this, our lab developed a novel method in which magnetic nanoparticles are used to apply localized pulling force on specific Piezo1 domains, while recording channel function electrophysiologically[49].

Notably, while most of the above manipulations efficiently activate Piezo1, several of them fail to activate Piezo2 to the same extent: For example, stimulation with “stretch” leads to only occasional Piezo2 activation (single channel openings occur in ~50% of Piezo2-expressing cells vs. ~90% of Piezo1-expressing cells) and negligible macroscopic currents [7, 13]. Likewise, compression with AFM and Yoda1 do not efficiently activate Piezo2 in HEK293t cells; Piezo2 shear stress responses have not yet been tested [13, 48]. The reasons for these observations are unclear, but may point towards fundamental differences in activation mechanisms. We believe the fact that Piezo1 is sensitive to a larger number of

stimuli may indicate that it is a polymodal sensor of diverse mechanical forces, whereas Piezo2 could be more narrowly tuned to specifically detect mechanical touch.

Two recent studies, including one from our lab, concluded that one activating stimulus of Piezo1 is lateral membrane tension [44, 45]. Both positive and negative pressure, which cause opposing global membrane curvature, efficiently activate Piezo1 ion channels with high sensitivity, with a half-maximal tension for activation ($T_{1/2}$) as low as 1.4 mN/m when resting tension is removed from the patch, as compared to mechanically activated ion channels MscS and MscL from bacteria ($T_{1/2}$ of ~ 5–10 mN/m) [44, 45, 50, 51]. This extremely low threshold of Piezo1 to membrane tension, together with its apparent polymodality, might explain why this unique mechanosensor evolved in metazoans.

Piezo channel structure

If tension is the primary activating stimulus for Piezo1, then how might the protein sense this force? A look at the overall architecture of the protein, recently revealed in a medium-resolution (4.8 Å) cryo-EM structure of mouse Piezo1, gives some clues [52]. The overall shape of the trimeric complex is that of a propeller, with three curved “blades” surrounding a central pore that is topped by a cap referred to as the C-terminal extracellular domain (CED) (Figure 4). Despite the large size of the protein (200 Å diameter), a much lower number (14) of TM domains are resolved in the Piezo1 structure than initially predicted through topology software (18–38) [7].

Although much of the primary sequence cannot be assigned to the structure due to its coarse resolution, the CED was crystallized separately at an atomic resolution (1.7 Å) and revealed an unusual beta sandwich fold that is not found elsewhere in nature [4, 52]. The CED is located between the last two TMs, termed outer helix (OH) and inner helix (IH), and together this region (OH-CED-IH) likely forms the permeation pathway. Evidence for this comes not only from a visible pore in the structure, but also from a series of chimeras and point mutations conferring changes in single-channel conductance, ion selectivity, and sensitivity to the pore blocker ruthenium red [7, 52, 53]. An intracellular coiled-coil beam structure aligns below each blade, and contacts an “anchor” domain at the interface of the three subunits. The anchor, which may contain the highly conserved $PF(X_2)E(X_6)W$ motif found in Piezos in all species, also results in clockwise swapping of the OH and CED of one monomer into the region of the neighboring monomer (Figure 4) [5, 52]. While no structure is yet available for Piezo2, its identical size, similar predicted topology, and high sequence similarity to the pore domain of Piezo1 (54% identity for the OH-CED-IH-CTD) suggest an overall identical architecture.

The activation mechanism

Several mechanisms have been proposed to explain how mechanical force can be coupled to opening of an ion channel (Figure 4) [54, 55]. Mechanical force can be directly transmitted to the channel through lateral tension in the membrane bilayer, whereby the conformation with the greater cross-sectional area is favored under higher tension [56]. For this elastic model of tension gating, the in-plane area change (ΔA) upon opening of Piezo1 has been estimated to be 6–20 nm^2, which is similar to that of MscL and MscS (~15 -20 nm^2) and

greater to that of TREK-1 and TRAAK channels (~3–5 nm^2) [24, 45, 57–59]. For MscL, ΔA is equivalent to the difference in cross-sectional area between the open and closed channels; for all channels, this value will be proportional to the work required to open the channel [54, 60]. .

Tension also causes changes in bilayer thickness and lateral pressure profiles, which creates hydrophobic mismatch and subsequent adaptive changes in protein conformation that could gate the pore [61]. While the direction of global curvature has no specific effect on Piezo channel gating, in theory, changes in global (radius > 100 nm) or local membrane curvature (radius < 100 nm, on the scale of caveolae or microvilli) could enact similar changes in the hydrophobic environment of polar and nonpolar residues [44, 62]. Moreover, based on the strongly curved shape of the blades, Piezo itself could induce locally distinct membrane curvature, thickness, and tension, and thereby create an equilibrium between externally and locally induced membrane properties [56].

For Piezo1, both the cap and the first two extracellular loops near the N terminus are mechanically sensitive, as pulling on them with magnetic force induces changes in channel activation and inactivation [49]. The curvature and large size of the peripheral blades may position them as particularly efficient sensors of membrane geometry.

Alternatively, mechanical force can be transmitted by tethering the channel to the extracellular matrix or the cytoskeleton. This mechanism seems unlikely for Piezo1, as when expressed in HEK293t cells, the channel was not found to interact strongly enough with any other proteins for them to be identified using mass spectroscopy [3]. Moreover, Piezo1 can be efficiently activated in cytoskeleton-deficient blebs [45]. However, the contribution of a tether to activation and/or inactivation of either Piezo1 or Piezo2 has not been tested in the vast number of cells in which they are expressed; a tethered mechanism remains an intriguing possibility for Piezo2 in particular, as robust macroscopic currents have thus far only been been elicited by the “poke” stimulus..

The coupling of mechanical energy to pore opening could also be mediated by interactions of a membrane lipid with a binding pocket on the protein, as has been established for the mechanosensitive channels TRAAK and MscS [63–65]. Depleting phosphoinositides, including PIP2, from the patch membrane inhibits Piezo1 and Piezo2 activity, indicating a similar mechanism could contribute to Piezo gating [66].

Again, the large, complex structure of Piezo1, coupled with its diverse expression patterns and activating stimuli *in vivo* and *in vitro,* points toward the possibility that several activation mechanisms exist, making Piezo1 a candidate for a mechanically polymodal ion channel.

Modulation of Piezo function

Having two mechanotransduction channels at their disposal allows cells to alter their mechanical sensitivity by expressing either of the two Piezo isoforms. Notably, in addition to the varying sensitivity to specific stimuli, the two isoforms have distinct inactivation properties, which will have a large influence on overall depolarization and calcium

signaling. Specifically, Piezo1 has slower inactivation kinetics than Piezo2 (decay time constant of ~15 ms vs ~7 ms, respectively, as measured with “poke”) (Figure 1) [1, 3].

Piezo activity can also be titrated by a diverse array of modulators, which can be broadly divided into two categories: those acting on membrane properties and thus indirectly modulating channel function, and those acting through direct interactions with the channel itself. Notably, the precise mechanism (passive versus active) has not yet been elucidated for many modulators, some of which could in theory act either on the channel or on the membrane.

Passive (indirect) modulation through membrane tension

As Piezo1 senses membrane tension through the lipid bilayer, alterations to the mechanical state of the membrane or its composition will affect Piezo1 function. First, although a cytoskeletal network is not required for mechanosensitivity of Piezo1, its bi-directional influence on tension sensitivity has been demonstrated in inside-out patches, cytoskeleton-deficient blebbed membranes, and by pharmacological disruption and osmotic swelling [44, 45, 67, 68]. Second, Piezo1 and Piezo2 are sensitized by the integral membrane protein STOML3, which recruits cholesterol to the membrane, thus likely increasing membrane stiffness and facilitating force transfer to the channel [47, 69]. The result highlights the possibility that lipid composition in general could affect Piezo function through alterations in membrane stiffness. Finally, our lab found that resting membrane tension itself modulates the fraction of Piezo1 channels in inactivated states, thereby shifting the apparent sensitivity of Piezo1 to stimulation [44].

Active (direct) modulation through channel structure

The channel itself is also a target for direct modulation. Multiple mutations, many of which are clustered in the pore region (OH-CED-IH), affect inactivation kinetics [24, 26, 40]. Interestingly, an irreversible loss of inactivation can be induced either by localized force application to two N-terminal extracellular loops or to the CED of Piezo1, or by repeated applications of tension [24, 49].

Inactivation kinetics are also affected by membrane voltage, divalent ions, and protonation, which highlights the channel’s sensitivity to electrostatic interactions, opening it up to continuous spatial and temporal modulation in a complex cellular context [1, 3, 67, 70]. We are still awaiting evidence for other potential and common direct channel modulators including phosphorylation, for which mass spectrometry identified 23 intracellular sites on Piezo1, and glycosylation [7].

Mechanisms that could be passive, active, or both

For other modulators, we do not yet have mechanistic insight into whether they influence channel activity directly or indirectly. The small molecule Yoda1, which activates Piezo1 in calcium-imaging assays and attenuates inactivation by stabilizing the open state when assayed with electrophysiology, could, in principle, act either on the channel or through the membrane, as could the Piezo1/Piezo2 inhibitory spider toxin peptide GsMTx4 [48, 71]. PIP2, whose depletion reduces current amplitudes, can directly activate ion channels as a

ligand, but also alters membrane properties directly and through the cytoskeleton by regulating actin- binding proteins, suggesting its effect on Piezos may be multimodal [72–75].

G protein-coupled pathways involving the bradykinin receptor and the cAMP receptor Epac1, as well as GTP itself, all sensitize Piezo1 and/or Piezo2 to stimulation, potentially through activation of PKA and PKC [76–78]. Whether any of these potentially connected means of regulation involve direct phosphorylation of the channel or indirect regulation of other cellular properties (e.g., the cytoskeleton) has yet to be established.

One current obstacle to understanding the mechanism of particular modulators is the lack of a stimulation method that directly controls membrane tension (i.e., a “tension clamp”), which would allow for the determination of whether a given modulator affects the sensitivity of Piezo to tension or, rather, alters the tension produced by a particular stimulation method.

Inactivation as a major mechanism of modulation

A recurring theme is that many modulators (e.g., voltage, pH, channelopathies, resting tension) specifically affect the process of channel inactivation. This brings up the intriguing possibility that regulation of inactivation is a major mechanism for calibrating Piezo activity to needed levels in diverse cells that are subject to a wide range of mechanical forces. Modulation of inactivation can also transiently amplify Piezo activity within a single cell, in particular, those with previous excitatory drive (e.g., due to synaptic transmission, temperature, or pH). For example, calcium entry will be further amplified at positive voltages (despite the loss of driving force) due to slowing of inactivation. A complete mechanistic understanding of the process of inactivation will therefore be crucial to further understand how diverse modulators alter inactivation and subsequently regulate Piezo function *in vivo*.

Concluding remarks

Our understanding of which mechanotransduction processes require Piezo ion channels, what molecules and processes modulate Piezo function, and the basic structural and functional properties of Piezo channels is growing rapidly. Yet, there remains much to be done (see Outstanding questions). In particular, little is known about the precise mechanism by which the channel senses such a wide variety of forces and transduces these forces into pore opening and subsequent inactivation. Additionally, there are many mechanotransduction processes for which a role of Piezos has not yet been explicitly tested. Finally, while we have identified many modulators, there is little information as to how they mechanistically alter the processes of activation and inactivation, or their physiological relevance. One key need going forward is therefore the development of new technologies that can specifically and quantitatively activate and measure the activity of Piezo channels.

Outstanding Questions

- What are all of the physiological roles played by Piezo1 and Piezo2? Multiple tissues express one or both Piezos (e.g. lung, colon) but the respective

contributions of each isoform to mechanotransduction have not yet been tested. Even for cell types with confirmed Piezo-mediated currents, we lack information on how these currents contribute to cell function and dysfunction, in the case of channelopathies. Filling these gaps will require additional cell-specific knockout models, as well as CRISPR lines to introduce and study specific mutations.

- Can Piezos be pharmacologically targeted for treatment of disease? The wide expression pattern of both Piezo1 and Piezo2 will require drugs to act in both an isoform-specific and tissue-specific manner.
- What are the activation mechanisms of Piezo1 and Piezo2? Additional, high-resolution structures that capture both channels in open, closed, and inactivated states will be crucial to elucidate the full spectrum of channel function. Detailed electrophysiological characterization of channels that are manipulated genetically (e.g., chimeras and point mutations) and chemically (e.g., magnetically labeled, crosslinked) will help in defining the functions of specific structural domains.
- What are the inactivation mechanisms for Piezo1 and Piezo2? Many channelopathies specifically affect inactivation, making this process an intriguing pharmacological target. This will require first understanding whether inactivation results from pore block or pore closure.
- How, mechanistically, do a rapidly growing list of proteins, chemical and physical modulators, and pathways alter Piezo function? The development of new stimulation methods will be instrumental in distinguishing between direct and indirect modulation, for example, by holding membrane tension constant (tension clamp).

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Glossary

Arthrogryposis
A family of disorders including Distal Arthrogryposis type 5, Gordon Syndrome, and Marden-Walker Syndrome. Patients with these disorders all exhibit congenital joint contractures (or abnormal stiffness of joints), but can be distinguished by other, specific symptoms.

Channelopathy
A disease caused by dysfunction of an ion channel, often resulting from a mutation in the channel gene.

Channel inactivation

A process in which a channel initially opens in response to a stimulus but over time, despite the continued presence of the stimulus, ceases to conduct ions (closes).

Congenital lymphatic dysplasia
A disease characterized by severe swelling, or lymphedema, in the limbs.

Dehydrated hereditary xerocytosis
A disease characterized by dehydration of red blood cells, resulting in increased fragility of these cells and subsequent anemia. The dehydrated cells have a cup shape, and are often referred to as stomatocytes.

Dorsal root ganglia (DRG) neurons
Sensory neurons with afferents that terminate in the spinal cord, and with sensitivity to mechanical touch, temperature and specific chemicals.

High-speed pressure clamp
An experimental device allowing precise (±1 mmHg) and rapid (~10 ms) control of pressure (both negative and positive). It is used to mechanically stimulate a channel-containing membrane patch within a pipette during an electrophysiological recording (Figure 1).

Hydrophobic mismatch
A difference in length between the hydrophobic segment of a protein and the hydrophobic thickness of a membrane, a situation that results in energetically unfavorable exposure of hydrophobic protein residues to the hydrophilic environment.

Mechanically activated ion channel
To fulfill this definition, a protein must form a channel, and confer mechanically activated currents when expressed heterologously. Further, to be considered a mechanosensor *in vivo*, the protein must additionally be expressed in mechanosensory cells, and be necessary and sufficient for mechanically activated currents in those cells.

Mechanotransduction
The process by which a mechanical stimulus is transduced into biological signals within a cell.

Membrane tension
The lateral (in-plane) force (N/m) in a membrane bilayer. Membrane tension has been demonstrated to be an activating stimulus for Piezo1 [44, 45].

Merkel cell
Specialized skin cell type that is sensitive to light mechanical touch.

Polymodal ion channel
An ion channel that is activated by multiple distinct stimuli through possibly distinct mechanisms.

Proprioception
The sense of one’s body position and movement in space.

Shear stress

Force resulting from the movement of fluid relative to an object (in units of force/cross-sectional area).

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Trends Box

- Piezo proteins were identified in 2010 as the pore-forming subunits of excitatory mechanosensitive ion channels.
- Piezo ion channels play essential roles in diverse physiological processes ranging from regulation of red blood cell volume to sensation of gentle touch, and are associated with a number of diseases.
- A recent medium-resolution structure gives insight to the overall architecture of Piezo1, but does not give straight answers as to how the channel transduces mechanical force into pore opening.
- The function of Piezos, including the inactivation mechanism, can be modulated by many factors both intrinsic and extrinsic to the channel.

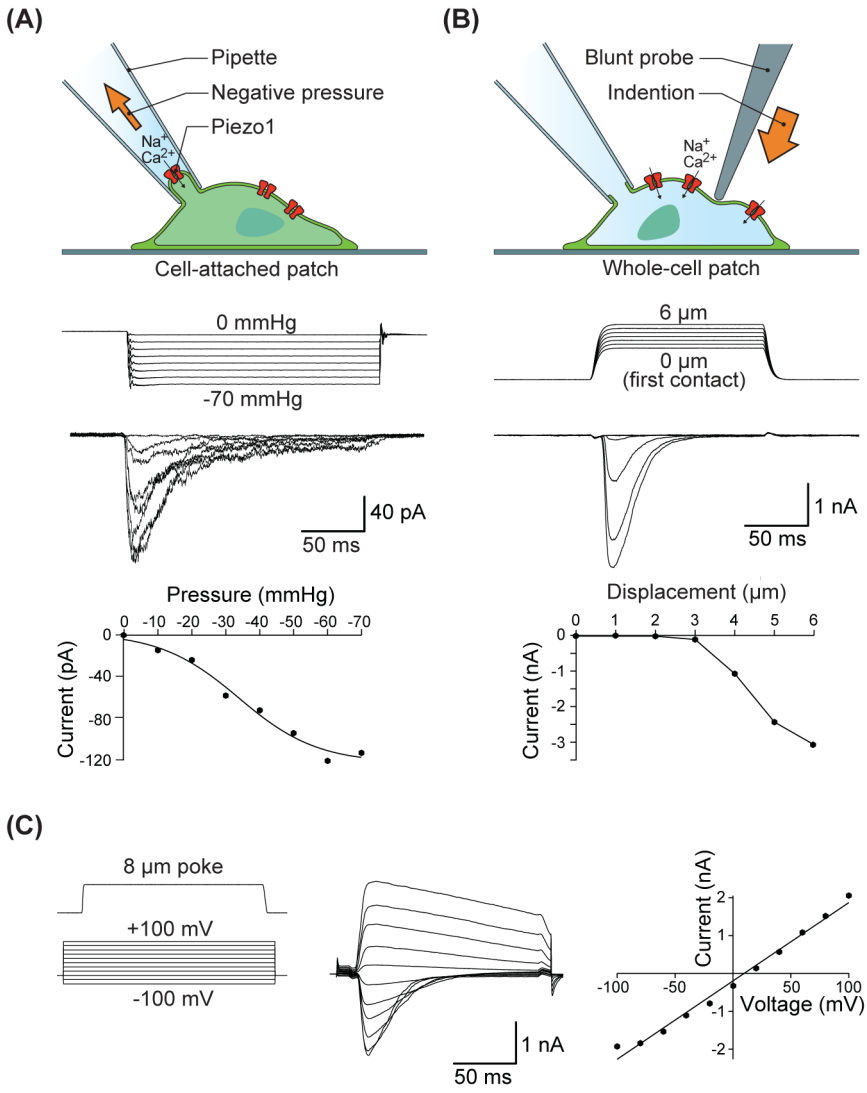

Figure 1. Piezos are mechanically activated ion channels

(A) Schematic of "stretch" setup, in which negative suction is applied to a cell-attached patch with a **high-speed pressure clamp** through the patch pipette, stimulating only those channels contained within the patch dome (*above*). Piezo1 peak current amplitudes initially rise with increasing magnitudes of pressure before reaching saturation (*middle*). The pressure-response relationship can be fit with a sigmoidal function to measure pressure sensitivity (*below*). Data are from Wu and Grandl, unpublished. (B) Schematic of "poke" setup depicting cell deformation by a blunt probe (typically a fire-polished glass pipette) during a whole-cell recording, which activates a larger population of channels throughout the cell (*above*). Piezo1 current amplitudes increase with increasing steps of displacement beginning a few micrometers beyond first contact of the probe with the cell membrane. From these experiments, a current-displacement curve can be generated. Typically, currents do not plateau before cell rupture (*below*). Data are from Lewis and Grandl, unpublished. (C) Voltage step protocol with a single "poke" displacement during each step (*left*). A family of currents from a single cell illustrates the voltage dependence of channel inactivation, with

severely slowed decay times at positive voltages (*middle*). An I-V curve plotted from peak current amplitudes reveals a reversal potential near 0 mV, demonstrating cationic non-selectivity (*right*). Data are from Lewis and Grandl, unpublished.

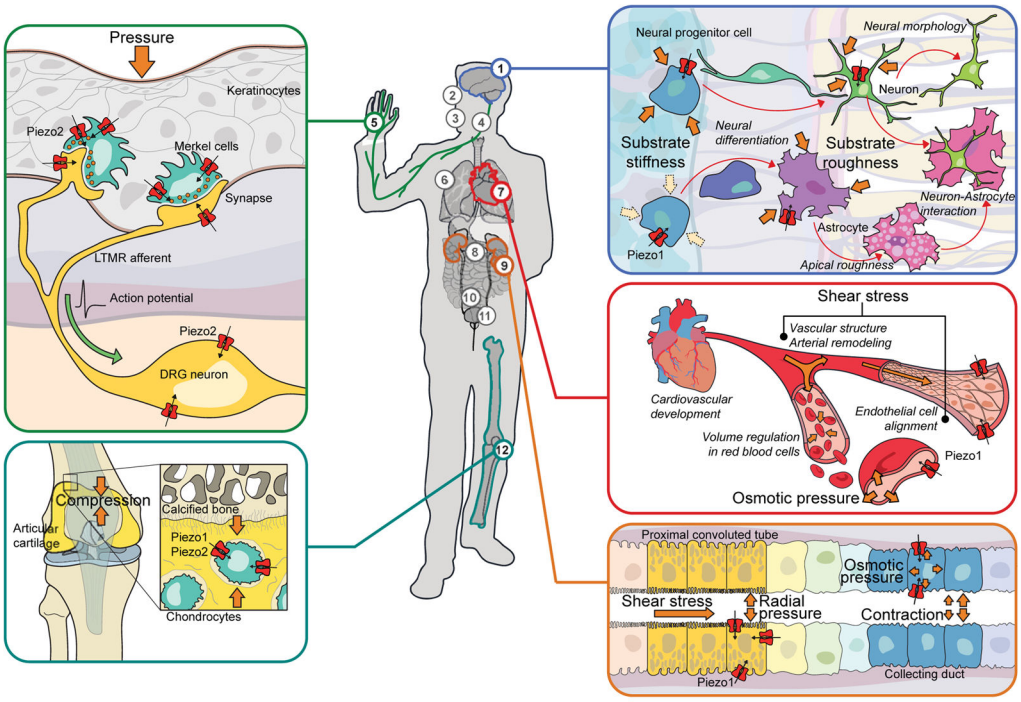

Figure 2. Expression and physiological roles of Piezos

Piezo1 and Piezo2 are expressed in a diverse set of organs and tissues within the human body, contributing to an equally diverse set of physiological roles [1, 9–11, 13, 15–22, 29, 33–35, 38, 81–83]. Numbered tissues are as follows: 1. Brain, 2. Optic nerve head, 3. Periodontal ligament, 4. Trigeminal ganglion, 5. Dorsal root ganglion and skin, 6. Lungs, 7. Cardiovascular system and red blood cells, 8. Gastrointestinal system, 9. Kidney, 10. Colon, 11. Bladder, 12. Articular cartilage. Tissues in which Piezo function has been extensively studied are expanded to show detail. *Top left* inset illustrates Piezo2 expressed in Merkel cells of the skin, where mechanical activation of Piezo mediates depolarization and activation of dorsal root ganglion cell afferents, which also express Piezo2. Together, these cells are involved in sensing light touch and proprioception. *Bottom left* inset highlights the expression of both Piezo1 and Piezo2 in chondrocytes of articular cartilage, where they activate under compressive force. *Top right* inset illustrates the role of Piezo1 in sensing mechanical properties of the environment of neural progenitor cells, thereby initiating signaling pathways that lead to neuronal differentiation and subsequent development of neurite morphology, neuronglia interactions, and nanoroughness of glial membranes. *Middle right* inset depicts the role of Piezo1 in regulating volume of red blood cells as well as sensing shear stress to regulate vascular branching and alignment of endothelial cells. *Bottom right* inset shows the role of Piezo1 in sensing fluid flow throughout the nephron of the kidney. Deficits in Piezo1 function in the kidney may lead to downstream effects on urinary osmolarity and renal pathologies.

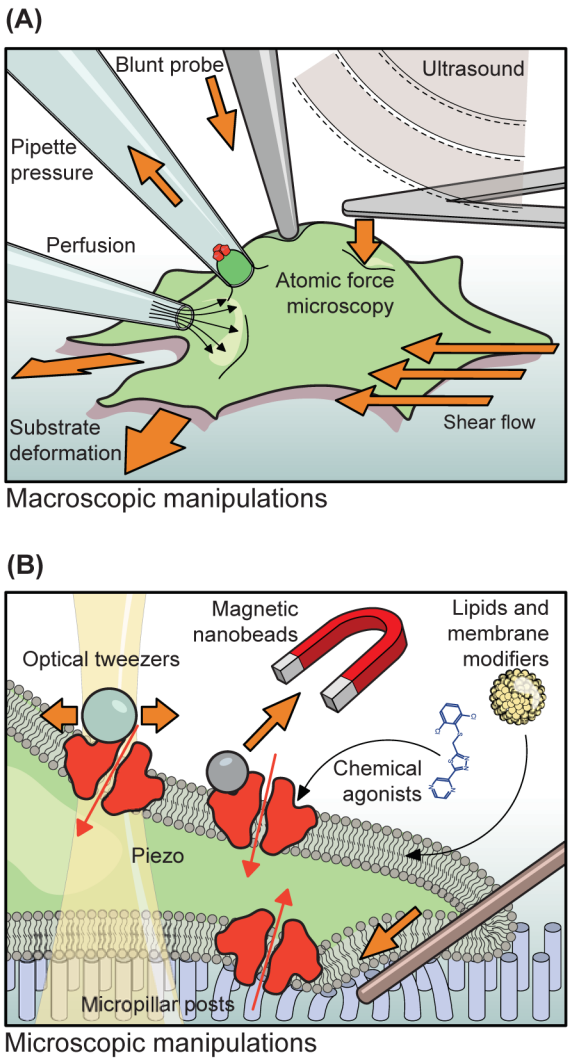

Figure 3. Current and future methods of stimulating Piezos

Orange arrows represent direction of force in relation to cell or channel, and Piezo ion channels are illustrated in red. (A) Macroscopic methods for stimulating large populations of Piezo channels, whose activity can be measured with electrophysiology or through calcium imaging [1, 13, 14, 18]. These include directly deforming the cell with a blunt probe ("poke" assay) or with atomic force microscopy. High pressure perfusion is an alternative method to deform the cell without physically contacting the membrane, while in contrast, shear flow achieved through microfluidic channels applies a parallel stress to the substrate surface. Both positive and negative pressure through a pipette ("stretch" assay) can stimulate single or many Piezo channels. Substrate deformation with flexible membranes and remote vibration of the cell and surrounding milieu through ultrasound are yet untested methods for directly stimulating Piezo channels. (B) Microscopic modes of Piezo stimulation are shown magnified in the context of the plasma membrane [47–49, 69]. Deflection of micropillars stimulates single or small populations of Piezo channels through membrane deformation. The agonist Yoda1 directly activates Piezo1, though the mechanism is unknown. Lipids such as cholesterol modulate Piezo function, but have not yet been shown to directly induce

activation. In theory, direct activation of the channel could be achieved through magnetic or optical control of nanoparticles bound to specific channel domains; application of force through magnetic nanobeads has been shown to perturb channel function, but neither technique has been shown to directly activate Piezo.

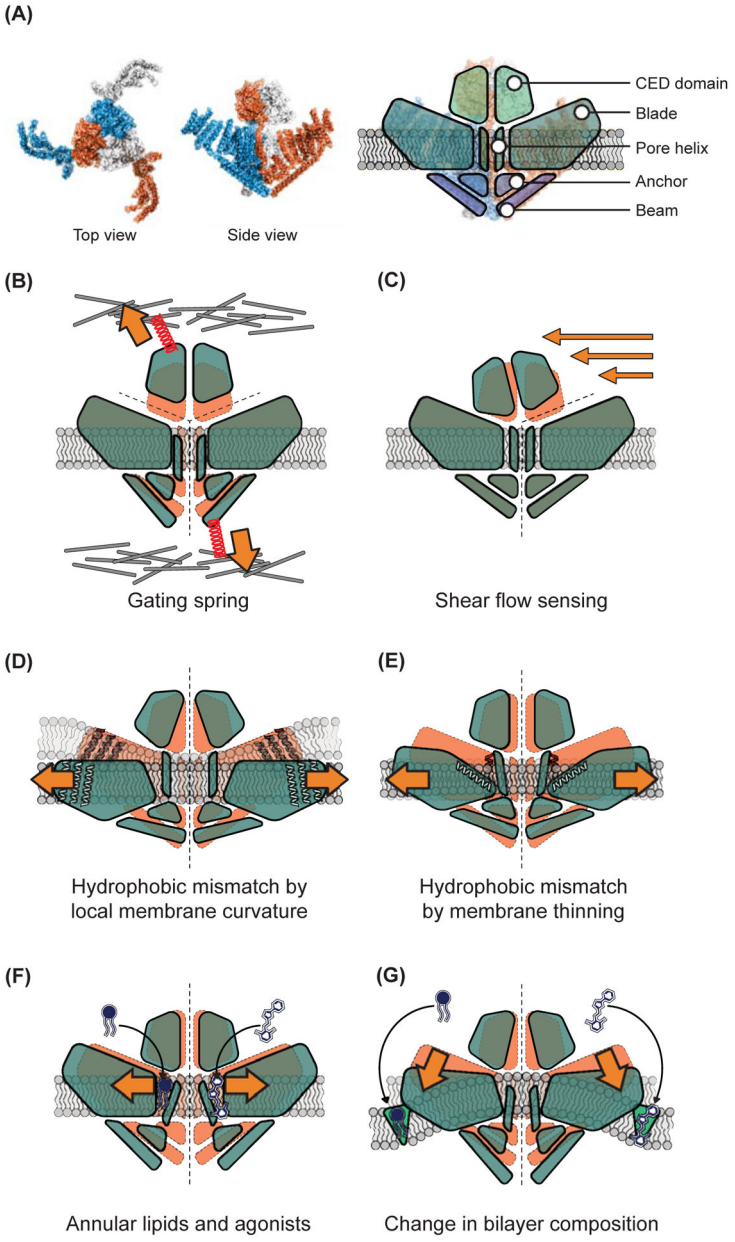

Figure 4. Potential mechanisms of mechanical sensing and activation

(A) The cryo-EM structure (PDB 3JAC) of Piezo1 (*left*) reveals possible structural domains (*right*) that may play a role in mechanosensing and channel activation. (B–E) Possible sensing mechanisms and conformational changes by which Piezo channels may activate in response to external forces. Potential ion permeation pathways are indicated with dashed lines; orange represents the closed channel conformation and green represents the open conformation upon applied force (B) Tethering of either the CED domain to the extracellular matrix or the “beams” to cytoskeletal elements may contribute to a gating spring mechanism of activation. (C) Similarly, local shear flow may displace the CED domain and expose an ion permeation pathway. (D) The curved architecture of the cryo-EM Piezo1 structure

supports the possibility that Piezo rests in a locally curved lipid bilayer environment. With rising membrane tension, the curvature is reduced, potentially causing hydrophobic mismatch of the “blades” and conformational changes in the “beam” and “anchor” domains to open the pore. (E) Hydrophobic mismatch may also occur due to changes in plasma membrane thickness by in-plane membrane stretch, by which a tilt in the pore helices might lead to pore opening. (F) Annular lipids, agonists, and inhibitors may insert directly within the channel structure to initiate changes in channel conformation. (G) Lipids and chemical modifiers may also insert directly into the membrane causing changes in membrane stiffness, tension, or curvature, leading to channel activation.

Table 1

List of mutations in Piezo1 and Piezo2 associated with human diseases

Piezo1	Channel Domain	Mutation	Disease	Effect on channel	Citation
		H702Y	CAP[a]	unknown	[83]
		G718S	DHS[b]	unknown	[28]
		E755X[c]	GLD[d]	unknown	[31]
		G782S	DHS	unknown	[28]
		R808Q	DHS	unknown	[28]
		L939M	GLD	unknown	[31]
		I1007M	CAP	unknown	[83]
		S1117L	DHS	unknown	[28]
		S1153Trpfs*21[e]	GLD	unknown	[30]
		R1358P	DHS	slowed inactivation	[26]
		E1630X	GLD	reduced expression	[31]
		V1712M	CAP	unknown	[83]
		Y1763X	CAP	unknown	[83]
		R1955C	CAP	unknown	[83]
		A2003D	DHS	unknown	[28]
		A2020T	DHS	slowed inactivation	[26]
		A2020V	DHS	unknown	[28]
		G2029R	GLD	reduced expression	[30]
	anchor	T2127M	DHS	slowed inactivation	[26, 28]
	OH[f]	K2166-2169 del[g]	DHS	unknown	[28]
	OH	V2171F	GLD	reduced expression	[31]
	CED[h]	M2225R	DHS	slowed inactivation	[27]
	CED	Q2228X	GLD	reduced expression	[31]
	CED-IH[i] linker	P2430L	GLD	unknown	[31]
	IH	R2456H	DHS	slowed inactivation	[27, 28]

Piezo1	Channel Domain	Mutation	Disease	Effect on channel	Citation
	IH	R2456C	GLD	unknown	[31]
	IH	F2458L	GLD	unknown	[31]
	CTD[i]	R2488Q	DHS	unknown	[28]
	CTD	E2496ELE[k]	DHS/HA[l]	slowed inactivation	[26]
Piezo2	**Channel Domain**	**Mutation**	**Disease**	**Effect on channel**	**Citation**
		M712V	DA5[m]	unknown	[39]
		I802F	DA5	faster recovery from inactivation	[40]
		M998T	DA5	unknown	[39]
		A1486P	DA5	unknown	[41]
		T2221I	DA5	unknown	[39]
		S2223L	DA5	unk	[39]
	anchor	T2356M	DA5	unknown	[39]
	IH	R2686H	GS[n]/DA5	unknown	[39]
	IH	R2686C	MWS[o]	unknown	[39]
	CTD	R2718L	DA5	unknown	[39]
	CTD	R2718P	DA5	unknown	[39]
	CTD	E2727del	DA5	slower onset, faster recovery from inactivation	[39, 40]
	CTD	Y2737Ilefs*7[p]	DA5	unknown	[39]
	CTD	S2739P	DA5	unknown	[39]
	CTD	W2746X	GS	unknown	[39]

[a] colorectal adenomatous polyposis;

[b] dehydrated hereditary stomatocytosis;

[c] premature stop codon;

[d] generalized lymphatic dysplasia;

[e] splice variant encoding truncated product at S1153;

[f] outer helix;

[g] deletion of 4 indicated residues;

[h] c-terminal extracellular domain;

[i] inner helix;

[j] c-terminal domain;

[k], insertion of two residues (LE);

[l] hemolytic anemia;

[m] distal arthrogryposis subtype 5;

[n] Gordon syndrome;

[o] Marden-Walker syndrome;

[p] single base pair deletion leading to premature stop codon