

## Review

# The functions of TRPP2 in the vascular system

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TRPP2 (polycystin-2, PC2 or PKD2), encoded by the *PKD2* gene, is a non-selective cation channel with a large single channel conductance and high Ca<sup>2+</sup> permeability. In cell membrane, TRPP2, along with polycystin-1, TRPV4 and TRPC1, functions as a mechanotransduction channel. In the endoplasmic reticulum, TRPP2 modulates intracellular Ca<sup>2+</sup> release associated with IP<sub>3</sub> receptors and the ryanodine receptors. Noteworthy, TRPP2 is widely expressed in vascular endothelial and smooth muscle cells of all major vascular beds, and contributes to the regulation of vessel function. The mutation of the *PKD2* gene is a major cause of autosomal dominant polycystic kidney disease (ADPKD), which is not only a common genetic disease of the kidney but also a systemic disorder associated with abnormalities in the vasculature; cardiovascular complications are the leading cause of mortality and morbidity in ADPKD patients. This review provides an overview of the current knowledge regarding the TRPP2 protein and its possible role in cardiovascular function and related diseases.

**Keywords:** TRPP2; polycystin-2; vascular smooth muscle cell; endothelial cell; hypertension; autosomal dominant polycystic kidney disease (ADPKD)

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## Introduction

TRPP2 (transient receptor potential polycystic 2, previously known as polycystin-2, PC2, or PKD2) is a nonselective cation channel protein encoded by the *PKD2* gene. It has a large single-channel conductance and high Ca<sup>2+</sup> permeability<sup>[1–3]</sup>. The TRPP2 protein comprises 968 amino acids, which form six transmembrane segments<sup>[1]</sup> with an endoplasmic reticulum (ER) retention signal in the C-terminal domain<sup>[4, 5]</sup>, two cytosolic extremities including two EF-hands (residues 720–797)<sup>[6]</sup>, a coiled-coil domain (residues 828–895)<sup>[6–8]</sup> and an acidic amino acid cluster (residues 798–827)<sup>[9, 10]</sup> (Figure 1). TRPP2 interacts with many proteins, primarily cytoskeletal components<sup>[11]</sup>. The acidic cluster can be recognized by phosphofurin acidic cluster sorting protein-1 (PACS-1) and PACS-2, and it regulates the trafficking of TRPP2 among the ER, the Golgi and the plasma membrane<sup>[9]</sup>. The coiled-coil domain contributes to homomeric assembly within TRPP2 or heteromeric assembly between TRPP2 and another TRPP family protein, polycystin-1<sup>[7, 12, 13]</sup>. Four TRPP2 subunits assemble into a functional ion channel (Figure 1). N- and C-termini of the TRPP2 protein contain dimerization domains, which are important

components of TRPP2 channel assembly<sup>[14, 15]</sup>. In addition to TRPP2, PKD2-like 1 (PKD2L1) and PKD2-like 2 (PKD2L2) are two other members of the TRPP subfamily of ion channels sharing structural homology with TRPP2<sup>[16]</sup>. TRPP2 has been implicated in various cellular processes including mechanosensation, polarity, apoptosis, cellular proliferation, mating behavior and directed sperm movement<sup>[17]</sup>.

Mutation of the *PKD2* gene is a major cause of autosomal dominant polycystic kidney disease (ADPKD), which is a common genetic disease of the kidney<sup>[18]</sup>. However, patients with ADPKD suffer from not only a defect in kidney function but also a systemic disorder associated with abnormalities in the vasculature, such as cerebral, intracranial, and aortic aneurysms and cardiac valvular dysfunction<sup>[19–21]</sup>. Therefore, cardiovascular complications are typically the leading cause of mortality and morbidity in ADPKD patients<sup>[22–26]</sup>. Evidence from the literature has shown that TRPP2 is widely expressed in the endothelial cells of mesenteric arteries<sup>[27]</sup> and the smooth muscle cells of cerebral arteries<sup>[28, 29]</sup>, the aorta<sup>[30–32]</sup>, and mesenteric arteries<sup>[31]</sup>, thus suggesting that TRPP2 may serve an important function in the cardiovascular system.

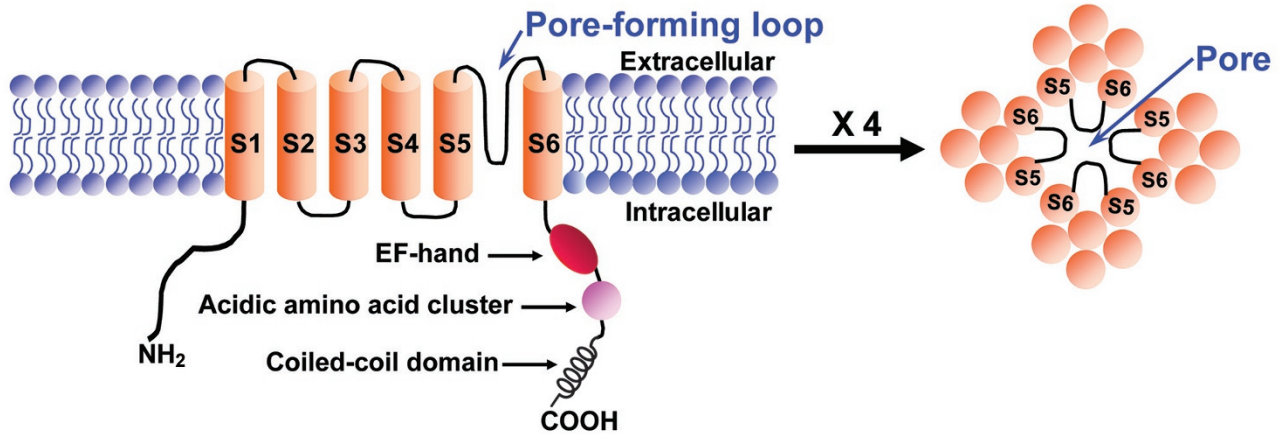
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## TRPP2's expression pattern and agonists and antagonists

TRPP2 is expressed in various tissues including epithelial cells, vascular smooth muscle cells, endothelial cells, cardiac



**Figure 1.** The structural features of a TRPP2 channel. A single TRPP2 subunit comprises six transmembrane segments (S1–S6) with a cation-permeable pore between S5 and S6. In the cytoplasmic C-terminus, there are EF-hands, a coiled-coil domain, and an acidic amino acid cluster. Four such units assemble into an ion channel.

myocytes, adrenal glands, and ovaries<sup>[33]</sup>. The subcellular localization of TRPP2 is dependent on its subsequent interaction with various trafficking proteins. TRPP2 contains an ER retention signal, which causes the protein to be retained in the ER compartment<sup>[4, 5]</sup>. TRPP2 is most prominently localized to the ER, plasma membrane, primary cilia, mitotic spindles, and lamellipodia<sup>[5, 34–41]</sup>. Numerous studies have indicated that transient receptor potential (TRP) isoforms have the ability to form homomultimeric or heteromultimeric channels with the same or other subfamilies. These new heteromultimeric channels typically have many novel biophysical properties, modes of activation, or simply more efficient trafficking of the ion conducting subunit to the plasma membrane<sup>[42]</sup>. To date, the literature has shown that the TRPP2 subunit might assemble into both homomeric and heteromeric complex channels with polycystin-1<sup>[43, 44]</sup>, TRPC1<sup>[29, 45–47]</sup>, TRPC3<sup>[48]</sup>, TRPC4<sup>[49]</sup>, TRPC5<sup>[50]</sup>, TRPC7<sup>[48]</sup> and TRPV4<sup>[51–53]</sup>.

The pharmacology associated with TRPP2 is poorly understood. So far, identification of a specific chemical antagonist acting on TRPP2 is still lacking. To examine the physiological function of TRPP2, Nauli *et al* have designed a specific antibody targeting the part of the first extracellular loop of mouse TRPP2 (residues 278–428) and thereby inhibiting pore function<sup>[34]</sup>. The authors found that inhibitory antibody completely prevented flow-induced  $\text{Ca}^{2+}$  influx into kidney cells, whereas a control antibody against the intracellular domain of TRPP2 (p96525) had no effect<sup>[34]</sup>. Amiloride,  $\text{La}^{3+}$ , and  $\text{Gd}^{3+}$  have been proposed to be TRPP2 inhibitors, although they do not specifically inhibit TRPP2. In addition, TRPP2 is inhibited by cytoplasmic pH and is modulated by the holding potential due to its  $\text{H}^+$  sensitive and voltage-dependent properties<sup>[54]</sup>. Abdi *et al* have reported that triptolide, an agonist of TRPP2, evokes TRPP2-mediated  $\text{Ca}^{2+}$  release from internal  $\text{Ca}^{2+}$  storage; however, the specificity of triptolide's action on TRPP2 is not well documented<sup>[28]</sup>. Therefore, the identification of the specific agonist and antagonist of TRPP2 should be a challenge for scientists in future studies.

### The function of TRPP2 in the regulation of intracellular $\text{Ca}^{2+}$ homeostasis

A debate exists with respect to the localization of TRPP2 in cells. Many studies have reported that TRPP2 located in the plasma membrane, along with polycystin-1, TRPV4 and TRPC1, bears a cationic current induced by mechanotransduction and is responsible for various physiological functions<sup>[27, 34, 43, 51, 55–57]</sup>. Other studies have shown that TRPP2 is expressed in the ER and either acts as a leak channel that lowers the  $\text{Ca}^{2+}$  concentration in the ER<sup>[58, 59]</sup> or acts in association with inositol 1,4,5-trisphosphate receptors ( $\text{IP}_3\text{R}$ ) and the ryanodine receptor (RyR) in modulating intracellular  $\text{Ca}^{2+}$  concentration ( $[\text{Ca}^{2+}]_i$ ) homeostasis<sup>[60–62]</sup>. A portion of the TRPP2 N-terminus interacts with RyR and thus reduces RyR-mediated  $\text{Ca}^{2+}$  release<sup>[60]</sup>. Additionally, the acidic cluster in the TRPP2 C-terminal cytoplasmic tail may interact with a cluster of positively charged residues in the  $\text{IP}_3\text{R}$  N-terminal ligand-binding domain. Through this protein-protein interaction, the local cytosolic  $\text{Ca}^{2+}$  concentration increases. This concentration increase is initiated by  $\text{Ca}^{2+}$  release via  $\text{IP}_3\text{R}$  and may activate TRPP2 and hence evoke further  $\text{Ca}^{2+}$  release<sup>[61, 62]</sup>. The decreased  $\text{Ca}^{2+}$  concentration in the ER then evokes the oligomerization of stromal interacting molecule 1 (STIM1), which is a  $\text{Ca}^{2+}$  sensor in the ER<sup>[63, 64]</sup>. Subsequently, oligomerized STIM1 activates a  $\text{Ca}^{2+}$ -permeable ion channel, Orai1, in the plasma membrane<sup>[65]</sup> and other store-operated channels<sup>[66]</sup>, thus resulting in  $\text{Ca}^{2+}$  entry. This process is referred to as store-operated  $\text{Ca}^{2+}$  entry (SOCE). The purpose of SOCE is to mediate  $\text{Ca}^{2+}$  influx to refill  $\text{Ca}^{2+}$  stores after depletion.

Much like the  $\text{Ca}^{2+}$  release channels in the ER, TRPP2 channels may partially contribute to the transient  $[\text{Ca}^{2+}]_i$  increase and the intensity of SOCE.  $\text{Ca}^{2+}$  entry after depletion of intracellular  $\text{Ca}^{2+}$  stores by treatment with caffeine and thapsigargin is significantly lower in TRPP2<sup>+/-</sup> mice than in wild type mice<sup>[32, 58]</sup>. Cytoplasmic and ER  $\text{Ca}^{2+}$  levels and SOCE have been found to be decreased in TRPP2-defective cholangiocytes compared with wild type cells, whereas the expression levels

of STIM1 and Orai1 were unchanged<sup>[67]</sup>.

### TRPP2's function in vascular smooth muscle cells

TRPP2 is broadly expressed in vascular smooth muscle cells, where it optimizes muscle contractility<sup>[30, 68]</sup>. Qian *et al* have found that, in vascular smooth muscle cells, TRPP2 is primarily located in the sarcoplasmic reticulum (SR) and in intracellular compartments, but polycystin-1 is located in the plasma membrane<sup>[32]</sup>. Despite these different subcellular localizations, the interaction between TRPP2 and polycystin-1 occurs across closely apposed plasmalemmal and SR membranes near the cell surface<sup>[32]</sup>. The interaction of TRPP2 and polycystin-1 have also been found at dense plaques, which are sites of cytoskeletal/plasma membrane/extracellular matrix interaction that provide a mechanical and functional link to the regulation of the elastic and contractile tension of the stretched vessel<sup>[32, 69, 70]</sup>.

The SR  $Ca^{2+}$  store and basal  $[Ca^{2+}]_i$  are decreased in *Pkd2*<sup>-/-</sup> arterial smooth muscle cells<sup>[58]</sup>. Furthermore, consistently with this finding, *Pkd2* mutations severely decrease the contractility of the visceral smooth muscle cells in *Drosophila*, which can be restored by expressing wild type *Pkd2* cDNA through a muscle-specific Gal4 driver<sup>[68]</sup>. Ren *et al* have also found that TRPP2 shRNA effectively suppresses TRPP2 protein expression as well as  $\alpha_1$ -adrenergic receptor agonist (phenylephrine)-induced vasoconstriction in the thoracic aorta and mesenteric arteries<sup>[31]</sup>. However, *Pkd2*<sup>-/-</sup> arteries exhibit exaggerated vasoconstriction and increased sensitivity to phenylephrine, which is caused by enhanced  $Ca^{2+}$ -independent force generation and increased contractile protein expression<sup>[71]</sup>. In high salt intake-induced hypertension, TRPP2 expression in vascular smooth muscle cells and phenylephrine-induced contractility of the aorta and mesenteric arteries are dramatically increased<sup>[31]</sup>. However, the weight of the aortic rings and the basal diameter of the mesenteric arteries is decreased<sup>[31]</sup>. Therefore, with the exception of blood vessel hypertrophy, the increase in the TRPP2 expression level in arterial smooth muscle cells appears to cause enhanced vasoconstriction induced by phenylephrine. One study has reported that in denuded aortic rings and mesenteric arteries, when  $Ca^{2+}$  stores are depleted through pretreatment with thapsigargin, no significant difference in the vasoconstriction between TRPP2 and control shRNA-transduced rats was observed<sup>[31]</sup>. These findings indicate that TRPP2 located in the ER membrane may be more important than TRPP2 located in the plasma membrane in terms of agonist-induced vasoconstriction. TRPP2 differentially regulates the myogenic response in different vascular beds. TRPP2 protein is primarily located in the plasma membrane of human and rat cerebral artery smooth muscle cells, in contrast to the expression observed in the ER or SR membrane in the mesenteric arteries and aorta<sup>[29, 31, 58]</sup>. TRPP2 knockdown by TRPP2 specific siRNA decreases swelling-activated cation currents in vascular smooth muscle cells and pressure-induced constriction in resistance-size cerebral arteries<sup>[29]</sup>. In addition, in vascular smooth muscle cells, an overabundance of free TRPP2 inhibits stretch-activated ion channels (SACs) through filamin A coupled to the actin cytoskeleton, but this inhibition can

be reversed by coexpression with polycystin-1<sup>[57]</sup>. This result indicates that the polycystin-1/TRPP2 ratio regulates SAC mechanosensitivity and pressure sensing.

### TRPP2's function in endothelial cells

In the circulatory system, the shear stress generated from hemodynamic blood flow is a major physiological stimulus inducing endothelial cell-dependent vascular dilation<sup>[72]</sup>. In the process of shear stress-induced vessel dilation, a key early event is the flow-stimulated  $[Ca^{2+}]_i$  increase in the vascular endothelial cells. TRPP2 is expressed in endothelial cells along with polycystin-1, TRPC1 and TRPV4, and it plays an important role as a mechanosensing  $Ca^{2+}$  channel. We have used a lentiviral construct carrying *TRPP2*<sup>D511V</sup> (dead mutant) to explore the functional role of TRPP2 in flow-induced vascular dilation<sup>[27]</sup>. Dilation was significantly reduced in the arteries isolated from lenti-*TRPP2*<sup>D511V</sup>-treated rats compared with arteries from empty lentivector-treated rats, which suggests the participation of TRPP2 in flow-induced vascular dilation.

TRPP2 shows mechanosensitive properties when it associates with polycystin-1 (formerly TRPP1), thereby functioning as a mechano-sensor. However, TRPP2 may lose its mechanosensitive activity when it is expressed alone<sup>[3, 34, 57]</sup>. TRPP2 may owe its mechanosensitive properties to coupling with other TRP subunits, presumably TRPC1 and/or TRPV4<sup>[27, 52, 73]</sup>. Knockdown of the TRPP2 protein significantly decreases the initial stretch-induced  $[Ca^{2+}]_i$  increase, whereas TRPC1 knockdown significantly decreases the later  $[Ca^{2+}]_i$  increase<sup>[45]</sup>. However, the combined knockdown of TRPP2 and TRPC1 is necessary to significantly decrease the stretch-induced  $[Ca^{2+}]_i$  changes<sup>[45]</sup>. These findings suggest that TRPP2 channels may substantially contribute to the  $[Ca^{2+}]_i$  increase in the first few minutes after stretch stimulus.

TRPP2 may associate with TRPV4 and TRPC1, thus forming heteromultimeric channels mediating flow-induced  $Ca^{2+}$  influx in TRPV4, TRPC1, and TRPP2 co-overexpressing HEK293 cells and rat mesenteric artery endothelial cells<sup>[27]</sup>. In this cross-subfamily assembly of TRPs, TRPV4 is essential to the flow-induced response. However, the heteromultimeric channels may display different properties from those of the homomultimeric channels. Flow-induced  $Ca^{2+}$  increases are transient in HEK293 cells overexpressing TRPV4 alone but become sustained when TRPV4 is co-expressed with TRPP2 or TRPC1<sup>[27]</sup>. PKG-mediated inhibition of TRPV4-TRPC1-TRPP2 channels might contribute to the transient nature of the flow-induced  $[Ca^{2+}]_i$  increase in vascular endothelial cells<sup>[27]</sup>. Through this negative feedback mechanism,  $Ca^{2+}$  influx would stimulate a nitric oxide (NO)-cGMP-PKG signaling cascade and cause the inhibition of  $Ca^{2+}$  entry channels<sup>[27]</sup>.

Mechanical stress can quickly lead to the dysfunction of the microvessel endothelial cells, including the disruption of the blood-brain barrier (BBB)<sup>[45]</sup>. Mechanical stress and  $[Ca^{2+}]_i$  elevation can produce functional modification of the tight junction complex, which leads to barrier leakiness<sup>[74, 75]</sup>. The mechanical stress delivered to the brain tissue during traumatic brain injury is associated with two ion channels: TRPP2

and TRPC1<sup>[45]</sup>. These channels appear to be largely responsible for the prolonged dynamics<sup>[45]</sup>.

An abnormal response to hyperemia and endothelium-dependent vasorelaxation has been found in ADPKD patients and in different animal models of ADPKD<sup>[20, 21]</sup>. ADPKD patients display a reduction in endothelium-dependent vessel dilatation in conduit arteries during the increase of sustained blood flow, which is associated with the complete loss of NO release<sup>[76]</sup>. Karen *et al* have found that the aorta in an autosomal recessive ARPKD animal model had abnormal endothelium-dependent vascular reactivity, likely due to a primary defect in the endothelial cells that occurred prior to the changes in mean arterial pressure or renal function. However, the endothelium-independent relaxation, for example, that in smooth muscle cells, was not impaired<sup>[19]</sup>.

### TRPP2 in blood pressure regulation

A portion (10%–20%) of children with ADPKD presents with hypertension symptoms, and the majority of adults exhibit hypertension before any loss of kidney function has occurred<sup>[77]</sup>. Hypertension relates to progressive kidney enlargement and is a significant independent risk factor for the progression toward end stage renal disease<sup>[78, 79]</sup>. The pathogenesis of hypertension in ADPKD is complex and dependent on many factors that influence each other. Defects in the primary cilia causing endothelial dysfunction and the activation of the renin-angiotensin-aldosterone system are central pathophysiologic explanations for the development of hypertension in ADPKD<sup>[80]</sup>. The treatment of hypertension effectively reduces cardiovascular mortality and may also slow the progression of kidney disease<sup>[81, 82]</sup>.

Decreased or absent polycystin-1 or TRPP2 protein expression is associated with abnormal vascular structure and function. A *Pkd1* (which encodes polycystin-1) /*Pkd2* deficiency results in decreased NO levels and altered endothelial response to shear stress with the attenuation in vascular relaxation in ADPKD patients<sup>[20, 21]</sup>.

Kocyyigit *et al* have demonstrated that ADPKD patients with hypertension have low levels of endothelial nitric oxide synthase (eNOS) expression compared with patients without hypertension and that eNOS expression is an independent predictive factor of hypertension in the ADPKD population<sup>[83]</sup>. In addition, TRPP2 protein expression has been shown to be dramatically increased in the vascular smooth muscle cells of the thoracic aorta and mesenteric arteries in high salt intake-induced hypertensive rats compared to age-controlled rats<sup>[31]</sup>.

### Conclusion and perspectives

TRPP2 is widely expressed in the endothelial cells and smooth muscle cells of diverse vascular beds. Even dysfunctional mutation of TRPP2 causes severe renal dysfunction, and vascular complications are the primary reason for mortality and morbidity. Therefore, TRPP2 is considered to be an important target for modulating vascular function. However, the physiological and pathological functions of TRPP2, as well as their underlying molecular mechanisms in the vascular system,

are still not completely understood. The conditional TRPP2 knockout model of the vascular system might be a powerful approach to address this issue. However, specific chemical agonists and antagonists of TRPP2 are largely lacking, thus hindering the elucidation of TRPP2's functional role in various tissues. Collaboration between researchers in various fields is needed for identifying the TRPP2-specific agonists and antagonists. These compounds will benefit both the investigation of physiological function and the development of therapeutic drugs in the future.

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